## Tessier sequential extraction on 17 elements from three marine sediment certified reference materials (HISS-1, MESS-4, and PACS-3)

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# Tessier sequential extraction on 17 elements from three marine sediment certified reference materials (HISS-1, MESS-4 and PACS-3)

**Paramee Kumkrong*, Ovi Mihai, Patrick H.J. Mercier, Indu Gedara Pihilligawa, Daniel D. Tyo, and Zoltan Mester**

National Research Council Canada, 1200 Montreal Road Ottawa, Ontario Canada, K1A 0R6

*Corresponding author : paramee.kumkrong@nrc-crnc.gc.ca

ORCID : 0000-0002-9281-9974



## Abstract

The four-step Tessier sequential extraction procedure is a well-known approach used for environmental and geochemical studies in soil and sediments. However, a lack of reference materials limits its use making implementation and quality control cumbersome. This study applied Tessier sequential extraction to three globally used marine sediment certified reference materials (CRMs) including HISS-1, MESS-4 and PACS-3 with varying levels of contamination.

The study analyzed the distribution of 17 elements throughout the extraction phases. Overall, the percent recovery (sum of steps vs total metal concentration) of all analyzed elements in Tessier extraction was 92 % ± 40 % in HISS-1, 101 % ± 12 % in MESS-4 and 102 % ± 10 % in PACS-3. The observed uncertainty of the individual elemental concentrations averaged of 13 %, which compares favorably with the 16 % target uncertainty derived from Horwitz equation. The reference data set produced here using the Tessier sequential extraction procedure will serve as a quality control and method development tool for laboratories.

Keywords: Distribution. Certified reference material. Sequential extraction. Recovery. Uncertainty.

# Table of contents

# Abbreviation

| | |
|---|---|
| °C | Degree Celsius |
| µm | Micrometer |
| BCR | Community Bureau of Reference |
| CI | Confidence interval |
| CRC | Collision reaction cell |
| CRM(s) | Certified reference material(s) |
| DIW | Deionized water |
| eV | Electron volt |
| Ext sol# | Extraction solution |
| g | Gram |
| h | Hour |
| ICP | Inductively coupled plasma |
| ISO | International Organization for Standardization |
| L | Litre |
| log | Logarithm |
| mol $L^{-1}$ | Mole per litre |
| mg | Milligram |
| min | Minute |
| mL | Millilitre |
| MS | Mass spectrometry |
| MΩ-cm | Megohm-centimeter |
| na | Not applicable |
| nd | Not detected |
| nr | Not reported |
| NRC | National Research Council Canada |

| | |
|---|---|
| OES | Optical emission spectrometry |
| rpm | Revolutions per minute |
| RSD | Relative standard deviation |
| SD | Standard deviation |
| $S_R$ | Reproducibility standard deviation |
| $u_{std}$ | Standard uncertainty |
| U | Expanded uncertainty |
| v | Volume |
| w | Weight |

# 1 Introduction

The association between metals bound to sediments is of interest because their potential release could pose significant environmental challenges (1,2). Marine sediments are mainly composed of biogenous, hydrogenous, and lithogenous sediments (3), and also receive foreign materials from inland activities such as weathering and anthropogenic inputs. A high accumulation of toxic metals in marine sediments is widely observed in areas of high human activity, for example harbors (4–8).

In general, assessment of sediment quality is achieved by quantitative analysis of total toxic metal content (9–11). However, not all measured total metal content in sediments is actually posing an environmental hazard, so better approaches that estimate the labile metal fraction and the conditions of their release are needed. The importance of such investigations brought the development of methodologies based on fractionating toxic metals from solid phases by partial dissolution / desorption / extraction (12–17).

Among sequential extraction procedures, the Tessier and the Community Bureau of Reference (BCR) sequential extractions are most commonly employed (18,19). The publications citing Tessier and BCR sequential extraction in Scopus database (https://www.scopus.com, accessed on June 7, 2020) showed results of 3,625 and 2,332, respectively. Tessier expressed significant growth until it reached a plateau in 2011, however, BCR extraction method has continued gaining popularity (**Fig.1**).

Tessier sequential extraction procedure was published in 1979 for the determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in river sediments from Quebec, Canada (18). The BCR method was developed with the objective to harmonize the sequential extraction procedure and be used in conjunction with the production of BCR 601 and BCR 701 CRMs (river sediment reference materials) that certified Cd, Cr, Cu, Ni, Pb, and Zn (19,20). There is a plethora of data available from the BCR on method characteristics (21–26) but the availability of such information is much limited for the Tessier extraction approach using reference materials.

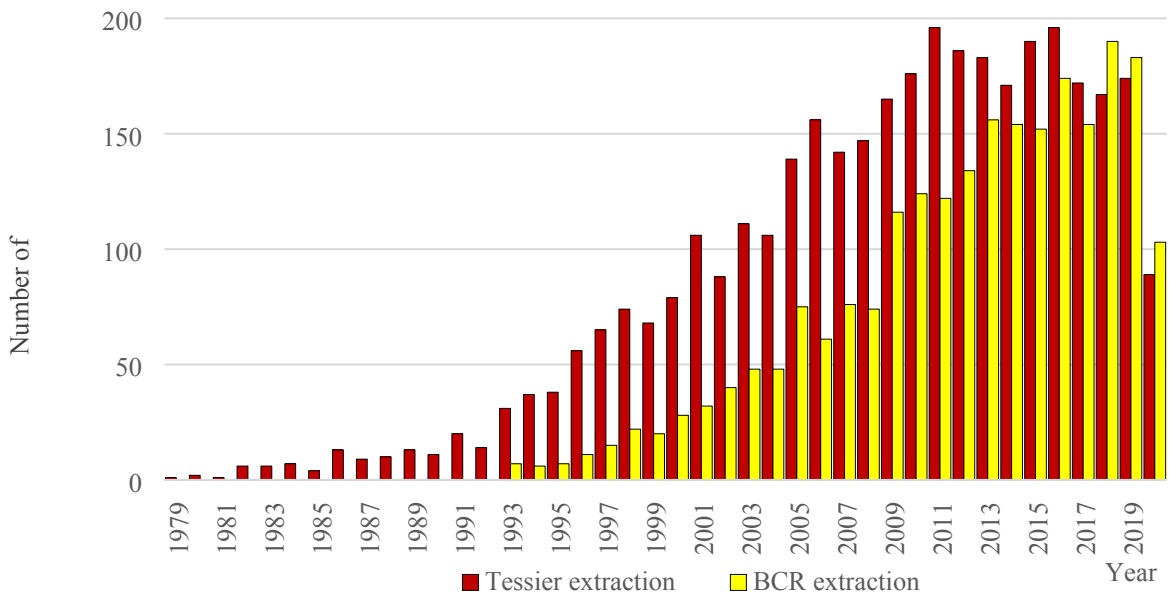


**Figure 1** : **The number of publications of Tessier and BCR sequential extraction procedure since 1979 to 2020 (Source: Scopus database, searched under keywords: "sequential extraction BCR" and "sequential extraction Tessier" (https://www.scopus.com, accessed on June 07, 2020)**

Considering the lack of CRMs and reference data on precision and accuracy upon application of a given extraction method, we have performed the Tessier extraction procedure on 17 elements (As, Be, Cd, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sn, Sr, U, V, and Zn) in three marine sediment CRMs produced by National Research Council Canada (NRC). The three CRMs included: HISS-1, a clean sediment; MESS-4, a moderately-contaminated sediment; and PACS-3, a highly-contaminated marine sediment (27–29). These were certified for total concentrations of numerous elements and have been used as quality control and for method validation purpose in analytical laboratories worldwide.

## 2. Materials and methods

HISS-1 CRM was sampled from Hibernia shelf, off the coast of Newfoundland, Canada (27) , MESS-4 from Beaufort Sea, Canadian Arctic (28) and PACS-3 from Esquimalt harbor, British Columbia, Canada (29). The CRMs were sieved to particle sizes of less than 125 µm. Homogeneity and stability was assessed. HISS-1 CRM has served as marine sediment reference materials since 1997, PACS-3 since 2013 and MESS-4 since 2014. The three marine sediment CRMs were used "as is" and the moisture content was corrected to obtain concentration based on dry weight.

Laboratory ware, chemicals, and instruments are listed in Table S1 of Electronic Supplementary Material. The extraction solution (Ext sol #) and its preparation in each step are provided in **Table 1**.

**Table 1: Extraction solutions for Tessier sequential extraction**

| Ext sol # | Reagent | Reagent preparation |
|---|---|---|
| 1 | 1 mol $L^{-1}$ sodium acetate solution (NaOAc), pH 8.2 | Dissolve 41 g of NaOAc in 450 mL of deionized water (DIW), adjust to pH 8.2 using acetic acid (AcOH) and dilute to 500 mL with DIW |
| 2 | 1 mol $L^{-1}$ NaOAc, pH 5.0 | Same as Ext sol# 1 but adjust to pH 5 using AcOH |
| 3 | 0.04 mol $L^{-1}$ hydroxylammonium chloride solution ($NH_2OH.HCl$) in 25 % (v/v) AcOH | Dissolve 1.4 g of $NH_2OH.HCl$ in 500 mL of 25 % (v/v) AcOH |
| 4 | 0.02 mol $L^{-1}$ nitric acid ($HNO_3$) | - Add 6.3 mL of 69 % (w/w) $HNO_3$ and make up to 1,000 mL with DIW to obtain 0.1 mol $L^{-1}$ $HNO_3$<br>- Pipette 20 mL of 0.1 mol $L^{-1}$ $HNO_3$ and make up to 100 mL with DIW to obtain 0.02 mol $L^{-1}$ $HNO_3$ |
| | 30 % (w/v) hydrogen peroxide ($H_2O_2$) at pH 2 | Add few drops of 69% (w/w) $HNO_3$ to 30% (w/v) $H_2O_2$ and check pH with pH strip to obtain pH 2 |
| | 3.2 mol $L^{-1}$ ammonium acetate solution ($NH_4OAc$) in 20 % (v/v) $HNO_3$ | Dissolve 24.7 g of $NH_4OAc$ in 100 mL of 20 % (v/v) of $HNO_3$ |

## 2.1 Method for sediment and residue digestion

The following digestion method is used for total sediment digestion of the raw material CRMs and reported results in Section 3.1 Total concentrations of elements and Section 3.2 Concentrations of elements by Tessier sequential extraction in Table 2 to 4 for the residues. Two laboratories (Lab A and Lab B) used their routine method for digestion as described by Kumkrong et.al. (21). In brief Lab A used 0.25 g of sample and a mixture of $HNO_3$, hydrochloric acid (HCl) and hydrogen fluoride (HF). The excess amount of HF was destroyed by adding

boric acid ($H_3BO_3$).After the microwave digestion, the samples were placed in a hot block, evaporated to obtain 5 mL volume and diluted to 50 g using 2 % (v/v) $HNO_3$.

Lab B used 0.25 g of raw material or 1 g of residue and digested using a mixture of $HNO_3$, HF, and $H_2O_2$. The opened digestion was performed after microwave digestion using a mixture of $HNO_3$ and HCl on a hot block and evaporated to approximately 1 mL and the digestrate was diluted to 100 g using DIW.

The elemental analysis of digestrates was conducted using inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS). The conditions of ICP-OES and ICP-MS are stated in the Electronic Supplementary Material Table S2.

## 2.2 Tessier sequential extraction procedure

The extraction procedure is presented in **Fig 2.** and detailed in Table S3 of Electronic Supplementary Material. The extraction tube was weighed before and after adding a new extraction solution for a weight correction. The extraction steps were labeled as Tessier step 1 - exchangeable (T # 1), step 2 - bound to carbonates (T # 2), step 3 - bound to Fe-Mn oxides (T # 3), step 4 - bound to organic matter and sulfide minerals (T # 4), and residue. The fractions were analyzed for the concentration of metals using ICP-OES or/and ICP-MS with an external or standard addition calibration depending on the laboratory procedure. Dilution with 2 % (v/v) $HNO_3$ was required if the concentration exceeded the calibration range. The reagent blank was used for background correction.

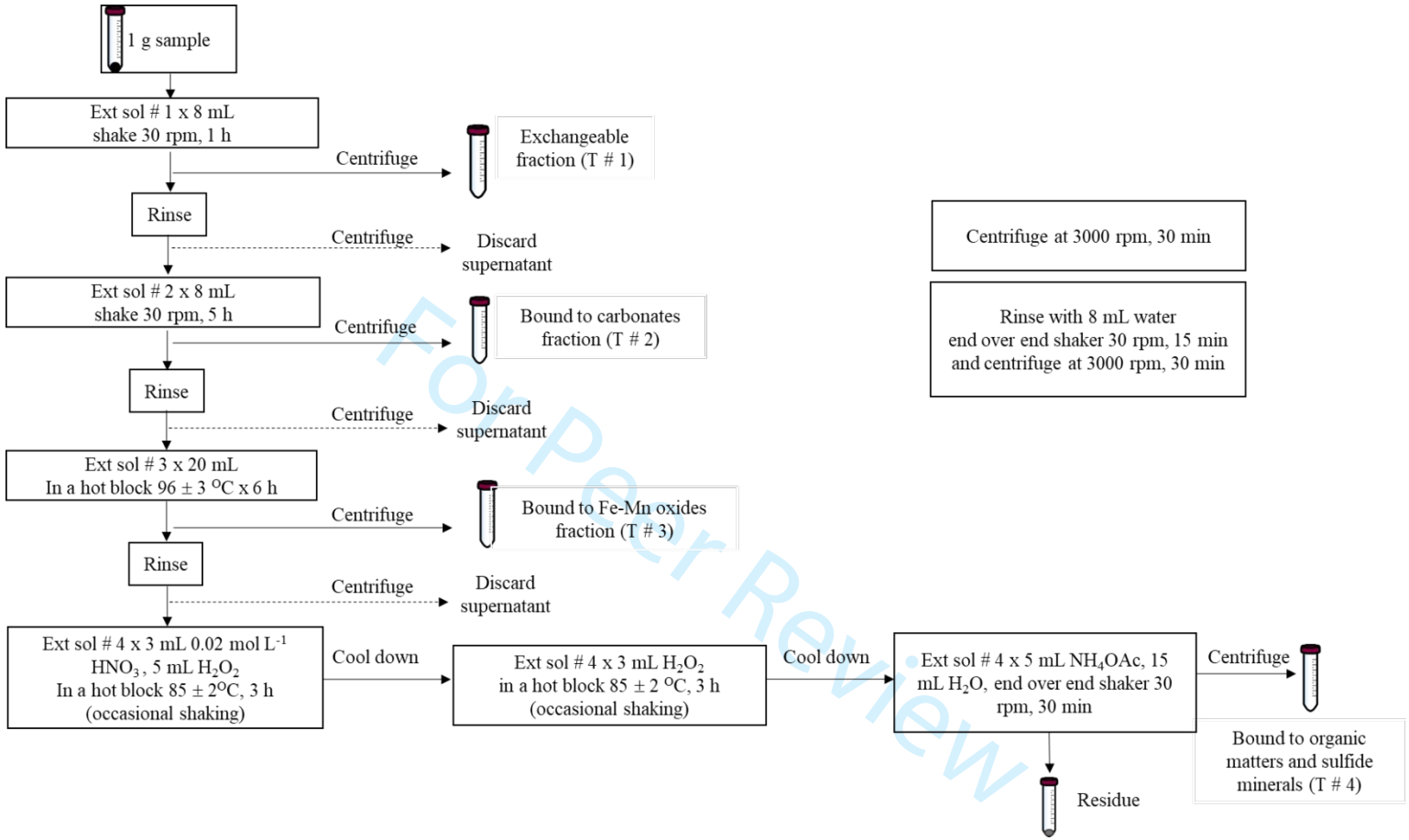


**Figure 2: Tessier sequential extraction procedure flowchart**

## 2.3 Data manipulation

A robust estimator based on the DerSimonian-Laird model was used for the assignment of the consensus value between the various laboratory data sets. NIST Consensus Builder software (https://consensus.nist.gov/app/nicob) was used for combining results from two laboratories using mean, standard deviation, and degrees of freedom from each laboratory (30). The DerSimonian-Laird model is often used for establishing an assigned value (*i.e.* key comparison reference value) and associated standard uncertainty for Consultative Committee for Amount of Substance (CCQM) key comparison data (31). The uncertainty values reported here are standard uncertainty at coverage factor k = 1 and expanded to cover 95 % confidence interval using k equals to 2. The approach of using DerSimonian-Laird model was also used for the assigned values of extracted elements determined recently for HISS-1, MESS-4 and PACS-3 CRMs by the BCR sequential extraction (21).

The percent distribution of each extraction step and residue was calculated according to Equation 1. The percent distribution fraction is used to evaluate the mobility of elements from the Tessier extraction.

$$\%\ Distribution\ fraction = \frac{100 \cdot\ C_{(i,x)}}{\sum(C_{1,x} + C_{2,x} + C_{3,x} + C_{4,x} + C_{residue,\,x})}$$

**Equation 1**

In the above equation, $C_{(i,x)}$ is the concentration of analyte *(x)* from Tessier step *i*. Denominator in Eq.1 (total combined concentration values) corresponds to the summation of concentrations ($C_i$) from steps 1 to 4 and residue ($C_{residue}$).

The standard uncertainty ($u_{std}$; k = 1) from DerSimonian-Laird model was compared to the target uncertainty ($u_{target}$) from the Horwitz equation (see Equation 2 below). Horwitz equation is widely used as a target uncertainty in inter-laboratory comparison (32,33), and as criteria for quality control in food and drug analysis (34). The calculation is based on the mass fraction of analyte *(C)* as follows :

$$\%\boldsymbol{u_{target}} = \mathbf{2}^{(\mathbf{1} - \mathbf{0.5}\boldsymbol{logC})} \qquad \textbf{Equation 2}$$

The accuracy of Tessier sequential extraction was calculated as a percent recovery (Equation 3) using the combination of the concentrations from four extraction steps and residue, then comparing against the total concentration from the

certified value. If no certified values were available, an average total concentration from the two laboratories was used as reference point.

$$Recovery\ (\%) = \frac{\sum(C_{1,x} + C_{2,x} + C_{3,x} + C_{4,x} + C_{residue,\,x})\ x\ 100}{C_{Certified\ value}}$$

**Equation 3**

# 3 Results

## 3.1 Total concentrations of elements in HISS-1, MESS-4, and PACS-3

The concentrations of 17 elements from the three CRMs were analyzed separately by two laboratories and previously reported by Kumkrong et.al (21) as indicated in Table S4 of Electronic Supplementary Material. The percent recovery of each element was calculated against the certified value and presented as a bar graph in Fig. S1 of Electronic Supplementary Material. The total concentrations of elements reported by the two laboratories shows no significant difference between the two digestion methods at 95% confidence interval level by paired T-test (21). The concentration of Cr was significantly low from both digestion methods when a mild digestion procedure was applied (27).

This study reported the concentrations associated with uncertainty (k=2) of some elements that are not certified in the three CRMs including Sb 0.119 ± 0.049 mg $kg^{-1}$, Sn 0.142 ± 0.020 mg $kg^{-1}$, and U 0.304 ± 0.116 mg $kg^{-1}$ for HISS-1, Se 1.18 ± 0.10 mg $kg^{-1}$ for MESS-4, and Se 1.03 ± 0.26 mg $kg^{-1}$, and U 2.58 ± 0.66 mg $kg^{-1}$ for PACS-3.

