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RESEARCH ARTICLE

Investigating geochemical factors affecting heavy metal bioaccessibility in surface sediment from Bernam River, Malaysia

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Abstract The present study applied the use of sequential extraction technique and simple bioaccessibility extraction test to investigate the bioavailable fractions and the human bioaccessible concentration of metals collected from nine stations in surface sediment of the Bernam River. The concentrations of total and bioaccessible metals from different stations were in the range of 0.30–1.43 μg g^{-1} and 0.04–0.14 μg g^{-1} for total cadmium and bioaccessibility of cadmium, respectively, 6.20–288 μg kg^{-1} and 2.06–8.53 μg kg^{-1} for total mercury and bioaccessibility of mercury, respectively, and 9.2–106.59 μg g^{-1} and 0.4–2.75 μg kg^{-1} for total tin and bioaccessibility of tin, respectively. The chemical speciation of Cd in most sampling stations was in the order of oxidisable-organic > acid-reducible > residual > exchangeable, while the chemical speciation of Hg was in the order of exchangeable > residual > oxidisable-organic > acid-reducible and the chemical speciation of Hg was in the order of residual > oxidisable-organic > acid-reducible > exchangeable. The principal component analysis showed that the main factors influencing the bioaccessibility of mercury in surface sediments were the sediment total organic matter, cation exchange capacity, and easily, freely, or leachable and exchangeable fraction, and the factors influencing the bioaccessibility of tin were the total tin and cation exchange capacity, while the bioaccessibility of Cd in surface sediments was influenced by the only factor which is the easily, freely, or leachable and exchangeable fraction.

Keywords Bioaccessibility · Sediment · Hg · Cd · Bernam River

Introduction

The Bernam River is among one of the most important rivers in Malaysia and has been identified as the ultimate and largest source of water supply from Sungai Bernam, especially for irrigation and supply for domestic use; Bernam River has been posed to be polluted by a variety of contaminant sources such as industrial and domestic wastewater; these are all likely to cause heavy metal pollution at this river which it can reach the water column and accumulate in sediments. Heavy metals from surface sediment may potentially transfer to humans via biota due to that these metals can be released from the surface sediment to overlying water which may cause many problems including health impacts (McCready et al. 2006; Chen et al. 2007; Gu et al. 2014b). Heavy metals can be associated with organic matter present in the thin fraction of the sediments as well as textural characteristic or adsorbed on Fe/Mn hydrous oxides which make aquatic sediments the main sink for toxic metals in the environment, and these parameters may affect the amount of metals bound to sediments (Calmano et al. 1993; Saeedi et al. 2013). In sediments, heavy metals are in existence in a number of chemical forms and generally exhibit different physical and chemical behaviors in terms of chemical interaction, identification of the main

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binding sites, mobility, biological availability, and potential toxicity (Sundaray et al. 2011). These chemical fractions can be obtained using four stages of the sequential extraction technique (SET) proposed by Yap et al. (2002) (exchangeable, acid-reducible, oxidisable-organic, and residual). Additional studies documented that the bioaccessibility of heavy metals (Bio-HMs), defined as the fraction that is soluble in the gastrointestinal environment and potentially available for absorption, is the major part that may render health risks (Luo et al. 2012b; Sauve et al. 2000). Thus, in this study, surface sediment was addressed to the SET and simple bioavailability tests (SEBT) to assess bioavailability in risk assessment study. Secondly, the occurrence and relative distribution of an element among these various phases, and the physical relation between the phases in the sediment, will control an element's dissolution properties and, hence, its bioavailability. Several researchers that have widely studied the factors influencing on metal bioaccessibility in the soil and sediment are listed as follows: Jinghua et al. (2015) in the Lake Taihu (China), Devesa-Rey et al. (2008) and Devesa-Rey et al. (2010) in Anllóns River (Spain), Xiaodong et al. (2015, 2016) in different soil sites (China), and Kadhum et al. (2016) in Langat River (Malaysia). Each study has its own theory foundation, and various factors influence in bioaccessibility depending on different anthropogenic sites. However, to date and the best of our knowledge, dominant factors which may influence the bioavailability and bioaccessibility for heavy metal analysis in surface sediment of Bernam River remain unknown. Therefore, this study were (1) to estimate the bioavailability and bioaccessibility of Hg and Cd in surface sediment using sequential extraction and SBET and (2) to investigate the potential influencing factors (sediment properties and heavy metal chemical forms) that may affect the bioaccessibility of metals.

Materials and methods

Study area

The Bernam River is sited in the west of Peninsular Malaysia between the states of Perak and Selangor, and it covers an area of 3335 km^2, as one of the most important in rice cultivation in the lower areas adjacent to peat swamps. Recently, at the upstream of Bernam River, the whole area is embedded with rubber and oil palm plantation. The Bernam River basin is changing from agriculture base to an industrial area where Proton city is located which is rapidly developing. Logging generates pollution through soil erosion, siltation, and sedimentation in the river. Bernam River basin is characterized by high temperatures and relative humidity with small seasonal variations. The mean relative humidity is about 77% on average, while the annual mean air temperature varied from minimum to maximum temperatures which are 26 to 32 °C, respectively. Rainfall maximum flows occur during the northeast monsoon from November to January and southwest monsoon from April to September, with maximum flows occuring from October to December, whereas minimum flows occur in the February, July, and August which are inter-seasonal periods and, hence, recording the lowest rainfall. The mean annual rainfall usually ranges from 2899 mm at the upstream catchment to 1632 mm at the downstream (Jamaluddin 2014).

Sample collection and preservation

A total of 27 surface sediment (0–5 cm) samples were collected at nine sites from downstream to upstream of Bernam Rivers from January to February in 2015. Figure 1 and Table 1 show the sampling collected locations and the sampling taken from surface sediments (0–5 cm depth); the Ekman grab was washed with distilled water after collecting the sample from each station to avoid possible metal contamination from the grab. They were placed in clean labeled plastic bags and transferred to the laboratory in the icebox. Sediment samples were deep frozen (−20 °C) prior to analysis.

Sequential extraction

Chemical speciation of heavy metal in surface sediments was analyzed by using modified sequential extraction technique described by Yap et al. (2002) and Badri and Aston (1983). The procedure for this method is as follows: Step 1: easily, freely, or leachable and exchangeable (EFLE)—about 10 g of sample was continuously shaken for 3 h with 50 ml of 1.0 M ammonium acetate (NH_4CH_3COO), pH 7.0 at room temperature. Step 2: acid-reducible—the residue from EFLE was continuously shaken for 3 h with 50 ml of 0.25 M hydroxylammonium chloride ($NH_2OH{\cdot}HCl$) acidified to pH 2 with HCl, at room temperature. Step 3: oxidisable-organic—the residue from acid-reducible was first oxidized with 15 ml H_2O_2 (R&M Chemicals 35%) in a water bath at 90 °C. After cooling, the metal released from the organic complexes was continuously shaken for 3 h with 50 ml of 1.0 M ammonium acetate (NH_4CH_3COO) acidified to pH 2.0 with HCl, at room temperature. Step 4: resistant—the residue from oxidisable-organic was digested in a 10-ml combination (ratio of 4:1) of concentrated HNO_3 and $HClO_4$. The samples were analyzed in triplicate, and the analysis was performed using ICP-MS (Perkin Elmer Model Elan DRC-e).

