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Title: Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform

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Abstract: A dynamic flow-through microcolumn extraction system based on extractant recirculation is herein proposed as a novel analytical approach for simplification of bioaccessibility tests of trace elements in sediments. On-line metal leaching is undertaken in the format of all-injection (AI) analysis, which is a sequel of flow injection analysis, but involving extraction under steady-state conditions. The minimum circulation times and flow rates required to determine the maximum bioaccessible pools of target metals (viz., Cu, Zn, Cd, and Pb) from lake and river sediment samples were estimated using the Tessier's sequential extraction scheme and an acid single extraction test. The on-line AIA method was successfully validated by mass balance studies of CRM and real sediment samples. Tessier's test in on-line AI format demonstrated to be carried out by one third of extraction time (6 h against more than 17 h by the conventional method), with better analytical precision (< 9.2% against > 15% by the conventional method) and significant decrease in blank readouts as compared with the manual batch counterpart.

Response to reviewer’s comments # TAL-D-15-00851

Dear Prof. Toshihiko Imato,

We are greatly appreciative of your decision regarding our article and thank you very much for your comments. We have revised the manuscript according to the comments made by the two reviewers. Corrected or deleted sentences appear in blue font in the new manuscript, including text, tables, figures, and figure captions. Please also find below the answers to these comments. We would be grateful if you could quickly inform us of any potential problems with the revised manuscript.

For reviewer #1:

The manuscript definitely presents improvements for the analysis of bio-accessible trace metals in sediment samples. However, this manuscript is not suitable for publication in a high quality journal such as 'Talanta', regarding the improvements presented in this work. For resubmission to another journal, several aspects should be considered:

We revised the manuscript substantially, according to comments of reviewer. The poor repeatability of the analytical data obtained by AI-based leaching before was a contamination of the flow-line. We must apologize that it took a while to find out the problems.

We believe that the revised version will be worth to be acceptable in Talanta. Please review our new achievements again and full re-writing of all sections of the manuscript including abstract, introduction, experimental and results and discussion.

Furthermore, we would like to respond each question and suggestion raised by the reviewer as follows

- First of all, the authors should consider thorough review of the manuscript with regards to English language and grammar. Especially abstract and introduction are in large passages hard to read, contain several mistakes, and some sentences are confusing, or unclear.

We largely revised the manuscript with thorough proof-reading by co-authors, re-writing of misleading sentences and editing by a native English speaker prior to resubmission. Moreover, we had edited by the English Editing Company.

- The conclusion states that the presented method represents improvements due to the decreased consumption of sample amount and solvent. Both factors are irrelevant, as sediment samples were analyzed which are usually available in large amounts. Also, decreasing the amount of extractants is not very important, as cheap and not very harmful solvents are used.

We deleted the consumption of sample amount in text. However, treatment of waste solutions in Japan is very strict. The waste with all heavy metals must be handled to the specialized waste liquid manager regardless the degree of toxicities of metals contained. The amount of waste solution that we might submit at one time is rather restricted, for example, the limitation is of 18 L in our university. This fact is inconvenient for researchers who do experiments on heavy metal analyses in water and soil. Therefore, the reduction in the generation of waste solution is strongly recommended and we do offer a good fractionation method along this line.
Accordingly, we described the reduction of generation of waste (extracts containing metal species) in conclusions on lines 10-11 in p. 21 in the revised text.

- Furthermore, it is stated that the analytical precision is improved. Based on the results presented in figure 6, I would not agree with that. The error bars of the AI method are sometimes larger, sometimes smaller than those of the batch results. At least these results do not indicate better precision. If the presented method indeed increases the precision of the method, respective results must be show to confirm this. No RSDs or LODs for the determinations are presented.

We have rechecked data and repeated experiments owing to the suggestion on the precisions and accuracies pointed out by reviewer #1. We listed the revised changes below:

1. **We changed all flow-lines of the AI device by new tubing, in order to check potential system contamination effects. The analytical performance of the FAAS detection system including LODs is given in the new Table S1.**
2. **We determined the concentration of analyte metals contained in the reagent solutions used in the extraction procedures as a blank test, that is, a test for which sediment samples were not contained in the analytical procedures. In Figures and Tables of the revised version, we reported actual metal concentrations after subtracting blank signals. Additionally, the values obtained in the blank test are summarized in Table S4 in Supplementary Materials.**
3. **Because the accuracy and precision of analyses could successfully be improved, we updated the text as follows;**
   <u>Fig. 4 in old MS → Fig. 3 in new MS</u>: "Investigation of the magnitude of bioaccessible pools of Cu and Pb in AI-based extraction as a function of circulation time using 2 mL of 1.0 mol L^{-1} $MgCl_2$ (a) and 1.5 mL of 1.0 mol L^{-1} HCl (b) as extractants.".

Fig. 5 in old MS → Fig. 4 in new MS: "Evaluation of data correlation of bioaccessible metal concentrations in 7 different river sediment samples as obtained by the leaching test with 1.0 mol L^{-1} HCl using both batchwise and AI methods.".

Fig. 5 in old MS → Fig. 4 in new MS: "Data comparison of sequential extraction of 4 metals from lake sediment CRM (top) and Watarase River sediment (bottom) by the batch against the AI method.".

4. **The RSDs of analyte metals in replicate experiments were listed on lines 8-9 in p. 16, and lines 8-10 in p. 17 in the revised text. Also, RSDs of Cu and Pb extracted from a lake sediment sample by the optimal AI method were summarized in Table S2 in Supplementary Materials.**

- The title containing the phrase 'on-line' suggests that on-line measurements are preformed. After reading the manuscript, it is totally clear that no on-line measurements have been carried out, due to the long reaction time required. The title should eventually be changed to delimit on-line measurements from the kind of analysis performed in this work. Suggestion: Circulating-flow leaching and fractionation or Dynamic leaching and fractionation…

According to reviewer's advice, we changed the title as following;

Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform

- Samples from seven different sampling sites were taken. What are the results of these investigations? In Figure 6 results for sample 7 are presented - what about the other samples? Even though the sampling sites are described in detail and even were devoted an own figure with their distribution along Watarase River, I cannot find a summary or discussion of the found results. If the aim of this study was to investigate the distribution profile of heavy metals along this river, the results have to be shown and discussed.

We performed the fractionation of analyte metals of sediment at Watarase River as the practical application of the AI method. This is now mentioned in the last paragraph of the introduction section of the revised text (lines 5-10 in p. 7). The new results and discussion thereof were described in detail on lines 5-17 in p. 20. The analytical data of metal partitioning in the 7 collected riverbed samples was added as Fig. 6 in the revised version.

Also, the "Collection sites of the various sediment samples from Watase River" in Fig. 3 of the old text was deleted in the revised text, and moved to Fig. S1 in the revised Supplementary

Materials.

- P.17, l.33-42: The authors state that the deviation of the AI and the batch values might be caused by the large RSD of the batch concentrations. In this case, the values should be randomly distributed around the regression with a slope not significantly different from 1. The slope of 0.84 rather indicates a systematic error, as it is also stated in the text. However, simply stating that the Zn concentrations might be affected by contaminations is not enough, in my point of view. The deviations might also be caused by other systematic errors, which would lead to the conclusion that the presented setup is not suitable for the reliable analysis of Zn. It must be possible to carry out the batch analysis without contaminations. Presentation of results without this bias, or clear experimental findings that contaminations are such a big problem have to be presented to underline the made assumptions.

As mentioned above, we rechecked the concentrations of metal extracted in the AI and batch methods, and reported the experimental RSD as repeatability tests. We changed all flow-lines in the flow-based device, and determined the concentrations extracted from sediment samples, as well as in blank tests. Consequently, because analytical errors were largely reduced, the figures except Figs. 1 and 2 were replaced by new ones with improved analytical performance and lack of bias. (see lines 7 - 15 in p. 19 of Results and Discussion).

- No experimental details for the F-AAS method are described. How have the standards been prepared, which analytical wavelengths were used, other measurement conditions, etc. Description of these parameters is crucial to reproduce the results.

Details for FAAS detection are now summarized in Table S1 in Supplementary Materials, and described in line 15 in p. 18 of the revised text.

Also, for clarification of the manuscript, some minor comments should be addressed:

- How was the determination of the particle size distributions performed? Figure 3 shows values for all samples, but the measurement is not described in the manuscript.

We have asked a Kusaki Dam Operation and Maintenance Office employee from Japan Water Agency (Numata, Gunma Pref., Japan) for collection of sediment samples at seven different river beds along the Watarase River in Gunma Prefecture, Japan. The particle size distribution was

carried out by the staff. This work could not be done by us because of legal regulations in the Watarase River area. We have however added a new Table (Table 1) summarizing the physicochemical characteristics of the seven sediment samples analysed (see fractionation results in Fig. 6) and appropriate acknowledgment for company assistance in the revised text.

- Please, describe in detail how the samples were taken. Were they taken from the surface, or below, etc. Representative sampling is the main prerequisite for reliable results.

As mentioned above, sampling was undertaken by Kusaki Dam Operation and Maintenance Officers at Japan Water Agency. We cannot describe further details in the text due to legal regulations in the Watarase River area. However, we added relevant information of the sampling method on line 16 in p. 10 – line 5 in p. 11 in the revised text and sediments characterization in Table 1.

- On p.11, l.46-53 the filling of the columns is described. How was the packing performed? Was the sample material just loosely filled in, or was it pressed? What was the flow rate for those experiments? Was there any back pressure? If so, how high was it?

The packing of the sediment sample in the column was not done by pressure because of the increase of column backpressure. The explanation of sample packing is described in details on lines 5 – 10 in p. 8 in the revised text. In order to illustrate the process of sample packing, the picture of the sediment laden microcolumn in Fig. 1 was slightly revised. Information about flow rate and experimentally measured backpressure is given in the text.

- P.12, l. 49: At which temperature was the evaporation step performed?

Thank you for your comment. The temperature in the evaporation step was 107 ^{o}C using a sand bath. We added it on line 1 in p. 13 in the revised text.

- P.12, l.55: What was the concentration of the HCl?

The concentration of HCl was 12 mol L^{-1}. We added it on line 3 in p. 13 in the revised text.

- P.16, l.12: What was the number of replicates for determination of the RSD?

Thank you for your comment. The number of replicates was five. We added it on lines 8-9 in p. 16 in the revised text.

- P.16, l.38: I guess, Pb and not Pd was analyzed.

We corrected from Pd to Pb on line 17 in p. 16 in the revised text.

- P.17, l.20-30: Why is the variability of the extracted metal concentrations higher if the analyte concentrations are lower? The higher deviations might rather be associated with the low signal intensities achieved for the low Cd concentrations. What was the limit of detection for Cd in this case, and compared to that, what were the signals/concentrations obtained for the sample extracts?

As mentioned above, we have now improved the variability of the extracted metal concentrations, including Cd, by eliminating contamination episodes of the flow-through system. The LODs of analyte metals by the FAAS are as follows: Cu: 11.7 mg L^{-1} at 324.8 nm, Zn: 3.5 mg L^{-1} at 213.9 nm, Cd: 1.3 mg L^{-1} at 228.8 nm, and Pb: 18.9 mg L^{-1} at 283.3 nm. The signal/concentration in LOD ratios (n = 5) were 2.9±0.1 in Cu, 1.4±0.4 in Zn, 0.23±0.02 in Cd, and 0.21±0.02 in Pb. Accordingly, with the new results reported we might conclude that the sensitivity and LOD of the FAAS do suffice for accurate quantification of leachate concentrations in both CRM and real sediment samples.

- Image quality of the figures is very weak

We have improved the image quality of the figures by changing from TIFF to JPEG.

For reviewer #2:

The authors should mention on the low R coefficient for the slope of the Cd calibration plot and how this can affect the reliability of the results.

We re-checked the whole figures and tables in order to improve the analytical performance. Consequently, the analytical data, including R of Cd was largely improved. In the revised version, the improved analytical data is given in Fig. 4.

Authors should add ultrasonication in future works to speed the extraction procedure.

The reviewer's suggestion is excellent good. We will try in future work to implement bathsonication in the AI-flow system.

Finally I would like to congratulate the authors for this nice work.

We were honored that this paper was well-estimated by reviewer #2. We will aim at further improvement of the analytical performance for extended applicability in ensuing papers.

Otherwise, we revised or replaced the references, figures or tables in the revised version, as the follow list.