## 3.2 Concentrations of elements by Tessier sequential extraction

The concentrations of elements in HISS-1, MESS-4 and PACS-3 from the extraction steps and residue along with the associated uncertainty (k=2) are reported in **Table 2** to **Table 4**. The distribution profiles of the three marine sediment CRMs was calculated for the purpose of interpreting the mobility of the elements and are shown in **Fig. 3**. The percent distribution was calculated based on the mass fraction of each extraction step and the combined concentration of the four extraction steps and the residue, as mentioned in Equation 1. The concentrations of Cd and Se in HISS-1 were removed from the results shown in **Fig. 3** due to an abnormal significantly higher recovery measured for these elements

in HISS-1. We note that these two elements in HISS-1 were presented in extremely low concentrations (0.024 mg $kg^{-1}$ for Cd and 0.050 mg $kg^{-1}$ for Se) (27).

167 **Table 2 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in HISS-1**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 2(8) | 0.105 | 0.028 | 0.123 | 0.035 | 0.322 | 0.130 | ≤ 0.01 | - | 3.05 | 2.64 | 3.60 | 2.64 |
| Be | 2(8) | ≤ 0.004 | - | ≤ 0.004 | - | 0.004 | 0.002 | 0.009 | 0.002 | 0.125 | 0.059 | 0.125 | 0.059 |
| V | 2(8) | 0.133 | 0.050 | ≤ 0.01 | - | 1.23 | 0.17 | 0.374 | 0.126 | 6.33 | 2.11 | 8.06 | 2.12 |
| Cr | 3(13) | ≤ 0.003 | - | < 0.003 | - | 1.18 | 0.21 | 0.596 | 0.110 | 16.8 | 0.6 | 18.5 | 0.6 |
| Mn | 2(8) | ≤ 0.1 | - | 1.64 | 0.44 | 4.99 | 0.42 | 0.502 | 0.005 | 59.8 | 7.1 | 67.0 | 7.1 |
| Fe | 2(8) | ≤ 2 | - | 6.30 | 0.15 | 333 | 27 | 18.7 | 1.0 | 2,003 | 110 | 2,361 | 113 |
| Ni | 2(8) | ≤ 0.01 | - | 0.575 | 0.107 | 0.752 | 0.112 | 0.345 | 0.048 | 1.24 | 0.29 | 2.91 | 0.33 |
| Cu | 3(13) | 0.113 | 0.002 | 0.279 | 0.069 | 0.751 | 0.059 | 0.711 | 0.019 | 0.627 | 0.311 | 2.48 | 0.32 |
| Zn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 1.52 | 0.14 | 0.412 | 0.091 | 3.14 | 0.71 | 5.07 | 0.72 |
| As | 2(8) | 0.050 | 0.026 | 0.124 | 0.0003 | 0.198 | 0.044 | 0.027 | 0.012 | 0.501 | 0.403 | 0.902 | 0.407 |
| Sr | 3(13) | 6.97 | 0.54 | 52.5 | 18.2 | 2.14 | 0.10 | 0.266 | 0.016 | 21.1 | 0.5 | 82.9 | 18.2 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | 0.054 | 0.003 | 0.138 | 0.114 | 0.192 | 0.114 |
| Sb | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 0.179 | 0.023 | ≤ 0.01 | - | 0.116 | 0.005 | 0.116 | 0.005 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pb | 2(8) | ≤ 0.01 | - | 0.682 | 0.135 | 0.622 | 0.029 | 0.186 | 0.069 | 1.99 | 0.24 | 3.48 | 0.29 |
| U | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 0.059 | 0.022 | 0.013 | 0.006 | 0.157 | 0.036 | 0.228 | 0.043 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

172 **Table 3 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in MESS-4**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 3(11) | 0.403 | 0.086 | 0.486 | 0.022 | 6.80 | 1.51 | 2.35 | 0.28 | 48.6 | 7.7 | 58.7 | 7.9 |
| Be | 2(8) | ≤ 0.004 | - | 0.041 | 0.024 | 0.472 | 0.180 | 0.070 | 0.010 | 1.72 | 0.13 | 2.30 | 0.22 |
| V | 2(8) | 0.276 | 0.044 | 0.471 | 0.121 | 21.9 | 2.3 | 0.764 | 0.205 | 199 | 25 | 222 | 25 |
| Cr | 3(11) | 0.005 | 0.001 | 0.159 | 0.030 | 3.55 | 0.69 | 1.84 | 0.40 | 89.0 | 10.4 | 94.5 | 10.5 |
| Mn | 3(11) | 2.95 | 0.26 | 75.7 | 7.1 | 71.5 | 10.4 | 11.4 | 0.24 | 111 | 16 | 273 | 20 |
| Fe | 2(8) | 4.26 | 1.23 | 327 | 31 | 6,152 | 235 | 508 | 108 | 27,826 | 6,756 | 34,818 | 6,761 |
| Ni | 3(11) | 0.055 | 0.009 | 0.829 | 0.067 | 12.3 | 3.3 | 5.82 | 0.89 | 26.8 | 4.6 | 45.8 | 5.8 |
| Cu | 3(11) | 0.463 | 0.015 | 1.44 | 0.29 | 4.42 | 0.27 | 7.13 | 0.93 | 20.9 | 2.7 | 34.4 | 2.9 |
| Zn | 3(11) | 0.101 | 0.044 | 11.7 | 0.5 | 49.4 | 10.4 | 13.6 | 2.1 | 81.8 | 8.1 | 157 | 13 |
| As | 3(11) | 0.102 | 0.014 | 0.257 | 0.050 | 2.57 | 0.27 | 0.264 | 0.060 | 18.0 | 3.7 | 21.2 | 3.7 |
| Se | 2(8) | 0.025 | 0.005 | 0.205 | 0.138 | 0.099 | 0.090 | 0.526 | 0.125 | 0.219 | 0.081 | 1.07 | 0.27 |
| Sr | 3(11) | 14.4 | 0.2 | 10.7 | 1.7 | 10.7 | 2.0 | 2.38 | 0.38 | 85.4 | 13.3 | 124 | 14 |
| Cd | 2(8) | ≤ 0.02 | - | 0.080 | 0.025 | 0.049 | 0.035 | 0.040 | 0.072 | 0.098 | 0.059 | 0.241 | 0.103 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | 3.23 | 0.48 | 3.23 | 0.48 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sb | 2(8) | 0.020 | 0.016 | 0.028 | 0.002 | 0.011 | 0.007 | ≤ 0.01 | - | 1.11 | 0.18 | 1.17 | 0.18 |
| Pb | 3(11) | 0.014 | 0.006 | 2.50 | 0.34 | 8.33 | 1.88 | 0.271 | 0.025 | 10.2 | 2.7 | 21.3 | 3.3 |
| U | 2(8) | 0.055 | 0.003 | 0.237 | 0.0312 | 0.353 | 0.224 | 0.162 | 0.004 | 2.28 | 0.93 | 3.09 | 0.95 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

176 **Table 4 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in PACS-3**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 3(11) | 0.518 | 0.012 | 0.277 | 0.083 | 7.76 | 0.88 | 2.37 | 0.03 | 19.3 | 4.9 | 30.2 | 5.0 |
| Be | 3(11) | ≤ 0.004 | - | 0.032 | 0.004 | 0.176 | 0.050 | 0.030 | 0.002 | 0.750 | 0.035 | 0.988 | 0.061 |
| V | 3(11) | 0.273 | 0.310 | 0.591 | 0.207 | 38.3 | 5.5 | 4.14 | 0.48 | 95.9 | 19.1 | 139 | 20 |
| Cr | 3(11) | 0.133 | 0.056 | 0.917 | 0.213 | 20.8 | 2.3 | 11.6 | 1.9 | 66.1 | 8.5 | 99.6 | 9.0 |
| Mn | 3(11) | 2.33 | 0.27 | 6.55 | 1.31 | 49.3 | 8.0 | 38.2 | 1.3 | 299 | 39 | 396 | 39 |
| Fe | 2(8) | 4.05 | 3.31 | 340 | 214 | 7,353 | 421 | 4,907 | 539 | 24,482 | 327 | 37,086 | 870 |
| Ni | 3(11) | 0.247 | 0.080 | 1.40 | 0.07 | 10.6 | 2.5 | 4.55 | 0.73 | 24.7 | 4.2 | 41.4 | 5.0 |
| Cu | 3(11) | 4.15 | 0.83 | 27.8 | 7.5 | 20.6 | 4.2 | 223 | 21 | 42.2 | 5.3 | 318 | 23 |
| Zn | 3(11) | 17.2 | 1.2 | 140 | 19 | 168 | 38 | 33.4 | 3.4 | 72.0 | 10.2 | 430 | 44 |
| As | 3(11) | 0.093 | 0.034 | 0.363 | 0.016 | 10.0 | 0.2 | 1.87 | 0.25 | 18.5 | 3.9 | 30.8 | 3.9 |
| Se | 2(8) | 0.024 | 0.008 | 0.039 | 0.011 | < 0.02 | - | 0.888 | 0.254 | 0.232 | 0.005 | 1.18 | 0.25 |
| Sr | 3(11) | 28.1 | 2.5 | 11.3 | 1.9 | 15.1 | 1.2 | 4.08 | 0.28 | 174 | 103 | 232 | 103 |
| Cd | 2(8) | ≤ 0.02 | - | 1.18 | 0.33 | 0.910 | 0.356 | 0.085 | 0.078 | 0.098 | 0.060 | 2.27 | 0.50 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 1.89 | 0.10 | ≤ 0.01 | - | 24.4 | 7.9 | 26.3 | 7.9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sb | 3(11) | 0.046 | 0.016 | 0.223 | 0.009 | 0.442 | 0.399 | 0.079 | 0.049 | 13.0 | 1.3 | 13.8 | 1.4 |
| Pb | 3(11) | 0.919 | 0.330 | 37.5 | 12.3 | 118 | 26 | 22.9 | 2.4 | 36.1 | 8.2 | 216 | 30 |
| U | 2(8) | ≤ 0.01 | - | 0.584 | 0.047 | 0.555 | 0.211 | 0.328 | 0.284 | 1.17 | 0.27 | 2.64 | 0.45 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

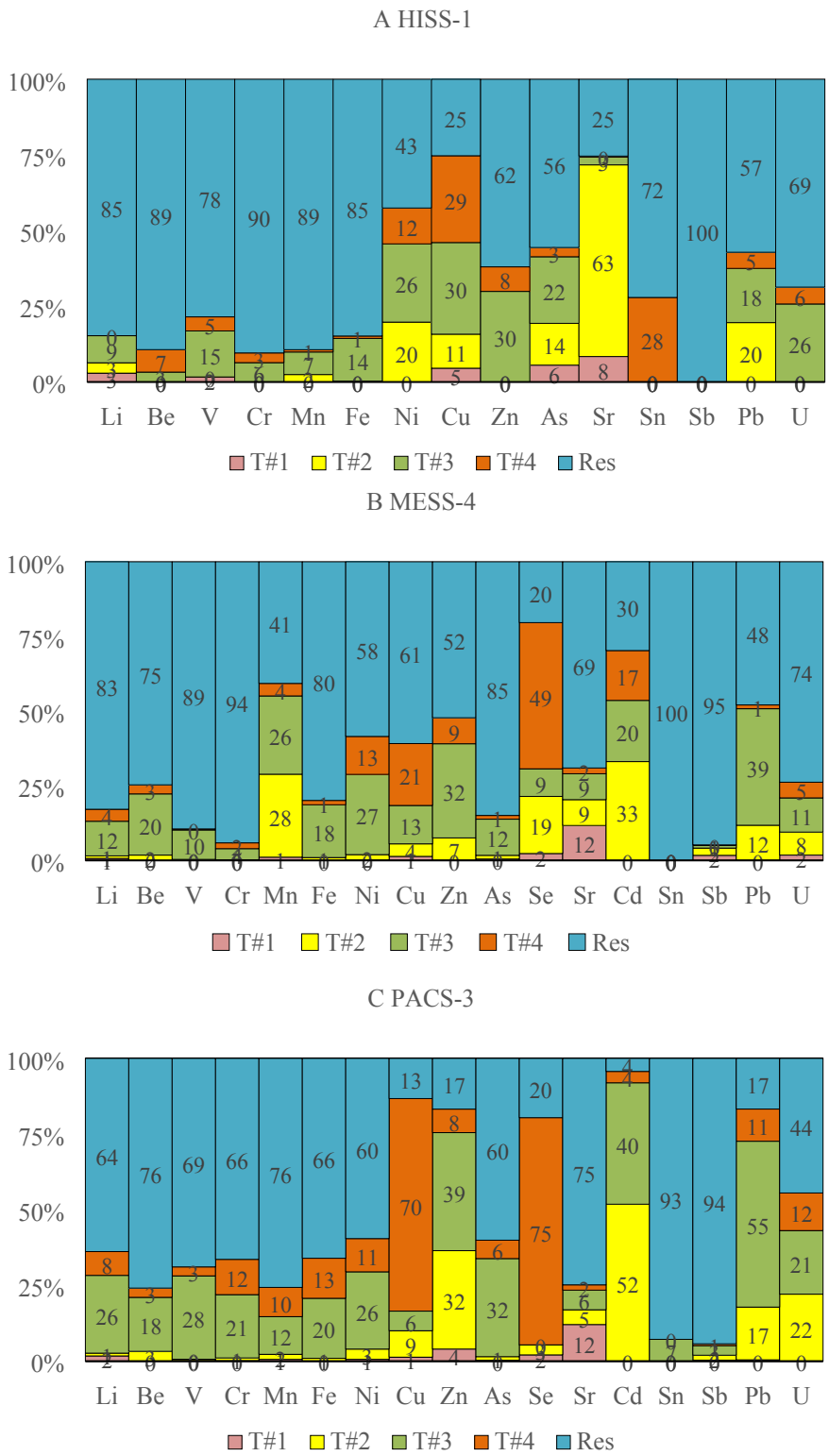


**Figure 3 : Percent distribution (y-scale) of elements (x-scale) by Tessier extraction from A HISS-1, B MESS-4 and C PACS-3**

# 4 Discussion

## 4.1 Total concentrations of elements in HISS-1, MESS-4, and PACS-3

**Fig. 4** plots the total concentrations of elements in the three marine sediment CRMs in comparison to the concentrations in Pelagic clay of the ocean (3) and the earth crust (36). Generally, in decreasing order of abundance, the concentrations of elements were: PACS-3 > MESS-4 > Pelagic clay > Earth crust >> HISS-1. The concentrations of Fe in MESS-4 and PACS-3 were similar and 80 times higher than in HISS-1. HISS-1 contains high amount of silica as quartz and has low accumulation of foreign matters (21,27). PACS-3 showed high concentrations of Zn, Cu, Pb, Sn, As, Sb, Se and Cd, possibly attributable to anthropogenic inputs. MESS-4 showed twice higher concentrations for V and Li when compared to PACS-3.

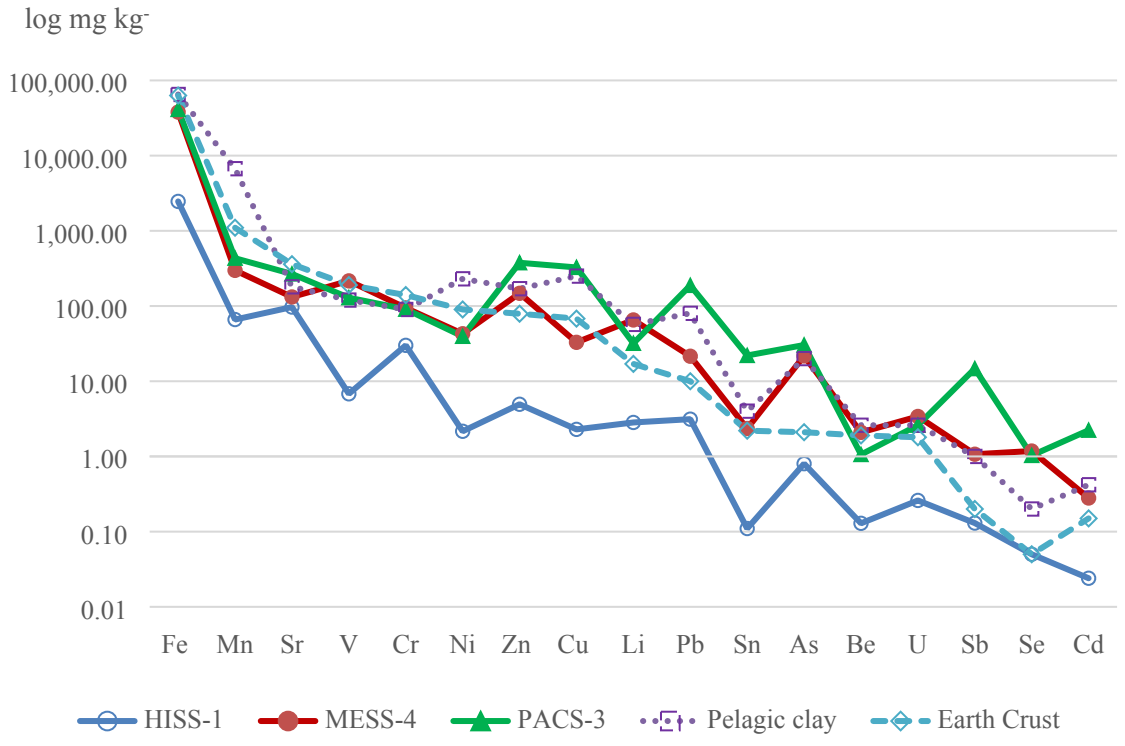


**Figure 4 : Element concentration and elements in HISS-1, MESS-4 and PACS-3 marine sediments, in comparison to Pelagic clay** (3) **and Earth crust** (36)

## 4.2 Distribution profile of elements from Tessier sequential extraction

### 4.2.1 HISS-1

Considering the released concentrations of elements in HISS-1 (**Table 2**), elements were detected in significant amounts from T # 1 and T # 2, except for Sr. Strontium likely associated with carbonate minerals and dissolved in the acidic solution of T # 2. The concentration of Sr in T # 2 appeared dominant of 63 % and left only 25 % in the residue (**Fig. 3A**). Very little Fe and Mn were extracted in T # 1. High concentrations of Fe and Mn were released in the reducing condition of T # 3; however, majority still remained in the residue (**Fig. 3A**). Additionally, many of the elements were released in T # 3 including Mn (4.95 mg $kg^{-1}$) > Sr > Zn ≈ V ≈ Cr > Ni ≈ Cu ≈ Pb (0.622 mg $kg^{-1}$). It is likely that most elements were released with Fe dissolution in reducing condition. Greater than 70 % fraction of Sb, Cr, Mn, Be, Fe, Li, V, and Sn remained in the residues.

