

Estimation and influence of physicochemical properties and chemical fractions of surface sediment on the bioaccessibility of Cd and Hg contaminant in Langat River, Malaysia

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Abstract This study applied the use of sequential extraction technique and simple bioaccessibility extraction test to quantify the bioavailable fractions and the human bioaccessible concentration of metals collected from nine stations in surface sediment of the Langat River. The concentrations of total and bioaccessible metals from different stations were in the range of 0.49–1.04, 0.10–0.32 $\mu\text{g g}^{-1}$ for T-Cd, Bio-Cd, respectively, and 12.9–128.03, 2.06–8.53 $\mu\text{g kg}^{-1}$ for T-Hg, Bio-Hg, respectively. The results revealed highest R-Bio-Cd in Banting station (55.3 %), while the highest R-Bio-Hg was in Kajang station (49.61 %). The chemical speciation of Cd in most sampling stations was in the order of oxidisable-organic > residual > exchangeable > acid-reducible, while speciation of Hg was in the order of exchangeable > residual

> oxidisable-organic > acid-reducible. The correlation matrix of mean content showed that the TOM, particle size and Mg^{++} in polluted surface sediments was highly correlated with total mercury. The PCA showed that the main factors influencing the bioaccessibility of Hg in surface sediments were the sediment TOM, F1 (EFLE) and F3 (oxidation-organic), while the factor influencing the bioaccessibility of Cd was the F3 (oxidation-organic) and T-Cd.

Keywords Bioaccessibility · Sequential extraction · SBET · Langat River

Introduction

Rapid economic development and urbanization bring prosperity worldwide including Malaysia. Langat River is one of the most important rivers in Malaysia that flows along major industrial and economic center in Selangor state and provides water for industries and agricultural sectors located along its banks apart from being among the most important location for fishery resources in Malaysia. However, rapid urbanization within Langat River basin has made the river vulnerable to different pollution sources such as industrial, domestic sewage and agricultural activities. These are all likely to cause heavy metal pollution in the Langat River and increase health risk problems for human. Generally, the heavy metal pollution on the aquatic ecosystems is increasing due to the effects of

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urbanization and industrialization and thus become supremely important pertaining to human health and welfare (Tzoulas et al. 2007; Hoanninen et al. 2014; Martin et al. 2015). Anthropogenic activities related to industry are usually more concentrated along urban rivers, and when heavy metals in sediment were released into water, long-term harmful effects on aquatic environment via resource consumption may cause many problems including health impacts (Sheng et al. 2008; Luo et al. 2012b; Gu et al. 2014). Heavy metals are serious pollutants of aquatic ecosystems, due to their nonbiodegradable, toxic characteristics and ability to be incorporated into food chain. Heavy metals tend to associate with organic matter present in the thin fraction of the sediments depending on the textural characteristic or adsorbed on Fe/Mn hydrous oxides which made aquatic sediments the main sink for toxic metals in the environment where these parameters may affect the amount of bounded metals (Calmano et al. 1993; Saeedi et al. 2013). However, using only the total metal content in potential risk assessments is a poor indicator of the actual hazard due to toxicity and bioaccumulation of metals that are related to their mobility and chemical speciation (Gu et al. 2016; Madrid et al. 2008; Camusso and Gasparella 2006). Heavy metals in sediments exist in a number of chemical forms and generally exhibit different physical and chemical behaviors in terms of chemical interaction, identification of the main binding sites, mobility, biological availability and potential toxicity (Sundaray et al. 2011). The simple bioaccessibility extraction test (SBET) application can mimic metal absorption in the stomach phase that may render health risks. SBET pays more attention to the risk assessment and obtaining the maximum amounts of metals bioavailability (Kim et al. 2002; Li et al. 2013; Wan et al. 2012). Thus, in this study, surface sediment was subjected to the sequential extraction technique (exchangeable, acid-reducible, oxidisable-organic and residual), with SBET to assess bioavailability in risk assessment studies. On top of that, investigation on sediment properties and parameter were done in assessing the mobility and bioavailability of metals in the sediment region, which in turn has implications on human health risk assessment. Hence, the occurrence and relative distribution of an element among these various phases and the sediment parameters in relation to the element phases in the sediment would be used to perform remediation and pollution control

measures. Researchers have extensively studied factors impacting the sequential extraction metals, physiologically based extraction tests (PBET) and SBET in the soil and sediment for example Jinghua et al. (2015) in the Lake Taihu (China), Devesa-Rey et al. (2008, 2010) in Anllóns River (Spain), Xiaodong et al. (2015) and Xiaodong et al. (2016) in different soil sites (China), where each study has its own theory foundation and various factors influencing the bioaccessibility depending on different anthropogenic sites. However, to date and to the best of our knowledge, dominant factors influencing the bioavailability and bioaccessibility for heavy metals analysis in surface sediment of Langat 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 metals