Simplified bioaccessibility extraction test (SBET)

Simplified bioaccessibility extraction test is a one-step in-vitro digestion test without pepsin (Oomen et al. 2002). The

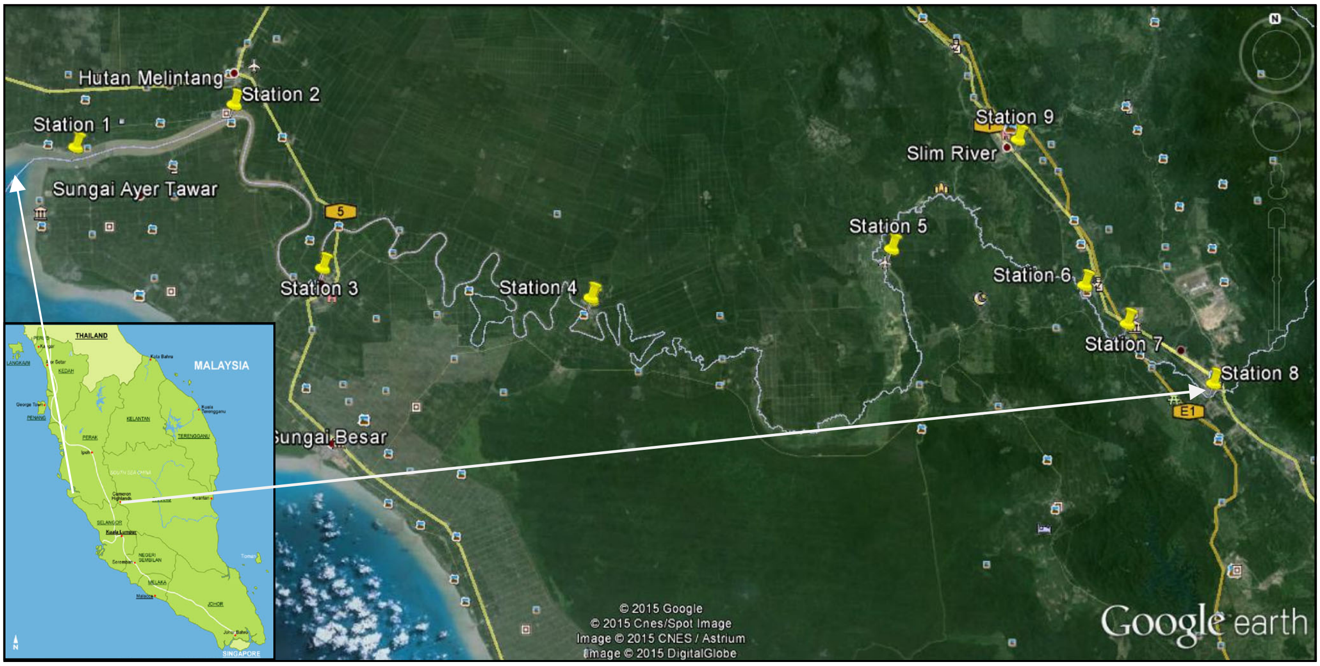

Fig. 1 Map of Bernam Rivers' catchment and sampling stations

procedure for this method was used to assess half gram (63 µm) air-dried sediment placed in a centrifuge tube and mixed with 50 ml of gastric solution (0.4 M glycine; pH = 1.5 pre-adjusted with concentrated HCl). The tube was rotated end-over-end at 30 rpm for 1 h at 37 °C and centrifuged at 3500 g for 15 min, and then, the extracted suspension was filtered through 0.45-µm cellulose acetate disc filter. After determination of pH, the gastric phase samples were stored in a refrigerator at 4 °C. Each sample was analyzed in triplicate, and the analysis was performed using ICP-MS (Perkin Elmer Model Elan DRC-e) within 1 week after the experiments. Relative heavy metal bioaccessibility was calculated by dividing the SBET of heavy metal concentrations by the total heavy metals:

$$\text{Heavy metal bioaccessibility } (\%) = \frac{\text{SBET heavy metals}}{\text{total heavy metals}} \times 100$$

Total heavy metal concentrations

The dried sediment samples (0.5) were digested in a 10-ml solution of a mixture HNO_3 (AnalaR grade, R&M 65%) and $HClO_4$ (AnalaR grade, R&M 70%) in the ratio of 4:1 (*v*/*v*), into a pre-heated block digester at low temperature (40 °C) for 1 h and then at 140 °C for 3 h (Ismail 1993). The digested samples were then diluted to 40 ml with double-distilled water (DDW) and then filtered through Whatman no.1 filter paper into pre-cleaned 40-ml volumetric flasks. The final samples were measured for metal concentration using an air-acetylene flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model A Analyst 800). The determination of total mercury in sediment samples is carried out by using ultrasound-assisted mercury extraction, which was proposed by Collasiol et al. (2004) and Looi et al. (2014) and analyzed using FIMS-100.

Table 1 The coordinates and description for each sampling station

Station	Sampling site	Coordinates	Description
1	Kampung Bagan	N 3° 50' 46.91" E 100° 50' 42.36"	Agricultural area, oil palm, boat harbor
2	Bagan Tepi Sungai	N 3° 52' 10.56" E 100° 55' 57.84"	Residential area, agricultural area, oil palm
3	Sabak Bernam	N 3° 46' 17.89" E 100° 59' 0.05"	Residential area, industry, agricultural area, domestic waste discharge, fisheries
4	Kampung Tanjung	N 3° 44' 45.37" E 101° 8' 41.09"	Industrial, agriculture area, oil palm
5	Ulu Bernam	N 3° 45' 57.21" E 101° 19' 44.19"	Agricultural area, air field
6	Selisek	N 3° 44' 20.02" E 101° 26' 44.06"	Agricultural area, oil palm, fisheries
7	Bandar Behrang	N 3° 42' 55.09" E 101° 28' 13.07"	Agriculture area, fisheries
8	Tanjong Malim	N 3° 40' 40.47" E 101° 31' 16.29"	Industrial, domestic waste discharge, car washing, fisheries
9	Slim River	N 3° 49' 35.46" E 101° 24' 32.26"	Residential area, agriculture area