1. We replaced the refs. 2–5, 9 and 10 in the old text into refs. 2-5, 8 and 9 in the revised text, respectively. Ref. 6 in old text was deleted by the suggestion from co-authors. Consequently, the reference numbers of 7 – 40 in old text were replaced into those of 6 – 39 in new text, respectively.

Old text

[2] G. Rauret, Extraction procedures for the determination of heavy metals in contaminated soil and sediment, Talanta, 46 (1998) 449–455.

[3] K.-J. Hong, S. Tokunaga, T. Kajiuchi, Evaluation of remediation process with plant-derived biosurfactant for recovery of heavy metals from contaminated soils, Chemosphere, 49 (2002) 379–387.

[4] W. Hartley, R. Edwards, N.W. Lepp, Arsenic and heavy metal mobility in iron oxide-amended contaminated soils as evaluated by short- and long-term leaching tests, Environ. Poll. 131 (2004) 495–504.

[5] D. Dermatas, X. Meng, Utilization of fly ash for stabilization/solidification of heavy metal contaminated soils, Eng. Geol. 70 (2003) 377–394.

[9] P. Svete, R. Milacic, B. Pihlar, Partitioning of Zn, Pb and Cd in river sediments from a lead and zinc mining area using the BCR three-step sequential extraction procedure, J. Environ. Monit. 3 (2001) 586–590.

[10] A. Smeda, W. Zyrnicki, Application of sequential extraction and the ICP-AES method for study of the partitioning of metals in fly ashes, Microchem. J. 72 (2002) 9–16.

New text

[2] C. R. M. Rao, A. Sahuquillo, J. F. Lopez-Sanchez, A Review of the Different Methods Applied in Environmental Geochemistry For Single and Sequential Extraction of Trace Elements in Soils and Related Materials. Water Air Soil Pollut. 189 (2008) 291-333.

[3] ISO/TS 17402:2008. Soil quality-Requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials. Geneva, Switzerland, 2008.

[4] A.V. Filgueiras, I. Lavilla, C. Bendicho, Chemical sequential extraction for metal partitioning in environmental solid samples, J Environ Monit. 4 (2002) 823-57.

[5] J.R. Bacon, C.M. Davidson, Is there a future for sequential chemical extraction? Analyst, 133 (2008) 25-46.

[8] T. Yoshida, Leaching of zinc oxide in acidic solution, Mater. Trans. 12 (2003) 2489–2493.

[9] K. Huang, K. Inoue, H. Harada, H. Kawakita, K. Ohto, Leaching behavior of heavy metals with hydrochloric acid from fly ash generated in municipal waste incineration plants, Trans. Nonferrous Met. Soc. China, 21 (2011) 1422–1427.

2. Table 1 in the old text was replaced into Table 2 in the revised text. Also the numbers of significant figures in the values were partially revised.

3. In the revised Supplementary Materials, Table 3, Comparative data on sequential extraction of Cu, Zn, Cd and Pb in sediment samples in a CRM sediment (NMIJ-CRM 7303-a) and a riverbed sediment in Watarase River (Gunma Pref., Japan) by AI and batch methods (n = 3), to assist comparison of accuracies obtained by AI and batch methods in the Fig. 5.

Highlights for

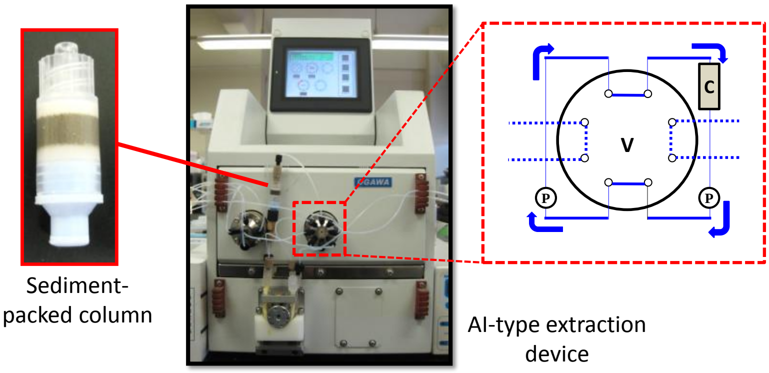
Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform

- ▶ An all injection method was utilized for extraction of metals from solid materials.
- ▶ Metals from sediment samples were extracted using inline circulation.
- ▶ Accuracy of metals extracted by this system was improved compared with the batch method.
- ▶ Four-step sequential extraction could be intermittently performed.

***Graphical Abstract (for review)**

Graphical Abstract for

Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform



Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform[1]

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[1] **Abbreviations:** AI, all injection; AIA, all injection analysis; CRM, certified reference material; FAAS, flame atomic absorption spectrometry; RSD, relative standard deviation.

Abstract

A dynamic flow-through microcolumn extraction system based on extractant recirculation is herein proposed as a novel analytical approach for simplification of bioaccessibility tests of trace elements in sediments. On-line metal leaching is undertaken in the format of all-injection (AI) analysis, which is a sequel of flow injection analysis, but involving extraction under steady-state conditions. The minimum circulation times and flow rates required to determine the maximum bioaccessible pools of target metals (viz., Cu, Zn, Cd, and Pb) from lake and river sediment samples were estimated using the Tessier's sequential extraction scheme and an acid single extraction test. The on-line AIA method was successfully validated by mass balance studies of CRM and real sediment samples. Tessier's test in on-line AI format demonstrated to be carried out by one third of extraction time (6 h against more than 17 h by the conventional method), with better analytical precision (< 9.2% against > 15% by the conventional method) and significant decrease in blank readouts as compared with the manual batch counterpart.

Keywords: bio-accessibility, leaching test, sequential extraction, trace element, sediment

1. Introduction

Elevated concentrations of metals in soils, sediments and airborne particulate matter might pose severe concerns to the public health. For example, the bioaccessible metal pools in sediments are known to influence the distribution and composition of benthic assemblages [1].

Risk assessment of trace element contamination in solid samples such as soils, solid wastes and river/ocean sediments is commonly undertaken by eluting the metals via leaching tests by utilizing water, electrolytes, acids (acetic acid or hydrochloric acid), reductants and oxidants as extractants so as to release target species associated to given mineralogical phases. Specifically, the purpose of leaching tests is to determine the aqueous phase concentrations of metals that are released from solids under environmentally simulated conditions, that is, the bioaccessible metal pools in environmental scenarios or alternatively when placed in land disposal units. The idea behind is to evaluate the potential harmful effects to biota and humans as a result of leaching of metal species into groundwater through a soil body or into marine ecosystems. Numerous laboratory leaching or bioaccessibility tests have been reported over the past few decades [2-5]. For example, in the US and Canada, the toxicity characteristic leaching procedure (TCLP) [6] is a commonly utilized leaching test that employs acetic acid or acetic acid/acetate buffer (pH 2.88 or 4.93, respectively) to evaluate metal leaching from solid wastes. Also, leaching tests that utilize HCl, with or without pepsin, mimicking gastric digestion, assess potential hazardous effects to human health when bringing toxic metals from the mouth to the stomach by directly taking solids such as soil, sediment, fly ash, or toys [6-10].

Single extraction methods are used to provide a rapid snapshot of metal leaching from environmental solids or solid wastes [11,12]. However, sequential extraction or fractionation tests have been commonly the method of choice so as to provide regarding detailed insight into different metal-soil associations [2,4-5,13-17], by exploiting a number of extractants of increasing elution strength. Fractionation studies are aimed at determining the chemical forms in which metals occur, because metal mobility, bioaccessibility and bioavailability strongly depend on their chemical and mineralogical forms [2,4-5, 13-17]. Sequential extraction protocols individually separate 4~6 pools of chemical forms of metal species that have different leachability, including the water soluble fraction, the ion-exchangeable fraction, the carbonate fraction, and metals bound to Fe-Mn oxyhydroxides and organic matter. The most widely accepted fractionation tests are the so-called 3-step BCR developed under the auspices of the Community Bureau of Reference (formerly BCR, but currently termed the Standard, Measurement and Testing

(SM&T) Programme of the Commission of the European Communities) [18, 19], and the Tessier scheme [20], where bioaccessible metals are associated to the exchangeable, acid soluble, reducible and oxidisable phases in soils and sediments. The classifications of chemical forms of metal species by sequential extractions are usually performed by batch-wise methods with tedious and time-consuming unit operations such as filtration, centrifugation, and reagent replacement. Those manual operations are likely prone to sample contamination, and they often lead to poor data accuracy.

To overcome the above issues of batchwise bio-accessibility tests, novel (semi)automatic or fully automatic flow systems for dynamic extraction procedures that may afford the addition of extractant(s), on-line extraction of metals, filtration of remaining solids, and alternatively, on-line analysis of extracts have been developed over the past few years [21-34].

A common procedure for on-line dynamic bioaccessibility tests of metal species in environmental solids is as follows [26]: 1) a soil sample is packed into a reactor, e.g., coil, column, or chamber; 2) an extractant is brought into the reactor using a pump; 3) the leachates are analyzed off- or on-line by an atomic spectrometric detector. A flow-based setup configurations for fractionation schemes have been developed so that the extractants of each specific chemical form of target metals could be intermittently loaded into the reactor from reagent reservoirs by switching valves [31-34]. Miró *et al*. developed sequential extraction methods in a dynamic flow-through format based on the distinct generations of flow injection analysis so as to ameliorate the extraction efficiency and assure leaching of bioaccessible pools of trace metals under “worst-case” extraction conditions [25-27, 30-34]. In the development of flow-based dynamic extraction systems, a great deal of attention has been focused on assembling analytical devices for repeatable unit operations and maximization of the reaction time between sample and reagent to get insight into the overall bioaccessible metal species.

In this study, a novel extraction system is proposed that could efficiently extract trace elements from riverbed sediment samples using a micro-column and by circulating the extract instead of shaking, stirring or continuous pumping of the extractant(s) toward and through the packed column. This idea is based on the principle of all injection analysis (AIA), which was first described by Itabashi *et al*. [35]. In AIA, all solutions are injected into a reaction coil and are circulated for a pre-set amount of time at a fixed flow rate. Because the solution (extractant) feed is stopped during the circulation process, the reagent consumption is significantly decreased as compared with conventional continuous-flow or flow injection manifolds.

Our goal is to demonstrate whether a circulating-flow platform based on the AIA principle is a suitable flow-based method to obtain reproducible patterns of leached metal species. The extraction performance of the AIA device was evaluated using an acidic single extraction test and the Tessier's sequential extraction procedure with the AIA features as a novel leaching approach critically compared against those of conventional batch leaching tests. As a practical application, sediments sampled along the Watarase River until Kusaki dam-lake located at the down-stream were on-line fractionated by the Tessier scheme in AIA fashion. As a result of mining activities from the Ashio copper area in Tochigi and Gunma Prefectures (1890s to 1990s), large amounts of metal species were discharged to the Watarase river, whereupon appropriate sediment and water management schemes has been continuously performed.

2. Experimental

2.1. All injection-type devise for metal leaching

The AI-type extraction device, AIA-IT3, was manufactured by Ogawa, Co., Ltd. (Kobe, Japan) and Kurahashi Giken, Ltd. (Kyoto, Japan) in cooperation with our research group. AIA-IT3 consisted of an 8-port rotary valve, a 4-port switching valve, a peristaltic pump, and a process control monitor (see details in Fig. 1). The tubes used to connect valves, sample-packed column, and reagent reservoirs were made of Teflon® (1 mm i.d.), and that of the pump was made of silicon (2 mm i.d.). The 4-port valve, which feeds the extractant into the re-circulation channel, was connected in series with the 8-port valve. The 8-port valve, in which the extractant leaches metals from the solid sample, as illustrated in Fig. 1 and 2. The sediment samples were packed into a microcolumn consisting of a Rezorian™ cartridge (1 cm^3 of inner volume, 5 cm long, Sigma-Aldrich, St. Louis, MO, US). The samples (0.25 g) were loosely packed to a thickness of 10 mm in the column, and PTFE membrane filters (pore size: 0.20 µm; thickness: 20 mm, Advantec) were placed at both ends. The column was connected to the manifold via luer-lock adaptors and nuts (see magnified view in Fig 1).

The sample-laden column was integrated in one of the loops of the 8-port rotary valve. The extractant was first loaded into the loop of the rotary valve (Fig. 2), and then circulated for a specific timeframe at a constant flow rate through the column.