Materials and methods

Study area

Langat River basin covers an area of 1300 km² in the Selangor with the length of Langat River is approximately about 120 km long (Sarmani 1989). The basin is also one of the most important agricultural lands consisting of oil palm, coconut and rubber plantation. The Langat River is an important water supply for approximately 50 % of Selangor population (Lim et al. 2013). Furthermore, Langat River basin covers most of the industrial areas in Selangor state that takes place along the banks of Langat River. However, according to DOE (2013), the slightly polluted Langat River is categorized as Class III of Malaysia National Water Quality Standards from 1997 to 2013 where it has been receiving effluent from different anthropogenic activities such as industrial discharge (9.09 %), sewage discharges (10.80 %), mining activities (0.24 %) and food service establishment (79 %). The waste water discharge from these activities in Langat River was reduced to 9.09 % in 2013 compared to 84.09 % in last decade (Ahmed et al. 2016). However, both food service establishment and chemical pollution are still the highest polluter for the river. The environment conditions of the study area are

characterized by uniform high humidity, high temperature and heavy rainfall with two major monsoon seasons, i.e., northeast and southwest monsoon. The average temperature of the study area varies from 23 to 33 °C all-round the year, whereas the relative humidity ranges from 62 to 96 % and averaged around 82 %. The mean annual rainfall of the study area is about 2400 mm. Heavier rainfall occurs in the month of November, which has a monthly mean rainfall of 270 mm. Besides that the area also occasionally experiences rainstorms generated by local convection and these rainstorms normally occur in late afternoons throughout the year which is generally of short duration with high intensity.

Sample collection and preservation

A total of 27 surface sediment (0–5 cm) samples were collected at nine sites from downstream to upstream of Langat Rivers from January to February 2015 (Fig. 1). The locations and description of sampling stations are presented in (Table 1). The Ekman grab was washed with distilled water after each sample collection on each station to avoid possible metal contamination from the grab. Samples were placed in clean labeled plastic bags and transferred to the laboratory in the icebox. Sediment samples were kept at –20 °C in the storage prior to analysis.

Sequential extraction

Chemical speciation of heavy metal in surface sediments was analyzed by using modified sequential extraction technique as 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 1.0 M ammonium acetate (NH₄CH₃COO), pH 7.0 at room temperature.
- Step 2: Acid-reducible. The residue from EFLE was continuously shaken for 3 h with 50 ml 0.25 M hydroxyl-ammonium chloride (NH₂OH·HCl) and acidified to pH 2 with HCl, at room temperature.
- Step 3: Oxidisable-organic. The residue from Acid-reducible was first oxidized with 15 ml H₂O₂ (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 (NH₄CH₃COO) 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 HNO₃ and HClO₄. The samples were analyzed in triplicate, and the analysis was performed using ICP-MS (PerkinElmer Model Elan DRC-e).

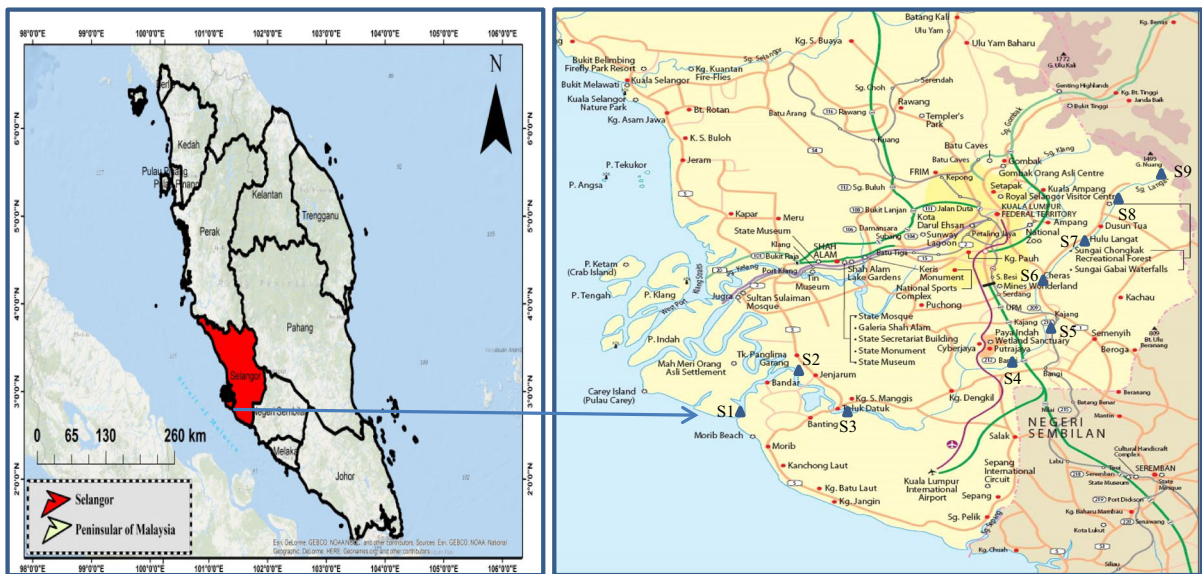


Fig. 1 Map of Langat River catchment and sampling stations. Source: (MOTAC 2007)

Table 1 Names and coordinates of the sampling stations for the surface sediment and water at Langat River