Quality assurance and quality control

To prevent uncertain contaminations, all laboratory equipment used were washed with phosphate-free soap, double rinsed with distilled water, left in 10% HNO_3 for 24 h, then rinsed two times with double distilled water, and left semi-closed to dry at room temperature. Certified reference material (CRM) was determined as a precision check used for sediment (PACS-2, Canada). The results for the contents of this experiment in regard to the three replicates and percentage of recoveries for the certified and measured concentration of Cd, Hg, and Sn were found with recovery percentages being about 104, 82, and 107%, respectively. The acceptance of the analytical result for the reference materials and its certified values for each metal was satisfactory. Calibration curves were prepared by making two appropriate dilutions in stock water solution (1000 mg l^{-1}) (BDH Spectrosol®) in 2% HNO_3. In addition, five standard solutions were prepared from a different stock standard source with detection limits of 0.1 μg l^{-}. A blank calibration solution was used for a zero. The linearity for the calibration lines of multi-elemental standards shows that the correlation coefficient values were at least 0.999 during analysis and considered as satisfaction.

Physicochemical properties of sediment analysis

Measurement of pH was conducted according to McLean (1982) by using distilled water with 1:2.5 solid/liquid ratio that is 25 ml of distilled water was added to 10 g of sediment in a glass beaker, which was covered with plastic film, put in orbital shaker for 4 h at 175 rpm, and then read with a digital electrode pH meter model WTW pH 330. The organic matter was expressed as a loss on ignition (LOI) by calculating the difference between the dry weight of sediment samples before and after ashing in a muffle furnace at 550 °C for 5 h (Arain et al. 2008; Kazi et al. 2005). Sediment particle sizes were determined by pipette method (Gee and Bauder 1986). The samples of sediment were classified into sand (>50 μm), silt (2 μm <size< 64 μm), and clay (<2 μm) fraction according to the USDA particle size classification (Soil Survey Staff 1992). Cation exchange capacity (CEC) was determined by measuring the exchangeable cation displaced from sediment and treated with NaCl which was used to determine Ca^{2+}, Mg^{2+}, and K^+, while NH_4Cl was used to determine the Na^+ (Apello and Postma 2005; Aris et al. 2010). The samples were analyzed for Na^+, Ca^{2+}, Mg^{2+}, and K^+ using ICP-MS (Perkin Elmer Model Elan DRC-e).

Results and discussion

Sediment properties

The soil properties, including pH, particle size, total organic matter content, and CEC, are presented in Table 2. pH and total organic matter values have been reported in the Bernam River (Safaa et al. 2015). The highest pH value was 7.50 (Kampung Bagan), but the lowest pH value was 5.91 (Kampung Tanjung). The mean TOM values were highest in station Bagan Tepi (31.47%) and lowest in station Selisek (9.63%). Organic matter levels in sediments can give a good indicator of metal bioavailability and mobility due to its great affinity for heavy metals (Hu et al. 2013). The particle size plays a vital role in controlling toxic concentrations in sediments whereby the pollutant concentrations tend to increase with declining particle size (Simpson et al. 2005). Thus, investigation of particle size is important to assess the anthropogenic influence on sediment properties. The mean percentage value of clay, silt, and sand fraction in surface sediment of Bernam River were found to range from 0.13 to 52.53%, 0.75 to 41.12%, and 6.24 to 98.39%, respectively. The sum of exchangeable cations (CEC) in the sediment ranged from 23.80 to 184.10 meq/100 g in Bernam River and the order exchangeable cations of decreased $Na^+ < Mg^{++} < Ca^{++} < K^+$.

The relationships among the examined physicochemical properties and heavy metals were tested using Spearman's correlation analysis (Table 3). The correlation matrix revealed that total Hg with K^+ in all the sampled stations of Bernam River had a negative relationship and total Hg had no significant relationship with other physicochemical properties. CEC and TOM had a strong positive correlation, and CEC in surface sediment of river could increase by increasing the TOM content (Camberato 2001; Shafie et al. 2013). Furthermore, total cadmium in Bernam River had significant relationship with TOM, Na^+, Mg^{++}, and CEC, and these physicochemical properties may contribute to increasing Cd concentration in the river. Total Cd had no significant relationship with other metals; this indicated that total Cd came from different sources such as industry effluent, agriculture activities around the rivers, and domestic sewage discharge. The correlation matrix revealed also that total Sn with total Hg in all the sampled stations of Bernam River had a negative relationship.

Total and bioaccessibility of heavy metals

The average concentrations of the total and bioaccessible Cd, Hg, and Sn from nine sampling sites are presented in Table 4. Total Cd, Hg, and Sn values have been reported in Bernam River (Safaa et al. 2015). T-Cd concentrations in the surface

Table 2 Physicochemical contents in the surface sediments of the Bernam Rivers

Sediment properties	Kampung Bagan	Bagan Tepi Sungai	Sabak Bernam	Kampung Tanjung	Ulu Bernam	Selisek	Bandar Behrang	Tanjong Malim	Slim River
pH	7.50	6.13	6.24	5.91	6.71	6.84	6.40	6.31	6.65
TOM (%)	24.67	31.47	29.27	20.91	11.57	9.63	14.81	14.11	10.30
Clay (%)	22.04	52.53	7.47	41.57	0.13	0.82	23.46	9.27	4.27
Silt (%)	20.41	41.12	14.29	6.47	1.55	0.75	3.49	74.24	43.06
Sand (%)	57.51	6.24	78.21	51.96	98.31	98.39	73.05	16.47	51.7
K^+ (meq/100 g)	11.97	10.11	10.74	5.07	4.41	4.56	5.16	4.58	3.16
Mg^{+2} (meq/100 g)	92.86	105.52	91.11	4.48	2.61	4.89	6.21	3.53	23.64
Ca^{+2} (meq/100 g)	17.27	17.90	24.85	11.11	8.85	21.92	40.09	10.52	34.06
Na^+ (meq/100 g)	21.09	19.79	57.38	3.13	19.25	19.05	18.62	19.04	18.58
CEC (meq/100 g)	143.21	153.34	184.10	23.80	35.14	50.44	70.06	37.69	79.46