2.2. Extraction procedure

The AI-based leaching procedure for leaching of trace elements from solids is schematically presented in Fig. 2. First, a given extractant is brought to the circulation channel at the 8-port valve (Fig. 2a), by activation of the peristaltic pump and selection of the appropriate port at the switching valve. By changing the valve position of the 8-port valve, the flow-line manifold is switched to a closed-line arrangement in order to circulate the extractant for a specific period, usually 0.5–5 h (Fig. 2b). During the extraction, the flow-line, except for the circulation channel, is stopped. Upon returning the valve to the original position, the re-circulated extract solution is flushed out from the 8-port valve with a pure water feed (Fig. 2c).

In our system, the circulation channel of the 8-port valve was filled with 1.5 or 2 mL solution, which was finally flushed out from the rotary valve and collected in a 20 mL volumetric flask. The volume was made up to 20 mL by adding pure water prior to analysis by flame atomic absorption spectrometry (FAAS, *ca*. 5 mL-per-injection). The detection instrument was a polarized Zeeman FAAS (Hitachi Z-5310, Japan). Hollow cathode lamps for FAAS were purchased from Hamamatsu Photonics, Ltd. (Hamamatsu, Japan). The analytical characteristics of the FAAS methods are summarized in Table S1 in Supplementary Materials.

2.3. Reagents and samples

All reagents used were of analytical grade, and were purchased from Wako Pure Chemical Industries, Ltd. The water used in this study was purified using a water distillation apparatus (ASK-2DS, Iwaki). Concentrated hydrochloric acid was diluted with water to a concentration of 1.0 mol L^{-1} for the single extraction test. Extractants for Tessier’s test were prepared as follows: A 1.0 mol L^{-1} solution of magnesium chloride was prepared by dissolution of the salt, and aqueous sodium hydroxide was added to adjust the pH to 7 (extractant 1, F1 in Fig. 2). A solution of 1.0 mol L^{-1} ammonium acetate at pH 5 was prepared by dissolving the appropriate amount of salt in water with pH adjustment using HCl (extractant 2, F2 in Fig. 2). A 0.04 mol L^{-1} solution of hydroxylamine hydrochloride was prepared by dissolving the reducing reagent in 25% (v/v) acetic acid (extractant 3, F3 in Fig. 2). An 8.8 mol L^{-1} solution of hydrogen peroxide was prepared by mixing 90 mL of the concentrated hydrogen peroxide and 8.5 mL of pure water; pH was adjusted to 2.0 using concentrated nitric acid before making up to 100 mL with pure water (extractant 4, F4 in Fig. 2).

A certified reference material (CRM), NMIJ CRM 7303-a, was purchased from the National Institute of Advanced Industrial Science and Technology (Tsukuba, Japan), and used as the reference lake sediment. Sediment samples were collected from seven different riverbed locations along the Watarase River in Gunma Prefecture, Japan (Table 1 and Fig. S1 in Supplementary Material). The riverbed sediments were collected from the surface using an Ekman-Birge grab by staff of Kusaki Dam Operation and Maintenance Office from Japan Water Agency (Numata, Gunma Pref., Japan), who provided us with measurements of particle sizes distributions in every sample using the Bouyoucos hydrometer method. The sediment samples were air-dried at ambient temperature for 2–3 days in our laboratory, pulverized using a mortar and pestle, and sieved through a 2-mm screen and the below 2-mm fraction collected for further analysis.

2.4. Acidic leaching test (single extraction procedure)

The leaching test in a batchwise format was performed according to the Soil Contamination Countermeasures Act of Japan [7]. To this end, one gram of the sediment sample was added to 6.0 mL of 1.0 mol L^{-1} HCl, and the mixture was shaken for 2 h. The residue was separated from the solution by centrifugation at 3000 rpm for 30 min (Kokusan H-200 Centrifugal Separator; Kokusan, Tokyo, Japan). The separated solution was filtered through a 0.45 µm PTFE membrane filter, before making up to 20 mL with pure water.

In the AI leaching method, 0.25 g of sediment was packed into the cylindrical column described above. Thereafter, 1.5 mL of 1.0 mol L^{-1} HCl was loaded in the circulation channel and circulated for a pre-set time interval (0.5, 1, 2, 3, 4, and 5 h). Then, the solution was collected, and made up to 20 mL using pure water.

2.5. Sequential extraction procedure (Tessier's fractionation scheme)

2.5.1. Batch sequential extraction

To estimate the bioaccessibility and mobility of trace elements from sediments the target metals were classified in four fractions, depending on the metal speciation in the sample, and in accordance with the sequential extraction method described by Tessier *et al.*: Fraction 1 (F1), ion exchangeable; Fraction 2 (F2), bound to carbonates; Fraction 3 (F3), bound to iron and manganese oxides; and Fraction 4 (F4), bound to organic matter [20].

The operational procedure for the batch method was as follows: F1 was generated by stirring (600 rpm) 1.0 g of the sample in 8 mL of 1.0 mol L^{-1} $MgCl_2$ (pH 7) at ambient temperature for 1 h. F2 was generated by stirring a mixture of F1 residue and 8.0 mL of 1.0 mol L^{-1} CH_3COONH_4 (pH 5) at ambient temperature for 5 h. F3 was generated by stirring a mixture of F2 residue and 20 mL of 0.04 mol L^{-1} $NH_2OH{\cdot}HCl$ (in 25% (v/v) CH_3COOH) at 96±3 °C for 6 h. F4 was generated by stirring a mixture of F3 residue and 8.0 mL of 8.8 mol L^{-1} H_2O_2 with nitric acid at 85±2 °C for 5 h. The final residue after F4 was dissolved in 10 mL of 46% (w/w) HF and 2.0 mL of 60% (w/w) $HClO_4$. The mixture was then evaporated to dryness, and 10 mL of 46% (w/w) HF and 1.0 mL of 60% (w/w) $HClO_4$ were added. The resulting mixture was again evaporated at 107 ^{o}C in a sand bath to dryness and 1.0 mL of 60% (w/w) $HClO_4$ was added. Finally, the residue was completely dissolved by 3 mL of 12 mol L^{-1} HCl.

The FI-F4 fractions were centrifuged at 3000 rpm for 30 min. The extracts (except for F3) were diluted with water to a final volume of 20 mL, and the concentrations of metals were determined by FAAS.

2.5.2. Sequential extraction by the AI method

The extractants in the AI fractionation method were the same as those indicated for the batch method. The extraction procedures for F1–F4 were successively carried out in the same way as the batch method is performed. The liquid-to-solid ratios of F1, F2, and F4 were also the same as those in the batch method; specifically, 2 mL of liquid (reagent) and 0.25 g of solid (sediment sample) were utilized. The liquid-to-solid ratio of F3 in the AI method differed from that of the batch method, because the channel loop of the rotary valve for loading and circulation of the extraction reagent was of fixed volume. All fractionations were performed at ambient temperature (*ca*. 22.0±0.5 °C).

In each fraction, 0.25 g of sediment sample reacted with 2 mL of extractant at a continuous circulation rate of 1 mL min^{-1} through the column. The concentrations of metals in the leachates were then determined by FAAS after the volume was made to 20 mL using pure water.

The analytical trueness of the AI method was evaluated by comparing the bioaccessible (leachable) concentrations plus immobile (residue) fractions in the CRM sediment (mass balance validation) to those certified. For this purpose, the residue obtained after the F4 procedure was dissolved by using 2.5 mL of HF and 0.5 mL $HClO_4$ using a virtually identical protocol than that described before for the batchwise method [20]. The solution containing the immobile metal fractions was diluted with water to a final volume of 20 mL, and subsequently subjected to FAAS analysis.

3. Results and discussion

3.1. Influence of the circulation time of the extractants in the AI-based leaching method

The circulation length and flow rates of the extractants for leaching of trace elements (namely, Cu and Pb) until steady-state extraction conditions was investigated using 1.0 mol L^{-1} $MgCl_2$ as the F1 agent in the sequential extraction protocol and 1.0 mol L^{-1} HCl in the leaching test. As shown in Fig. 3, the average circulation times required to reach the maximum concentrations of bioaccessible Cu and Pb with 1.0 mol L^{-1} $MgCl_2$ were 4 h at 0.5 mL min^{-1}, 1–2 h at 1.0 mL min^{-1}, and 0.5–1 h at 2.0 mL min^{-1}, regardless of the metal species, and those with 1.0 mol L^{-1} HCl were 5 h at 0.5 mL min^{-1}, 3 h at 1.0 mL min^{-1}, and

1-2 h at 2.0 mL min^{-1}. Therefore, the average circulation times required to reach the maximum concentrations of Cu and Pb with 1.0 mol L^{-1} HCl were longer than those obtained with 1.0 mol L^{-1} $MgCl_2$, because the greater extractability of the acidic extractant toward metals generates larger amounts of leached metals. Dilute strong acids such as HCl can dissolve metals strongly attached to sediment particles associated to carbonates or acid-soluble mineralogical phases, but such metals usually would require longer extraction times to elute because of phase dissolution as compared to ion-exchange reactions on sediment particle surfaces under steady circulation rates.

Ideally, faster circulation rates should lead to the decrease of circulation times because of the increase in the number of extraction cycles for a fixed extractant volume within a pre-set timeframe. However, circulation rates faster than 2 mL min^{-1}caused undue column back pressure with subsequent extract leaking. Because column back pressures t were estimated as 53 kg cm^{-2} at 2.1 mL min^{-1} and 46 kg cm^{-2} at 2.0 mL min^{-1}, the flow back pressure in our system should be limited to ca. 50 kg cm^{-2} to prevent mechanical damages and ensure column durability.

The effects of the circulation time upon metal extractability in 1.0 mol L^{-1} CH_3COONH_4, 0.040 mol L^{-1} NH_2OH-HCl, and 8.8 mol L^{-1} H_2O_2-HNO_3, used as reagents for generating F2, F3, and F4 in the sequential extraction protocol, were also evaluated.

The timeframes required to reach steady-state bioaccessible concentrations were 1 h for 1.0 mol L^{-1} CH_3COONH_4, 2 h for 0.04 mol L^{-1} NH_2OH-HCl, and 2 h for 8.8 mol L^{-1} H_2O_2-HNO_3 at 2.0 mL min^{-1}, regardless of the target metals (Fig. S2 in Supplementary Materials).

For subsequent extractions in real-life sample analysis, the circulation time was set to 2 h for the leaching test with HCl, and 1 h for F1 and F2, and 2 h for F3 and F4 for sequential extraction tests at a circulation rate of 2.0 mL min^{-1}, taking into account that the intermediate precisions (RSD, n = 5) of bioaccessible metal concentrations were in all instances below 9.2% (Fig. S2 and Table S2 in Supplementary Materials).

3.2. Data comparison between AI leaching/fractionation and batchwise extraction counterparts

3.2.1. Leaching test

The concentrations of Cu, Zn, Cd, and Pb in river sediments as determined by the AI extraction method upon circulating of 1.0 mol L^{-1} HCl were compared to those obtained by the batchwise method [7]. As shown in Fig. 4, the concentrations of bioaccessible Cu, Zn, Cd, and Pb obtained with the AI method were virtually identical to those determined using the batchwise method, as indicated by the slopes and the intercepts of the linear least-squares regressions that were not significantly different from 1 and 0, respectively, at the 0.05 significance levels. In fact, 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) from the plots obtained in Fig. 4 were in all cases far below the critical *t*-value (t_{crit}), that is, 1.11 for Cu, 0.18 for Zn, 0.35 for Cd, and 1.62 for Pb against the t_{crit} of 2.45 [36]. The average of RSDs of the acid bioaccessible metal concentrations in CRM sediment as obtained in five consecutive measurements by the AI method were in all instances better than those of the batch method (AI method: 3.9% for Cu; 6.3% for Zn; 5.4% for Cd; 8.0% for Pb vs batch method: 8.4% for Cu; 17.3% for Zn; 13.6% for Cd; 13.0% for Pb). The RSD values by the AI method were even far below than those reported in previous continuous flow leaching methods for trace elements using acid extractants [34,37,38].

3.2.2. Sequential extraction

The bioaccessible pools of Cu, Zn, Cd, and Pb from sediment samples and the CRM using the AI-based sequential extraction method were compared to those of the batch sequential method.