### 4.2.2 MESS-4

The total concentrations of released elements in MESS-4 are 3 to 45 times higher than in HISS-1. The higher concentrations observed in T # 1 were for Sr (14.4 mg $kg^{-1}$) >> Fe > Mn (2.95 mg $kg^{-1}$), and trace amounts of Cu (0.463 mg $kg^{-1}$) > Li > V (0.276 mg $kg^{-1}$) were observed. At T # 2, significantly high concentrations of Fe and Mn were released, in contrast with HISS-1 where virtually no Fe and Mn were released in T # 1, as noted above. A high fraction of 28 % Mn released at T # 2 suggests that Mn in carbonate minerals was dominant in MESS-4 marine sediment. Besides, high concentrations of Fe (327 mg $kg^{-1}$) >>> Zn >> Pb > Cu (1.4 mg $kg^{-1}$) were also observed with carbonate mineral dissolution at T # 2. Other elements that were observed in trace amounts (from 0.1 mg $kg^{-1}$ to 1 mg $kg^{-1}$) in T # 2, included As, Cr, Li, Ni, V and U. Several elements were released in T # 3, similarly to HISS-1, but 3 to 116 times larger concentrations were detected in MESS-4 as follows : Fe (6,152 mg $kg^{-1}$) >>> Mn > Zn > V > Ni ≈ Sr > Pb > Li > Cu > Cr > As (2.57 mg $kg^{-1}$). At T # 4, apart from high concentrations of Fe, Zn, and Mn, a large fraction (21 %) of the Cu (**Fig. 3B**) was released (7.13 mg $kg^{-1}$) followed by Ni (5.82 mg $kg^{-1}$) >> Sr ≈ Li > Cr (1.84 mg $kg^{-1}$). A significant portion of Sn (100 %), Sb, Cr, V, Li, Fe, Be, and U (74 %) remained in the residue, demonstrating minimal mobility of these elements in MESS-4.

#### 4.2.3 PACS-3

PACS-3 is a highly-contaminated marine sediment with 3 to 200 times higher concentration of the elements analyzed than in HISS-1 (**Fig 4**). At T # 1, elevated concentrations were observed for most elements (except for Be, Cd, Sb, Se, Sn, and U), with particularly high concentrations measured for Zn, Cu, and Pb. At T # 2, higher concentrations of Zn, Pb and Cu were released with carbonate dissolution in comparison to T #1. Similar to HISS-1 and MESS-4, high concentration of Fe (7,353 mg $kg^{-1}$) was observed at T # 3 together with many elements including Zn (168 mg $kg^{-1}$) > Pb >> Mn > V > Cr, Cu > Sr > Ni ≈ As (10 mg $kg^{-1}$). It seems the highest fractions of Pb, Zn, and As are observed at T # 3. Interestingly, Sn was firstly observed in PACS-3 at T # 3 (**Fig. 3C**), suggesting that high accumulation of Sn from the harbor input existed as mono-, di- and tri butyltin in PACS-3 (29); in particular, butyltin is widely used as an antifouling agent in marine paint (37). At T # 4, surprisingly, a high concentration of Fe (4,907 mg $kg^{-1}$) was observed, indicating Fe associated with sulfide minerals in PACS-3, which is different from HISS-1 and MESS-4. The largest fractions of Cu (70 %) and Se (59 %) were observed at T # 4 (**Fig 3C**), similarly to HISS-1 (**Fig 3A**) and MESS-4 (**Fig 3B**). The most stable elements which remained in the residue are Sb (94 %), Sn, Be, Mn, and Sr (75 %).

## 4.3 Analytical performance of Tessier sequential extraction on three marine sediment CRMs

#### 4.3.1 Assessment of precision

Since there are no CRM reference data sets readily available on Tessier sequential extraction procedure, assessment of precision and accuracy is needed to benchmark the procedure. The reproducibility from Horwitz formulation (33) was chosen as a target to assess observed precisions in this study. **Fig. 5** illustrates that the majority of standard uncertainty ($u_{std}$, k=1) of element concentrations determined with the Tessier sequential extractions on HISS-1, MESS-4 and PACS-3 were below the target uncertainty specified by Horwitz equation (Equation 2). Higher than target uncertainties were occasionally observed for lower concentration elements of the three CRMs, which might reflect either stability of the extracts and/or the laboratories ability to perform the measurements at these concentration regimes. Larger uncertainties observed for elements extracted with high concentrations from PACS-3 are unexpected and non-rationalized.

The distribution of observed relative uncertainties from the four Tessier extraction steps and residue of the three CRMs ranged from 1% to 91% (Cd). The distribution histogram in comparison with target uncertainty from Horwitz model was presented in Fig S2 of Electronic Supplementary Material. Majority (72 %) of uncertainty values in the data set was below the average uncertainty of 13 %, which compares favorably with the average target uncertainty of 16 % (from Horwittz equation, Equation 2).

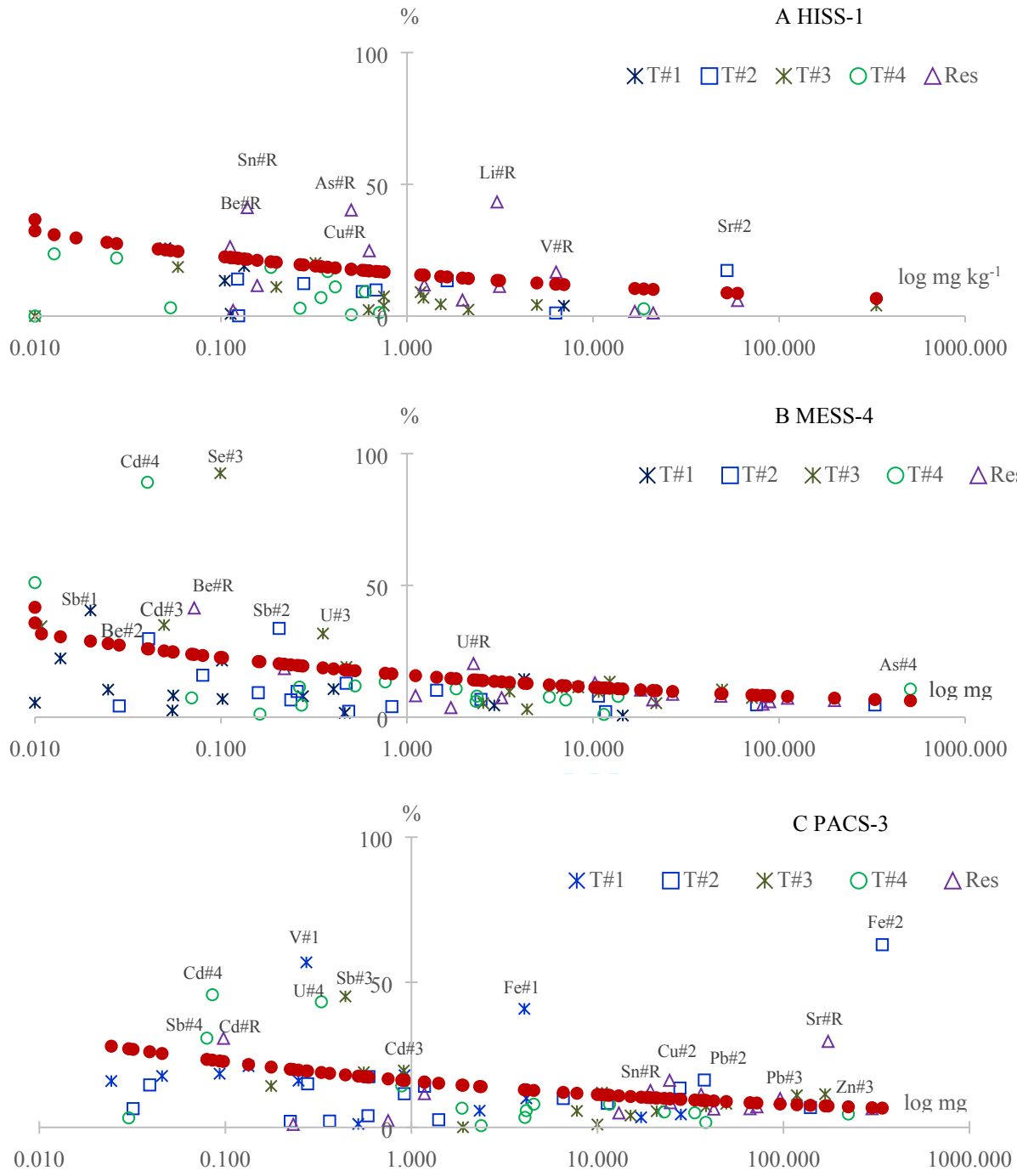


**Figure 5 : Standard uncertainty (%, at k=1) of A HISS-1, B MESS-4, and C PACS-3 from Step T # 1, T # 2, T # 3, T # 4 and residue in comparison with target uncertainty from Horwitz equation (red dot).**

### 4.3.2 Assessment of accuracy

The assessment of accurary of the Tessier extraction procedure is non trivial as no quality control materials/ CRMs are available. The accuracy was assessed as recovery by combining the elemental concentrations measured from the four extraction steps and the residue and then comparing against the total concentrations of analytes from either certified values or measured total concentrations obtained here (see Tables S5 to Table S7 of Electronic Supplementary Material). The results of the recovery study are presented as a bar graph in **Fig. 6**. The recovery of HISS-1 was 92 % $\pm$ 40 %, MESS-4 of 101 % $\pm$ 12 % and PACS-3 of 102 % $\pm$ 10 %. A large deviation of recovery values ($\pm$ 40 %) for HISS-1 is due to a low recovery of Cr (62 %), an element which is known to be problematic for analysis of this particular CRM (21,38).

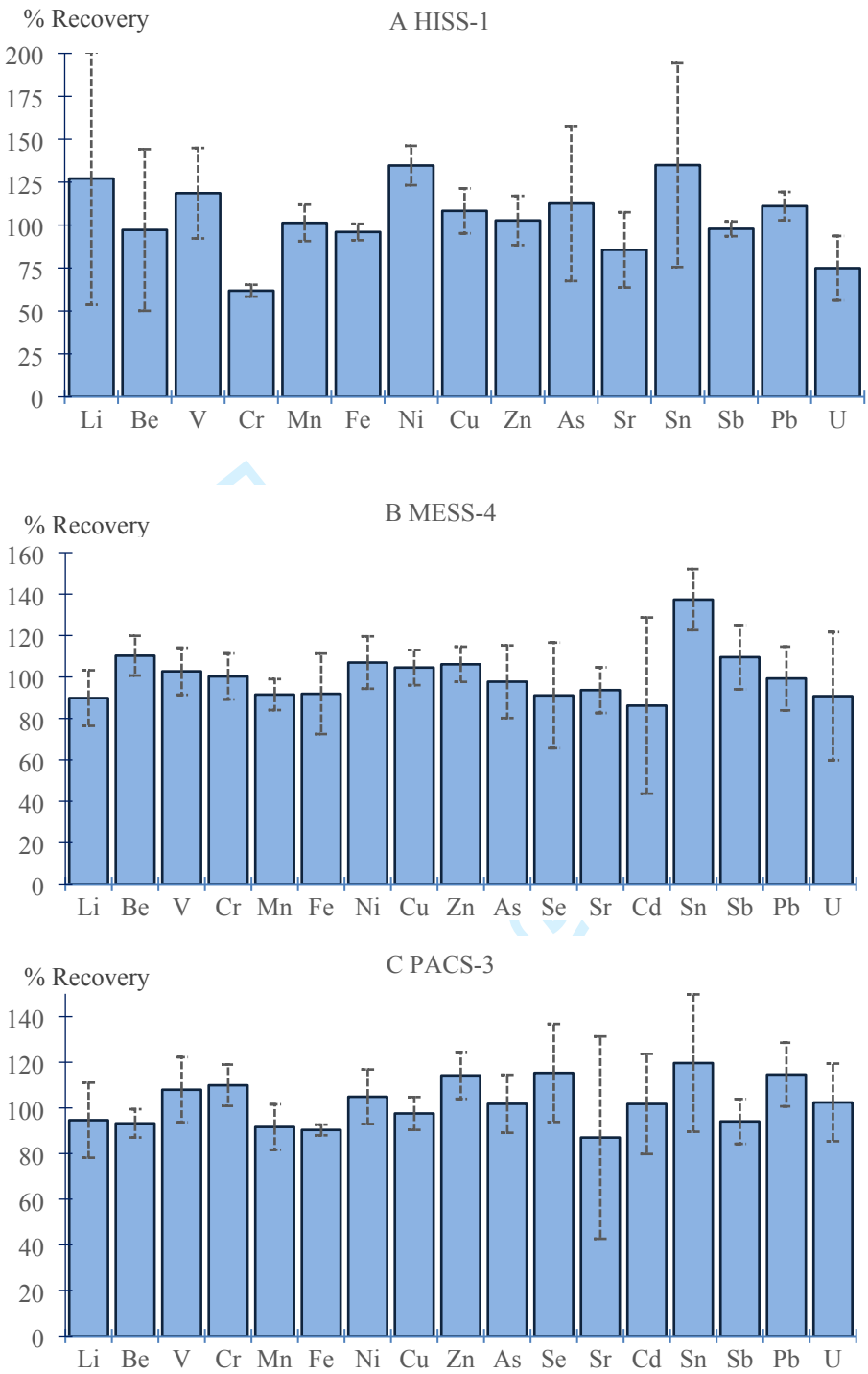


**Figure 6 : Percent recovery of combined concentrations of four extraction steps and residue vs elements of A HISS-1, B MESS-4 and C PACS-3 with observed uncertainty (k=2)**

# 5 Conclusions

The four-step Tessier sequential extraction procedure was applied to three widely used marine sediment CRMs and 17 elements were analysed. Different dissolution profiles were obseved for HISS-1, MESS-4 and PACS-3. Elements that showed mobility in all the extraction steps included Ni, Cu, Zn, As, Se, Sr, Cd, Pb, and U.

In T # 1, a high amount of Sr was released from the three CRMs, particularly in the highly-contaminated marine sediment PACS-3, which released significant amount of Sr, as well as Zn. In the acidic conditions of T # 2, Sr preferentially released from HISS-1, Mn from MESS-4, and Zn and Cd from PACS-3. Most metals mimic the dissolution pattern of Fe with a significant release under the reducing conditions of extraction step T # 3, particularly Pb, Ni and Zn. Interestingly, in oxidizing conditions (T # 4), Cu and Se are relased likely because of their association with iron sulfide, which is being selectively dissolved in this step. In all three sediments, Be, Sb, and Sn remained in the residue. Other elements found in high amounts within the residues of HISS-1 and MESS-4 included Cr, V, Li and Fe.

The precision of Tessier sequential extraction in this study was benchmarked against Horwitz model and was found largely satisfactory. However some elements, which were present in low concentrations, consistently showed high uncertainty. Overall, the percent recovery by the combined element concentrations from the four Tessier extraction steps and residue compared against the certified values shows satisfactory performance of 101 % $\pm$ 12 % for MESS-4 and 102 % $\pm$ 10 % for PACS-3, but poor recovery with high uncertainty for HISS-1 of 92 % $\pm$ 40 %. The reference data set produced here using the Tessier sequential extraction procedure carried out on HISS-4, MESS-4 and PACS-3 CRMs will serve as a quality control and method development tool for laboratories.



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## Compliance with ethical standards

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Tessier sequential extraction on 17 elements from three marine sediment certified reference materials (HISS-1, MESS-4 and PACS-3)

**Paramee Kumkrong*, Ovi Mihai, Patrick H.J. Mercier, Indu Gedara Pihilligawa, Daniel D. Tyo, and Zoltan Mester**

National Research Council Canada, 1200 Montreal Road Ottawa, Ontario Canada, K1A 0R6

*Corresponding author : paramee.kumkrong@nrc-crnc.gc.ca

ORCID : 0000-0002-9281-9974



## Abstract

The four-step Tessier sequential extraction procedure is a well-known approach used for environmental and geochemical studies in soil and sediments. However, a lack of reference materials limits its use making implementation and quality control cumbersome. This study applied Tessier sequential extraction to three globally used marine sediment certified reference materials (CRMs) including HISS-1, MESS-4 and PACS-3 with varying levels of contamination.

The study analyzed the distribution of 17 elements throughout the extraction phases. Overall, the percent recovery (sum of steps vs total metal concentration) of all analyzed elements in Tessier extraction was 92 % ± 40 % in HISS-1, 101 % ± 12 % in MESS-4 and 102 % ± 10 % in PACS-3. The observed uncertainty of the individual elemental concentrations averaged of 13 %, which compares favorably with the 16 % target uncertainty derived from Horwitz equation. The reference data set produced here using the Tessier sequential extraction procedure will serve as a quality control and method development tool for laboratories.

Keywords: Distribution. Certified reference material. Sequential extraction. Recovery. Uncertainty.

# Table of contents

# Abbreviation

| | |
|---|---|
| °C | Degree Celsius |
| µm | Micrometer |
| BCR | Community Bureau of Reference |
| CI | Confidence interval |
| CRC | Collision reaction cell |
| CRM(s) | Certified reference material(s) |
| DIW | Deionized water |
| eV | Electron volt |
| Ext sol# | Extraction solution |
| g | Gram |
| h | Hour |
| ICP | Inductively coupled plasma |
| ISO | International Organization for Standardization |
| L | Litre |
| log | Logarithm |
| mol $L^{-1}$ | Mole per litre |
| mg | Milligram |
| min | Minute |
| mL | Millilitre |
| MS | Mass spectrometry |
| MΩ-cm | Megohm-centimeter |
| na | Not applicable |
| nd | Not detected |
| nr | Not reported |
| NRC | National Research Council Canada |

| | |
|---|---|
| OES | Optical emission spectrometry |
| rpm | Revolutions per minute |
| RSD | Relative standard deviation |
| SD | Standard deviation |
| $S_R$ | Reproducibility standard deviation |
| $u_{std}$ | Standard uncertainty |
| U | Expanded uncertainty |
| v | Volume |
| w | Weight |

# 1 Introduction

The association between metals bound to sediments is of interest because their potential release could pose significant environmental challenges (1,2). Marine sediments are mainly composed of biogenous, hydrogenous, and lithogenous sediments (3), and also receive foreign materials from inland activities such as weathering and anthropogenic inputs. A high accumulation of toxic metals in marine sediments is widely observed in areas of high human activity, for example harbors (4–8).

In general, assessment of sediment quality is achieved by quantitative analysis of total toxic metal content (9–11). However, not all measured total metal content in sediments is actually posing an environmental hazard, so better approaches that estimate the labile metal fraction and the conditions of their release are needed. The importance of such investigations brought the development of methodologies based on fractionating toxic metals from solid phases by partial dissolution / desorption / extraction (12–17).

Among sequential extraction procedures, the Tessier and the Community Bureau of Reference (BCR) sequential extractions are most commonly employed (18,19). The publications citing Tessier and BCR sequential extraction in Scopus database (https://www.scopus.com, accessed on June 7, 2020) showed results of 3,625 and 2,332, respectively. Tessier expressed significant growth until it reached a plateau in 2011, however, BCR extraction method has continued gaining popularity (**Fig.1**).

Tessier sequential extraction procedure was published in 1979 for the determination of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in river sediments from Quebec, Canada (18). The BCR method was developed with the objective to harmonize the sequential extraction procedure and be used in conjunction with the production of BCR 601 and BCR 701 CRMs (river sediment reference materials) that certified Cd, Cr, Cu, Ni, Pb, and Zn (19,20). There is a plethora of data available from the BCR on method characteristics (21–26) but the availability of such information is much limited for the Tessier extraction approach using reference materials.