No.	Sampling site	Coordinates	Description
1	Jugra	N 2°49'27.94" E 101°24'52.39"	Agriculture area, boat harbor, oil palm
2	Jenjarom	N 2°52'57.81" E 101°29'20.06"	Fisheries, residential area, oil palm agriculture
3	Banting	N 2°48'56.87" E 101°30'46.66"	Residential area, factory
4	UKM	N 2°55'52.99" E 101°46'31.22"	Residential area, bridge
5	Jalan sungai Kajang	N 2°59'35.75" E 101°47'07.58"	Waste dumping, fisheries, industrial/human settlements nearby
6	Cheras Bandar	N 3°03'06.55" E 101°46'46.76"	Domestic waste discharge, industrial or human settlements nearby, fisheries
7	Jalan Hulu Langat	N 3°06'32.55" E 101°48'41.48"	Waste dumping, sand mining, fisheries
8	Batu, Hulu Langat	N 3°08'46.2" E 101°50'22.3"	Agricultural area
9	Pangsun	N 3°12'45.63" E 101°52'42.17"	Dam river, water recreational area

Simplified bioaccessibility extraction test (SBET)

SBET is a one-step *in vitro* digestion test without pepsin (Oomen et al. 2002). The procedure for this method was used to assess half gram (63 μm) air-dried sediment that was 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, centrifuged at 3500 g for 15 min, and then the extracted suspension was filtered through 0.45 μm cellulose acetate disk 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. The relative heavy metals bioaccessibility was calculated by dividing the SBET of heavy metals concentrations with the total heavy metals, as follows:

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

Total heavy metals concentrations

The dried sediment samples (0.5 g) were digested in 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 preheated 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 metals concentration using an air-acetylene flame Atomic Absorption Spectrophotometer (PerkinElmer Model A Analyst 800). The determination of total mercury in sediment samples is carried out by using ultrasound-assisted mercury extraction as proposed by Collasiol et al. (2004) and Looi et al. (2014) and analyzed using FIMS-100 mercury analyzer.

Quality assurance and quality control

To avoid any contaminations, all laboratory equipment used were washed with phosphate-free soap, double rinsed with distilled water and left in 10 % HNO_3 for 24 h and were then rinsed two times with double-distilled water and left semi-closed to dry at room temperature. Certified Reference Material (CRM) was determined during precision check using sediment certified reference materials (PACS-2, Canada). The results of three replicates and percentage of recoveries for the certified and measured concentration of Cd and Hg were 104 % and 82 % recovery percentages, respectively. Therefore, the analytical result for the reference materials and its certified values for each metal were considered satisfactory. Calibration curves were prepared by making two appropriate dilutions in stock water solution (1000 mg l^{-1}) (BDH Spectrosol[®]) stock solution in 2 % HNO_3 . In addition, five standard solutions were prepared from a different stock standard source with detection limits of 0.1 $\mu\text{g/l}$. A blank calibration solution was used for a

zero point. The linearity for the calibration lines of multi-elemental standards shows that the correlation coefficient values were at least 0.999 during analysis and is considered as satisfactory.

Physicochemical properties of sediment analysis

Measurement of pH was taken 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 sediment in a glass beaker, covered with plastic film and 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 (L.O.I) 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 based on 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) which gives measures of the exchangeable cations displaced from sediment was determined by treating sediment with NaCl and were used to determine Ca²⁺, Mg²⁺, K⁺, while NH₄Cl was used to determine the Na⁺ (Apello and Postma 2005; Aris et al. 2010). The samples were analysed for Na⁺, Ca²⁺, Mg²⁺ and K⁺ using ICP-MS (PerkinElmer Model Elan DRC-e).

Results and discussion

Sediment properties

The soil properties, including pH, particle size and total organic matter content are presented in Table 2. The pH and total organic matter values in the Langat River have been reported by Safaa et al. (2015). The highest pH value was at 6.77 (Kajang) but the lowest pH value was at 4.38 (Hulu Langat). Sediment pH was slightly acidic in Langat River, this is due to sand mining activities in upstream and downstream of river and also the use of fertilizers for crops (oil palm) within the area that could contribute to the decrease in pH in the sediment as fertilizer may contain monosodium and disodium methylarsonate (Sarmani 1989).

The mean TOM values were highest in station Jugra (34.6 %) and lowest in station Pangsun (8.7 %). 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 on particle size is important in order to assess the anthropogenic influence on sediment properties. The mean percentage value of clay, silt and sand fraction in surface sediment of Langat River were found to range from 0.85 to 22.89, 1.09–61.29, and 19.30–98.02 %, respectively. The sum of exchangeable cations (CEC) in the sediment ranged from 130.36 to 14.91 meq/100 g in Langat River and the descending order of exchangeable cations was in the order of Na < Mg < Ca < K. High CEC in Langat River is probably related to the high content of available clay minerals (Spisto 1989; El-Radaideh et al. 2014). Consequently, the increase in CEC could also increase the TOM in the sediment of Langat River similar to other studies as reported by Camberato (2001) and Shafie et al. (2013).