As shown in Fig. 5, the concentrations of metals leached in each fraction from

Watarase River sediments were much greater than those from the CRM lake sediment (also see in Table S3 in Supplementary Materials). This is most likely attributed to the influence of a copper mine, Ashio Mine, located near the upper stream of the Watarase River. The magnitudes of the bioaccessible pools of Cu, Zn, and Pb by the AI method were as follows: F4 > F3 > F2 > F1 and those for Cd were F1 > F2 ≅ F3 ≅ F4 for both CRM and Watarase River sediment samples. These values were on a par with the batch method, as well as with those reported in previous studies on Watarase River sediments [39] indicating greater mobility of Cd.

The comparison of the fractionation results from the two extraction methods revealed that there were not large different, though metal concentrations in F1 obtained by the AI method were slightly larger and those in F4 slightly lower than those obtained by the batch method. In fact, the contact efficiency between the sediment and the extraction reagents within the circulation channel in the AI method is expected to be higher than that in the batch method. For example, the leachability of Pb in F1 for the AI method was greater than the limit of detection of FAAS (> 18.9 ± 0.2 μg L^{-1} in Pb), whereas with the batch method, Pb in F1 could not be extracted sufficiently to be measured by FAAS. In regard to F4, a lower metal extractability might be expected by resorting to the AI method because of the differences in reaction temperature (room temperature (*ca*. 22.0±0.5 °C) for the AI method against 85±2 °C for the batch method). As is the case with F4, the extraction temperatures of metals in F3 were different for both methods (room temperature for the AI method against 96±3 °C for the batch method). However, the concentrations of extracted metal ions were in this case not significantly different. This shows that the extractability of the reducible forms of target metals from the sediment samples herein analyzed is not affected by temperature.

On the other hand, differences in analytical results between the AI and batch methods might be also attributed to potential systematic errors caused by sample contaminations during the extraction procedures. In fact, we compared metal concentrations encountered in blank tests by the batch and AI methods. Metal concentrations found when reagent solutions were on-line delivered through the empty column were two- to ten-fold lower than those by the batchwise when shaking the reagents without sediment (Table S4 in Supplementary Materials). This is the result of the large number of manual operations involved in the batch protocol, which is in turn makes the manual bio-accessibility tests prone to contamination episodes.

The trueness of the AI method was evaluated by mass balance assessment in CRM as detailed in the Experimental section. As summarized in Table 2, in the AI method, the total amount of bioaccessible metals in the FI-F4 fractions plus residue were in good agreement with the certified metal concentrations in CRM, with recoveries ranging from 86% to 94%. The analytical reliability of the AI extraction method is supported by the fact that the t-values of the experimental results (t_{exp}) were in all cases smaller than that of the t critical value (t_{cri}).

The application of on-line AI fractionation to the seven sediment samples from upper- to down-stream (dam-lake) revealed that the concentrations of bioaccessible metals increased toward down-stream. This would be dependent upon the average particle sizes, that is, the smaller particles indicate the larger specific surface area (see Table 1 for details). The comparison of the AI fractionation profiles was somewhat different between samples 1 – 4 and samples 5 – 7. As shown in Fig. 6, the percentages of F4 of Cu, Zn, and Pb in samples 5 – 7 were higher than those in samples 1 – 4. These sampling points of samples 5 – 7 were much deeper than those of samples 1 – 4, as summarized in Table 1, and it thus seemed in which the environments were reductive atmosphere. Because chemical form in F4 is defined to be oxidisable compounds, the metal concentrations in F4 would be higher. In addition, because the bioaccessible pool of Cd in F1, which is defined as a chemical form easily eluted to water environments, was found to be the highest in every sediment, further research is underway so as to evaluate remediation protocols.

In brief, the AI fractionation method is herein demonstrated to be undertaken with a

4-fold decrease in sample amount, a 5-fold decrease in extractant volume, and one third of extraction time, along with better analytical precision than that of the conventional Tessier's batch sequential extraction for bioaccessibility tests of trace elements in sediments.

4. Conclusion

This work demonstrates that AIA is an appealing flow approach to conduct leaching and fractionation protocols of trace elements in environmental solids in which the extraction times could be significantly reduced compared to the conventional batch leaching and fractionation methods. Also, waste generation (viz., metal laden extracts) is reduced by about 4-times as compared to the manual counterpart. Based on a microcolumn system and circulation process, the AI protocol is regarded as a facile leaching method to obtain the concentrations of bioaccessible metal pools under steady-state conditions reliably. Further investigations are underway to determine the applicability of the AI method for fractionation of trace elements in real soils, fly ash, bottom ash, airborne particulate matter and food products, as well as sediments collected from various places.

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Table 1 Characterization of the riverbed sediment samples collected along Watarase River (Japan)

Sampling No.	1	2	3	4	5	6	7
Distance from dam / km	14.2	10.2	5.7	4.0	2.2	1.2	0.2
Water depth / m	< 0.5	< 0.5	12.3	17.3	50.9	76.6	82.0
Particle size distribution / µm	75 – 2000	75 – 2000	15 – 2000	5 – 2000	< 5 – 850	<5 – 250	< 5 – 250
Main property	Gravel	Gravel	Sand	Sand	Silt	Silt	Silt

Table 2 Total concentration and recoveries in the fractionation of Cu, Zn, Cd, and Pb in CRM (NMIJ-CRM 7303-a, lake sediment) by the AI method (n = 5).[a]

Fraction	Cu, mg kg^{-1}	Zn, mg kg^{-1}	Cd, mg kg^{-1}	Pb, mg kg^{-1}
F1	0.20±0.06	1.8±0.6	0.17±0.02	0.09±0.02
F2	0.30±0.18	0.8±0.3	0.029±0.008	0.6±0.2
F3	1.2±0.2	3.5±0.5	0.038±0.007	1.3±0.4
F4	8.1±0.4	10.1±0.8	0.024±0.007	10.7±0.7
F5[c]	12±2	81±3	0.05±0.01	14.2±0.6
Total	22±2	97±4	0.304±0.009	27±1
Certified values	23.1±3.1	107±5	0.342±0.017	31.3±1.1
Recovery to certified values, %	94	91	89	86
t_{exp} (α = 0.05) [d]	1.42	1.32	1.40	1.90

[a] Experimental conditions are described in section 2.5.2. [b] Mean value ± standard deviation. [c] F5 (immobilized fraction) was determined by batchwise acid dissolution (section 2.5.2). [d] t_{crit} at the 0.05 confidence level = 2.77.

Figure captions

Fig. 1 Close-up of the AI-type extraction device (AI device) furnished with a sample-packed column for leaching tests of metal species in sediments. The inset illustrates a magnified view of the sediment containing microcolumn

Fig. 2 Illustration of the analytical AI-based leaching protocol for single extraction and fractionation protocols based on extractants re-circulation.

C: column; P: peristaltic pump; F1-F4: extraction reagents in the fractionation procedure; W: water; V: valve. F1 consists of 1 mol L^{-1} HCl in the single-extraction test.

Fig. 3 Investigation of the magnitude of bioaccessible pools of Cu and Pb in AI-based extraction as a function of circulation time using 2 mL of 1.0 mol L^{-1} $MgCl_2$ (a) and 1.5 mL of 1.0 mol L^{-1} HCl (b) as extractants. Experimental conditions of the AI extraction method: sediment sample, 0.25 g of CRM sediment (< 2 mm ϕ); circulation rate, 0.5, 1.0, and 2.0 mL min^{-1}; circulation timeframe, 0.5–5 h; temperature, room temperature; measurement, FAAS.

Fig. 4 Evaluation of data correlation of bioaccessible metal concentrations in 7 different river sediment samples as obtained by the leaching test with 1.0 mol L^{-1} HCl using both batchwise and AI methods. Sediment samples: Watarase River sediment samples collected at 7 different point locations shown in Table 1 and Fig. S1. Experimental conditions of the AI leaching method: sediment sample, 0.25 g (< 2 mmϕ); volume of 1 mol L^{-1} HCl, 1.5 mL; circulation rate, 2.0 mL min^{-1}; circulation time, 2 h; temperature, room temperature; measurement, FAAS.

Fig. 5 Data comparison of sequential extraction of 4 metals from lake sediment CRM (top) and Watarase River sediment (bottom) by the batch against the AI method. The river sediment sample analyzed was No. 7 collected at the sampling site indicated in Table 1 and Fig. S1. Experimental conditions of the AI leaching method: sediment sample, 0.25 g (< 2 mm ϕ); circulation rate, 2.0 mL min^{-1}; volume of extraction reagents, 2.0 mL; circulation time, 1 h for F1 and F2, and 2 h for F3 and F4; temperature, room temperature; measurement, FAAS.

Fig. 6 Tessier fractionation of metals in riverbed sediment samples collected along Watarase River by the AI method. The physicochemical characterization of the sediment samples is summarized in Table 1. The experimental conditions are the same as those in Fig. 4.

Dynamic leaching and fractionation of trace elements from environmental solids exploiting a novel circulating-flow platform[1]

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[1] **Abbreviations:** AI, all injection; AIA, all injection analysis; CRM, certified reference material; FAAS, flame atomic absorption spectrometry; RSD, relative standard deviation.

Abstract

A dynamic flow-through microcolumn extraction system based on extractant recirculation is herein proposed as a novel analytical approach for simplification of bioaccessibility tests of trace elements in sediments. On-line metal leaching is undertaken in the format of all-injection (AI) analysis, which is a sequel of flow injection analysis, but involving extraction under steady-state conditions. The minimum circulation times and flow rates required to determine the maximum bioaccessible pools of target metals (viz., Cu, Zn, Cd, and Pb) from lake and river sediment samples were estimated using the Tessier’s sequential extraction scheme and an acid single extraction test. The on-line AIA method was successfully validated by mass balance studies of CRM and real sediment samples. Tessier’s test in on-line AI format demonstrated to be carried out by one third of extraction time (6 h against more than 17 h by the conventional method), with better analytical precision (< 9.2% against > 15% by the conventional method) and significant decrease in blank readouts as compared with the manual batch counterpart.

Keywords: bio-accessibility, leaching test, sequential extraction, trace element, sediment

1. Introduction

Elevated concentrations of metals in soils, sediments and airborne particulate matter might pose severe concerns to the public health. For example, the bioaccessible metal pools in sediments are known to influence the distribution and composition of benthic assemblages [1].

Risk assessment of trace element contamination in solid samples such as soils, solid wastes and river/ocean sediments is commonly undertaken by eluting the metals via leaching tests by utilizing water, electrolytes, acids (acetic acid or hydrochloric acid), reductants and oxidants as extractants so as to release target species associated to given mineralogical phases. Specifically, the purpose of leaching tests is to determine the aqueous phase concentrations of metals that are released from solids under environmentally simulated conditions, that is, the bioaccessible metal pools in environmental scenarios or alternatively when placed in land disposal units. The idea behind is to evaluate the potential harmful effects to biota and humans as a result of leaching of metal species into groundwater through a soil body or into marine ecosystems. Numerous laboratory leaching or bioaccessibility tests have been reported over the past few decades [2-5]. For example, in the US and Canada, the toxicity characteristic leaching procedure (TCLP) [6] is a commonly utilized leaching test that employs acetic acid or acetic acid/acetate buffer (pH 2.88 or 4.93, respectively) to evaluate metal leaching from solid wastes. Also, leaching tests that utilize HCl, with or without pepsin, mimicking gastric digestion, assess potential hazardous effects to human health when bringing toxic metals from the mouth to the stomach by directly taking solids such as soil, sediment, fly ash, or toys [6-10].

Single extraction methods are used to provide a rapid snapshot of metal leaching from environmental solids or solid wastes [11,12]. However, sequential extraction or fractionation tests have been commonly the method of choice so as to provide regarding detailed insight into different metal-soil associations [2,4-5,13-17], by exploiting a number of extractants of increasing elution strength. Fractionation studies are aimed at determining the chemical forms in which metals occur, because metal mobility, bioaccessibility and bioavailability strongly depend on their chemical and mineralogical forms [2,4-5, 13-17]. Sequential extraction protocols individually separate 4~6 pools of chemical forms of metal species that have different leachability, including the water soluble fraction, the ion-exchangeable fraction, the carbonate fraction, and metals bound to Fe-Mn oxyhydroxides and organic matter. The most widely accepted fractionation tests are the so-called 3-step BCR developed under the auspices of the Community Bureau of Reference (formerly BCR, but currently termed the Standard, Measurement and Testing

(SM&T) Programme of the Commission of the European Communities) [18, 19], and the Tessier scheme [20], where bioaccessible metals are associated to the exchangeable, acid soluble, reducible and oxidisable phases in soils and sediments. The classifications of chemical forms of metal species by sequential extractions are usually performed by batch-wise methods with tedious and time-consuming unit operations such as filtration, centrifugation, and reagent replacement. Those manual operations are likely prone to sample contamination, and they often lead to poor data accuracy.