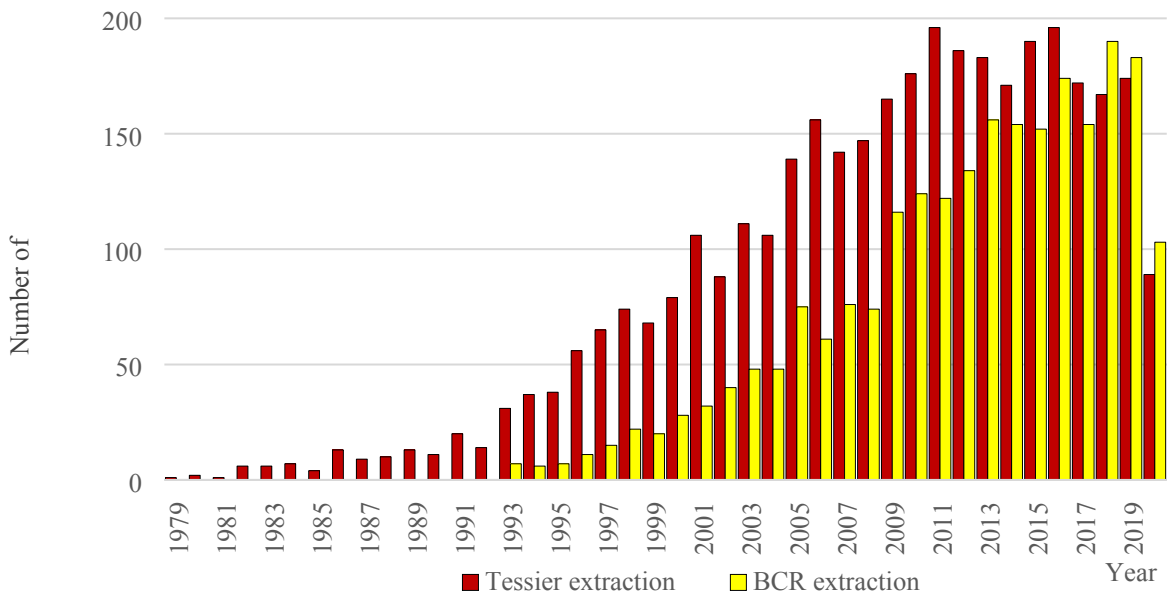


**Figure 1** : **The number of publications of Tessier and BCR sequential extraction procedure since 1979 to 2020 (Source: Scopus database, searched under keywords: "sequential extraction BCR" and "sequential extraction Tessier" (https://www.scopus.com, accessed on June 07, 2020)**

Considering the lack of CRMs and reference data on precision and accuracy upon application of a given extraction method, we have performed the Tessier extraction procedure on 17 elements (As, Be, Cd, Cr, Cu, Fe, Li, Mn, Ni, Pb, Sb, Se, Sn, Sr, U, V, and Zn) in three marine sediment CRMs produced by National Research Council Canada (NRC). The three CRMs included: HISS-1, a clean sediment; MESS-4, a moderately-contaminated sediment; and PACS-3, a highly-contaminated marine sediment (27–29). These were certified for total concentrations of numerous elements and have been used as quality control and for method validation purpose in analytical laboratories worldwide.

## 2. Materials and methods

HISS-1 CRM was sampled from Hibernia shelf, off the coast of Newfoundland, Canada (27) , MESS-4 from Beaufort Sea, Canadian Arctic (28) and PACS-3 from Esquimalt harbor, British Columbia, Canada (29). The CRMs were sieved to particle sizes of less than 125 µm. Homogeneity and stability was assessed. HISS-1 CRM has served as marine sediment reference materials since 1997, PACS-3 since 2013 and MESS-4 since 2014. The three marine sediment CRMs were used "as is" and the moisture content was corrected to obtain concentration based on dry weight.

Laboratory ware, chemicals, and instruments are listed in Table S1 of ~~Supplementary Material~~Electronic Supplementary Material. The extraction solution (Ext sol #) and its preparation in each step are provided in **Table 1**.

**Table 1: Extraction solutions for Tessier sequential extraction**

| Ext sol # | Reagent | Reagent preparation |
|---|---|---|
| 1 | 1 mol $L^{-1}$ sodium acetate solution (NaOAc), pH 8.2 | Dissolve 41 g of NaOAc in 450 mL of deionized water (DIW), adjust to pH 8.2 using acetic acid (AcOH) and dilute to 500 mL with DIW |
| 2 | 1 mol $L^{-1}$ NaOAc, pH 5.0 | Same as Ext sol# 1 but adjust to pH 5 using AcOH |
| 3 | 0.04 mol $L^{-1}$ hydroxylammonium chloride solution ($NH_2OH.HCl$) in 25 % (v/v) AcOH | Dissolve 1.4 g of $NH_2OH.HCl$ in 500 mL of 25 % (v/v) AcOH |
| 4 | 0.02 mol $L^{-1}$ nitric acid ($HNO_3$) | - Add 6.3 mL of 69 % (w/w) $HNO_3$ and make up to 1,000 mL with DIW to obtain 0.1 mol $L^{-1}$ $HNO_3$<br>- Pipette 20 mL of 0.1 mol $L^{-1}$ $HNO_3$ and make up to 100 mL with DIW to obtain 0.02 mol $L^{-1}$ $HNO_3$ |
| | 30 % (w/v) hydrogen peroxide ($H_2O_2$) at pH 2 | Add few drops of 69% (w/w) $HNO_3$ to 30% (w/v) $H_2O_2$ and check pH with pH strip to obtain pH 2 |
| | 3.2 mol $L^{-1}$ ammonium acetate solution ($NH_4OAc$) in 20 % (v/v) $HNO_3$ | Dissolve 24.7 g of $NH_4OAc$ in 100 mL of 20 % (v/v) of $HNO_3$ |

## 2.1 Method for sediment and residue digestion

The following digestion method is used for total sediment digestion of the raw material CRMs and reported results in Section 3.1 Total concentrations of elements and Section 3.2 Concentrations of elements by Tessier sequential extraction in Table 2 to 4 for the residues. Two laboratories (Lab A and Lab B) used their routine method for digestion~~. Lab A and Lab B used the microwaved digestion method~~ as described by Kumkrong

et.al. (21). In brief Lab A used 0.25 g of sample and a mixture of $HNO_3$, hydrochloric acid (HCl) and hydrogen fluoride (HF) ~~rammed~~. The excess amount of HF was destroyed by adding boric acid ($H_3BO_3$). ~~After~~andre-digested ~~in~~ the microwave digestion, the samples were placed in a hot block, evaporated ~~at~~ to obtain 5 mL volume and diluted to 50 g using 2 % (v/v) $HNO_3$.

~~Lab B modified the U.S. EPA. Method 3052 (31) as~~ Lab B used ~~follows: 0.25 g of sample or 1.0 g of residue was weighed in pre-cleaned Teflon microwave digestion vessels, then~~ 0.25 g of raw material or 1 g of residue and digested using a mixture of ~~10 mL of 69 % (w/w)~~ $HNO_3$, ~~3 mL of 48 % (w/v)~~ HF, and ~~3 ml of 30 % (w/w)~~ $H_2O_2$ ~~were added and the vessels were placed in a fume hood overnight. The next day, the vessels were heated on a hot plate at 80 °C for 24 h. 1 mL of 37 % (w/w) HCl was added and digested using the microwave programmed at 5 min ramping to 360 Watts and hold for 5 min, another 5 min ramping to 850 Watts and hold for 20 min, then 0 Watt for the 35 min cool-down cycle The digested solution was transferred to a Teflon tube.~~ The opened digestion was performed after microwave digestion using a mixture of $HNO_3$ and HCl on a hot block and evaporated to approximately~~near~~ 1 mL~~. Another 2 mL of 69 % (w/w) $HNO_3$ and 3 mL of 37 % (w/w) HCl were added to the tube and heated at 80 °C for 60 min. Finally, the~~ and the digestrate~~samples~~ was~~ere~~ diluted to 100 g using DIW.

The elemental analysis of digestrates was conducted using inductively coupled plasma-optical emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS). The conditions of ICP-OES and ICP-MS are stated in the Electronic Supplementary Material Table S2.

## 2.2 Tessier sequential extraction procedure

The extraction procedure is presented in **Fig 2.** and detailed in Table S3~~2~~ of ~~Supplementary Material~~Electronic Supplementary Material. The extraction tube was weighed before and after adding a new extraction solution for a weight correction. The extraction steps were labeled as Tessier step 1 - exchangeable (T # 1), step 2 - bound to carbonates (T # 2), step 3 - bound to Fe-Mn oxides (T # 3), step 4 - bound to organic matter and sulfide minerals (T # 4), and residue. The fractions were analyzed for the concentration of metals using ~~an inductively coupled plasma optical emission spectrometer (~~ICP-OES~~)~~ or/and ~~inductively coupled plasma mass spectrometer (~~ICP-MS~~)~~ with an external or standard addition calibration depending on the laboratory procedure. Dilution with 2 % (v/v) $HNO_3$ was required if the concentration exceeded the calibration range. The reagent blank was used for background correction.

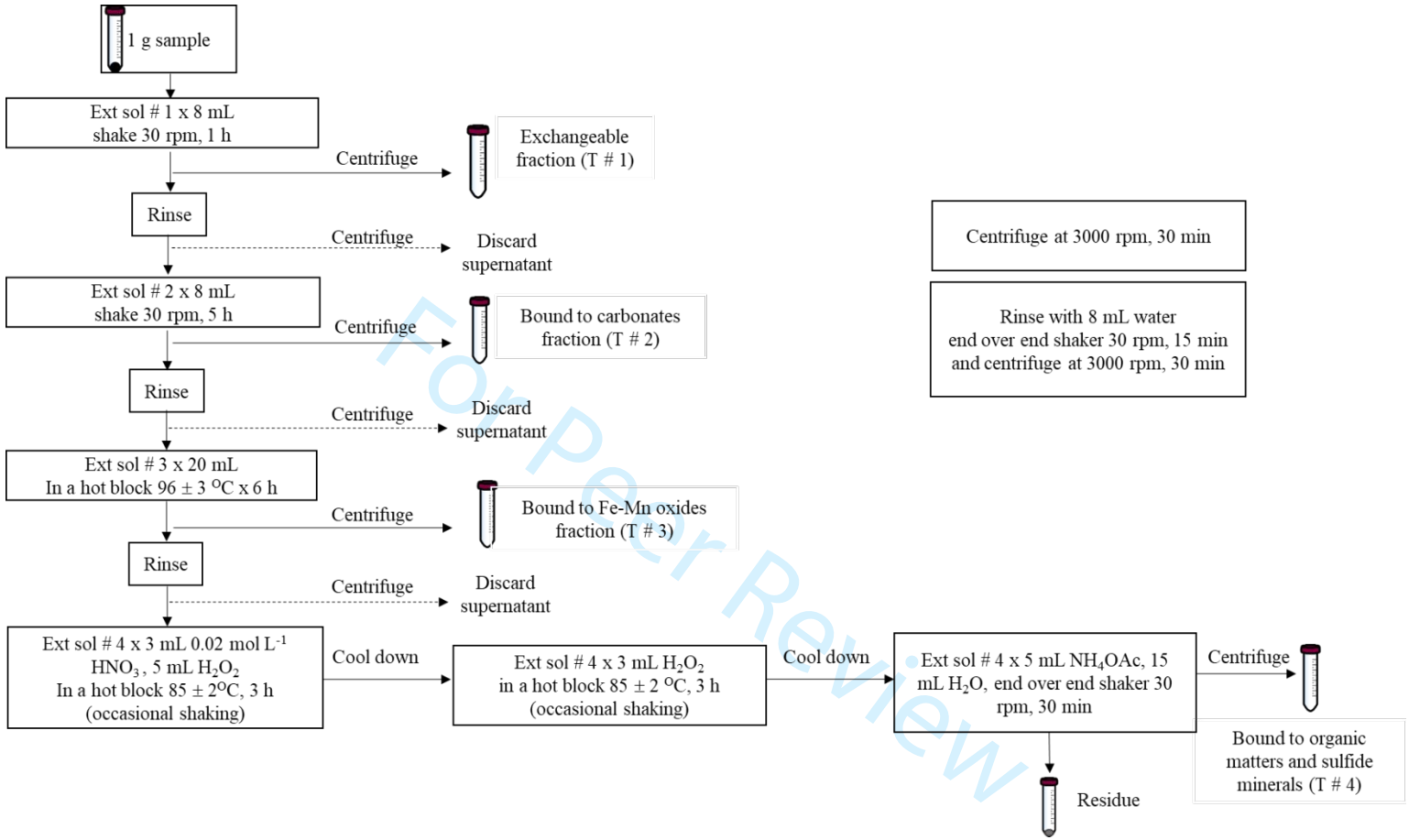


**Figure 2: Tessier sequential extraction procedure flowchart**

## 2.3 Data manipulation

A robust estimator based on the DerSimonian-Laird model was used for the assignment of the consensus value between the various laboratory data sets. NIST Consensus Builder software (https://consensus.nist.gov/app/nicob) was used for combining results from two laboratories using mean, standard deviation, and degrees of freedom from each laboratory (30). The DerSimonian-Laird model is often used for establishing an assigned value (*i.e.* key comparison reference value) and associated standard uncertainty for Consultative Committee for Amount of Substance (CCQM) key comparison data (31). The uncertainty values reported here are standard uncertainty at coverage factor k = 1 and expanded to cover 95 % confidence interval using k equals to 2. The approach of using DerSimonian-Laird model was also used for the assigned values of extracted elements determined recently for HISS-1, MESS-4 and PACS-3 CRMs by the BCR sequential extraction (21).

The percent distribution of each extraction step and residue was calculated according to Equation 1. The percent distribution fraction is used to evaluate the mobility of elements from the Tessier extraction.

$$\%\ Distribution\ fraction = \frac{100 \cdot\ C_{(i,x)}}{\sum(C_{1,x} + C_{2,x} + C_{3,x} + C_{4,x} + C_{residue,\,x})}$$

**Equation 1**

In the above equation, $C_{(i,x)}$ is the concentration of analyte *(x)* from Tessier step *i*. Denominator in Eq.1 (total combined concentration values) corresponds to the summation of concentrations ($C_i$) from steps 1 to 4 and residue ($C_{residue}$).

The standard uncertainty ($u_{std}$; k = 1) from DerSimonian-Laird model was compared to the target uncertainty ($u_{target}$) from the Horwitz equation (see Equation 2 below). Horwitz equation is widely used as a target uncertainty in inter-laboratory comparison (32,33), and as criteria for quality control in food and drug analysis (34). The calculation is based on the mass fraction of analyte *(C)* as follows :

$$\%\boldsymbol{u_{target}} = \mathbf{2}^{(\mathbf{1} - \mathbf{0.5}\boldsymbol{logC})} \qquad \textbf{Equation 2}$$

The accuracy of Tessier sequential extraction was calculated as a percent recovery (Equation 3) using the combination of the concentrations from four extraction steps and residue, then comparing against the total concentration from the

certified value. If no certified values were available, an average total concentration from the two laboratories was used as reference point.

$$Recovery\ (\%) = \frac{\sum(C_{1,x} + C_{2,x} + C_{3,x} + C_{4,x} + C_{residue,\,x})\ x\ 100}{C_{Certified\ value}}$$

**Equation 3**

# 3 Results

## 3.1 Total concentrations of elements in HISS-1, MESS-4, and PACS-3

The concentrations of 17 elements from the three CRMs were analyzed ~~by two digestions performed~~ separately by ~~each of~~ the two laboratories as previously ~~and~~ reported by Kumkrong et.al (21) and indicated ~~are reported~~ in ~~-~~Table S~~4~~3 of ~~Supplementary Material~~Electronic Supplementary Material. The percent recovery of each element was calculated against the certified value and presented as a bar graph in Fig. S1 of ~~Supplementary Material~~Electronic Supplementary Material. ~~Paired t-test was used to access the equivalence of the results and showed~~The total concentrations of elements reported by the two laboratories shows no significant difference between the two digestion methods~~, and between each digestion and certified values~~ at 95% confidence interval level by paired T-test ~~(Table S4 of Supplementary Material)~~(21). The concentration of Cr was significantly low from both digestion methods~~. The low concentration of Cr in HISS-1 is reported~~ when a mild digestion procedure was applied~~.~~ (27).

This study reported the concentrations associated with uncertainty (k=2) of some elements that are not certified in the three CRMs including Sb 0.119 ± 0.049 mg $kg^{-1}$, Sn 0.142 ± 0.020 mg $kg^{-1}$, and U 0.304 ± 0.116 mg $kg^{-1}$ for HISS-1, Se 1.18 ± 0.10 mg $kg^{-1}$ for MESS-4, and Se 1.03 ± 0.26 mg $kg^{-1}$, and U 2.58 ± 0.66 mg $kg^{-1}$ for PACS-3.

## 3.2 Concentrations of elements by Tessier sequential extraction

The concentrations of elements in HISS-1, MESS-4 and PACS-3 from the extraction steps and residue along with the associated uncertainty (k=2) are reported in **Table 2** to **Table 4**. The distribution profiles of the three marine sediment CRMs was calculated for the purpose of interpreting the mobility of the elements and are shown in **Fig. 3**. The percent distribution was calculated based on the mass fraction of each extraction step and the combined concentration of the

four extraction steps and the residue, as mentioned in Equation 1. The concentrations of Cd and Se in HISS-1 were removed from the results shown in **Fig. 3** due to an abnormal significantly higher recovery measured for these elements in HISS-1. We note that these two elements in HISS-1 were presented in extremely low concentrations (0.024 mg/kg$^{-1}$ for Cd and 0.050 mg/kg$^{-1}$ for Se) (27).