sediments from nine stations varied from 0.49 to 1.04 μg g^{-1}, T-Hg concentration was in the range of 3.96 to 65.46 μg kg^{-1}, and T-Sn varied from 106.59 to 9.2 μg g^{-1}. Bio-Cd, Bio-Hg, and Bio-Sn concentrations in sediment varied considerably among different sampling sites; Bio-Cd, Bio-Hg, and Bio-Sn in all nine sampling stations were 0.04–0.14 μg g^{-1}, 5.7–33.3 μg kg^{-1}, and 0.4–2.75 μg g^{-1}, respectively. The highest Bio-Hg and Bio-Sn concentrations were recorded in the sediment collected from Kampung Bagan station while the highest Bio-Cd value was in Kampung Tanjung station. There was an increase of Cd and Sn concentrations from upstream (Slim River) to the downstream (Kampung Bagan); the highest of Cd concentrations in these parts of the sediment profiles may indicate increased metal discharges from upstream to downstream due to settled anthropogenic sources surrounding the rivers into downstream such as local industrial, agricultural development, and oil palm plantation (Chen et al. 2007). Slim River had the lowest T-Cd whereas the lowest Bio-Cd was in Ulu Bernam, while Bio-Hg had the lowest concentrations on in Tanjong Malim station. The relative bioaccessibility of HMs (R-Bio-HMs) was defined as the percentage of bioaccessibility of metals to the total metals; the highest R-Bio-Cd among these sampling stations appeared at Bandar Behrang (39.9%), and the highest R-Bio-Sn was in Sabak Bernam (6.06%). Slim River had the very highest R-Bio-Hg (89.91%). R-Bio-Hg showed higher than R-Bio-Cd and Bio-Sn. There are limited studies determining the bioaccessibility of Cd, Hg, and Sn in sediment or soil from Southeast Asian region. Thus, we compared our results with different universal studies. Relative bioaccessibility of Cd which is showed less than the bioaccessibility of Cd (75.96%) was previously recorded from soil urban parks in

Table 3 Pearson correlation of heavy metal concentrations with physicochemical parameters from Bernam River (*n* = 27)

	pH	TOM	Clay	Silt	Sand	Cd	Sn	Hg	K^+	Ca^{++}	Mg^{++}	Na^+	CEC
pH	1												
TOM	−0.203	1											
Clay	−0.390*	−0.657**	1										
Silt	−0.135	0.061	0.059	1									
Sand	0.336	−0.428*	−0.624**	−0.817**	1								
Cd	−0.354	0.693**	0.234	0.065	−0.187	1							
Sn	−0.049	0.152	0.281	0.382*	−0.454*	0.052	1						
Hg	−0.249	−0.187	−0.022	0.054	0.040	−0.019	0.472*						
K^+	0.317	0.288	0.096	−0.003	−0.015	0.108	−0.065	−0.458	1				
Ca^{++}	0.083	0.204	−0.190	−0.076	0.098	0.041	0.498**	−0.281	0.505**	1			
Mg^{++}	−0.117	0.933**	−0.150	−0.488**	−0.454*	0.641**	0.141	−0.254	0.577**	0.308	1		
Na^+	−0.494**	0.424*	−0.157	−0.234	0.240	0.404*	−0.025	−0.202	−0.178	0.358	0.235	1	
CEC	−0.348	0.691**	−0.205	−0.354	0.351	0.524**	0.152	−0.309	0.263	0.640**	0.625**	0.863**	1

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Table 4 The total of Cd (µg/g), Hg (µg/kg), Sn (µg/g), bioaccessible (µg/g), relative bioaccessible (%), and sequential chemical concentration (µg/g) (mean ± standard deviation)

Stations	Total HMs	Bioaccessible	R-Bio	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Kampung Bagan							
Cd	0.53 ± 0.1	0.08 ± 0.06	16.2	0.05 ± 0.01 (8.6)	0.25 ± 0.02 (37.3)	0.19 ± 0.05 (28.6)	0.17 ± 0.08 (25.2)
Hg	14.36 ± 0.03	5.7 ± 0.1	39.9	14.13 ± 0.15 (77.5)	1.7 ± 0.05 (9.5)	1.15 ± 0.01 (6.3)	1.19 ± 0.09 (6.5)
Sn	54.49 ± 2.8	2.75 ± 0.05	5.05	0.43 ± 0.4 (1.30)	0.63 ± 0.02 (1.91)	0.27 ± 0.1 (0.82)	31.7 ± 0.5 (95.9)
Bagan Tepi Sungai							
Cd	1.05 ± 0.03	0.10 ± 0.04	9.62	0.05 ± 0.04 (6.6)	0.24 ± 0.08 (29.3)	0.12 ± 0.08 (14.6)	0.41 ± 0.04 (49.2)
Hg	41.36 ± 0.01	5.5 ± 0.05	13.46	18.8 ± 0.4 (42.2)	1.56 ± 0.05 (3.5)	1.06 ± 0.03 (2.3)	23.1 ± 0.1 (51.8)
Sn	106.59 ± 1.0	1.29 ± 0.01	1.21	0.15 ± 0.05 (0.38)	0.59 ± 0.06 (1.49)	6.92 ± 0.06 (17.5)	31.7 ± ±0.2 (80.5)
Sabak Bernam							
Cd	1.43 ± 0.02	0.04 ± 0.01	2.93	0.06 ± 0.04 (4.5)	0.14 ± 0.01 (10.6)	0.84 ± 0.6 (60.3)	0.34 ± 0.05 (24.5)
Hg	53.13 ± 0.03	5.06 ± 0.1	9.53	13.73 ± 1.67 (43.3)	4.53 ± 0.2 (13.8)	0.92 ± 0.05 (2.8)	13.6 ± 0.1 (41.4)
Sn	28.54 ± 1.6	1.73 ± 0.01	6.06	0.12 ± 0.05 (0.50)	0.71 ± 0.01 (2.91)	3.59 ± 0.09 (14.6)	20.0 ± 0.2 (81.8)
Kampung Tanjung							
Cd	0.66 ± 0.4	0.14 ± 0.06	22	0.04 ± 0.03 (8.6)	0.19 ± 0.01 (37)	0.21 ± ±0.03 (40.3)	0.07 ± 0.02 (13.9)
Hg	62.40 ± 0.02	4.56 ± 0.05	7.31	7.26 ± 0.15 (80.9)	0.11 ± 0.01 (0.6)	8.4 ± 0.1 (50)	1.0 ± 0.04 (5.9)
Sn	39.2 ± 0.6	1.66 ± 0.01	4.23	0.17 ± 0.05 (0.58)	0.53 ± 0.01 (1.81)	1.89 ± 0.05 (6.42)	26.9 ± 0.4 (91.1)
Ulu Bernam							
Cd	0.59 ± 0.4	0.04 ± 0.06	8.24	0.0004 ± 0.2 (0.09)	0.19 ± 0.01 (47.5)	0.11 ± 0.03 (28.2)	0.10 ± 0.05 (24.06)
Hg	6.20 ± 0.03	3.90 ± 0.1	62.9	11.46 ± 0.6 (14.9)	0.12 ± 0.09 (0.8)	0.71 ± 0.03 (5)	1.87 ± 0.06 (13.1)
Sn	39.4 ± 4.5	1.90 ± 0.04	4.83	0.67 ± 0.05 (2.12)	0.57 ± 0.05 (1.81)	1.32 ± 0.01 (4.16)	29.1 ± 0.1 (91.8)
Selisek							
Cd	0.28 ± 0.2	0.10 ± 0.01	37.4	0.05 ± 0.08 (12.8)	0.07 ± 0.02 (15.7)	0.22 ± 0.04 (47.9)	0.10 ± 0.01 (23.3)
Hg	65.46 ± 0.02	3.86 ± 0.1	5.90	12.2 ± 0.3 (51.5)	0.56 ± 0.2 (0.6)	67.6 ± 0.6 (82.6)	1.41 ± 0.08 (1.7)
Sn	92.94 ± 0.7	1.8 ± 0.01	1.93	0.62 ± 0.05 (2.02)	1.06 ± 0.01 (3.48)	7.12 ± 0.1 (23.1)	22.0 ± 0.3 (71.4)
Bandar Behrang							
Cd	0.24 ± 0.1	0.08 ± 0.06	39.9	0.07 ± 0.03 (19.5)	0.17 ± 0.05 (44.8)	0.09 ± 0.02 (25.6)	0.03 ± 0.02 (9.92)
Hg	8.83 ± 0.09	3.36 ± 0.05	38.11	6.70 ± 0.4 (40.4)	1.38 ± 1.4 (10.6)	0.51 ± 0.01 (3.9)	4.41 ± 0.09 (33.8)
Sn	36.63 ± 0.8	1.59 ± 0.03	4.33	0.92 ± 0.03 (3.60)	0.69 ± 0.05 (2.70)	7.76 ± 0.01 (30.2)	16.2 ± 0.3 (63.4)
Tanjong Malim							
Cd	0.55 ± 0.04	0.10 ± 0.01	18.9	0.06 ± 0.02 (9.98)	0.04 ± 0.05 (7.2)	0.17 ± 0.02 (28.1)	0.33 ± 0.04 (54.5)
Hg	288 ± 0.02	3.33 ± 0.05	1.19	8.09 ± 0.1 (43.2)	2.9 ± 0.3 (14.4)	0.43 ± 0.05 (2.1)	8.59 ± 0.1 (42.9)
Sn	100.2 ± 2.4	1.270.02	1.27	0.38 ± 0.01 (1.60)	4.51 ± 0.1 (18.5)	0.16 ± 0.05 (0.67)	19.2 ± 0.3 (79.1)
Slim River							
Cd	0.30 ± 0.07	0.08 ± 0.06	29.2	0.0002 ± 0.01 (0.06)	0.08 ± 0.1 (20.2)	0.27 ± 0.02 (64.6)	0.06 ± 0.02 (15)
Hg	59.26 ± 0.01	3.56 ± 0.05	6.01	2.53 ± 0.5 (48.4)	1.56 ± 0.05 (26.7)	0.48 ± 0.01 (8.2)	1.27 ± 0.01 (21.7)
Sn	9.2 ± 0.2	0.4 ± 0.05	4.95	0.54 ± 0.03 (5.11)	1.09 ± 0.1 (10.2)	5.67 ± 0.1 (53)	3.36 ± ±1.6 (31.5)