The relationships among the examined physicochemical properties and heavy metals were tested using Spearman's correlation analysis (Table 3). The correlation matrix revealed that Hg in all sampled stations of Langat River showed strong positive relationship with TOM, clay, silt, sand and Mg⁺⁺. This indicates that mercury is associated with TOM, clay, silt, sand and Mg⁺⁺ and suggests that TOM content, clay, silt, sand and Mg⁺⁺ may contribute to the increasing metal concentrations in the river although total Hg had no significant relationship with Cd. CEC and silt had a weakly correlation with TOM due to the high level of silt and CEC in surface sediment of river that could increase the TOM content (Camberato 2001; Shafie et al. 2013). Furthermore, the high CEC observed in Langat River is probably related to the high content of available silt minerals similar to other studies reported by Spisto (1989) and El-Radaideh et al. (2014). Total cadmium in Langat River had no significant relationship with physicochemical contents and T-Hg. This indicates that T-Cd could come from different sources such as industrial effluent, agriculture activities around the rivers and domestic sewage discharge.

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

Sediment properties	Jugra	Jenjarom	Banting	UKM	Kajang	Cheras	Hulu Langat	Batu, Hulu Langat	Pangsun
pH	5.27	5.54	5.33	5.48	6.77	4.79	4.38	6.09	6.60
TOM (%)	34.9	28.2	22.17	10.45	16.21	13.98	9.42	10.92	8.7
Clay (%)	4.86	22.89	20.16	19.38	2.56	9.46	21.48	0.85	5.27
Silt (%)	7.67	19.20	23.62	61.29	4.21	14.16	41.69	1.09	7.59
Sand (%)	87.43	57.87	56.22	19.30	93.23	76.35	36.82	98.02	87.11
K ⁺ (meq/100 g)	8.44	5.14	7.23	7.30	4.23	8.72	4.59	2.56	4.68
Mg ⁺² (meq/100 g)	29.95	33.43	34.35	26.97	5.63	13.15	7.36	6.96	5.30
Ca ⁺² (meq/100 g)	18.77	24.17	19.36	14.07	12.83	33.42	24.95	10.75	2.22
Na ⁺ (meq/100 g)	17.74	58.35	69.40	18.55	19.92	19.20	71.46	23.36	2.69
CEC (meq/100 g)	65.08	121.11	130.36	66.91	42.63	74.50	108.38	43.65	14.91

Total and bioaccessibility of heavy metals

The average concentrations of the total and bioaccessible Cd and Hg from nine sampling sites are presented in (Table 4). Total Cd value has been reported in Langat River (Safaa et al. 2015). T-Cd concentrations in the surface sediments from nine stations were varied from 0.49 to 1.04 µg g⁻¹, and T-Hg concentration were in the range of 12.9–128.05 µg kg⁻¹. Bio-Cd and Bio-Hg concentrations in sediment varied considerably among different sampling sites, with the range of Bio-Cd and Bio-Hg in all nine sampling stations were 0.10–0.32 µg g⁻¹, 8.53–2.06 µg kg⁻¹, respectively. The highest T-Hg, T-Cd and Bio-Hg concentrations were recorded in the sediment

collected from Jugra and Jenjarom stations, while the highest Bio-Cd value was recorded in Batu Hulu Langat station. There was an increasing trend of Cd and Hg concentrations from upstream (Pangsun) to the downstream (Jugra) and the highest Cd concentrations in these parts of the sediment profiles may indicate increasing discharges of these metals from upstream to downstream due to anthropogenic sources surrounding the river such as small and medium industries, agricultural development and oil palm plantation (Chen et al. 2007). Furthermore, steel factories located in the upstream area which are among one of largest of that type of industries in Malaysia could increase source of Cd concentration in the Batu Hulu Langat (Forstner and Wittmann 1981). Stainless steel,

Table 3 Pearson correlation of heavy metals concentrations with physicochemical parameters from Langat River (n = 27)

	Hg	pH	TOM	Clay	Silt	Sand	Na	Ca	Mg	K	CEC	Cd
Hg	1											
pH	-0.165	1										
TOM	0.888**	-0.172	1									
Clay	-0.587**	0.172	-0.439*	1								
Silt	-0.714**	0.165	-0.655**	0.870**	1							
Sand	0.702**	-0.169	0.637**	-0.897**	-0.998**	1						
Na	-0.004	-0.600**	0.187	-0.212	-0.316	0.319	1					
Ca	0.178	-0.708**	0.293	-0.190	-0.076	0.098	0.491**	1				
Mg	0.563**	-0.366	0.729**	-0.150	-0.488**	0.454*	0.377	0.308	1			
K	0.304	-0.442*	0.438*	0.096	-0.003	-0.015	-0.051	0.505**	0.577**	1		
CEC	0.241	-0.709**	0.457*	-0.229	-0.387*	0.382*	0.898**	0.690**	0.683**	0.331	1	
Cd	0.364	-0.091	0.326	-0.273	-0.296	0.305	0.294	0.208	0.091	-0.358	0.253	1