To overcome the above issues of batchwise bio-accessibility tests, novel (semi)automatic or fully automatic flow systems for dynamic extraction procedures that may afford the addition of extractant(s), on-line extraction of metals, filtration of remaining solids, and alternatively, on-line analysis of extracts have been developed over the past few years [21-34].

A common procedure for on-line dynamic bioaccessibility tests of metal species in environmental solids is as follows [26]: 1) a soil sample is packed into a reactor, e.g., coil, column, or chamber; 2) an extractant is brought into the reactor using a pump; 3) the leachates are analyzed off- or on-line by an atomic spectrometric detector. A flow-based setup configurations for fractionation schemes have been developed so that the extractants of each specific chemical form of target metals could be intermittently loaded into the reactor from reagent reservoirs by switching valves [31-34]. Miró *et al*. developed sequential extraction methods in a dynamic flow-through format based on the distinct generations of flow injection analysis so as to ameliorate the extraction efficiency and assure leaching of bioaccessible pools of trace metals under “worst-case” extraction conditions [25-27, 30-34]. In the development of flow-based dynamic extraction systems, a great deal of attention has been focused on assembling analytical devices for repeatable unit operations and maximization of the reaction time between sample and reagent to get insight into the overall bioaccessible metal species.

In this study, a novel extraction system is proposed that could efficiently extract trace elements from riverbed sediment samples using a micro-column and by circulating the extract instead of shaking, stirring or continuous pumping of the extractant(s) toward and through the packed column. This idea is based on the principle of all injection analysis (AIA), which was first described by Itabashi *et al*. [35]. In AIA, all solutions are injected into a reaction coil and are circulated for a pre-set amount of time at a fixed flow rate. Because the solution (extractant) feed is stopped during the circulation process, the reagent consumption is significantly decreased as compared with conventional continuous-flow or flow injection manifolds.

Our goal is to demonstrate whether a circulating-flow platform based on the AIA principle is a suitable flow-based method to obtain reproducible patterns of leached metal species. The extraction performance of the AIA device was evaluated using an acidic single extraction test and the Tessier's sequential extraction procedure with the AIA features as a novel leaching approach critically compared against those of conventional batch leaching tests. As a practical application, sediments sampled along the Watarase River until Kusaki dam-lake located at the down-stream were on-line fractionated by the Tessier scheme in AIA fashion. As a result of mining activities from the Ashio copper area in Tochigi and Gunma Prefectures (1890s to 1990s), large amounts of metal species were discharged to the Watarase river, whereupon appropriate sediment and water management schemes has been continuously performed.

2. Experimental

2.1. All injection-type devise for metal leaching

The AI-type extraction device, AIA-IT3, was manufactured by Ogawa, Co., Ltd. (Kobe, Japan) and Kurahashi Giken, Ltd. (Kyoto, Japan) in cooperation with our research group. AIA-IT3 consisted of an 8-port rotary valve, a 4-port switching valve, a peristaltic pump, and a process control monitor (see details in Fig. 1). The tubes used to connect valves, sample-packed column, and reagent reservoirs were made of Teflon® (1 mm i.d.), and that of the pump was made of silicon (2 mm i.d.). The 4-port valve, which feeds the extractant into the re-circulation channel, was connected in series with the 8-port valve. The 8-port valve, in which the extractant leaches metals from the solid sample, as illustrated in Fig. 1 and 2. The sediment samples were packed into a microcolumn consisting of a Rezorian™ cartridge (1 cm^3 of inner volume, 5 cm long, Sigma-Aldrich, St. Louis, MO, US). The samples (0.25 g) were loosely packed to a thickness of 10 mm in the column, and PTFE membrane filters (pore size: 0.20 μm; thickness: 20 mm, Advantec) were placed at both ends. The column was connected to the manifold via luer-lock adaptors and nuts (see magnified view in Fig 1).

The sample-laden column was integrated in one of the loops of the 8-port rotary valve. The extractant was first loaded into the loop of the rotary valve (Fig. 2), and then circulated for a specific timeframe at a constant flow rate through the column.

2.2. Extraction procedure

The AI-based leaching procedure for leaching of trace elements from solids is schematically presented in Fig. 2. First, a given extractant is brought to the circulation channel at the 8-port valve (Fig. 2a), by activation of the peristaltic pump and selection of the appropriate port at the switching valve. By changing the valve position of the 8-port valve, the flow-line manifold is switched to a closed-line arrangement in order to circulate the extractant for a specific period, usually 0.5–5 h (Fig. 2b). During the extraction, the flow-line, except for the circulation channel, is stopped. Upon returning the valve to the original position, the re-circulated extract solution is flushed out from the 8-port valve with a pure water feed (Fig. 2c).

In our system, the circulation channel of the 8-port valve was filled with 1.5 or 2 mL solution, which was finally flushed out from the rotary valve and collected in a 20 mL volumetric flask. The volume was made up to 20 mL by adding pure water prior to analysis by flame atomic absorption spectrometry (FAAS, *ca*. 5 mL-per-injection). The detection instrument was a polarized Zeeman FAAS (Hitachi Z-5310, Japan). Hollow cathode lamps for FAAS were purchased from Hamamatsu Photonics, Ltd. (Hamamatsu, Japan). The analytical characteristics of the FAAS methods are summarized in Table S1 in Supplementary Materials.

2.3. Reagents and samples

All reagents used were of analytical grade, and were purchased from Wako Pure Chemical Industries, Ltd. The water used in this study was purified using a water distillation apparatus (ASK-2DS, Iwaki). Concentrated hydrochloric acid was diluted with water to a concentration of 1.0 mol L^{-1} for the single extraction test. Extractants for Tessier's test were prepared as follows: A 1.0 mol L^{-1} solution of magnesium chloride was prepared by dissolution of the salt, and aqueous sodium hydroxide was added to adjust the pH to 7 (extractant 1, F1 in Fig. 2). A solution of 1.0 mol L^{-1} ammonium acetate at pH 5 was prepared by dissolving the appropriate amount of salt in water with pH adjustment using HCl (extractant 2, F2 in Fig. 2). A 0.04 mol L^{-1} solution of hydroxylamine hydrochloride was prepared by dissolving the reducing reagent in 25% (v/v) acetic acid (extractant 3, F3 in Fig. 2). An 8.8 mol L^{-1} solution of hydrogen peroxide was prepared by mixing 90 mL of the concentrated hydrogen peroxide and 8.5 mL of pure water; pH was adjusted to 2.0 using concentrated nitric acid before making up to 100 mL with pure water (extractant 4, F4 in Fig. 2).

A certified reference material (CRM), NMIJ CRM 7303-a, was purchased from the National Institute of Advanced Industrial Science and Technology (Tsukuba, Japan), and used as the reference lake sediment. Sediment samples were collected from seven different riverbed locations along the Watarase River in Gunma Prefecture, Japan (Table 1 and Fig. S1 in Supplementary Material). The riverbed sediments were collected from the surface using an Ekman-Birge grab by staff of Kusaki Dam Operation and Maintenance Office from Japan Water Agency (Numata, Gunma Pref., Japan), who provided us with measurements of particle sizes distributions in every sample using the Bouyoucos hydrometer method. The sediment samples were air-dried at ambient temperature for 2–3 days in our laboratory, pulverized using a mortar and pestle, and sieved through a 2-mm screen and the below 2-mm fraction collected for further analysis.

2.4. Acidic leaching test (single extraction procedure)

The leaching test in a batchwise format was performed according to the Soil Contamination Countermeasures Act of Japan [7]. To this end, one gram of the sediment sample was added to 6.0 mL of 1.0 mol L^{-1} HCl, and the mixture was shaken for 2 h. The residue was separated from the solution by centrifugation at 3000 rpm for 30 min (Kokusan H-200 Centrifugal Separator; Kokusan, Tokyo, Japan). The separated solution was filtered through a 0.45 µm PTFE membrane filter, before making up to 20 mL with pure water.

In the AI leaching method, 0.25 g of sediment was packed into the cylindrical column described above. Thereafter, 1.5 mL of 1.0 mol L^{-1} HCl was loaded in the circulation channel and circulated for a pre-set time interval (0.5, 1, 2, 3, 4, and 5 h). Then, the solution was collected, and made up to 20 mL using pure water.

2.5. Sequential extraction procedure (Tessier's fractionation scheme)

2.5.1. Batch sequential extraction

To estimate the bioaccessibility and mobility of trace elements from sediments the target metals were classified in four fractions, depending on the metal speciation in the sample, and in accordance with the sequential extraction method described by Tessier *et al.*: Fraction 1 (F1), ion exchangeable; Fraction 2 (F2), bound to carbonates; Fraction 3 (F3), bound to iron and manganese oxides; and Fraction 4 (F4), bound to organic matter [20].

The operational procedure for the batch method was as follows: F1 was generated by stirring (600 rpm) 1.0 g of the sample in 8 mL of 1.0 mol L^{-1} $MgCl_2$ (pH 7) at ambient temperature for 1 h. F2 was generated by stirring a mixture of F1 residue and 8.0 mL of 1.0 mol L^{-1} CH_3COONH_4 (pH 5) at ambient temperature for 5 h. F3 was generated by stirring a mixture of F2 residue and 20 mL of 0.04 mol L^{-1} $NH_2OH{\cdot}HCl$ (in 25% (v/v) CH_3COOH) at 96±3 °C for 6 h. F4 was generated by stirring a mixture of F3 residue and 8.0 mL of 8.8 mol L^{-1} H_2O_2 with nitric acid at 85±2 °C for 5 h. The final residue after F4 was dissolved in 10 mL of 46% (w/w) HF and 2.0 mL of 60% (w/w) $HClO_4$. The mixture was then evaporated to dryness, and 10 mL of 46% (w/w) HF and 1.0 mL of 60% (w/w) $HClO_4$ were added. The resulting mixture was again evaporated at 107 ^{o}C in a sand bath to dryness and 1.0 mL of 60% (w/w) $HClO_4$ was added. Finally, the residue was completely dissolved by 3 mL of 12 mol L^{-1} HCl.

The FI-F4 fractions were centrifuged at 3000 rpm for 30 min. The extracts (except for F3) were diluted with water to a final volume of 20 mL, and the concentrations of metals were determined by FAAS.

2.5.2. Sequential extraction by the AI method

The extractants in the AI fractionation method were the same as those indicated for the batch method. The extraction procedures for F1–F4 were successively carried out in the same way as the batch method is performed. The liquid-to-solid ratios of F1, F2, and F4 were also the same as those in the batch method; specifically, 2 mL of liquid (reagent) and 0.25 g of solid (sediment sample) were utilized. The liquid-to-solid ratio of F3 in the AI method differed from that of the batch method, because the channel loop of the rotary valve for loading and circulation of the extraction reagent was of fixed volume. All fractionations were performed at ambient temperature (*ca*. 22.0±0.5 °C).

In each fraction, 0.25 g of sediment sample reacted with 2 mL of extractant at a continuous circulation rate of 1 mL min^{-1} through the column. The concentrations of metals in the leachates were then determined by FAAS after the volume was made to 20 mL using pure water.

The analytical trueness of the AI method was evaluated by comparing the bioaccessible (leachable) concentrations plus immobile (residue) fractions in the CRM sediment (mass balance validation) to those certified. For this purpose, the residue obtained after the F4 procedure was dissolved by using 2.5 mL of HF and 0.5 mL $HClO_4$ using a virtually identical protocol than that described before for the batchwise method [20]. The solution containing the immobile metal fractions was diluted with water to a final volume of 20 mL, and subsequently subjected to FAAS analysis.