179 **Table 2 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in HISS-1**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 2(8) | 0.105 | 0.028 | 0.123 | 0.035 | 0.322 | 0.130 | ≤ 0.01 | - | 3.05 | 2.64 | 3.60 | 2.64 |
| Be | 2(8) | ≤ 0.004 | - | ≤ 0.004 | - | 0.004 | 0.002 | 0.009 | 0.002 | 0.125 | 0.059 | 0.125 | 0.059 |
| V | 2(8) | 0.133 | 0.050 | ≤ 0.01 | - | 1.23 | 0.17 | 0.374 | 0.126 | 6.33 | 2.11 | 8.06 | 2.12 |
| Cr | 3(13) | ≤ 0.003 | - | < 0.003 | - | 1.18 | 0.21 | 0.596 | 0.110 | 16.8 | 0.6 | 18.5 | 0.6 |
| Mn | 2(8) | ≤ 0.1 | - | 1.64 | 0.44 | 4.99 | 0.42 | 0.502 | 0.005 | 59.8 | 7.1 | 67.0 | 7.1 |
| Fe | 2(8) | ≤ 2 | - | 6.30 | 0.15 | 333 | 27 | 18.7 | 1.0 | 2,003 | 110 | 2,361 | 113 |
| Ni | 2(8) | ≤ 0.01 | - | 0.575 | 0.107 | 0.752 | 0.112 | 0.345 | 0.048 | 1.24 | 0.29 | 2.91 | 0.33 |
| Cu | 3(13) | 0.113 | 0.002 | 0.279 | 0.069 | 0.751 | 0.059 | 0.711 | 0.019 | 0.627 | 0.311 | 2.48 | 0.32 |
| Zn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 1.52 | 0.14 | 0.412 | 0.091 | 3.14 | 0.71 | 5.07 | 0.72 |
| As | 2(8) | 0.050 | 0.026 | 0.124 | 0.0003 | 0.198 | 0.044 | 0.027 | 0.012 | 0.501 | 0.403 | 0.902 | 0.407 |
| Sr | 3(13) | 6.97 | 0.54 | 52.5 | 18.2 | 2.14 | 0.10 | 0.266 | 0.016 | 21.1 | 0.5 | 82.9 | 18.2 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | 0.054 | 0.003 | 0.138 | 0.114 | 0.192 | 0.114 |
| Sb | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 0.179 | 0.023 | ≤ 0.01 | - | 0.116 | 0.005 | 0.116 | 0.005 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pb | 2(8) | ≤ 0.01 | - | 0.682 | 0.135 | 0.622 | 0.029 | 0.186 | 0.069 | 1.99 | 0.24 | 3.48 | 0.29 |
| U | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 0.059 | 0.022 | 0.013 | 0.006 | 0.157 | 0.036 | 0.228 | 0.043 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

184 **Table 3 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in MESS-4**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 3(11) | 0.403 | 0.086 | 0.486 | 0.022 | 6.80 | 1.51 | 2.35 | 0.28 | 48.6 | 7.7 | 58.7 | 7.9 |
| Be | 2(8) | ≤ 0.004 | - | 0.041 | 0.024 | 0.472 | 0.180 | 0.070 | 0.010 | 1.72 | 0.13 | 2.30 | 0.22 |
| V | 2(8) | 0.276 | 0.044 | 0.471 | 0.121 | 21.9 | 2.3 | 0.764 | 0.205 | 199 | 25 | 222 | 25 |
| Cr | 3(11) | 0.005 | 0.001 | 0.159 | 0.030 | 3.55 | 0.69 | 1.84 | 0.40 | 89.0 | 10.4 | 94.5 | 10.5 |
| Mn | 3(11) | 2.95 | 0.26 | 75.7 | 7.1 | 71.5 | 10.4 | 11.4 | 0.24 | 111 | 16 | 273 | 20 |
| Fe | 2(8) | 4.26 | 1.23 | 327 | 31 | 6,152 | 235 | 508 | 108 | 27,826 | 6,756 | 34,818 | 6,761 |
| Ni | 3(11) | 0.055 | 0.009 | 0.829 | 0.067 | 12.3 | 3.3 | 5.82 | 0.89 | 26.8 | 4.6 | 45.8 | 5.8 |
| Cu | 3(11) | 0.463 | 0.015 | 1.44 | 0.29 | 4.42 | 0.27 | 7.13 | 0.93 | 20.9 | 2.7 | 34.4 | 2.9 |
| Zn | 3(11) | 0.101 | 0.044 | 11.7 | 0.5 | 49.4 | 10.4 | 13.6 | 2.1 | 81.8 | 8.1 | 157 | 13 |
| As | 3(11) | 0.102 | 0.014 | 0.257 | 0.050 | 2.57 | 0.27 | 0.264 | 0.060 | 18.0 | 3.7 | 21.2 | 3.7 |
| Se | 2(8) | 0.025 | 0.005 | 0.205 | 0.138 | 0.099 | 0.090 | 0.526 | 0.125 | 0.219 | 0.081 | 1.07 | 0.27 |
| Sr | 3(11) | 14.4 | 0.2 | 10.7 | 1.7 | 10.7 | 2.0 | 2.38 | 0.38 | 85.4 | 13.3 | 124 | 14 |
| Cd | 2(8) | ≤ 0.02 | - | 0.080 | 0.025 | 0.049 | 0.035 | 0.040 | 0.072 | 0.098 | 0.059 | 0.241 | 0.103 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | ≤ 0.01 | - | 3.23 | 0.48 | 3.23 | 0.48 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sb | 2(8) | 0.020 | 0.016 | 0.028 | 0.002 | 0.011 | 0.007 | ≤ 0.01 | - | 1.11 | 0.18 | 1.17 | 0.18 |
| Pb | 3(11) | 0.014 | 0.006 | 2.50 | 0.34 | 8.33 | 1.88 | 0.271 | 0.025 | 10.2 | 2.7 | 21.3 | 3.3 |
| U | 2(8) | 0.055 | 0.003 | 0.237 | 0.0312 | 0.353 | 0.224 | 0.162 | 0.004 | 2.28 | 0.93 | 3.09 | 0.95 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

188 **Table 4 : Assigned value (mg kg$^{-1}$ dry weight) and associated uncertainty (U, k = 2) of elements in PACS-3**

| Elements | N(n)* | Tessier # 1 | | Tessier # 2 | | Tessier # 3 | | Tessier # 4 | | Residue | | Combined value ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U | Assigned value | U |
| Li | 3(11) | 0.518 | 0.012 | 0.277 | 0.083 | 7.76 | 0.88 | 2.37 | 0.03 | 19.3 | 4.9 | 30.2 | 5.0 |
| Be | 3(11) | ≤ 0.004 | - | 0.032 | 0.004 | 0.176 | 0.050 | 0.030 | 0.002 | 0.750 | 0.035 | 0.988 | 0.061 |
| V | 3(11) | 0.273 | 0.310 | 0.591 | 0.207 | 38.3 | 5.5 | 4.14 | 0.48 | 95.9 | 19.1 | 139 | 20 |
| Cr | 3(11) | 0.133 | 0.056 | 0.917 | 0.213 | 20.8 | 2.3 | 11.6 | 1.9 | 66.1 | 8.5 | 99.6 | 9.0 |
| Mn | 3(11) | 2.33 | 0.27 | 6.55 | 1.31 | 49.3 | 8.0 | 38.2 | 1.3 | 299 | 39 | 396 | 39 |
| Fe | 2(8) | 4.05 | 3.31 | 340 | 214 | 7,353 | 421 | 4,907 | 539 | 24,482 | 327 | 37,086 | 870 |
| Ni | 3(11) | 0.247 | 0.080 | 1.40 | 0.07 | 10.6 | 2.5 | 4.55 | 0.73 | 24.7 | 4.2 | 41.4 | 5.0 |
| Cu | 3(11) | 4.15 | 0.83 | 27.8 | 7.5 | 20.6 | 4.2 | 223 | 21 | 42.2 | 5.3 | 318 | 23 |
| Zn | 3(11) | 17.2 | 1.2 | 140 | 19 | 168 | 38 | 33.4 | 3.4 | 72.0 | 10.2 | 430 | 44 |
| As | 3(11) | 0.093 | 0.034 | 0.363 | 0.016 | 10.0 | 0.2 | 1.87 | 0.25 | 18.5 | 3.9 | 30.8 | 3.9 |
| Se | 2(8) | 0.024 | 0.008 | 0.039 | 0.011 | < 0.02 | - | 0.888 | 0.254 | 0.232 | 0.005 | 1.18 | 0.25 |
| Sr | 3(11) | 28.1 | 2.5 | 11.3 | 1.9 | 15.1 | 1.2 | 4.08 | 0.28 | 174 | 103 | 232 | 103 |
| Cd | 2(8) | ≤ 0.02 | - | 1.18 | 0.33 | 0.910 | 0.356 | 0.085 | 0.078 | 0.098 | 0.060 | 2.27 | 0.50 |
| Sn | 2(8) | ≤ 0.01 | - | ≤ 0.01 | - | 1.89 | 0.10 | ≤ 0.01 | - | 24.4 | 7.9 | 26.3 | 7.9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sb | 3(11) | 0.046 | 0.016 | 0.223 | 0.009 | 0.442 | 0.399 | 0.079 | 0.049 | 13.0 | 1.3 | 13.8 | 1.4 |
| Pb | 3(11) | 0.919 | 0.330 | 37.5 | 12.3 | 118 | 26 | 22.9 | 2.4 | 36.1 | 8.2 | 216 | 30 |
| U | 2(8) | ≤ 0.01 | - | 0.584 | 0.047 | 0.555 | 0.211 | 0.328 | 0.284 | 1.17 | 0.27 | 2.64 | 0.45 |

* N is number of Tessier extraction experiments

n is number of repeated measurements of element concentration

** A combined value is summation of concentrations of an element from T # 1, # 2, # 3, # 4 and residue, and a combined uncertainty is calculated from $u_{std} = 2 \cdot \sqrt{u_{step\,1}^2 + u_{step\,2}^2 + u_{step\,3}^2 + u_{Residue}^2}$ (35)

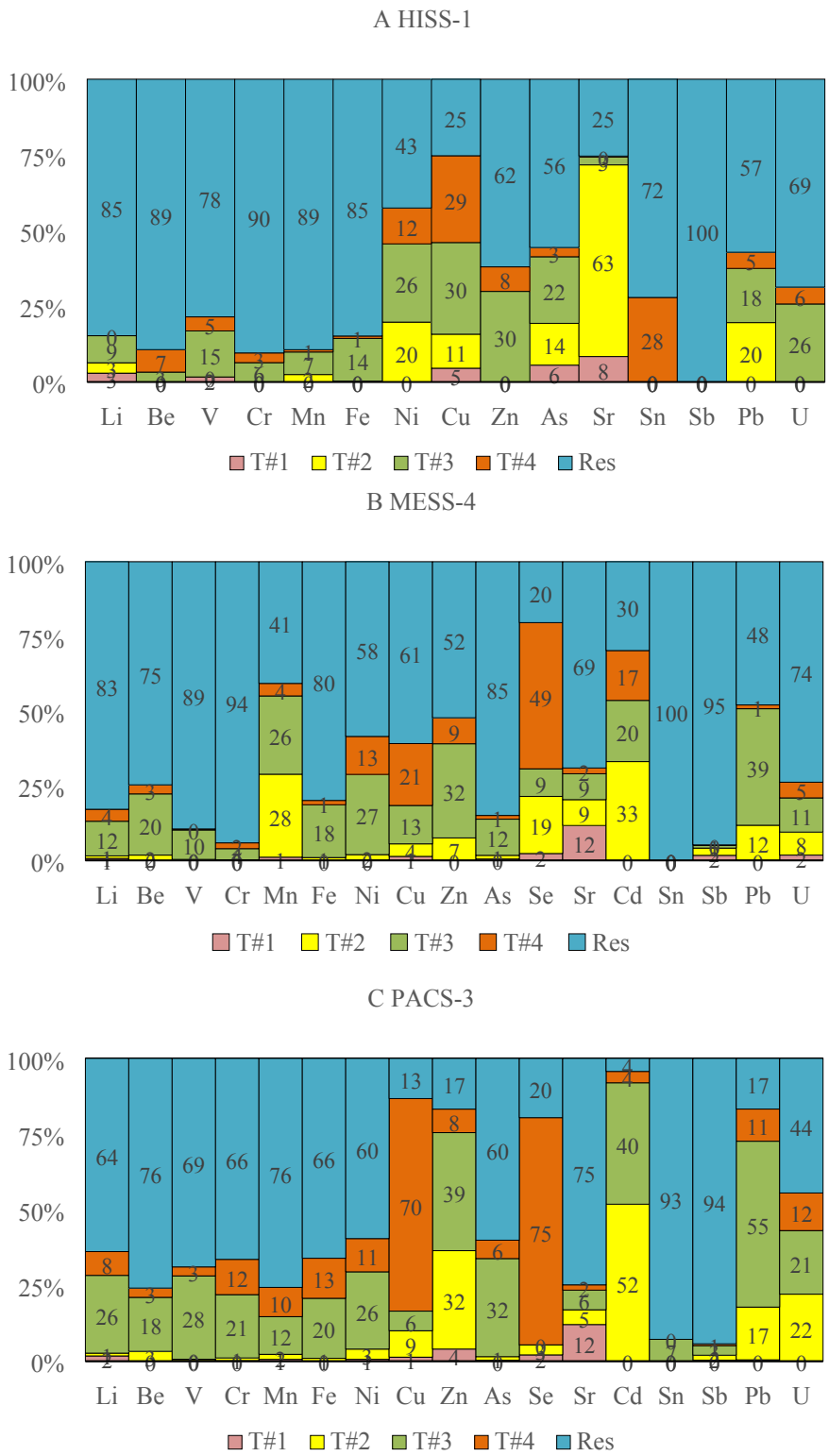


**Figure 3 : Percent distribution (y-scale) of elements (x-scale) by~~in~~ Tessier extraction from A HISS-1, B MESS-4 and C PACS-3**

# 4 Discussion

## 4.1 Total concentrations of elements in HISS-1, MESS-4, and PACS-3

**Fig. 4** plots the total concentrations of elements in the three marine sediment CRMs in comparison to the concentrations in Pelagic clay of the ocean (3) and the earth crust (36). Generally, in decreasing order of abundance, the concentrations of elements were: PACS-3 > MESS-4 > Pelagic clay > Earth crust >> HISS-1. The concentrations of Fe in MESS-4 and PACS-3 were similar and 80 times higher than in HISS-1. HISS-1 contains high amount of silica as quartz and has low accumulation of foreign matters (21,27). PACS-3 showed high concentrations of Zn, Cu, Pb, Sn, As, Sb, Se and Cd, possibly attributable to anthropogenic inputs. MESS-4 showed twice higher concentrations for V and Li when compared to PACS-3.

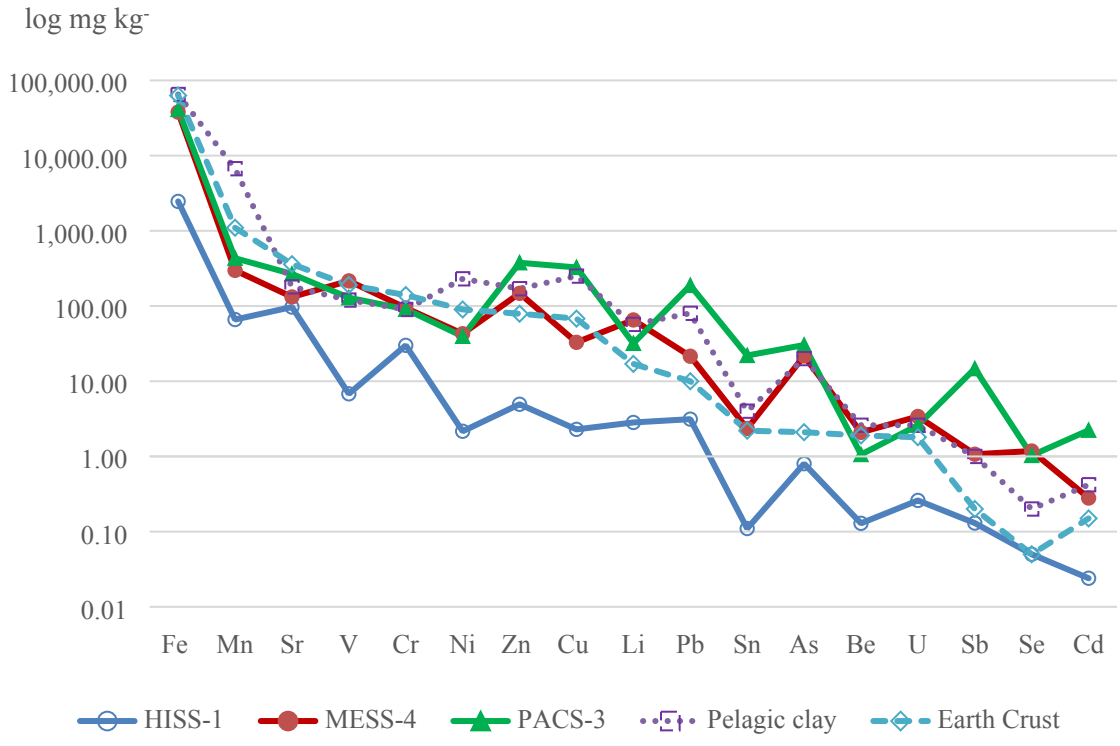


**Figure 4 : Element concentration and elements in ~~profile of~~ HISS-1, MESS-4 and PACS-3 marine sediments, in comparison to Pelagic clay** (3) **and Earth crust** (36)

## 4.2 Distribution profile of elements from Tessier sequential extraction

### 4.2.1 HISS-1

Considering the released concentrations of elements in HISS-1 (**Table 2**), elements were detected in significant amounts from T # 1 and T # 2, except for Sr. Strontium likely associated with carbonate minerals and dissolved in the acidic solution of T # 2. The concentration of Sr in T # 2 appeared dominant of 63 % and left only 25 % in the residue (**Fig. 3A**). Very little Fe and Mn were extracted in T # 1. High concentrations of Fe and Mn were released in the reducing condition of T # 3; however, majority still remained in the residue (**Fig. 3A**). Additionally, many of the elements were released in T # 3 including Mn (4.95 mg $kg^{-1}$) > Sr > Zn ≈ V ≈ Cr > Ni ≈ Cu ≈ Pb (0.622 mg $kg^{-1}$). It is likely that most elements were released with Fe dissolution in reducing condition. Greater than 70 % fraction of Sb, Cr, Mn, Be, Fe, Li, V, and Sn remained in the residues.

### 4.2.2 MESS-4

The total concentrations of released elements in MESS-4 are 3 to 45 times higher than in HISS-1. The higher concentrations observed in T # 1 were for Sr (14.4 mg $kg^{-1}$) >> Fe > Mn (2.95 mg $kg^{-1}$), and trace amounts of Cu (0.463 mg $kg^{-1}$) > Li > V (0.276 mg $kg^{-1}$) were observed. At T # 2, significantly high concentrations of Fe and Mn were released, in contrast with HISS-1 where virtually no Fe and Mn were released in T # 1, as noted above. A high fraction of 28 % Mn released at T # 2 suggests that Mn in carbonate minerals was dominant in MESS-4 marine sediment. Besides, high concentrations of Fe (327 mg $kg^{-1}$) >>> Zn >> Pb > Cu (1.4 mg $kg^{-1}$) were also observed with carbonate mineral dissolution at T # 2. Other elements that were observed in trace amounts (from 0.1 mg $kg^{-1}$ to 1 mg $kg^{-1}$ ) in T # 2, included As, Cr, Li, Ni, V and U. Several elements were released in T # 3, similarly to HISS-1, but 3 to 116 times larger concentrations were detected in MESS-4 as follows : Fe (6,152 mg $kg^{-1}$) >>> Mn > Zn > V > Ni ≈ Sr > Pb > Li > Cu > Cr > As (2.57 mg $kg^{-1}$). At T # 4, apart from high concentrations of Fe, Zn, and Mn, a large fraction (21 %) of the Cu (**Fig. 3B**) was released (7.13 mg $kg^{-1}$) followed by Ni (5.82 mg $kg^{-1}$) >> Sr ≈ Li > Cr (1.84 mg $kg^{-1}$). A significant portion of Sn (100 %), Sb, Cr, V, Li, Fe, Be, and U (74 %) remained in the residue, demonstrating minimal mobility of these elements in MESS-4.