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

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

Table 4 Total of Cd ($\mu\text{g/g}$), Hg ($\mu\text{g/kg}$), bioaccessible ($\mu\text{g/g}$), relative bioaccessible (%) and sequential chemical concentration ($\mu\text{g/g}$) (mean \pm SD)

Stations	Total HMs	Bioaccessible	R-Bio	Fraction 1	Fraction 2	Fraction 3	Fraction 4
Jugra							
Cd	0.57 \pm 0.06	0.22 \pm 0.001	38.83	0.023 \pm 0.001 (3.22)	0.087 \pm 0.008 (5.36)	0.47 \pm 0.005 (66.13)	0.133 \pm 0.1 (18.48)
Hg	128.03 \pm 0.1	8.53 \pm 0.06	6.66	38.66 \pm 2.6 (39.28)	2.53 \pm 0.29 (5.51)	11.23 \pm 0.12 (24.47)	14.1 \pm 0.06 (30.71)
Jenjarom							
Cd	1.04 \pm 0.48	0.18 \pm 0.002	17.19	0.05 \pm 0.003 (6.23)	0.10 \pm 0.006 (6.29)	0.51 \pm 0.003 (63.11)	0.149 \pm 0.01 (18.27)
Hg	55 \pm 0.066	8.06 \pm 0.06	14.66	69.33 \pm 1.2 (81.21)	1.16 \pm 0.18 (1.36)	6.73 \pm 0.06 (7.88)	8.13 \pm 0.03 (9.52)
Banting							
Cd	0.4 \pm 0.11	0.22 \pm 0.006	55.33	0.022 \pm 0.005 (4.24)	0.39 \pm 0.001 (4.04)	0.05 \pm 0.009 (10.44)	0.06 \pm 0.04 (11.30)
Hg	56.43 \pm 0.033	5.93 \pm 0.06	10.51	18.0 \pm 2.5 (58.12)	2.96 \pm 0.12 (9.58)	4.36 \pm 0.18 (14.10)	5.63 \pm 0.03 (18.19)
UKM							
Cd	0.43 \pm 0.01	0.16 \pm 0.04	39.22	0.054 \pm 0.01 (15.87)	0.04 \pm 0.01 (15.36)	0.10 \pm 0.08 (29.09)	0.16 \pm 0.02 (43.63)
Hg	74.73 \pm 0.033	5.60 \pm 0.1	7.49	32.33 \pm 0.33 (85.91)	0.8 \pm 0.17 (2.12)	3.43 \pm 0.03 (9.12)	1.06 \pm 0.03 (2.83)
Kajang							
Cd	0.47 \pm 0.19	0.24 \pm 0.006	50.62	0.040 \pm 0.02 (6.52)	0.09 \pm 0.004 (2.94)	0.42 \pm 0.06 (68.41)	0.061 \pm 0.04 (9.93)
Hg	12.9 \pm 0.03	6.40 \pm 0.1	49.61	40.33 \pm 0.3 (83.12)	0.29 \pm 0.01 (0.59)	3.53 \pm 0.03 (7.28)	4.36 \pm 0.03 (8.99)
Cheras							
Cd	0.39 \pm 0.09	0.18 \pm 0.01	47.86	0.040 \pm 0.004 (9.80)	0.037 \pm 0.003 (9.88)	0.20 \pm 0.01 (49.17)	0.13 \pm 0.09 (32.01)
Hg	103.6 \pm 0.03	6.63 \pm 0.06	6.11	44.0 \pm 2.5 (73.86)	7.16 \pm 0.92 (12.03)	2.03 \pm 0.33 (3.41)	6.36 \pm 0.03 (10.68)
Hulu Langat							
Cd	0.49 \pm 0.14	0.25 \pm 0.001	51.02	0.031 \pm 0.003 (3.39)	0.038 \pm 0.02 (3.28)	0.58 \pm 0.4 (61.97)	0.28 \pm 0.03 (30.51)
Hg	43.96 \pm 0.06	5.46 \pm 0.1	12.43	46.30 \pm 0.3 (73.30)	3.13 \pm 2.43 (4.96)	1.7 \pm 0.05 (2.68)	12.03 \pm 0.06 (19.03)
Batu, Hulu Langat							
Cd	0.63 \pm 0.02	0.320 \pm 0.001	50.26	0.036 \pm 0.003 (3.52)	0.099 \pm 0.02 (3.49)	0.74 \pm 0.01 (72.82)	0.143 \pm 0.03 (13.94)
Hg	76.7 \pm 0.03	2.06 \pm 0.06	2.69	62.33 \pm 0.33 (84.03)	0.14 \pm 0.01 (0.19)	1.43 \pm 0.03 (1.93)	10.26 \pm 0.03 (13.84)
Pangsun							
Cd	0.35 \pm 0.05	0.10 \pm 0.001	29.71	0.038 \pm 0.02 (11.20)	0.10 \pm 0.003 (11.06)	0.074 \pm 0.002 (21.39)	0.131 \pm 0.06 (37.71)
Hg	44.43 \pm 1.3	4.93 \pm 0.1	11.10	81.34 \pm 0.88 (91.22)	3.05 \pm 2.48 (3.42)	1.66 \pm 0.03 (1.86)	4.4 \pm 0.05 (4.86)

chemical and paint-based industries such as what have been observing at Cheras area is likely to increase the Hg concentration in Cheras site (Ahmed and Chamhuri 2001). Pangsun had the lowest T-Cd as well as Bio-Cd, while Hg showed lowest concentrations on both the total and bioaccessible fractions at Kajang station.