3. Results and discussion

3.1. Influence of the circulation time of the extractants in the AI-based leaching method

The circulation length and flow rates of the extractants for leaching of trace elements (namely, Cu and Pb) until steady-state extraction conditions was investigated using 1.0 mol L^{-1} $MgCl_2$ as the F1 agent in the sequential extraction protocol and 1.0 mol L^{-1} HCl in the leaching test. As shown in Fig. 3, the average circulation times required to reach the maximum concentrations of bioaccessible Cu and Pb with 1.0 mol L^{-1} $MgCl_2$ were 4 h at 0.5 mL min^{-1}, 1–2 h at 1.0 mL min^{-1}, and 0.5–1 h at 2.0 mL min^{-1}, regardless of the metal species, and those with 1.0 mol L^{-1} HCl were 5 h at 0.5 mL min^{-1}, 3 h at 1.0 mL min^{-1}, and

1-2 h at 2.0 mL min^{-1}. Therefore, the average circulation times required to reach the maximum concentrations of Cu and Pb with 1.0 mol L^{-1} HCl were longer than those obtained with 1.0 mol L^{-1} $MgCl_2$, because the greater extractability of the acidic extractant toward metals generates larger amounts of leached metals. Dilute strong acids such as HCl can dissolve metals strongly attached to sediment particles associated to carbonates or acid-soluble mineralogical phases, but such metals usually would require longer extraction times to elute because of phase dissolution as compared to ion-exchange reactions on sediment particle surfaces under steady circulation rates.

Ideally, faster circulation rates should lead to the decrease of circulation times because of the increase in the number of extraction cycles for a fixed extractant volume within a pre-set timeframe. However, circulation rates faster than 2 mL min^{-1}caused undue column back pressure with subsequent extract leaking. Because column back pressures t were estimated as 53 kg cm^{-2} at 2.1 mL min^{-1} and 46 kg cm^{-2} at 2.0 mL min^{-1}, the flow back pressure in our system should be limited to ca. 50 kg cm^{-2} to prevent mechanical damages and ensure column durability.

The effects of the circulation time upon metal extractability in 1.0 mol L^{-1} CH_3COONH_4, 0.040 mol L^{-1} NH_2OH-HCl, and 8.8 mol L^{-1} H_2O_2-HNO_3, used as reagents for generating F2, F3, and F4 in the sequential extraction protocol, were also evaluated.

The timeframes required to reach steady-state bioaccessible concentrations were 1 h for 1.0 mol L^{-1} CH_3COONH_4, 2 h for 0.04 mol L^{-1} NH_2OH-HCl, and 2 h for 8.8 mol L^{-1} H_2O_2-HNO_3 at 2.0 mL min^{-1}, regardless of the target metals (Fig. S2 in Supplementary Materials).

For subsequent extractions in real-life sample analysis, the circulation time was set to 2 h for the leaching test with HCl, and 1 h for F1 and F2, and 2 h for F3 and F4 for sequential extraction tests at a circulation rate of 2.0 mL min^{-1}, taking into account that the intermediate precisions (RSD, n = 5) of bioaccessible metal concentrations were in all instances below 9.2% (Fig. S2 and Table S2 in Supplementary Materials).

3.2. Data comparison between AI leaching/fractionation and batchwise extraction counterparts

3.2.1. Leaching test

The concentrations of Cu, Zn, Cd, and Pb in river sediments as determined by the AI extraction method upon circulating of 1.0 mol L^{-1} HCl were compared to those obtained by the batchwise method [7]. As shown in Fig. 4, the concentrations of bioaccessible Cu, Zn, Cd, and Pb obtained with the AI method were virtually identical to those determined using the batchwise method, as indicated by the slopes and the intercepts of the linear least-squares regressions that were not significantly different from 1 and 0, respectively, at the 0.05 significance levels. In fact, 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) from the plots obtained in Fig. 4 were in all cases far below the critical *t*-value (t_{crit}), that is, 1.11 for Cu, 0.18 for Zn, 0.35 for Cd, and 1.62 for Pb against the t_{crit} of 2.45 [36]. The average of RSDs of the acid bioaccessible metal concentrations in CRM sediment as obtained in five consecutive measurements by the AI method were in all instances better than those of the batch method (AI method: 3.9% for Cu; 6.3% for Zn; 5.4% for Cd; 8.0% for Pb vs batch method: 8.4% for Cu; 17.3% for Zn; 13.6% for Cd; 13.0% for Pb). The RSD values by the AI method were even far below than those reported in previous continuous flow leaching methods for trace elements using acid extractants [34,37,38].

3.2.2. Sequential extraction

The bioaccessible pools of Cu, Zn, Cd, and Pb from sediment samples and the CRM using the AI-based sequential extraction method were compared to those of the batch sequential method.

As shown in Fig. 5, the concentrations of metals leached in each fraction from

Watarase River sediments were much greater than those from the CRM lake sediment (also see in Table S3 in Supplementary Materials). This is most likely attributed to the influence of a copper mine, Ashio Mine, located near the upper stream of the Watarase River. The magnitudes of the bioaccessible pools of Cu, Zn, and Pb by the AI method were as follows: F4 > F3 > F2 > F1 and those for Cd were F1 > F2 ≅ F3 ≅ F4 for both CRM and Watarase River sediment samples. These values were on a par with the batch method, as well as with those reported in previous studies on Watarase River sediments [39] indicating greater mobility of Cd.

The comparison of the fractionation results from the two extraction methods revealed that there were not large different, though metal concentrations in F1 obtained by the AI method were slightly larger and those in F4 slightly lower than those obtained by the batch method. In fact, the contact efficiency between the sediment and the extraction reagents within the circulation channel in the AI method is expected to be higher than that in the batch method. For example, the leachability of Pb in F1 for the AI method was greater than the limit of detection of FAAS ($> 18.9 \pm 0.2$ μg L^{-1} in Pb), whereas with the batch method, Pb in F1 could not be extracted sufficiently to be measured by FAAS. In regard to F4, a lower metal extractability might be expected by resorting to the AI method because of the differences in reaction temperature (room temperature (*ca*. 22.0±0.5 °C) for the AI method against 85±2 °C for the batch method). As is the case with F4, the extraction temperatures of metals in F3 were different for both methods (room temperature for the AI method against 96±3 °C for the batch method). However, the concentrations of extracted metal ions were in this case not significantly different. This shows that the extractability of the reducible forms of target metals from the sediment samples herein analyzed is not affected by temperature.

On the other hand, differences in analytical results between the AI and batch methods might be also attributed to potential systematic errors caused by sample contaminations during the extraction procedures. In fact, we compared metal concentrations encountered in blank tests by the batch and AI methods. Metal concentrations found when reagent solutions were on-line delivered through the empty column were two- to ten-fold lower than those by the batchwise when shaking the reagents without sediment (Table S4 in Supplementary Materials). This is the result of the large number of manual operations involved in the batch protocol, which is in turn makes the manual bio-accessibility tests prone to contamination episodes.

The trueness of the AI method was evaluated by mass balance assessment in CRM as detailed in the Experimental section. As summarized in Table 2, in the AI method, the total amount of bioaccessible metals in the FI-F4 fractions plus residue were in good agreement with the certified metal concentrations in CRM, with recoveries ranging from 86% to 94%. The analytical reliability of the AI extraction method is supported by the fact that the t-values of the experimental results (t_{exp}) were in all cases smaller than that of the t critical value (t_{cri}).

The application of on-line AI fractionation to the seven sediment samples from upper- to down-stream (dam-lake) revealed that the concentrations of bioaccessible metals increased toward down-stream. This would be dependent upon the average particle sizes, that is, the smaller particles indicate the larger specific surface area (see Table 1 for details). The comparison of the AI fractionation profiles was somewhat different between samples 1 – 4 and samples 5 – 7. As shown in Fig. 6, the percentages of F4 of Cu, Zn, and Pb in samples 5 – 7 were higher than those in samples 1 – 4. These sampling points of samples 5 – 7 were much deeper than those of samples 1 – 4, as summarized in Table 1, and it thus seemed in which the environments were reductive atmosphere. Because chemical form in F4 is defined to be oxidisable compounds, the metal concentrations in F4 would be higher. In addition, because the bioaccessible pool of Cd in F1, which is defined as a chemical form easily eluted to water environments, was found to be the highest in every sediment, further research is underway so as to evaluate remediation protocols.

In brief, the AI fractionation method is herein demonstrated to be undertaken with a

4-fold decrease in sample amount, a 5-fold decrease in extractant volume, and one third of extraction time, along with better analytical precision than that of the conventional Tessier's batch sequential extraction for bioaccessibility tests of trace elements in sediments.

4. Conclusion

This work demonstrates that AIA is an appealing flow approach to conduct leaching and fractionation protocols of trace elements in environmental solids in which the extraction times could be significantly reduced compared to the conventional batch leaching and fractionation methods. Also, waste generation (viz., metal laden extracts) is reduced by about 4-times as compared to the manual counterpart. Based on a microcolumn system and circulation process, the AI protocol is regarded as a facile leaching method to obtain the concentrations of bioaccessible metal pools under steady-state conditions reliably. Further investigations are underway to determine the applicability of the AI method for fractionation of trace elements in real soils, fly ash, bottom ash, airborne particulate matter and food products, as well as sediments collected from various places.

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Table 1 Characterization of the riverbed sediment samples collected along Watarase River (Japan)

Sampling No.	1	2	3	4	5	6	7
Distance from dam / km	14.2	10.2	5.7	4.0	2.2	1.2	0.2
Water depth / m	< 0.5	< 0.5	12.3	17.3	50.9	76.6	82.0
Particle size distribution / µm	75 – 2000	75 – 2000	15 – 2000	5 – 2000	< 5 – 850	<5 – 250	< 5 – 250
Main property	Gravel	Gravel	Sand	Sand	Silt	Silt	Silt

Table 2 Total concentration and recoveries in the fractionation of Cu, Zn, Cd, and Pb in CRM (NMIJ-CRM 7303-a, lake sediment) by the AI method (n = 5).[a]

Fraction	Cu, mg kg^{-1}	Zn, mg kg^{-1}	Cd, mg kg^{-1}	Pb, mg kg^{-1}
F1	0.20±0.06	1.8±0.6	0.17±0.02	0.09±0.02
F2	0.30±0.18	0.8±0.3	0.029±0.008	0.6±0.2
F3	1.2±0.2	3.5±0.5	0.038±0.007	1.3±0.4
F4	8.1±0.4	10.1±0.8	0.024±0.007	10.7±0.7
F5[c]	12±2	81±3	0.05±0.01	14.2±0.6
Total	22±2	97±4	0.304±0.009	27±1
Certified values	23.1±3.1	107±5	0.342±0.017	31.3±1.1
Recovery to certified values, %	94	91	89	86
t_{exp} (α = 0.05) [d]	1.42	1.32	1.40	1.90

[a] Experimental conditions are described in section 2.5.2. [b] Mean value ± standard deviation. [c] F5 (immobilized fraction) was determined by batchwise acid dissolution (section 2.5.2). [d] t_{crit} at the 0.05 confidence level = 2.77.

Figure captions

Fig. 1 Close-up of the AI-type extraction device (AI device) furnished with a sample-packed column for leaching tests of metal species in sediments. The inset illustrates a magnified view of the sediment containing microcolumn

Fig. 2 Illustration of the analytical AI-based leaching protocol for single extraction and fractionation protocols based on extractants re-circulation.

C: column; P: peristaltic pump; F1-F4: extraction reagents in the fractionation procedure; W: water; V: valve. F1 consists of 1 mol L^{-1} HCl in the single-extraction test.

Fig. 3 Investigation of the magnitude of bioaccessible pools of Cu and Pb in AI-based extraction as a function of circulation time using 2 mL of 1.0 mol L^{-1} $MgCl_2$ (a) and 1.5 mL of 1.0 mol L^{-1} HCl (b) as extractants. Experimental conditions of the AI extraction method: sediment sample, 0.25 g of CRM sediment (< 2 mm ϕ); circulation rate, 0.5, 1.0, and 2.0 mL min^{-1}; circulation timeframe, 0.5–5 h; temperature, room temperature; measurement, FAAS.

Fig. 4 Evaluation of data correlation of bioaccessible metal concentrations in 7 different river sediment samples as obtained by the leaching test with 1.0 mol L^{-1} HCl using both batchwise and AI methods. Sediment samples: Watarase River sediment samples collected at 7 different point locations shown in Table 1 and Fig. S1. Experimental conditions of the AI leaching method: sediment sample, 0.25 g (< 2 mmϕ); volume of 1 mol L^{-1} HCl, 1.5 mL; circulation rate, 2.0 mL min^{-1}; circulation time, 2 h; temperature, room temperature; measurement, FAAS.