Guangzhou, China (Gu et al. 2015), while our report for R-Bio-Hg was higher than R-Bio-Hg of 1.2 and 3.0% for total mercury using PBET (gastric phase and gastric + intestinal phase) methodologies, respectively in residential soils in the Flin Flon, Canada (Safruk et al. 2015). In Malaysia, only one study by Kadhum et al. (2016) reported on R-Bio-Cd and R-Bio-Hg in surface sediment of Langat River (13.47 and 42.23%, respectively). Our results on Bernam River showed that R-Bio-Hg was more than R-Bio-Hg in Langat River while R-Bio-Cd was less than R-Bio-Cd in Langat River.

Cadmium speciation

The mean Cd concentrations and percentages of four chemical speciation fractions for each sampling station are tabulated in Table 4 and Fig. 2. The EFLE fraction for Cd ranged from 0.0004 to 0.07 µg g^{-1} with mean percentage of 7.89%. The acid-reducible fraction ranged from 0.04 to 0.25 µg g^{-1} with mean percentage of 27.7%. The oxidisable-organic fraction ranged from 0.09 to 0.84 µg g^{-1} with mean percentage of 37.6%. The residue (resistant) fraction ranged from 0.06 to

0.41 μg g^{-1} with mean percentage of 26.6%. Cd contained a relatively lower EFLE fraction F1, while the rest fractions displayed greater percentages with Cd. The Cd concentration in most sampling stations was dominated by the non-resistant steps (anthropogenic). The non-resistant fractions (the sum of fractions 1, 2, and 3) are highly potentially toxic for organisms because it is easily removed and used by organisms (fraction 1). Further, fractions 2 and 3 under certain physicochemical conditions, like the presence of oxygen, variations in redox potential, and bacterial activity, can become soluble (Morillo et al. 2004; Yap et al. 2006; Dou et al. 2013). In most of sampling sites, Cd concentrations were mostly in oxidisable-organic fraction, indicating that the oxidisable-organic fraction of Cd in this study was influenced mainly by the organic contents in sediments due to Cd strong positive correlation with phosphate fertilizers and irrigation water discharge (Dou et al. 2013; Liu et al. 2012). Cd was high in non-resistant forms from the most sites, and these results are not in agreement with the literature, which indicates that Cd was mostly present in resistant forms (Sungur et al. 2014; Eyupoglu et al. 2012). However, the results are in agreement with Sungur et al. (2016) which they find that Cd was mostly present in non-resistant forms and easy mobile due to anthropogenic impacts. The percentages of each chemical form of Cd as well as the R-Bio-Cd are presented in Fig. 2. The R-Bio-Cd was evidently less than the non-resistant fractions (F1 + F2 + F3). However, most of resistant forms (F4) in all stations have notably higher content than R-Bio-Cd, indicating that Cd in sediment could be easily mobilized and become easy accessible under anthropogenic condition to aquatic biota and human consumption.