For the relative bioaccessibility of HMs (R-Bio-HMs), defined as the percentage of bioaccessible of metals to the total metals, the highest R-Bio-Cd among these sampling stations appeared at Banting (55.3 %) and the highest R-Bio-Hg was in Kajang (49.61 %). R-Bio-Cd showed higher value than R-Bio-Hg, where R-Bio-Cd value ranges from 17.9 to 55.3 %, while R-Bio-Hg value ranges from 2.69 to 49.61 % (Table 3). There were no reported on the bioaccessibility of Cd and Hg in sediment or soil from Malaysia in order for us to compare with other study. Thus, we compared our results with different universal studies. The relative bioaccessibility of Cd from this study was less than the Cd bioaccessibility value of 75.96 % recorded from soil of urban parks in Guangzhou, China by Gu et al. (2016). This study reported higher R-Bio-Hg value 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 reported by Safruk et al. (2015)

Cadmium speciation

The mean Cd concentrations and percentages of four chemical speciation fractions for each sampling station are tabulated in (Table 3; Fig. 2). The EFLE fraction for Cd ranged from 0.022 to 0.054 $\mu\text{g g}^{-1}$

with a mean percentage of 7 %. The acid-reducible fraction ranged from 0.037 to 0.39 $\mu\text{g g}^{-1}$ with a mean percentage of 6.86 %. The oxidisable-organic fraction ranged from 0.74 to 0.05 $\mu\text{g g}^{-1}$ with a mean percentage of 49.17 %. The residue (resistant) fraction ranged from 0.28 to 0.061 $\mu\text{g g}^{-1}$ with a mean percentage of 24 %.

The Cd concentration in most sampling stations was dominated by the nonresistant steps (anthropogenic). The nonresistant fractions (the sum of fractions 1, 2 and 3) are likely to be potentially toxic for organisms because it is easily removed and used by organisms (fraction 1). Furthermore, fractions 2 and 3 under certain physico-chemical conditions, such as 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 found 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). The percentages of each chemical form of Cd as well as the R-Bio-Cd are presented in Fig. 1. The R-Bio-Cd were evidently less than the nonresistant fractions (F1 + F2 + F3), suggesting that nonresistant forms of Cd in sediment could be contributing little to the bioaccessibility of Cd in the sediment of Langat River with exception of Kajang, Banting, Hulu Langat and Batu Hulu Langat stations. However, most of R-Bio-Cd in all stations were notably higher than the resistant forms (F4), indicating that Cd in sediment could be easily mobilized and become easy accessible to aquatic biota and human consumption.

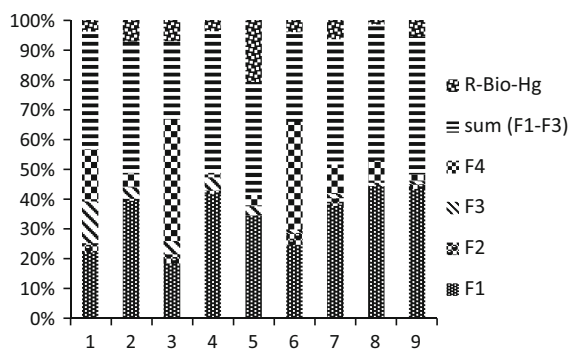


Fig. 2 Extraction percentage of Hg at sampling station

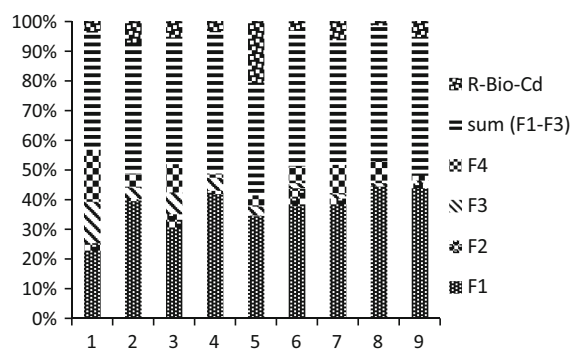


Fig. 3 Extraction percentage of Cd at sampling station

Table 5 Total variance of Hg values explained and matrix of principal component analysis (significant factor loadings are marked in bold) for 27 variables on Langat 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	4.765	39.710	39.710	4.765	39.710	39.710	3.208	26.737	26.737
2	2.719	22.655	62.365	2.719	22.655	62.365	3.203	26.689	53.425
3	1.662	13.853	76.218	1.662	13.853	76.218	2.233	18.611	72.037
4	1.246	10.380	86.599	1.246	10.380	86.599	1.747	14.562	86.599
5	0.558	4.648	91.247						
6	0.442	3.687	94.934						
7	0.322	2.685	97.619						
8	0.123	1.023	98.642						
9	0.099	0.827	99.469						
10	0.059	0.493	99.962						
11	0.004	0.036	99.999						
12	0.000	0.001	100.000						