Fig. 5 Data comparison of sequential extraction of 4 metals from lake sediment CRM (top) and Watarase River sediment (bottom) by the batch against the AI method. The river sediment sample analyzed was No. 7 collected at the sampling site indicated in Table 1 and Fig. S1. Experimental conditions of the AI leaching method: sediment sample, 0.25 g (< 2 mm ϕ); circulation rate, 2.0 mL min^{-1}; volume of extraction reagents, 2.0 mL; circulation time, 1 h for F1 and F2, and 2 h for F3 and F4; temperature, room temperature; measurement, FAAS.

Fig. 6 Tessier fractionation of metals in riverbed sediment samples collected along Watarase River by the AI method. The physicochemical characterization of the sediment samples is summarized in Table 1. The experimental conditions are the same as those in Fig. 4.

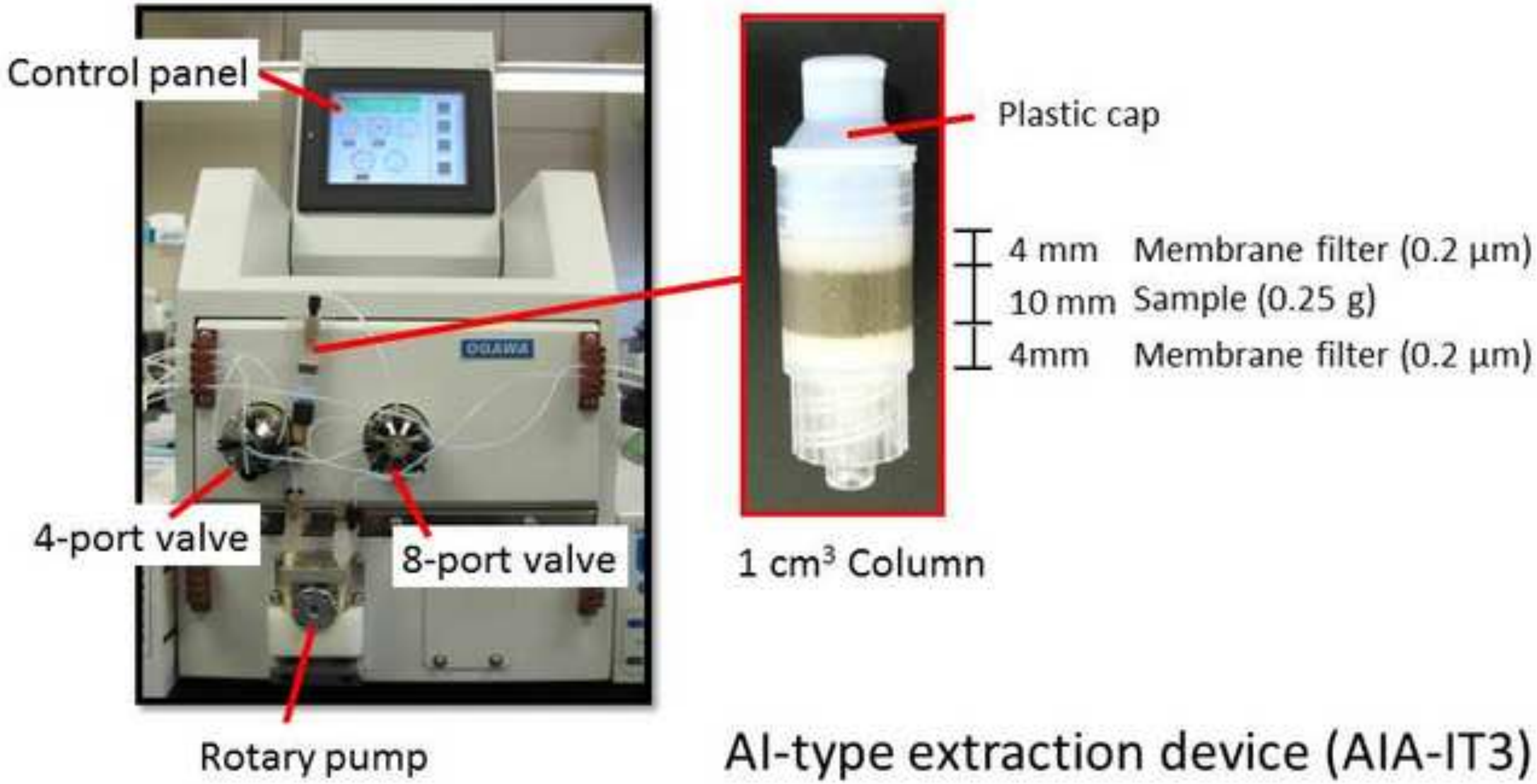

Control panel
4-port valve
8-port valve
Rotary pump
Plastic cap
4 mm Membrane filter (0.2 µm)
10 mm Sample (0.25 g)
4mm Membrane filter (0.2 µm)
1 cm³ Column
AI-type extraction device (AIA-IT3)

Fig. 1

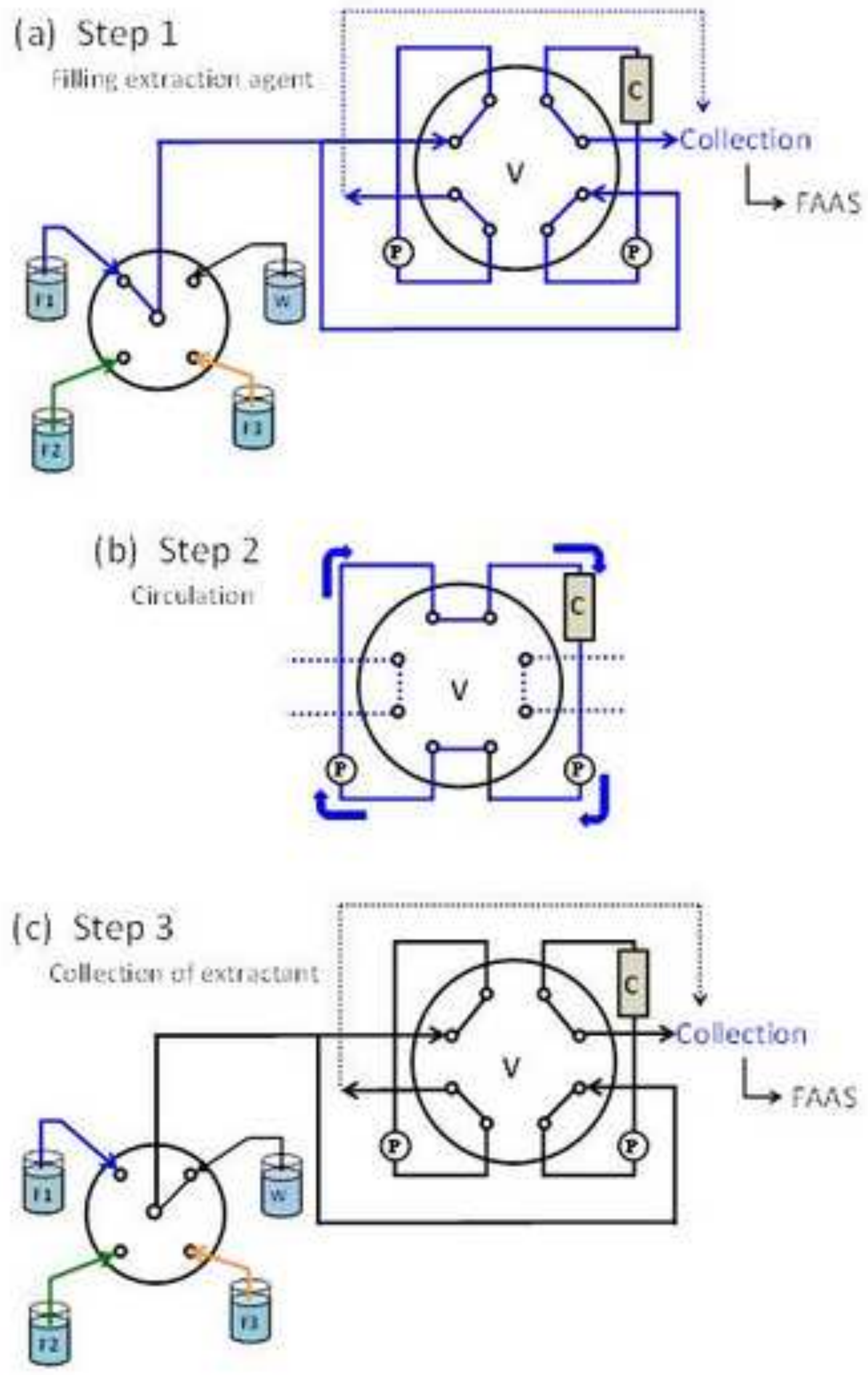

(a) Step 1
Filling extraction agent
C
V
Collection
FAAS
P
P
F1
W
F2
F3
(b) Step 2
Circulation
C
V
P
P
(c) Step 3
Collection of extractant
C
V
Collection
FAAS
P
P
F1
W
F2
F3

Fig. 2

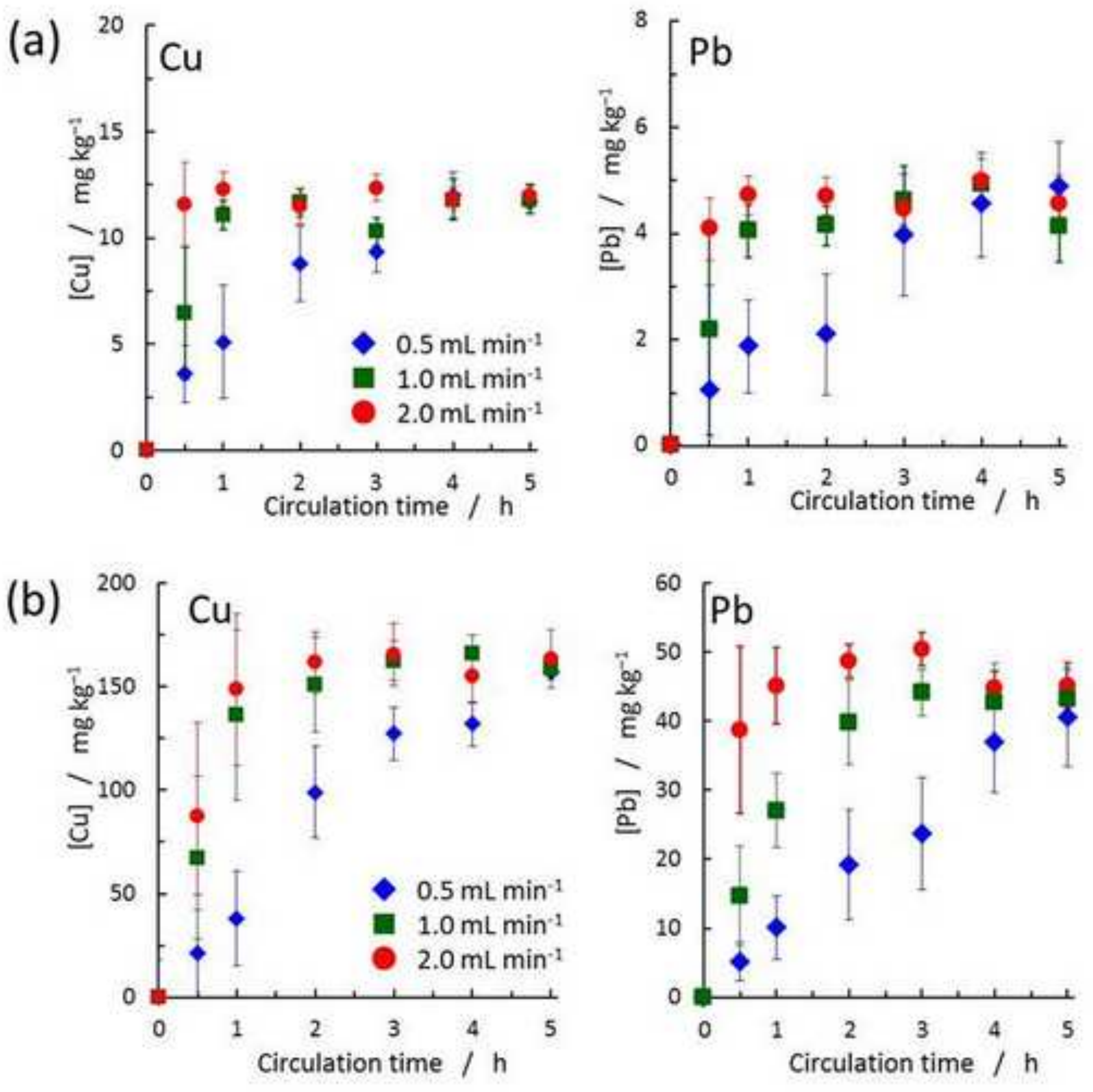

(a)
Cu
[Cu] / mg kg⁻¹
0
5
10
15
20
0
1
2
3
4
5
Circulation time / h
◆ 0.5 mL min⁻¹
■ 1.0 mL min⁻¹
● 2.0 mL min⁻¹
Pb
[Pb] / mg kg⁻¹
0
2
4
6
8
0
1
2
3
4
5
Circulation time / h
(b)
Cu
[Cu] / mg kg⁻¹
0
50
100
150
200
0
1
2
3
4
5
Circulation time / h
◆ 0.5 mL min⁻¹
■ 1.0 mL min⁻¹
● 2.0 mL min⁻¹
Pb
[Pb] / mg kg⁻¹
0
10
20
30
40
50
60
0
1
2
3
4
5
Circulation time / h