Mercury speciation

The mean concentrations and percentages of sequential extraction of Hg for each sampling station are shown in Table 4 and Fig. 3. Hg in the EFLE fraction was higher than those in the other fractions in most of sampling stations, especially in Kampung Bagan and Ulu Bernam which were approximately 77 and 80%, respectively, implying high potential toxicity for these stations. The EFLE step had high Hg bioavailability in the present study ranged from 2.5 to 18.8 μg/kg with mean percentage of 48.4%; this is due to the relationship between metal adsorption and changes in the ionic composition of the water, which is capable of altering the processes of adsorption–desorption as well as the transport of metals in the soil (Fuentes et al. 2008). Again, this can greatly affect the aquatic environment relative to the other fractions. The acid-reducible fraction ranged from 0.11 to 4.5 μg/kg with the mean percentage of 8.9%. These results are not in agreement with Parthasarathi and Raghunadh (2015) which they found that Hg content was the highest with organic phases of the sediments. The oxidisable-organic fraction ranged from 0.43 to 67.6 μg/kg with the mean percentage of 18.1%, and the residue fraction ranged from 1.0 to 23.1 μg/kg with the mean percentage of 24.3% and the low percentage of acid-reducible, oxidisable-organic, and residue fractions from all stations, suggesting poor bioavailabilities of Hg. The percentages of each chemical form of Hg as well as the R-Bio-Hg are presented in Fig. 3. The R-Bio-Hg was evidently less than the non-resistant fractions (F1 + F2 + F3) in all stations of sediment with exception of Slim River station and a little higher than resistant fractions (F4) in most stations of Bernam River, suggesting that non-resistant forms of Hg could be contributed to the bioaccessibility of T-Hg under anthropogenic conditions in the sediment of Bernam River.

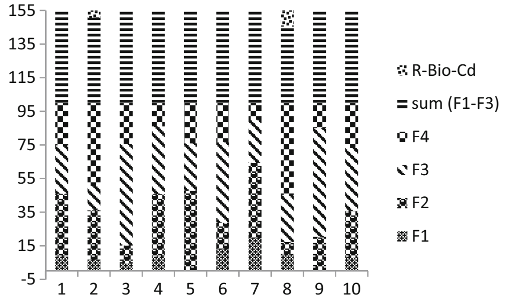

Fig. 2 Extraction percentage of Cd at sampling station

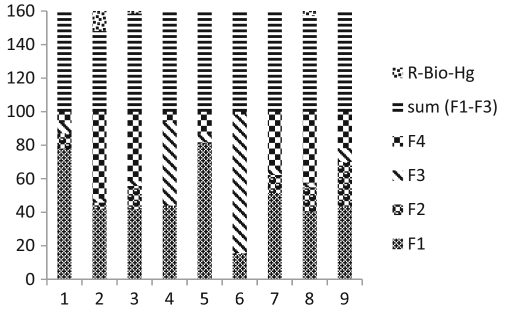

Fig. 3 Extraction percentage of Hg at sampling station

Tin speciation

The distribution of Sn in the different binding phases of the sediments is shown in Table 4 and Fig. 4. The concentrations of EFLE fraction of Sn were observed to be very low bioavailable and were found to vary from 0.12 to 0.92 μg g^{-1} with

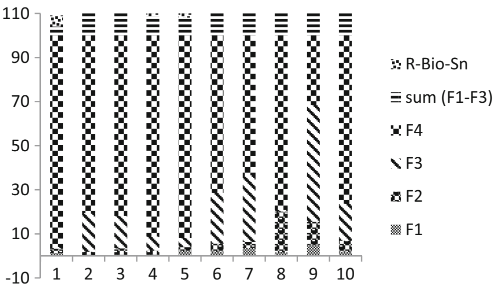

Fig. 4 Extraction percentage of Sn at sampling station

mean percentage of 1.91%. The acid-reducible fraction ranged from 0.53 to 4.9 μg g^{-1} with mean percentage of 4.9%. The oxidisable-organic fraction ranged from 0.17 to 7.76 μg g^{-1} with mean percentage of 16.75%. Greatest amounts of Sn distribution are found in the residual fraction which ranged from 3.36 to 31.7 μg g^{-1} with the mean percentage of 76.3%. Tin was mostly present in non-resistant forms across all locations of Bernam River due to natural sources such as chemical weathering of igneous and metamorphic rocks, as well as decomposition of biota detritus (Badri and Aston 1983). These results are in agreement with the literature, which indicates that Sn was mostly present in resistant forms (>80%) (Hussein et al. 2014). Thus, the residual fraction is the most stable with Sn and considered to be long-term stable with a very low risk of contaminant release into the environment. The comparative percentages of each chemical form of Hg as well as the R-Bio-Hg are presented in Fig. 3. The R-Bio-Sn was evidently less than the non-resistant fractions (F1 + F2 + F3) in all stations of sediment with exception of Kampung Bagan and resistant fractions (F4) in all stations of Bernam River, suggesting that non-resistant forms of Hg could be contributed to the bioaccessibility of T-Hg under natural conditions in the sediment of Bernam River.

Principal component analysis

A principal component analysis was run on nine stations where physicochemical properties, T-Hg, T-Cd, Bio-Hg, Bio-Cd, Bio-Sn, and chemical fractions were determined. The suitability of PCA was assessed prior to the analysis. Bartlett's test of sphericity was statistically significant ($p < 0.000$), indicating that the data was likely factorizable. PCA for Hg revealed four components that had eigenvalues greater than one and which explained 35.080, 24.527, 13.896, and 9.282% of the total variance, respectively (Table 5). Visual inspection of the scree plot indicated that four components should be retained. The four component solution explained 82.78% of the total variance. A varimax orthogonal rotation was employed to aid interpretability and to further identify the factors responsible for each one. PCA results from Bernam River sediment are represented by loadings and score plot, respectively. In this case, the data set relating to physicochemical properties of sand and pH, represented by PC1, explained 31.86% of the total variance (Fig. 5) and had strong loading on TOM, F1 (EFLE), CEC, and Bio-Hg indicating that F1 (EFLE), CEC, and TOM could play important role in the increasing of Bio-Hg in surface sediment due to anthropogenic activities which can be directly responsible for risks on human health. PC2 explained 20.43% of the total variance, which had strong loadings on T-Hg, silt, and F2 (acid-reducible). PC3 explained 19.02% of the total variance and had strong loadings on pH, clay, sand, and F4 (resistant). PC4 explained 11.45% of the total variance, which had only strong loadings on F4 (resistant) of the total variance.

The T-Cd, fractions, physicochemical properties, and Bio-Cd could mainly be divided into four categories. PCA for Cd revealed also four components that had eigenvalues >1 and which explained 34.693, 18.078, 13.639, and 10.601% of the total variance, respectively.

Visual inspection of the scree plot indicated that four components should be retained. The four component solution explained 77.01% of the total variance. A varimax orthogonal rotation was employed to aid interpretability and to further identify the factors responsible for each one. The PCA (rotated components) presented four PCs with eigenvalues >1, explaining 23.45% (PC1), 19.97% (PC2), 19.46% (PC3), and 12.52% (PC4) of total variance, respectively (Table 6).