Variables	Component matrix				Rotated component matrix			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
pH	-0.420	-0.590	0.425				-0.806	-0.319
Bio-Hg	0.773		0.341		0.820			
Clay	-0.669	0.480	0.474			-0.951		
F1	-0.333	-0.643	-0.460	0.315	-0.608	0.438	-0.522	
Silt	-0.808	0.499			-0.321	-0.916		
Sand	0.802	-0.500				0.935		
TOM	0.902		0.337		0.863	0.392		
F3	0.846		0.487		0.927	0.312		
Hg	0.427	0.389		0.675	0.539			0.717
F2		0.575	-0.477	0.490				0.859
F4		0.759	-0.343				0.622	0.555
CEC	0.613	0.361		-0.578			0.879	

Mercury speciation

The mean concentrations and percentages of sequential extraction of Hg for each sampling station are shown in (Table 3; Fig. 3). Hg in the EFLE fraction was higher than the others fractions in most of sampling stations, especially in Jenjarom, UKM, Kajang and Pangsun which were approximately 81, 85, 83 and 84 %, respectively, implying the high potential of toxicity for these stations. The EFLE step had high Hg bioavailability in the present study ranged from 18 to 81.34 µg/kg with a mean percentage of 66.92 % 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 0.14 to 7.16 µg/kg with a mean percentage of 3.20 %. The oxidisable-organic fraction ranged from 1.43 to 11.23 µg/kg with a mean percentage of 7.08 %, and the residue fraction ranged from 3.11 to 63.66 µg/kg with a mean percentage of 22.78 %. These low percentage of acid-reducible, oxidisable-organic and residue fractions from all stations are

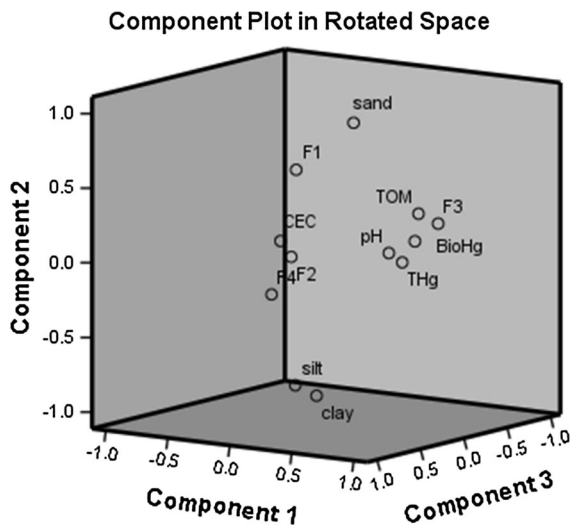


Fig. 4 Plot of loading of three principle components in PCA for Hg

suggesting poor bioavailability of Hg. The percentages of each chemical form of Hg as well as the R-Bio-Hg are presented in Fig. 1. The R-Bio-Cd were evidently less than the nonresistant fractions (F1 + F2 + F3) fractions and a little higher than the resistant fractions (F4), suggesting that nonresistant forms of Hg in all stations of sediment with exception Pangsun station, could be contributing little to the bioaccessibility of T-Hg in the sediment of Langat River.

Principal component analysis

A principal component analysis was run on nine stations where physicochemical properties, T-Hg, T-Cd, Bio-Hg, Bio-Cd and fractions were determined. The suitability of PCA was assessed prior to the analysis. The Bartlett's test of sphericity showed statistically significant ($p < 0.0005$), indicating that the data was likely factorisable. PCA for Hg revealed four components that had an eigenvalues greater than one and which explained 39.710, 22.655, 13.853, 10.380 % of the total variance, respectively. Visual inspection of the scree plot indicated that four components should be retained. The four component solution explained 86.599 % 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 26.73 % (PC1), 26.689 % (PC2), 18.61 % (PC3) and 14.56 % (PC4) of total variance, respectively (Table 5). 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 physicochemical properties of sand and pH, represented by PC1, explained 26.73 % of the total variance (Fig. 4) and had strong loading on TOM, F3 (oxidation-organic), F1 (EFLE), and Bio-Hg indicating that TOM, F3 (oxidation-organic), and F1 (EFLE) had a good relationship and strong influence on Bio-Hg which in turn is affected by anthropogenic activities such as industrial waste discharge and agricultural fertilizer run off where these can directly increase the risks on human health, whereas the PC2, explaining 26.68 % of the total variance, had strong loadings on clay, silt and sand. PC3 explained 18.61 % of the total variance and had strong loadings on pH, CEC, and F4 (resistant) while the PC4 explained 14.56 % of the total variance and had strong loadings on F2 (acid-reducible) and T-Hg.