Fig. 3

Figure

Click here to download high resolution image

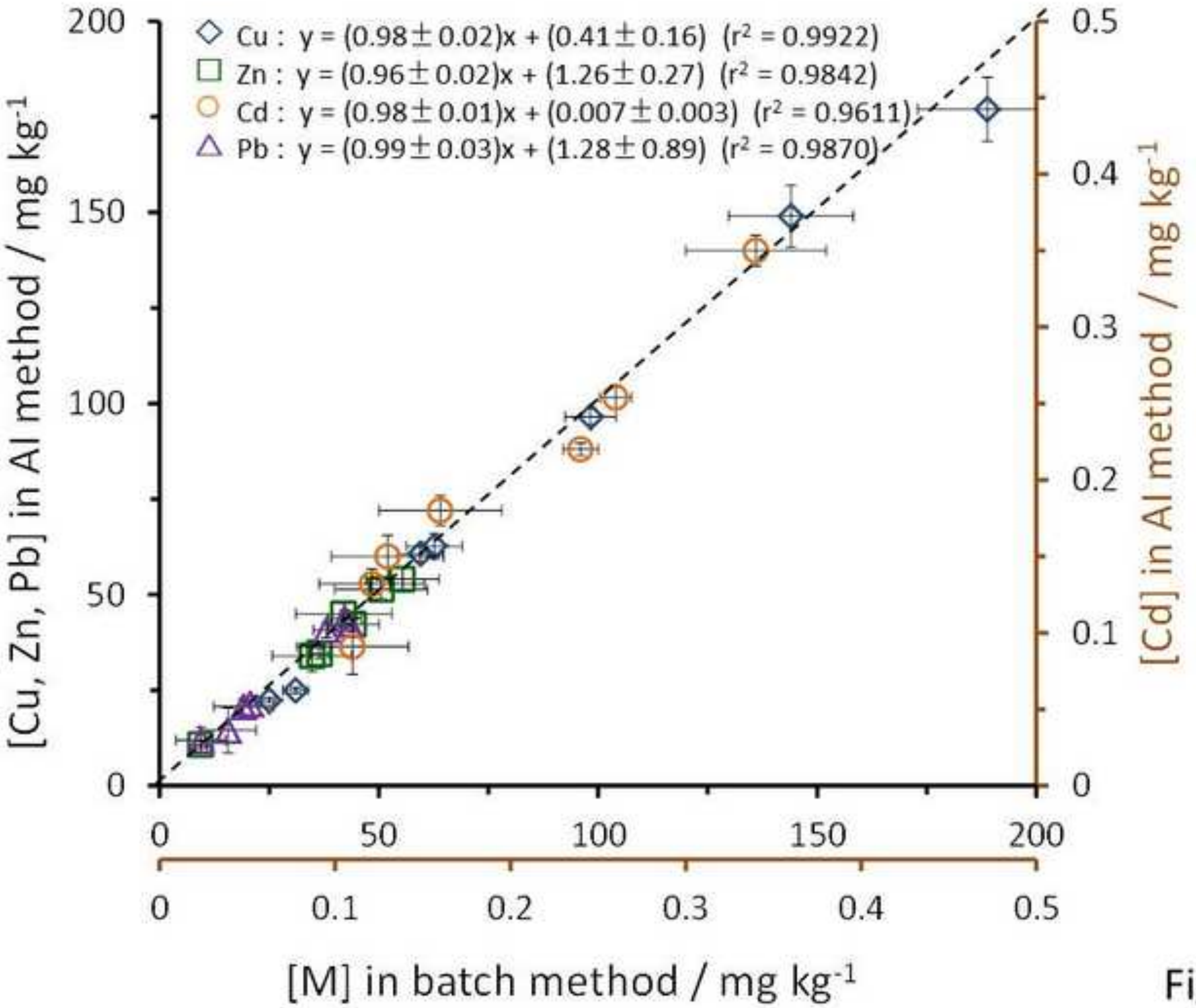
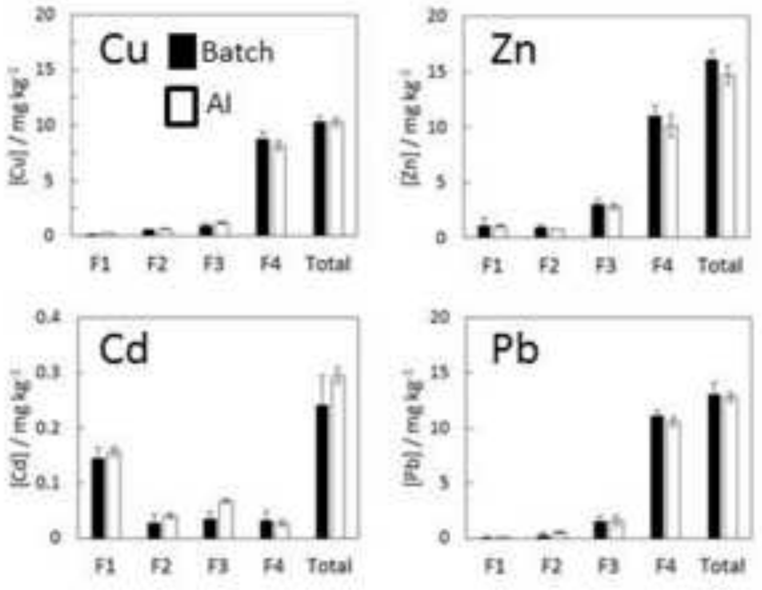


Fig. 4

Lake sediment CRM



Watarase River sediment (no. 7)

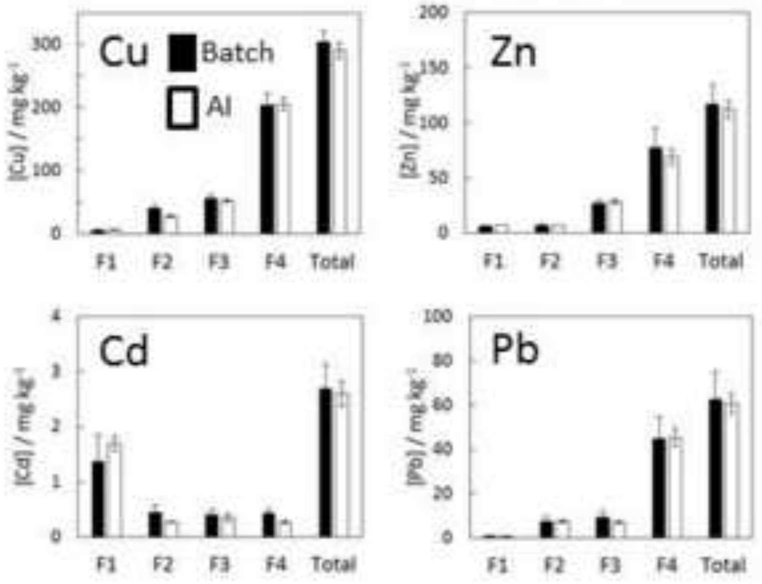


Fig. 5

Figure

Click here to download high resolution image

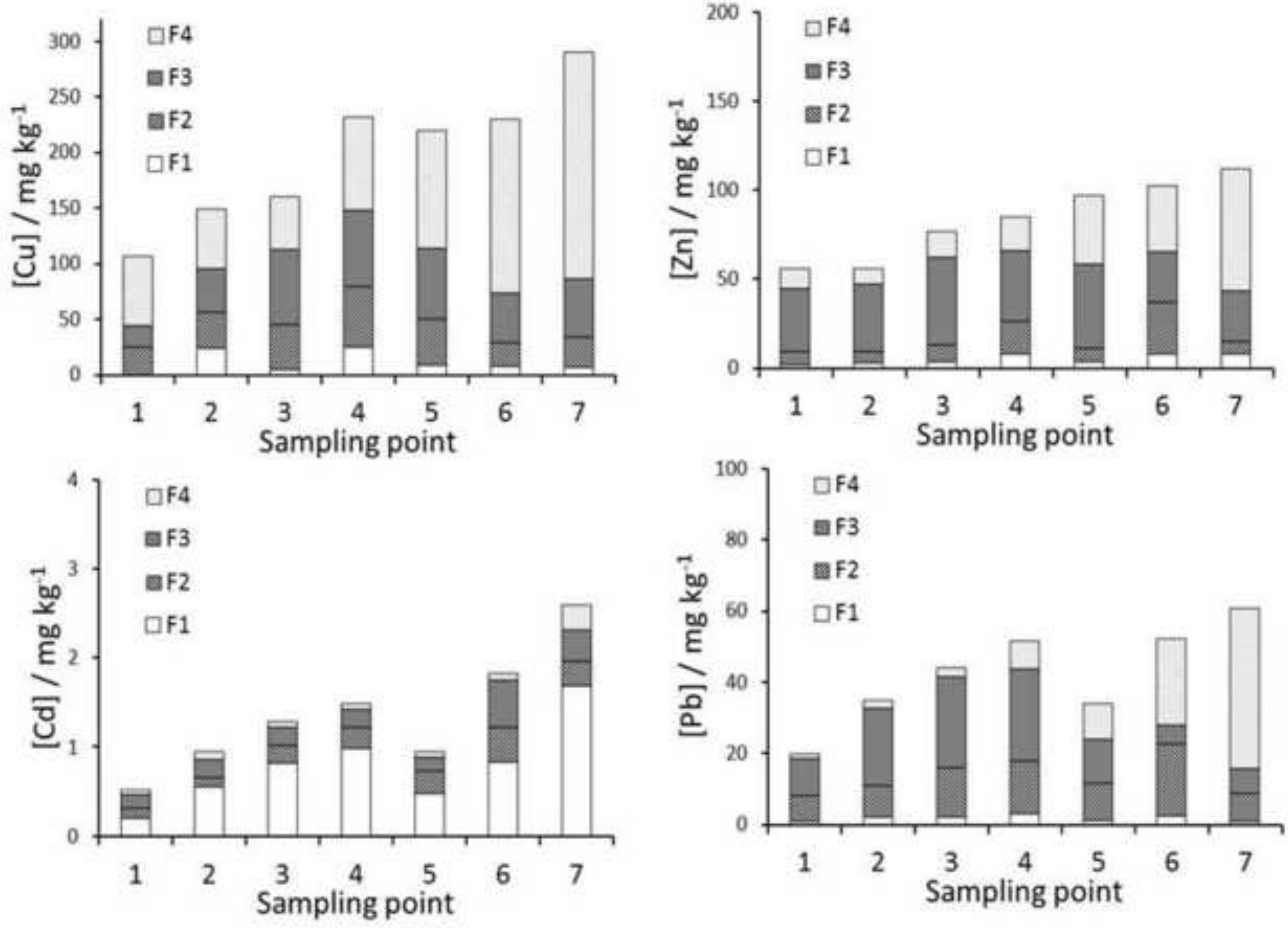


Fig. 6