To explain the patterns presented by the data, PCA is represented by loadings and score plot, respectively. In this case, the data set relating to TOM, F3 (oxidation-organic), T-Cd, F4 (resistant), and CEC represented by PC1 explained 23.4% of the total variance (Fig. 6). CEC and TOM could play important role in the increasing of T-Cd in F4 (resistant) and F3 (oxidation-organic). Therefore, it was observed that Cd indicates a tendency to accumulate in F4 and F3 along with the increase in the amount of CEC and TOM. PC2 explained 19.9% of the total variance which had strong loadings on clay, and F2 (acid-reducible) PC3 explained 19.4% of the total variance and had strong loadings on silt and sand; PC4 explained 12.5% of the total variance, which had strong loadings on Bio-Cd and F1 (EFLE), indicating that F1 (EFLE) in surface sediment had the largest and positive influence on Bio-Cd.

The T-Sn, fractions, physicochemical properties, and Bio-Sn could mainly be divided into four categories. PCA for Sn revealed four components that had eigenvalues >1 and which explained 30.184, 25.451, 16.647, and 10.500% of the total variance, respectively. Visual inspection of the scree plot indicated that three components should be retained. The four component solution explained 82.78% of the total variance. A varimax orthogonal rotation was employed to aid interpretability and to further identify the factors responsible for each one. The PCA (rotated components) presented four PCs with eigenvalues >1, explaining 23.943% (PC1), 21.637% (PC2), 18.063% (PC3), and 15.604% (PC4) of the total variance, respectively (Table 7). To explain the patterns presented by the data, PCA is represented by loadings and score plot, respectively. In this case, the data set relating to silt, sand, T-Sn, and F2 (acid-reducible), represented by PC1, explained 23.9% of the total variance represented by PC1 and explained 29.99% of the total variance (Fig. 7). It is obvious that silt and sand are responsible to increase T-Sn in acid-reducible fraction. PC2 explained 21.6% of the total variance which had strong loadings on TOM, CEC, and F1 (EFLE). TOM increases with the increasing of CEC in EFLE fraction, and this explains that CEC is entirely due to the organic colloid fractions because the presence of humic substances in these organic materials may enhance CEC in the soil (Lax et al.

Table 5 Total variance of Hg values explained and matrix of principal component analysis for 27 variables on Bernam River surface sediment

Component	Initial eigenvalues			Extraction sum of square loadings			Rotation sum of square loadings			
	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	
Total variance explained										
1	4.210	35.080	35.080	4.210	35.080	35.080	3.824	31.865	31.865	
2	2.943	24.527	59.606	2.943	24.527	59.606	2.453	20.438	52.303	
3	1.667	13.896	73.502	1.667	13.896	73.502	2.283	19.024	71.327	
4	1.114	9.282	82.784	1.114	9.282	82.784	1.375	11.457	82.784	
5	0.897	7.474	90.258							
6	0.684	5.703	95.961							
7	0.187	1.556	97.517							
8	0.161	1.345	98.862							
9	0.103	0.857	99.719							
10	0.029	0.246	99.964							
11	0.004	0.036	100.000							
12	7.521E−6	6.267E−5	100.000							
Variables	Component matrix						Rotated component matrix			
	PC1	PC2	PC3	PC4			PC1	PC2	PC3	PC4
pH		0.424	0.401						*−0.592*	
Bio-Hg	*0.782*	*0.511*					*0.910*			
Clay	*0.691*		*−0.673*				0.482		*0.763*	
F1	*0.729*	0.338		0.472			*0.859*			0.342
Silt	0.359	*−0.805*	0.316					0.858		
Sand	*−0.676*	*0.634*						−0.522	*−0.660*	0.324
TOM	*0.944*						*0.917*			
F3	−0.399	0.332		*0.803*						*0.931*
Hg		*−0.866*		0.323			−0.302	*0.853*	0.307	
F2	0.497		*0.722*				0.449	*0.711*		
F4		−0.460	−0.348	0.343					*0.681*	
CEC	*0.760*	0.416	0.417				*0.902*			

Significant factor loadings are set in italics

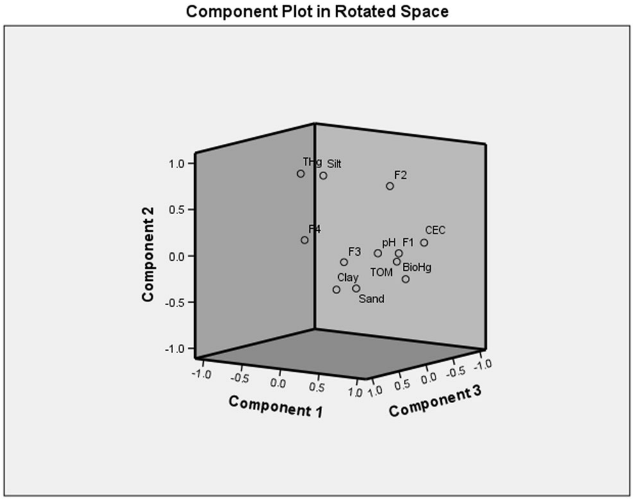

Fig. 5 Plot of loading of three principal components in PCA for Hg

1986). PC3 explained 18% of the total variance and had strong loadings on T-Sn, F4 (resistant), and Bio-Sn; it is seen that F4 (resistant) and T-Sn factors can provide significant influencing on Bio-Sn in natural form. In addition, PC4 explained 15.6% of the total variance, which had strong loadings on pH and clay.

It seems that F1 (EFLE) in surface sediment had the largest and positive influence on Bio-Hg, while F4 (resistant) had the largest and positive influence on the Bio-Sn. The bioaccessibility of Hg and Cd was generally more affected by non-resistant fractions (F1-F3) than resistant fraction (F4). In contrast, the bioaccessibility of Sn was generally more affected by resistant fraction (F4) than non-resistant fractions (F1-F3).