The T-Cd, Fractions, physicochemical properties and Bio-Hg could mainly be divided into four categories where the PCA for Cd revealed four components that had an eigenvalues greater than one and which explained 34.064, 20.303, 14.631, 10.936 % of the total variance, respectively. Visual inspection of the scree plot indicated that three components should be retained. The four component solution explained 79.28 % 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 28.61 % (PC1), 18.23 % (PC2), 16.70 % (PC3) and 15.72 % (PC4) of total variance, respectively (Table 6). The PCA representation by loadings and score plot was used to explain the patterns of the data, respectively. In our study, the data set relating to clay, silt and sand represented by PC1, explained 28.61 % of the total variance (Fig. 5), whereas PC2 explained 18.23 % of the total variance had strong loadings on T-Cd and F3 (oxidation-organic) and Bio-Cd and it appears that T-Cd and F3 (oxidation-organic) in surface sediment, had the largest and positive influence on Bio-Cd. Meanwhile, the PC3, explained 16.70 % of the total variance and had strong loadings on F2 (acid-reducible) and F4 (resistant). The PC4 explained 15.72 % of the total variance, with

Table 6 Total variance of Cd values explained and matrix of principal component analysis (significant factor loadings are marked in bold) for 27 variables on Langat 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	4.088	34.064	34.064	4.088	34.064	34.064	3.434	28.616	28.616
2	2.436	20.303	54.367	2.436	20.303	54.367	2.188	18.236	46.853
3	1.756	14.631	68.998	1.756	14.631	68.998	2.005	16.707	63.560
4	1.312	10.936	79.934	1.312	10.936	79.934	1.887	15.728	79.288
5	1.061	8.845	88.779						
6	0.486	4.047	92.826						
7	0.316	2.635	95.461						
8	0.220	1.830	97.291						
9	0.154	1.285	98.576						
10	0.089	0.740	99.316						
11	0.082	0.683	99.998						
12									

Variables	Component matrix				Rotated component matrix			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
pH	-0.386	-0.676	0.397	0.388			0.463	-0.836
Bio-Cd		0.592	0.674			0.881		
Clay	-0.840			-0.368	-0.937			
F1				0.422				
Silt	-0.912				-0.959			
Sand	0.918				0.967			
TOM	0.704	-0.369		-0.380	0.537		0.554	0.457
F3	0.501	0.460	0.602			0.904		
T-Cd	0.520		0.516			0.721		
F2		-0.760	0.346		0.378		0.780	
F4		0.610	-0.460	0.440	0.314		-0.877	
CEC	0.578			-0.622				0.902

strong loadings on pH and CEC. The bioaccessibility of Hg and Cd was generally more affected by the nonresistant fractions (F1–F3) rather than the resistant fraction (F4). The Bio-Hg was affected more by TOM, F1 (EFLE) and F3 (oxidation-organic) of sediment, while the Bio-Cd was affected more by F3 (oxidation-organic) and T-Cd of sediment. The represented metals are largely embedded in the crystal lattice of the minerals in sediment (Ikem et al. 2003) where bioavailability is related to be mobilized (Luoma and Rainbow 2008; Luo et al. 2012a). This suggests that the bioaccessible Hg and Cd in the sediment could

strongly be contributed by the anthropogenic input in the sediment of Langat 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 Langat River. The Cd and Hg in the Langat River

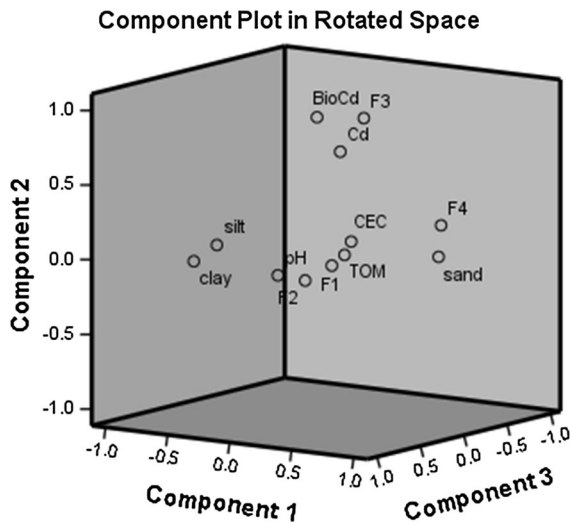


Fig. 5 Plot of loading of three principle components in PCA for Cd

surface sediments were greater in the nonresistant fractions (anthropogenic) than in the resistant fractions (natural origin). The results of R-Bio-Cd indicated greater concentrations than R-Bio-Hg in the surface sediment. The R-Bio-HMs concentrations were greater than the resistant fraction concentrations, indicating that Cd and Hg were highly accessible in surface sediment of Langkat River. The PCA revealed that the main factors influencing the bioaccessibility of Hg in surface sediments were the sediment TOM, F1 (EFLE) and F3 fraction (oxidation-organic), while the factor influencing the bioaccessibility of Cd was the F3 fraction (oxidation-organic) and T-Cd. Our 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 be improved ecological risk assessment and pollution control

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