### 4.2.3 PACS-3

PACS-3 is a highly-contaminated marine sediment with 3 to 200 times higher concentration of the elements analyzed than in HISS-1 (**Fig 4**). At T # 1, elevated concentrations were observed for most elements (except for Be, Cd, Sb, Se, Sn, and U), with particularly high concentrations measured for Zn, Cu, and Pb. At T # 2, higher concentrations of Zn, Pb and Cu were released with carbonate dissolution in comparison to T #1. Similar to HISS-1 and MESS-4, high concentration of Fe (7,353 mg $kg^{-1}$) was observed at T # 3 together with many elements including Zn (168 mg $kg^{-1}$) > Pb >> Mn > V > Cr, Cu > Sr > Ni ≈ As (10 mg $kg^{-1}$). It seems the highest fractions of Pb, Zn, and As are observed at T # 3. Interestingly, Sn was firstly observed in PACS-3 at T # 3 (**Fig. 3C**), suggesting that high accumulation of Sn from the harbor input existed as mono-, di- and tri butyltin in PACS-3 (29); in particular, butyltin is widely used as an antifouling agent in marine paint (37). At T # 4, surprisingly, a high concentration of Fe (4,907 mg $kg^{-1}$) was observed, indicating Fe associated with sulfide minerals in PACS-3, which is different from HISS-1 and MESS-4. The largest fractions of Cu (70 %) and Se (59 %) were observed at T # 4 (**Fig 3C**), similarly to HISS-1 (**Fig 3A**) and MESS-4 (**Fig 3B**). The most stable elements which remained in the residue are Sb (94 %), Sn, Be, Mn, and Sr (75 %).

## 4.3 Analytical performance of Tessier sequential extraction on three marine sediment CRMs

### 4.3.1 Assessment of precision

Since there are no CRM reference data sets readily available on Tessier sequential extraction procedure, assessment of precision and accuracy is needed to benchmark the procedure. The reproducibility from Horwitz formulation (33) was chosen as a target to assess observed precisions in this study. **Fig. 5** illustrates that the majority of standard uncertainty ($u_{std}$, k=1) of element concentrations determined with the Tessier sequential extractions on HISS-1, MESS-4 and PACS-3 were below the target uncertainty specified by Horwitz equation (Equation 2). Higher than target uncertainties were occasionally observed for lower concentration elements of the three CRMs, which might reflect either stability of the extracts and/or the laboratories ability to perform the measurements at these concentration regimes. Larger uncertainties observed for elements extracted with high concentrations from PACS-3 are unexpected and non-rationalized.

The distribution of observed relative uncertainties from the four Tessier extraction steps and residue of the three CRMs ranged from 1% to 91% (Cd). The distribution histogram in comparison with target uncertainty from Horwitz model was presented in Fig S2 of ~~Supplementary Material~~Electronic Supplementary Material. Majority (72 %) of uncertainty values in the data set was below the average uncertainty of 13 %, which compares favorably with the average target uncertainty of 16 % (from Horwittz equation, Equation 2).

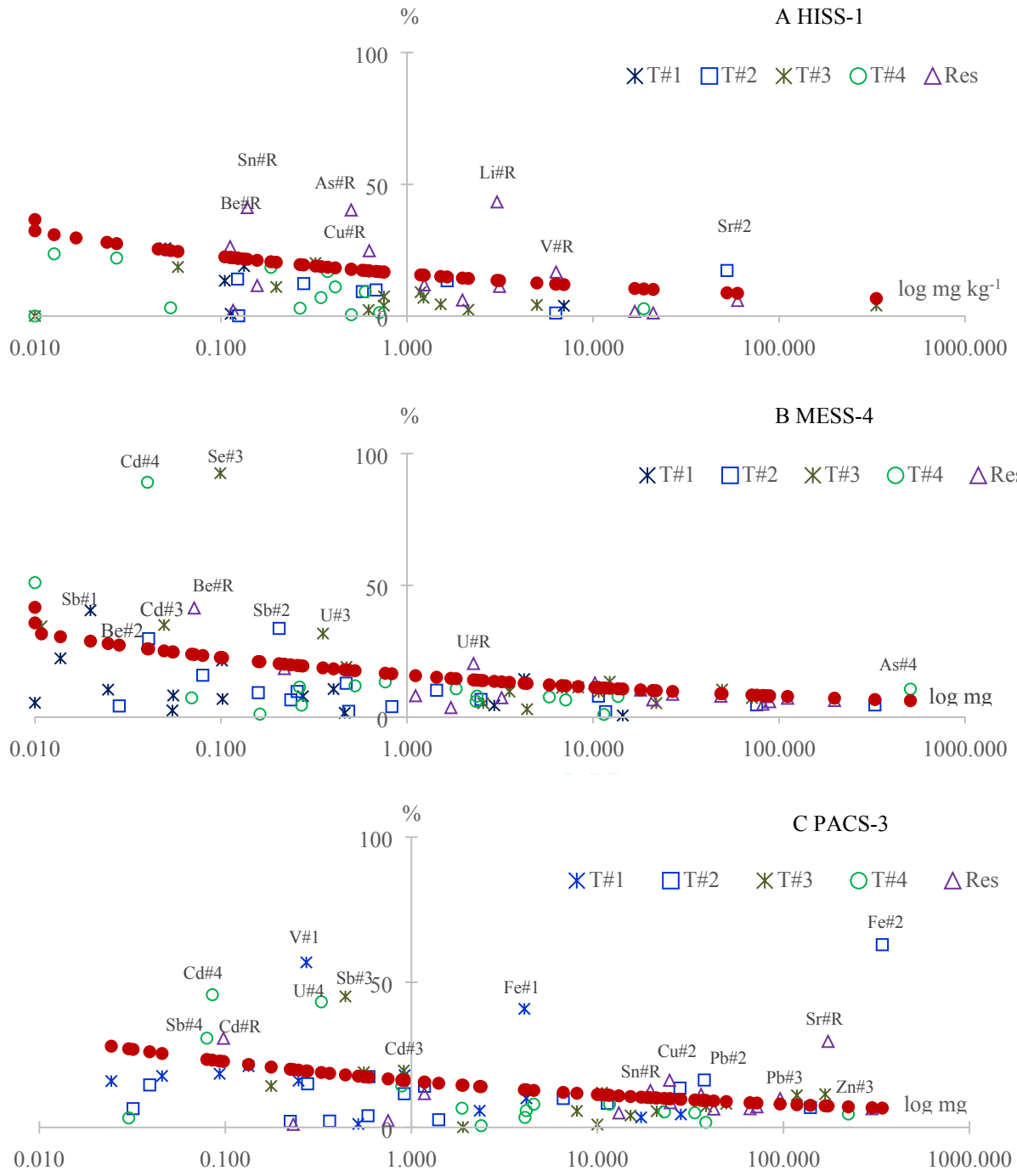


**Figure 5 : Standard uncertainty (%, at k=1) of A HISS-1, B MESS-4, and C PACS-3 from Step T # 1, T # 2, T # 3, T # 4 and residue in comparison with target uncertainty from Horwitz equation (red dot).**

### 4.3.2 Assessment of accuracy

The assessment of accurary of the Tessier extraction procedure is non trivial as no quality control materials/ CRMs are available. The accuracy was assessed as recovery by combining the elemental concentrations measured from the four extraction steps and the residue and then comparing against the total concentrations of analytes from either certified values or measured total concentrations obtained here (see Tables S5 to Table S7 of ~~Supplementary Material~~Electronic Supplementary Material). The results of the recovery study are presented as a bar graph in **Fig. 6**. The recovery of HISS-1 was 92 % ± 40 %, MESS-4 of 101 % ± 12 % and PACS-3 of 102 % ± 10 %. A large deviation of recovery values (± 40 %) for HISS-1 is due to a low recovery of Cr (62 %), an element which is known to be problematic for analysis of this particular CRM (21,38).

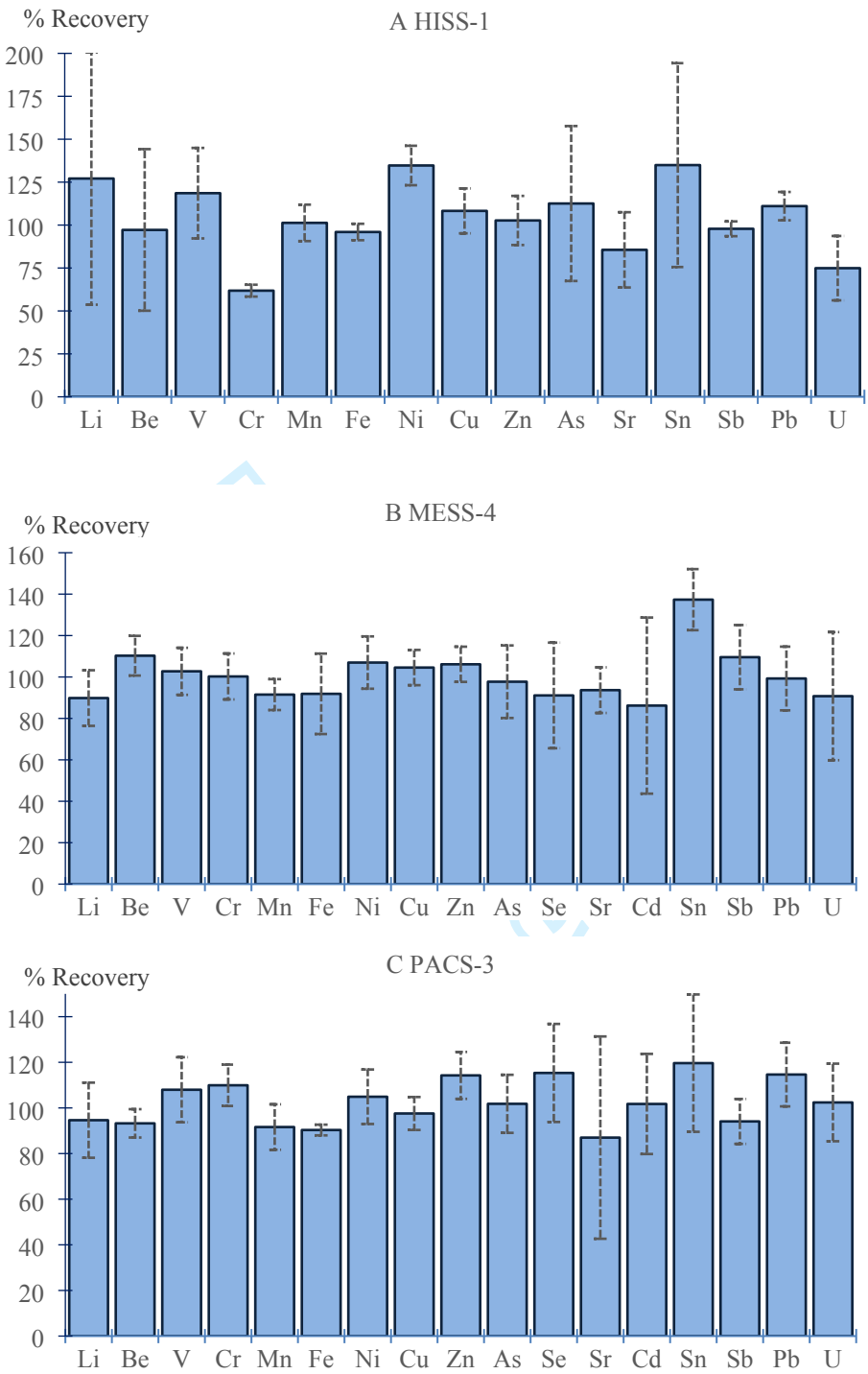


**Figure 6 : Percent recovery of combined concentrations of four extraction steps and residue vs elements of A HISS-1, B MESS-4 and C PACS-3 with observed uncertainty (k=2)**

## 5 Conclusions

The four-step Tessier sequential extraction procedure was applied to three widely used marine sediment CRMs and 17 elements were analysed. Different dissolution profiles were obseved for HISS-1, MESS-4 and PACS-3. Elements that showed mobility in all the extraction steps included Ni, Cu, Zn, As, Se, Sr, Cd, Pb, and U.

In T # 1, a high amount of Sr was released from the three CRMs, particularly in the highly-contaminated marine sediment PACS-3, which released significant amount of Sr, as well as Zn. In the acidic conditions of T # 2, Sr preferentially released from HISS-1, Mn from MESS-4, and Zn and Cd from PACS-3. Most metals mimic the dissolution pattern of Fe with a significant release under the reducing conditions of extraction step T # 3, particularly Pb, Ni and Zn. Interestingly, in oxidizing conditions (T # 4), Cu and Se are relased likely because of their association with iron sulfide, which is being selectively dissolved in this step. In all three sediments, Be, Sb, and Sn remained in the residue. Other elements found in high amounts within the residues of HISS-1 and MESS-4 included Cr, V, Li and Fe.

The precision of Tessier sequential extraction in this study was benchmarked against Horwitz model and was found largely satisfactory. However some elements, which were present in low concentrations, consistently showed high uncertainty. Overall, the percent recovery by the combined element concentrations from the four Tessier extraction steps and residue compared against the certified values shows satisfactory performance of 101 % $\pm$ 12 % for MESS-4 and 102 % $\pm$ 10 % for PACS-3, but poor recovery with high uncertainty for HISS-1 of 92 % $\pm$ 40 %. The reference data set produced here using the Tessier sequential extraction procedure carried out on HISS-4, MESS-4 and PACS-3 CRMs will serve as a quality control and method development tool for laboratories.

## Acknowledgement

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## Compliance with ethical standards

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Figure

## Tessier sequential extraction on 17 elements from three marine sediment certified reference materials (HISS-1, MESS-4 and PACS-3)

**Paramee Kumkrong*, Ovi Mihai, Patrick H.J. Mercier, Indu Gedara Pihilligawa, Daniel Tyo, and Zoltan Mester**

National Research Council Canada, 1200 Montreal Road Ottawa, Ontario Canada, K1A 0R6

*Corresponding author : Paramee.Kumkrong@nrc-crnc.gc.ca



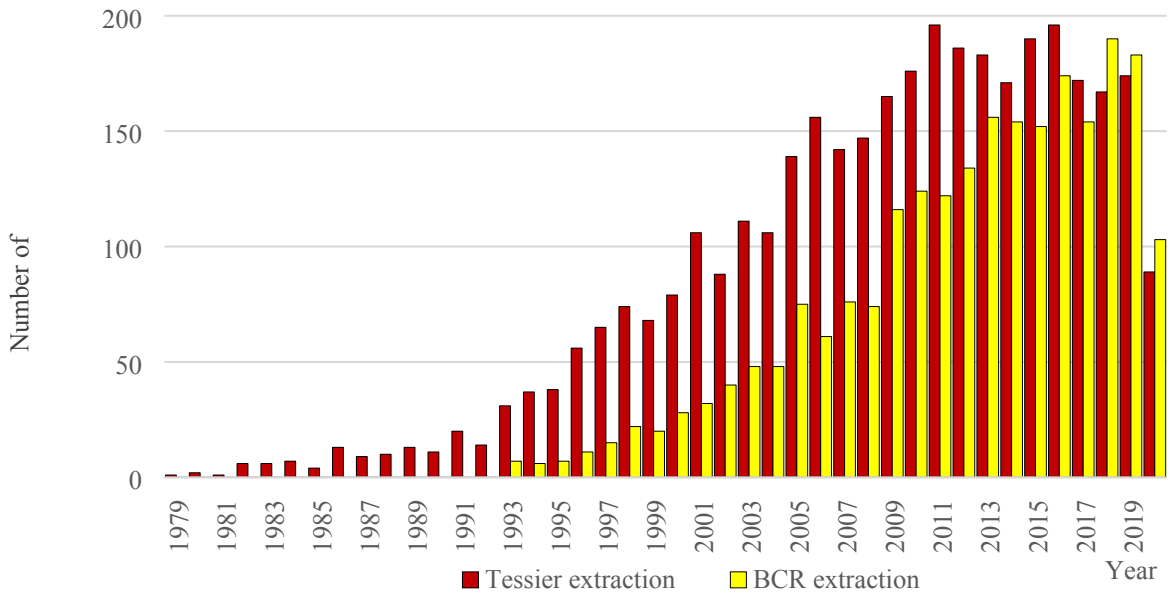



**Figure 1** : **The number of publications of Tessier and BCR sequential extraction procedure since 1979 to 2020 (Source: Scopus database, searched under keywords: "sequential extraction BCR" and "sequential extraction Tessier" (https://www.scopus.com, accessed on June 07, 2020)**