Bio-Hg was more affected by TOM, F1 (EFLE), and CEC of sediment. In addition, Bio-Cd was affected more by F1 (EFLE) of sediment and F4 (resistant) and T-Sn factors can provide significant influence on Bio-Sn, which represents

Table 6 Total variance of Cd values explained and matrix of principal component analysis for 27 variables on Bernam River surface sediment

Component	Initial eigenvalues			Extraction sum of square loadings			Rotation sum of square loadings			
	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	
Total variance explained										
1	4.163	34.693	34.693	4.163	34.693	34.693	2.815	23.458	23.458	
2	2.169	18.078	52.772	2.169	18.078	52.772	2.397	19.977	43.435	
3	1.637	13.639	66.410	1.637	13.639	66.410	2.336	19.466	62.901	
4	1.272	10.601	77.011	1.272	10.601	77.011	1.503	12.522	77.011	
5	1.122	9.352	86.363							
6	0.780	6.501	92.864							
7	0.516	4.296	97.160							
8	0.171	1.424	98.585							
9	0.117	0.973	99.557							
10	0.034	0.287	99.844							
11	0.019	0.156	100.000							
12	9.042E−7	7.53E−6	100.000							
Variables	Component matrix						Rotated component matrix			
	PC1	PC2	PC3	PC4			PC1	PC2	PC3	PC4
pH	−0.355									
Bio-Cd		−0.434	0.354	*0.689*						*0.858*
Clay	*0.679*		*−0.643*					*0.875*		
F1	0.437			*0.651*						*0.800*
Silt	0.345	*−0.842*							*0.963*	
Sand	*−0.657*	*0.557*	0.434					−0.334	*−0.894*	
TOM	*0.935*	0.312					*0.737*	*0.617*		
F3			*0.630*	−0.379			*0.648*	−0.374		
T-Cd	*0.714*						*0.749*			
F2	0.461	*0.699*	−0.340					*0.859*		
F4	*0.825*	−0.349					*0.663*		*0.599*	
CEC	*0.715*	0.342	0.366				*0.853*			

Significant factor loadings are set in italics

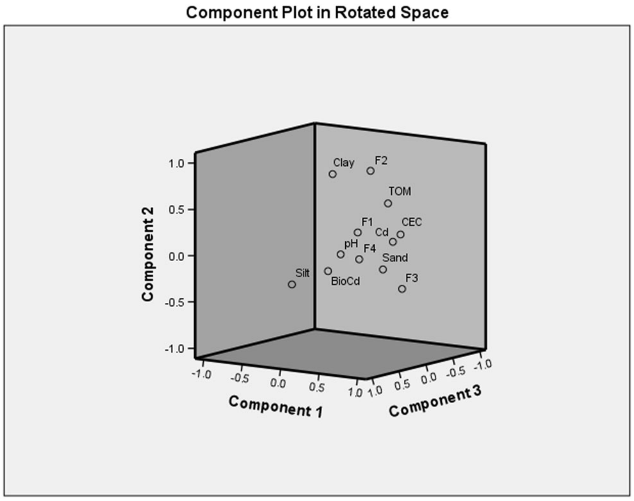

Fig. 6 Plot of loading of three principal components in PCA for Cd

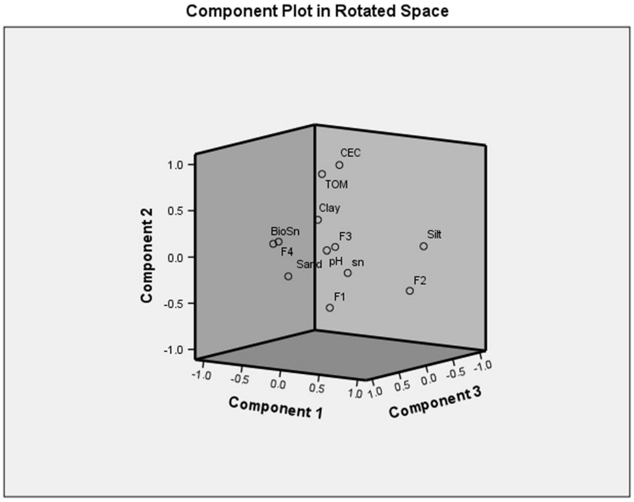

Fig. 7 Plot of loading of three principal components in PCA for Sn

Table 7 Total variance of Sn values explained and matrix of principal component analysis for 27 variables on Bernam River surface sediment

Component	Initial eigenvalues			Extraction sum of square loadings			Rotation sum of square loadings			
	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	Total	% of variance	Cumulative (%)	
Total variance explained										
1	3.622	30.184	30.184	3.622	30.184	30.184	2.873	23.943	23.943	
2	3.054	25.451	55.635	3.054	25.451	55.635	2.596	21.637	45.579	
3	1.998	16.647	72.282	1.998	16.647	72.282	2.168	18.063	63.643	
4	1.260	10.500	82.781	1.260	10.500	82.781	1.872	15.604	82.781	
5	1.035	8.627	91.409							
6	.565	4.706	96.115							
7	.312	2.599	98.714							
8	.124	1.031	99.746							
9	.019	.159	99.905							
10	.011	.092	99.997							
11	.000	.003	100.000							
12	6.005E−7	5.00E−6	100.000							
Variables	Component matrix						Rotated component matrix			
	PC1	PC2	PC3	PC4			PC1	PC2	PC3	PC4
pH	−0.358	0.335	0.483	−0.321						*0.904*
Bio-Sn		*0.788*	*0.561*				−0.415		*0.656*	0.418
Clay	*0.751*			0.390				0.427	0.481	*−0.624*
F1	*−0.765*							*−0.629*		0.437
Silt	0.478	*−0.782*					*0.957*			
Sand	*−0.802*	*0.502*					*−0.770*	−0.353		0.412
TOM	*0.833*	0.443						*0.893*	0.320	
F3			*−0.756*	0.333						
Sn	0.495		0.410	*0.518*			*0.591*		*0.710*	
F2		−0.793	*0.524*				*0.856*	−0.336		
F4	0.489	*0.630*	0.441	0.355					*0.896*	
CEC	0.497	0.401		*−0.551*				*0.942*		

Significant factor loadings are set in italics

metals largely embedded in the crystal lattice in the minerals in sediment (Ikem and Nyavor 2003). Thus, bioavailability is related to be mobilized (Luoma and Rainbow 2008; Luo et al. 2012a), suggesting that bioaccessibility of Hg and Cd in the sediment can contribute to the anthropogenic input in the sediment of Bernam River.

Conclusion

This study made use of the sequential extraction technique (SET) and simple bioaccessibility extraction test (SBET) to quantify the concentration of bioavailable metals and the human bioaccessible fractions collected from nine stations in the surface sediment of Bernam River. The Cd and Hg in the Bernam River surface sediments were greater in the non-resistant fractions (anthropogenic) than in the resistant fractions (natural origin). The relative bioaccessibility of Hg and Cd concentrations were greater than the resistant fraction concentrations, indicating that Cd and Hg were highly accessible in surface sediment of Bernam River. The principal component analysis showed that the main factors influencing the bioaccessibility of mercury in surface sediments were the sediment total organic matter, cation exchange capacity, and easily, freely, or leachable and exchangeable fraction, and the factors influencing the bioaccessibility of tin were the total tin and cation exchange capacity, while the bioaccessibility of Cd in surface sediments was influenced by the only factor which is the easily, freely, or leachable and exchangeable fraction. The results can be useful to build models in the future through incorporating these factors to predict the bioaccessibility of Cd and Hg in the surface sediment based on physicochemical properties and fractions. Therefore, these results could improve ecological risk assessment and pollution control.

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