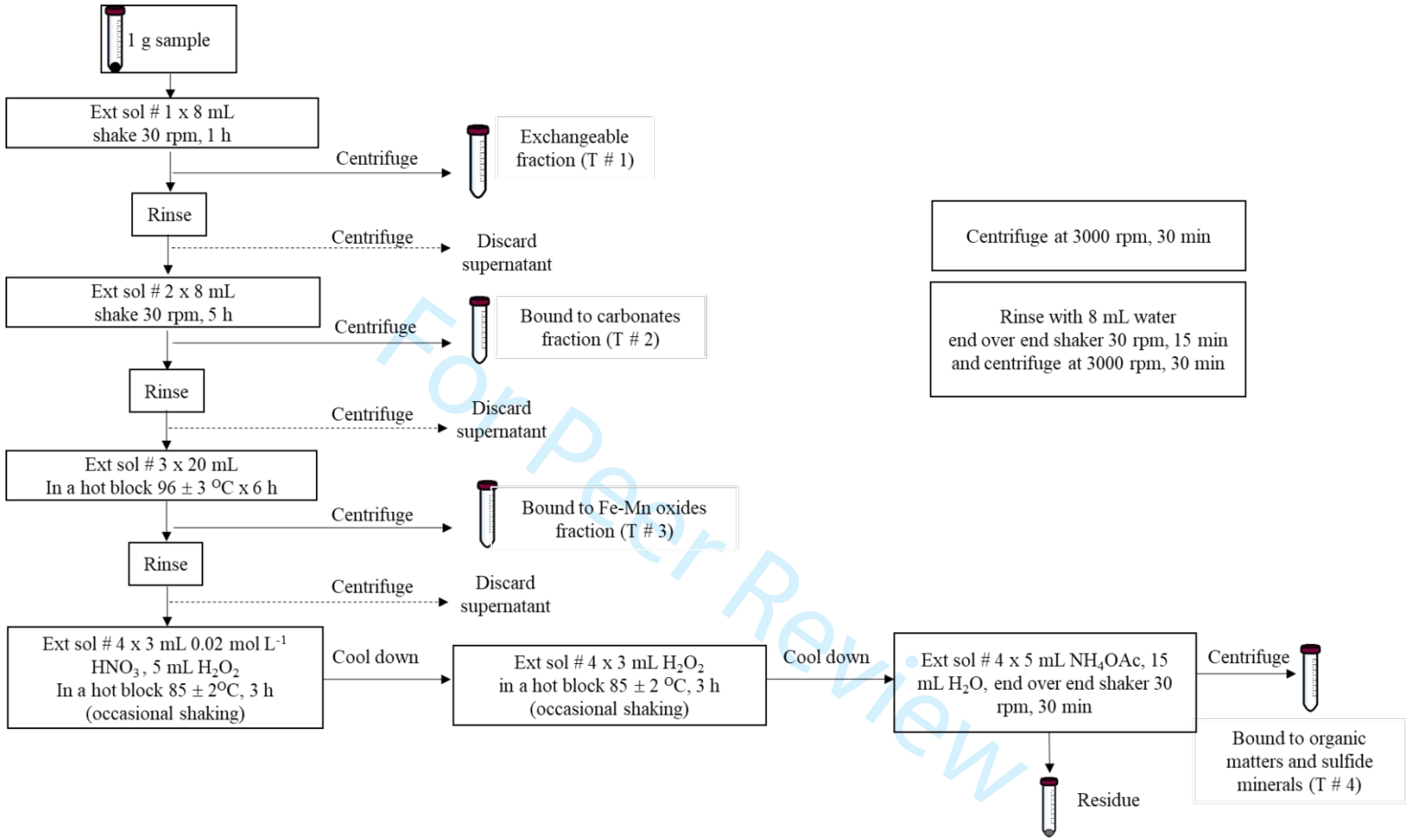



**Figure 2 : Tessier sequential extraction procedure flowchart**

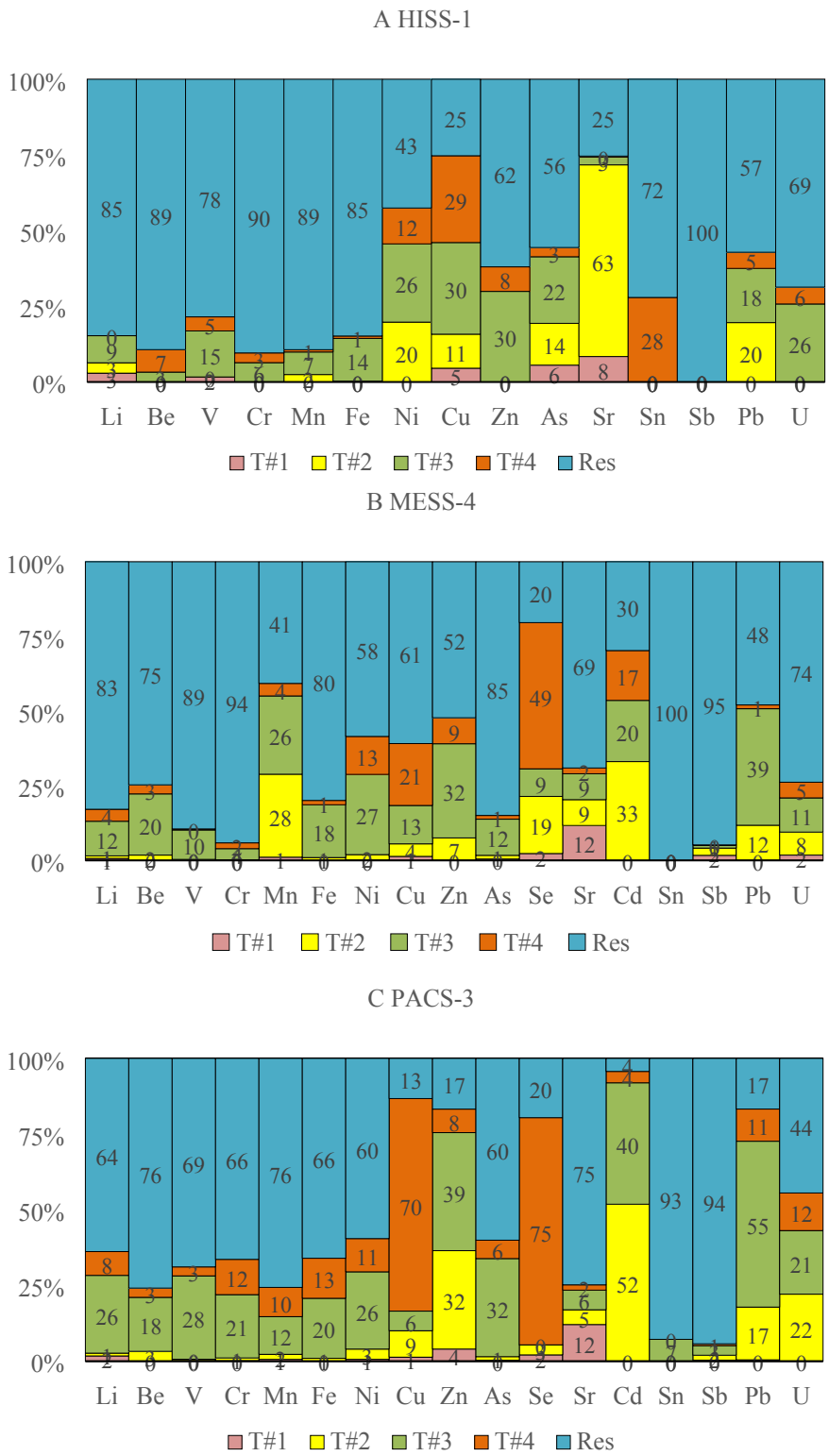



**Figure 3 : Percent distribution (y-scale) of elements (x-scale) in Tessier extraction from A HISS-1, B MESS-4 and C PACS-3**

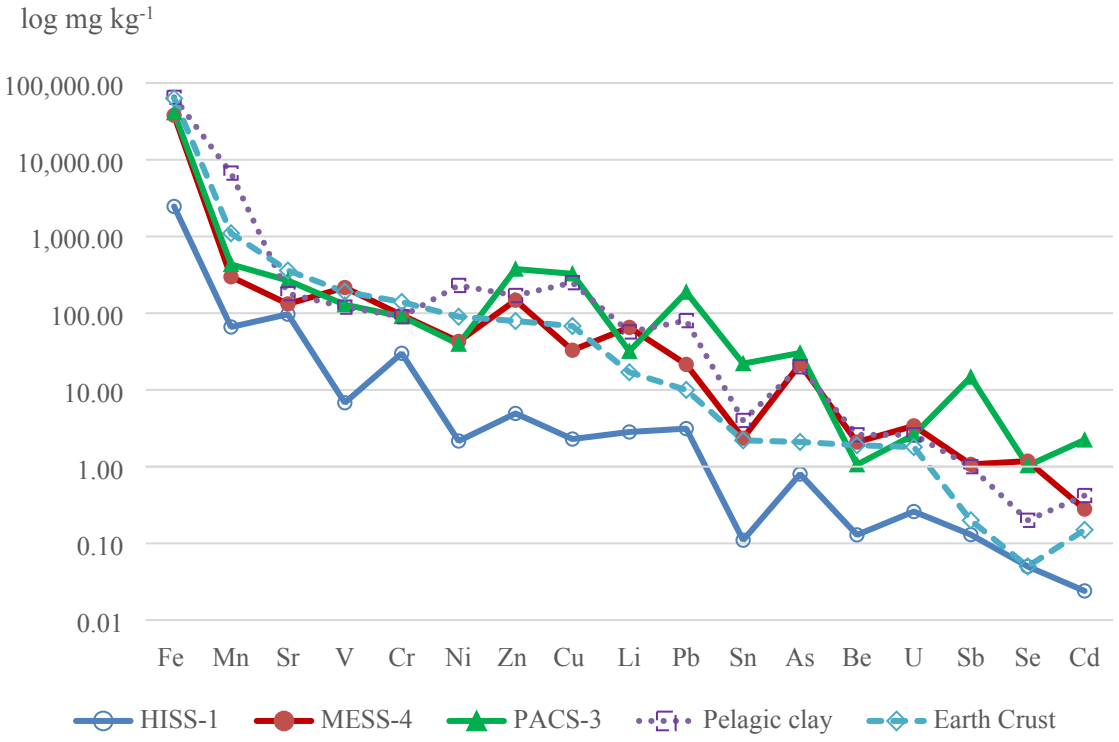



**Figure 4 : Element concentration and elements in HISS-1, MESS-4 and PACS-3 marine sediments, in comparison to Pelagic clay [3] and Earth crust [38]**

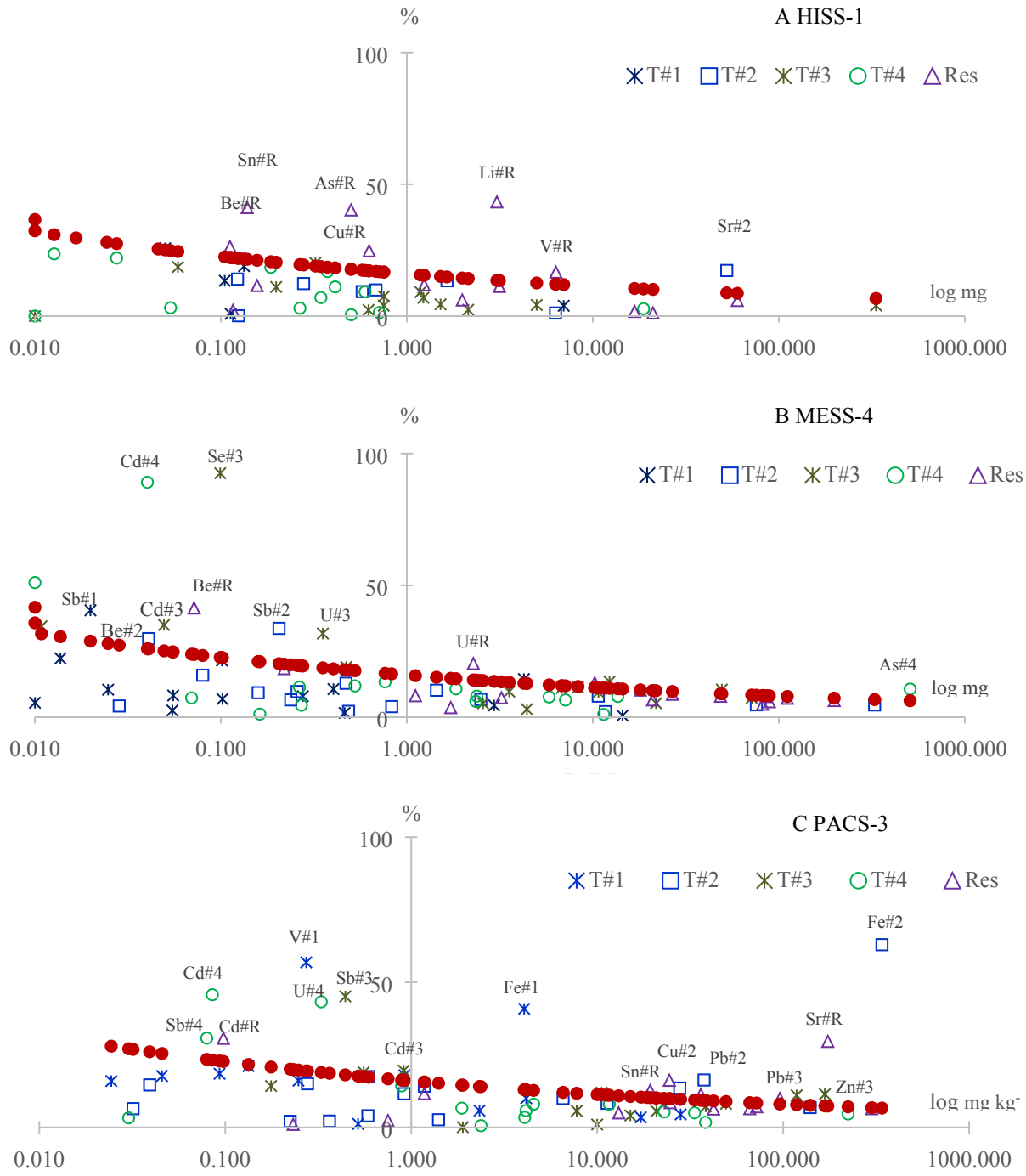
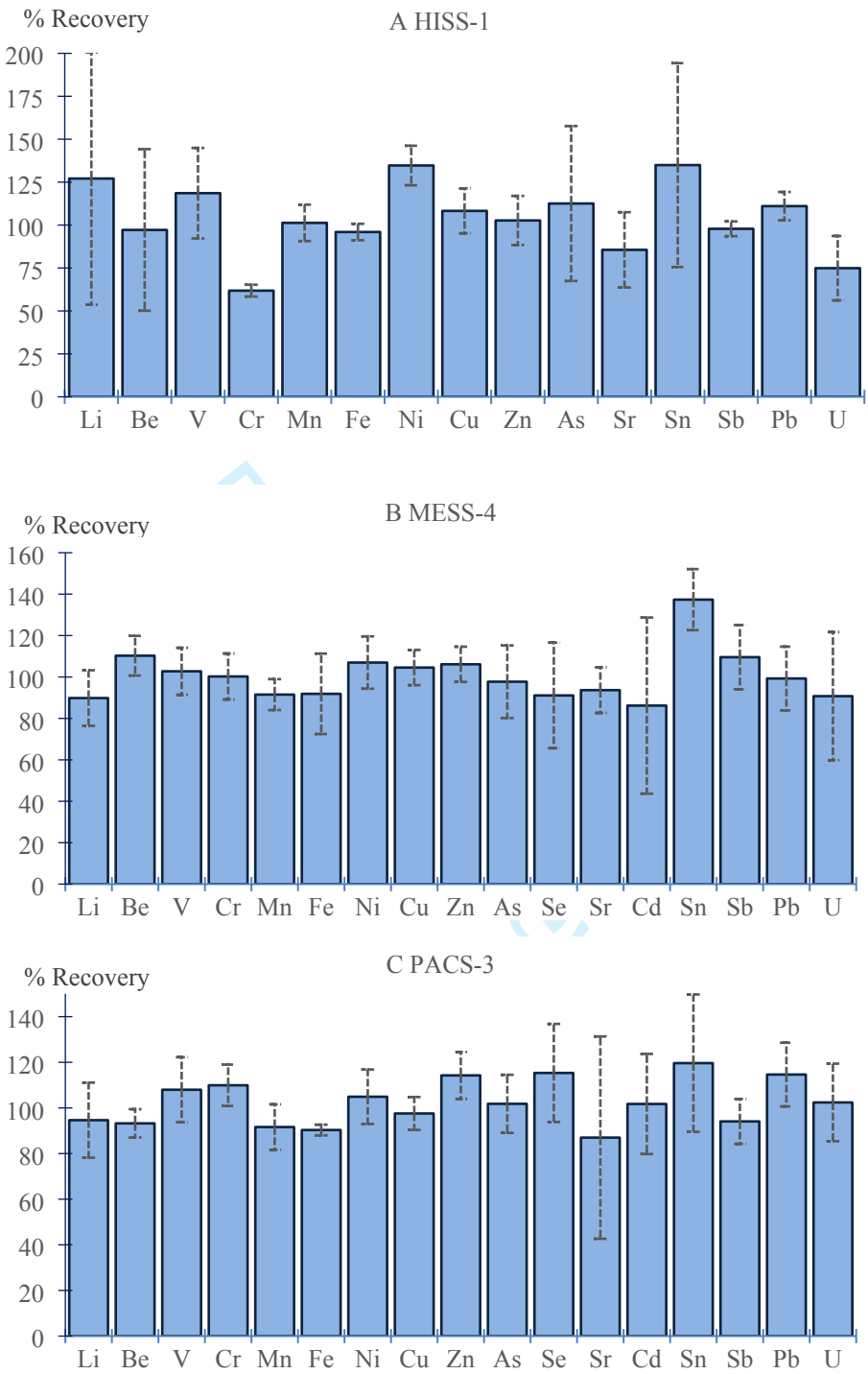



**Figure 5 : Standard uncertainty (%, at k=1) of A HISS-1, B MESS-4, and C PACS-3 from Step T # 1, T # 2, T # 3, T # 4 and residue in comparison with target uncertainty from Horwitz equation (red dot).**

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# Figure captions

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**Supplementary Material**

# Tessier sequential extraction on 17 elements from three marine sediment certified reference materials (HISS-1, MESS-4 and PACS-3)

**Paramee Kumkrong*, Ovi Mihai, Patrick H.J. Mercier, Indu Gedara Pihilligawa, Daniel D. Tyo, and Zoltan Mester**

National Research Council Canada, 1200 Montreal Road Ottawa, Ontario Canada, K1A 0R6

*Corresponding author : paramee.kumkrong@nrc-cnrc.gc.ca

Table S1 : Laboratory ware, chemicals and instruments

| **Laboratory ware** | **Lab A** | **Lab B** |
|---|---|---|
| Extraction tube | A 50 mL polypropylene conical tube-Falcon with a screw cap from ThermoFisher Scientific Canada<br>Pre-cleaned by soaking in 5% (v/v) nitric acid overnight, washing with double-distilled deionized water (later call water), and rinsed three times with water. | A 50 mL ultra-high performance centrifuge tube with a screw cap from VWR Canada.<br>Pre-cleaned was conducted the same as Lab A |
| **Chemicals** | **Lab A** | **Lab B** |
| Deionized water (DIW) | Reverse osmosis connected to the ultrapure water type I by Millipore Synergy UV-R, Canada | Reverse osmosis connected to a Milli-Q Advantage A10, Canada. |
| Sodium acetate anhydrous (NaOAc Cas number 127-09-3) | ACS ≥99 %, Sigma-Aldrich, Germany | ≥ 99 %, Fisher Bio-reagent, Canada |
| 100 % (v/v) acetic acid (AcOH, Cas number 64-19-7) | Suprapur, Merck, Canada | ACS Grade, VWR (BDH) Chemicals, Canada |
| Hydroxylammonium chloride ($NH_2OH.HCl$ Cas number 5470-11-1) | ACS, Sigma-Aldrich, Canada | ≥ 96 % (w/w), Anachemia, Canada |
| 30 % (w/w) hydrogen peroxide ($H_2O_2$, Cas number 7722-84-1) | Aristar Ultra, BDH, VWR Analytical, Canada | Optima Grade, ThermoFisher Chemicals Scientific, Canada |
| 69 % (w/w) nitric acid ($HNO_3$, Cas number 7697-37-2) | Aristar Plus, BDH, VWR Analytical, Canada | J.T. Baker, Canada Distilled in house |
| ammonium acetate ($NH_4OAc$, Cas number 631-61-8) | ≥ 99 % (w/w) ACS, ThermoFisher Scientific, Canada. | ≥ 98 % (w/w) Sigma Aldrich, Canada. |
| 48 % (w/v) hydrofluoric acid (HF, Cas number 7664-39-3) | Aristar Ultra, BDH, VWR Analytical, Canada. | Trace Metal Grade, ThermoFisher Chemical Scientific, Canada. |
| ≥ 99.5 % (w/w) boric acid ($H_3BO_3$, Cas number 10043-35-3) | Analar Grade, Chemique ACP Chemical Inc, Montreal Quebec Canada | Not use |
| 37 % (w/w) hydrochloric acid (HCl, Cas number 7647-01-0) | Aristar Plus, BDH, VWR Analytical, Canada. | J.T. Baker, Canada, Distilled in house |
| Standard solution | - As, Co, Cr, Cu, Pb, Mn, Ni, Se, V, and Zn were ICP grade from SCP Science, Canada<br>- Be, Cd, and Sb were ICP grade from ISO Spec, Delta, Canada<br>- U was ICP grade from High Purity Standard, Canada.<br>All standard solutions were traceable to SI unit via NIST. | CRMs from the NRC Canada for As, Cr, Mn, Ni, and Pb; and the rest of elements were based on CRMs from NIST. |
| **Instruments** | **Lab A** | **Lab B** |

| | | |
|---|---|---|
| Analytical balance | Mettler Toledo AB304-S (Mettler Toledo GmbH, Laboratories & Weighing Technologies, Switzerland) | Mettler Toledo XPR305D5 (Mettler Toledo GmbH, Laboratories & Weighing Technologies, Switzerland) |
| Double distilled deionized water | Reverse osmosis connected to Millipore Synergy UV-R | Reverse osmosis connected to a Milli-Q Advantage A10 |
| Shaker | End over end shaker Genie SI-1100 Roto-Shake Rotator (Scientific Industries Inc. USA) | Same as Lab A |
| Centrifugation* | Thermo Scientific Sorvall Legend XFR centrifuge at a speed of 3000 rpm or 2000xg (Thermo Fisher Scientific, Germany) | Thermo Scientific Sorvall Legend X1R Benchtop a speed of 3000 rpm or 1500xg (Thermo Fisher Scientific, Germany) |
| Hot block | Environmental express Model SC100 (Environmental express Inc., UA) | Same as Lab A |
| Microwave | Anton Paar Multiwave PRO with eight digestion tubes (Anton Paar GmbH, Austria) | Anton Paar Multiwave 3000 with 16 digestion tubes (Anton Paar GmbH, Austria) |
| Inductively coupled plasma-optical emission spectrometry (ICP-OES)** | Varian 720 (Agilent Technologies Inc, Australia) | - Agilent 5110 (SVDV) (Agilent Technologies Inc, USA), and<br>- Varian 720 (Agilent Technologies Inc, Australia) |
| Inductively coupled plasma-mass spectrometry (ICP-MS)** | Agilent 7900 with collision reaction cell and MassHunter software (Agilent Technologies Inc, USA) | - Agilent 8800 triple Quadrupole ICP-MS (ICP-QQQ) with collision reaction cell and MassHunter software (Agilent Technologies Inc, USA), and<br>- Perkin Elmer ELAN 9000 (PerkinElmer, Inc. USA) |

*modified from the Tessier by using a speed of 3,000 rpm instead of 10,000 rpm (1,200 x g). The extraction solution after centrifugation was separated clearly from the sediment. Pasteur pipette or a narrow tip dropper was used to collect the extraction solution and to decant supernatant from the rinsing step. Care was taken not to disturb the sediments when the extraction solution level was close to the surface of sediments.

** Because of wide range concentrations of elements and matrix interferences, ICP-OES (axial and dual view) and various ICP-MS (single and triple quadrupole mass spectrometers) with a collision reaction cell were selected for comparison.

Table S2 : Instrumental conditions of elemental analysis

| Condition | ICP-OES Varian 720 | ICP-MS Agilent 7900 | ICP-MS Agilent 8800 |
|---|---|---|---|
| Radio frequency power (W) | 1,200 | 1,550 | 1,560 |
| Auxiliary argon flow rate (L/min) | 0.7 | 1.0 | 0.9 |
| Plasma gas flow (L/min) | 12 | 15 | 15 |
| Nebulizer flow (L/min) | 0.7 | 1.04 | 1.1 |
| Spray chamber | cyclonic | quartz Scott double pass | cyclonic |
| Sample pump rate (rpm) | 12 | 18 | 15 |
| Wavelength or mass | Nanometer (nm) | Isotopic mass | Isotopic mass |
| Arsenic (As) | 193.696 | 75 | 75 |
| Beryllium (Be) | * | 9 | 9 |
| Cadmium (Cd) | * | 111 | 111 |
| Chromium (Cr) | 205.552 | 52 | 52 and 68 ($O_2$) |
| Copper (Cu) | 324.754 | 63 | 63 |
| Iron (Fe) | 259.940 | - | - |
| Lithium (Li) | * | 7 | 7 |
| Manganese (Mn) | 257.610 | 55 | 55 |
| Nickel (Ni) | 231.604 | 60 | 58 and 74 ($O_2$) and 60 and 94 ($NH_3$) |
| Lead (Pb) | 220.353 | 206, 207, 208 | 208 |
| Antimoni (Sb) | * | 121, 123 | 121 |
| Selenium (Se) | * | 77, 78 | 82 ($O_2$) |
| Tin (Sn) | * | 118 | 118 and 135 ($NH_3$) |
| Strontium (Sr) | 421.552 | - | 88 and 104 ($O_2$) |
| Uranium (U) | * | 238 | 238 and 254 ($O_2$) |
| Vanadium (V) | 292.402 | 51 | 51 and 67 ($O_2$) |
| Zinc (Zn) | 213.856 | 66 | 64 and 80 ($O_2$) |
| Auxiliary gas | | No gas, $H_2$ and He | No gas, $O_2$ and $NH_3$ |

*Analyzed by ICP-MS

Table S3 : Tessier sequential extraction procedure

| Ext sol # | Reagent | Extraction Procedure |
|---|---|---|
| 1 | 1 mol $L^{-1}$ NaOAc, pH 8.2 | -Extract 1 g of dried sample with 8 mL of Ext sol # 1, continuous end over end shaking at 30 ± 10 rpm for 1 h, centrifuge at 3000 rpm for 30 min, and collect the supernatant<br>-Rinse the residue with 8 mL of DIW, shake for 15 min, centrifuge at 3000 rpm for 30 min, and discard the supernatant |
| 2 | 1 mol $L^{-1}$ NaOAc, pH 5 | -Add 8 mL of Ext sol # 2, end over end shaking at 30 ± 10 rpm for 5 h, centrifuge at 3000 rpm for 30 min, and collect the supernatant<br>- Rinse the residue with 8 mL of DI W, shake for 15 min, centrifuge at 3000 rpm for 30 min, and discard the supernatant |
| 3 | 0.04 mol $L^{-1}$ $NH_2OH.HCl$ in 25 % (v/v) AcOH | -Add 20 mL of Ext sol # 3, place in 96 ± 3 $^{O}$C hot block for 6 h with occasionally shaking, cool down, centrifuge at 3000 rpm for 30 min, and collect the supernatant<br>- Rinse the residue with 8 mL of DIW, shake for 15 min, centrifuge at 3000 rpm for 30 min, and discard the supernatant |
| 4 | 1) 0.02 mol $L^{-1}$ $HNO_3$<br>2) 30 % $H_2O_2$ at pH 2<br>3) 3.2 mol $L^{-1}$ $NH_4OAc$ in 20 % (v/v) $HNO_3$ | -Add 3 mL of 0.02 mol $L^{-1}$ $HNO_3$ and 5 mL of 30 % $H_2O_2$, stand for 15 min, place in 85 ± 2 $^{O}$C hot block for 3 h with occasionally shaking and cool down<br>-Add 3 mL of 30 % $H_2O_2$ at pH 2, place in 85 ± 2 $^{O}$C hot block for 3 h with occasionally shaking and cool down,<br>-Add 5 mL of 3.2 mol $L^{-1}$ $NH_4OAc$ and 15 mL of DIW, end over end shaking at 30 ± 10 rpm for 30 min, centrifuge at 3000 rpm for 30 min, and collect the supernatant. |

Table S4 : Total concentrations and standard deviation, as mg $kg^{-1}$ dry weight, of elements in HISS-1, MESS-4 and PACS-3 as measured by two laboratories and the certified values where applicable

| Elements | HISS-1 (mg $kg^{-1}$) | | | MESS-4 (mg $kg^{-1}$) | | | PACS-3 (mg $kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lab A Found ± SD | Lab B Found ± SD | Certified value ± U | Lab A Found ± SD | Lab B Found ± SD | Certified value ± U | Lab A Found ± SD | Lab B Found ± SD | Certified value ± U |
| As | 0.863 ± 0.007 | 0.885 ± 0.031 | 0.801 ± 0.099 | 19.1 ± 2.0 | 23.6 ± 0.5 | 21.7 ± 2.8 | 24.6 ± 4.0 | 29.4 ± 1.0 | 30.3 ± 2.4 |
| Be | 0.110 ± 006 | 0.121 ± 0.010 | 0.129 ± 0.023 | 2.16 ± 0.07 | 1.93 ± 0.03 | 2.09 ± 0.28 | 1.03 ± 0.15 | 0.902 ± 0.102 | 1.06 ± 0.12 |
| Cd | < 0.04 | 0.020 ± 0.006 | 0.024 ± 0.009 | 0.264 ± 0.042 | 0.316 ± 0.039 | 0.28 ± 0.04 | 2.49 ± 0.46 | 2.29 ± 0.16 | 2.23 ± 0.06 |
| Cr | 15.4 ± 2.2 | 15.9 ± 0.5 | 30 ± 6.8 | 86.7 ± 5.7 | 93.5 ± 3.5 | 94.3 ± 1.8 | 85.8 ± 6.3 | 89.5 ± 3.5 | 90.6 ± 4.0 |
| Cu | 1.97 ± 0.02 | 2.03 ± 0.25 | 2.29 ± 0.37 | 35.3 ± 5.8 | 30.1 ± 1.5 | 32.9 ± 1.8 | 292 ± 11 | 322 ± 7 | 326 ± 10 |
| Fe | 2,406 ± 203 | 2,363 ± 40 | 2,460 ± 90 | 36,067 ± 740 | 36,435 ± 1,770 | 37,900 ± 1,600 | 37,361 ± 1759 | 39,813 ± 199 | 41,060 ± 640 |
| Li | 2.80 ± 0.11 | 3.02 ± 0.05 | 2.83 ± 0.54 | 69.7 ± 6.0 | 76.8 ± 3.6 | 65.3 ± 6.8 | 36.9 ± 3.2 | 35.6 ± 0.8 | 31.9 ± 4.2 |
| Mn | 60.7 ± 4.0 | 63.7 ± 2.1 | 66.1 ± 4.2 | 289 ± 23 | 301 ± 15 | 298 ± 14 | 409 ± 28 | 424 ± 14 | 432 ± 16 |
| Ni | 2.55 ± 0.62 | 2.17 ± 0.21 | 2.16 ± 0.29 | 45.1 ± 4.2 | 41.5 ± 1.7 | 42.8 ± 1.6 | 41.7 ± 4.0 | 39.2 ± 2.0 | 39.5 ± 2.2 |
| Pb | 3.78 ± 0.88 | 3.00 ± 0.56 | 3.13 ± 0.40 | 19.8 ± 1.4 | 21.0 ± 0.9 | 21.5 ± 1.2 | 179.4 ± 16.1 | 189.4 ± 7.4 | 188.0 ± 7.4 |
| Sb | 0.092 ± 0.023 | 0.134 ± 0.009 | 0.13 * | 1.13 ± 0.13 | 1.00 ± 0.16 | 1.07 ± 0.16 | 11.2 ± 1.4 | 12.0 ± 0.4 | 14.7 ± 2.2 |
| Se | 0.049 ± 0.009 | 0.056 ± 0.007 | 0.050 ± 0.007 | 0.963 ± 0.028 | 1.07 ± 0.04 | 1.5 * | 0.955 ± 0.018 | 1.097 ± 0.186 | Not reported |
| Sn | 0.185 ± 0.006 | 0.132 ± 0.008 | 0.11 * | 2.50 ± 0.11 | 2.50 ± 0.12 | 2.35 ± 0.12 | 26.4 ± 1.1 | 20.5 ± 1.7 | 22.0 ± 0.2 |
| Sr | 92.3 ± 3.3 | 100.6 ± 1.4 | 96.9 ± 11.2 | 115 ± 3 | 133 ± 4 | 132 ±8 | 230 ± 3 | 268 ± 6 | 267 ± 10 |
| U | 0.352 ± 0.081 | 0.301 ± 0.019 | 0.26 * | 3.20 ± 0.42 | 3.70 ± 0.22 | 3.4 ± 0.4 | 2.55 ± 0.32 | 2.59 ± 0.08 | 2.6 * |
| V | 5.99 ± 0.22 | 7.22 ± 0.31 | 6.8 ± 0.78 | 197 ± 9 | 211 ± ±5 | 216 ± 8 | 127 ± 4 | 129 ± 4 | 129 ± 8 |
| Zn | 6.87 ± 1.82 | 4.84 ± 0.45 | 4.94 ± 0.79 | 134 ± 17 | 142 ± 6 | 147 ± 6 | 352 ± 31 | 374 ± 6 | 376 ± 12 |

* an indicative value


**Figure S1: Percent recovery (y-scale) of total element (x-scale) determination by two labortaories in comparsion with certified values**

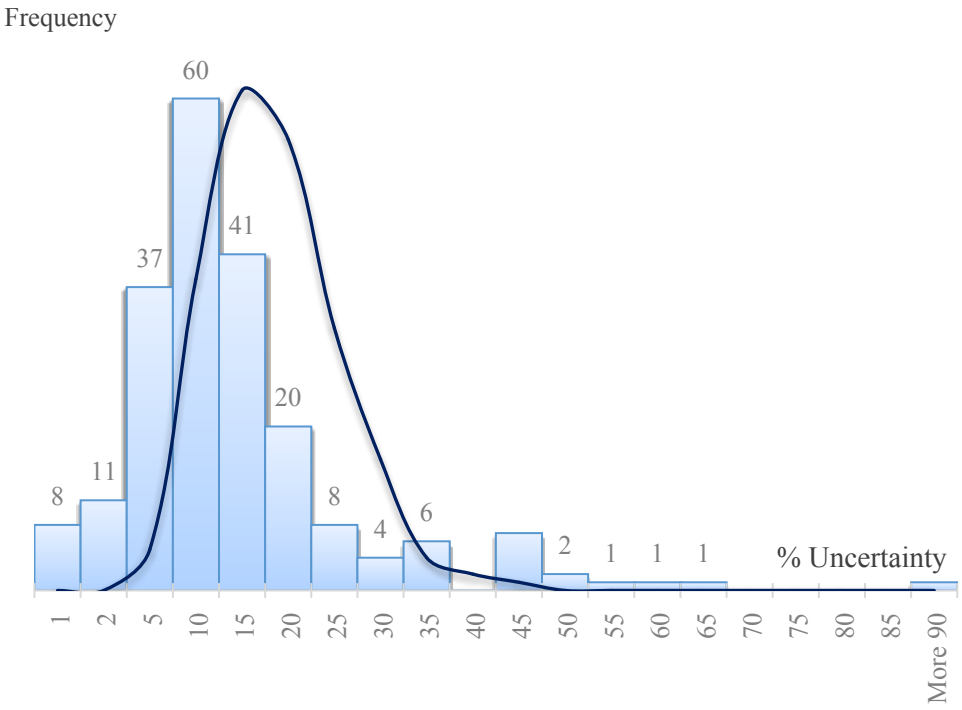


**Figure S2 : Distribution histogram of observed standard uncertainties of elements in HISS-1, MESS-4 and PACS-3 (bar chart) and target uncertainty (line plot) by Horwitz equation**

Table S5 : Combined concentrations (mg $kg^{-1}$ dry weight) of elements from four extraction steps and residue and associated uncertainties (U, k=2) in comparison to certified values with associated uncertainties (k=2) of HISS-1 and percent recovery

| Metals | Combined value | U | Certified value | Certified U | Recovery (%) |
|---|---|---|---|---|---|
| Li | 3.60 | 2.64 | 2.83 | 0.54 | 127 |
| Be | 0.125 | 0.059 | 0.129 | 0.023 | 97 |
| V | 8.06 | 2.12 | 6.80 | 0.78 | 119 |
| Cr | 18.5 | 0.6 | 30.0 | 6.8 | 62 |
| Mn | 67.0 | 7.1 | 66.1 | 4.2 | 101 |
| Fe | 2,361 | 13 | 2,460 | 90 | 96 |
| Ni | 2.91 | 0.33 | 2.16 | 0.29 | 135 |
| Cu | 2.479 | 0.324 | 2.290 | 0.370 | 108 |
| Zn | 5.07 | 0.73 | 4.94 | 0.79 | 103 |
| As | 0.902 | 0.407 | 0.801 | 0.099 | 113 |
| Sr | 82.9 | 18.2 | 96.9 | 11.2 | 86 |
| Sn | 0.192 | 0.114 | 0.142 | 0.020 | 135 |
| Sb | 0.116 | 0.005 | 0.119 | 0.049 | 98 |
| Pb | 3.48 | 0.29 | 3.13 | 0.40 | 111 |
| U | 0.228 | 0.043 | 0.304 | 0.166 | 75 |
| Total concentration of U used average concentration of two laboratories | | | | | |

Table S6 : Combined concentrations (mg $kg^{-1}$ dry weight) of elements from four extraction steps and residue and associated uncertainties (U, k=2) in comparison to certified values with associated uncertainties (k=2) of MESS-4 and percent recovery

| Metals | Combined value | U | Certified value | Certified U | Recovery (%) |
|---|---|---|---|---|---|
| Li | 58.7 | 7.9 | 65.3 | 6.8 | 90 |
| Be | 2.30 | 0.22 | 2.09 | 0.28 | 110 |
| V | 222 | 25 | 216 | 8 | 103 |
| Cr | 94.5 | 10.5 | 94.3 | 1.8 | 100 |
| Mn | 273 | 20 | 298 | 14 | 91 |
| Fe | 34818 | 6761 | 37900 | 1600 | 92 |
| Ni | 45.8 | 5.8 | 42.8 | 1.6 | 107 |
| Cu | 34.4 | 2.9 | 32.9 | 1.8 | 105 |
| Zn | 157 | 13 | 148 | 6 | 106 |
| As | 21.2 | 3.7 | 21.7 | 2.8 | 98 |
| Se* | 1.074 | 0.274 | 1.179 | 0.101 | 91 |
| Sr | 124 | 14 | 132 | 8 | 94 |
| Cd | 0.241 | 0.103 | 0.280 | 0.040 | 86 |
| Sn | 3.23 | 0.48 | 2.35 | 0.12 | 137 |
| Sb | 1.172 | 0.182 | 1.070 | 0.160 | 110 |
| Pb | 21.3 | 3.3 | 21.5 | 1.2 | 99 |
| U | 3.09 | 0.95 | 3.40 | 0.40 | 91 |
| *Total concentration of Se used an average concentration of two laboratories | | | | | |

Table S7 : Combined concentrations (mg $kg^{-1}$ dry weight) of elements from four extraction steps and residue and associated uncertainties (U, k=2) in comparison to certified values with associated uncertainties (k=2) of PACS-3 and percent recovery

| Metals | Combined value | U | Certified value | Certified U | Recovery (%) |
|---|---|---|---|---|---|
| Li | 30.2 | 5.0 | 31.9 | 4.2 | 95 |
| Be | 0.99 | 0.06 | 1.06 | 0.12 | 93 |
| V | 139 | 20 | 129 | 8 | 108 |
| Cr | 99.6 | 9.0 | 90.6 | 4.0 | 110 |
| Mn | 396 | 39 | 432 | 16 | 92 |
| Fe | 37086 | 870 | 41060 | 640 | 90 |
| Ni | 41.4 | 5.0 | 39.5 | 2.2 | 105 |
| Cu | 318 | 23 | 326 | 10 | 98 |
| Zn | 430 | 44 | 376 | 12 | 114 |
| As | 30.8 | 3.9 | 30.3 | 2.4 | 102 |
| Se* | 1.18 | 0.25 | 1.03 | 0.26 | 115 |
| Sr | 232 | 103 | 267 | 10 | 87 |
| Cd | 2.27 | 0.50 | 2.23 | 0.16 | 102 |
| Sn | 26.3 | 7.9 | 22.0 | 2.20 | 120 |
| Sb | 13.8 | 1.4 | 14.7 | 2.200 | 94 |
| Pb | 216 | 30 | 188 | 7.4 | 115 |
| U* | 2.64 | 0.45 | 2.58 | 0.66 | 102 |
| *Total concentrations of Se and U used an average concentration of two laboratories | | | | | |

## Graphical Abstract