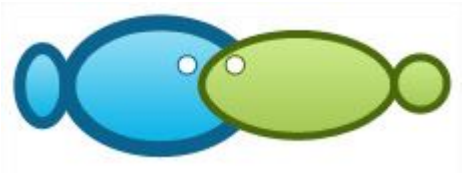

Sequential extraction analysis of heavy metals in relation to bioaccumulation in mangrove, *Rhizophora mucronata* from Kelantan Delta, Malaysia

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Abstract. The bioavailability of sediment As, Cr and Pb to the mangrove, *Rhizophora mucronata* in the Kelantan Delta, Malaysia, was studied. The average total concentration of the metals was 24.93 µg/g dry wt. for As, 52.77 µg/g dry wt. for Cr and 55.05 µg/g dry wt. for Pb. The majority of Cr (91.5%), Pb (88.8%) and As (78.7%) was found in the residual fraction. The root of *R. mucronata* accumulated the most metals compared to bark and leaves, with 9.37±3.33 µg/g dry wt. for Cr, 0.34±0.18 µg/g dry wt. for Pb and 0.34±0.18 µg/g dry wt. for As. Thus, *R. mucronata* restricted uptake of As, Cr and Pb (Bioaccumulation Factor, BAF<1). Interestingly, As and Pb was found to be efficiently translocated from root to leaf with a Translocation Factor, TF>1. The bioavailable metals in sediment from the Kelantan Delta comprise of less than 20% of the total metal concentration in sediment and were determined to be of low availability to the mangrove *R. mucronata*.
Key Words: bioavailability, sediment, arsenic, chromium, lead, BAF, TF.

Introduction. Globally, there has been growing concern over the increase of contamination of arsenic, chromium and lead in sediments (Liang et al 2015; Mahimairaja et al 2005). Contamination of sediments by these heavy metals may pose risks and hazards to humans and the ecosystem through the food chain interaction (sediment-plant-human). Since the last century, legislative authorities worldwide have established guideline values for heavy metals to deal with many environmental concerns (Fishwick 2009; Buchman 2008; CCME 1999; MOEJ 1994). Traditionally, heavy metal concentrations of the individual compound were compared with the guideline values to assess contamination. However, this may overestimate the potential risk as heavy metals may occur naturally in high concentrations in some areas of the world (Violante et al 2010; Burton 2002).

It is now widely recognized that the adverse impact of heavy metals does not simply depend on their concentrations but critically on their bioavailable fraction of the total metal concentration in sediment (Kim et al 2015; Tokalioglu et al 2000). Bioavailability is determined by conducting fractionation using sequential extraction procedure (Jain 2004; Gleyzes et al 2002; Tessier et al 1979). Sequential extraction procedure employed in this study partitioned the heavy metals into five fractions namely; (1) water-soluble, (2) exchangeable, (3) bound to Fe-Mn oxide, (4) bound to organic matter, and (5) residual.

The bioavailable amount in the sediment that can be incorporated into bioaccumulation in plants matrix constitutes by the first four fractions. Two comparative measures were chosen to assess the heavy metals uptake and distribution within the plant. Bioaccumulation Factor (BAF; ratio of heavy metal in root to sediment total metal concentration) was calculated to assess concentrations in root relative to sediment loadings. Translocation Factor (TF; ratio of heavy metal in leaves to root metal

concentration) was calculated to assess the transport of accumulated metal from root to shoot (Nouri et al 2009; MacFarlene et al 2007).

This study concentrates on the sediments from Kelantan Delta, located in the northeast of Malaysia. This area is an important natural capital for the livelihood of the local communities. Examples of economic activities in Kelantan Delta are fishing, production of fermented anchovy sauce, fish cracker and salted fish. In recent years, several studies concerning total metal concentration of As, Cr and Pb have been conducted in this area (Khairiah et al 2014). However, the total concentration of metals often does not accurately represent their risks. Bioavailability data can give accurate and reliable risk assessment of contaminants in relation to their adverse effects. Therefore, the aims of this study were to determine the bioavailability of As, Cr, and Pb in the sediment of Kelantan Delta and their accumulation in *Rhizophora mucronata* (roots, barks, and leaves). In addition, knowledge and understanding of bioavailability will encourage avoidance of overestimation or underestimation of risk by heavy metals in sediments to the ecosystem.

Material and Method

Study area. Kelantan Delta is located in the northeast of Malaysia and is bound to the South China Sea and Kelantan River. The Kelantan River is the main source of fresh water draining into the Kelantan Delta and with the length of about 248 km this river flows northward passing through major cities such as Kuala Krai, Tanah Merah, Pasir Mas, Tumpat and Kota Bharu. The sampling was conducted two times in July 2015 and November 2015 during dry and wet season respectively. Three stations were established in Kelantan Delta and marked using global positioning system (GPS). The study area experiences semi-diurnal tide and the sampling activity were conducted during low tide. Four environmental parameters namely temperature (29-32ºC), salinity (6-21 psu), redox potential (-223 mV to -238 mV) and pH (7.07-7.51) were measured using YSI multiparameter. There were no statistically significant differences between the two seasons except for salinity. The salinity changes from brackish water in the dry season (July) to fresh water in the wet season (November).

Sample collection and preparation. The sample collected consisted of sediment and *R. mucronata* sample. Three replicates of sediment samples were collected to a depth of 5 cm at each site using a plastic shovel at a distance of one meter from the tree trunk. At each site, *R. mucronata* samples were collected from three randomly selected trees. The circumference of the trees was measured to ensure that the trees to be sampled were of the similar size of about 30 cm circumference. The samples were segregated into barks, roots, and leaves (young, mature and senescent). Root that is considered to be nutritive roots for absorption was chosen rather than anchoring roots. The barks were collected two meters from the ground surface. The leaves were handpicked whereas roots and barks were collected using a ceramic knife.

To prepare the samples, the *R. mucronata* samples were washed thoroughly with deionized water to remove attached soil and other foreign particles. The *R. mucronata* and sediment samples were dried at 50ºC to constant weight. The samples were then ground to a fine powder using a porcelain mortar and pestle. Next, the samples were stored in plastic vials with labels and kept in desiccators.

Sample analysis

Sediment. Sediment metal concentrations were measured following total digestion as well as sequential extraction.

Total metal concentration. Total digestion of sediments followed the published method of Abdullah et al (2014) and Marchand et al (2006). Aliquots of 0.05 g sample were digested in 1.5 mL of mixed acid (HCl, HNO_3, HF) in closed Teflon vessel. After cooled down to room temperature, the digested sample was transferred into 15 mL centrifuge tube and diluted to 10 mL with deionized water. For quality assurance, standard reference material NIST 1646a Estuarine Sediment was digested following the same procedure.

Sequential extraction. The sediments sample was partitioned into five fractions following Tessier et al (1979) with some modifications (Figure 1).

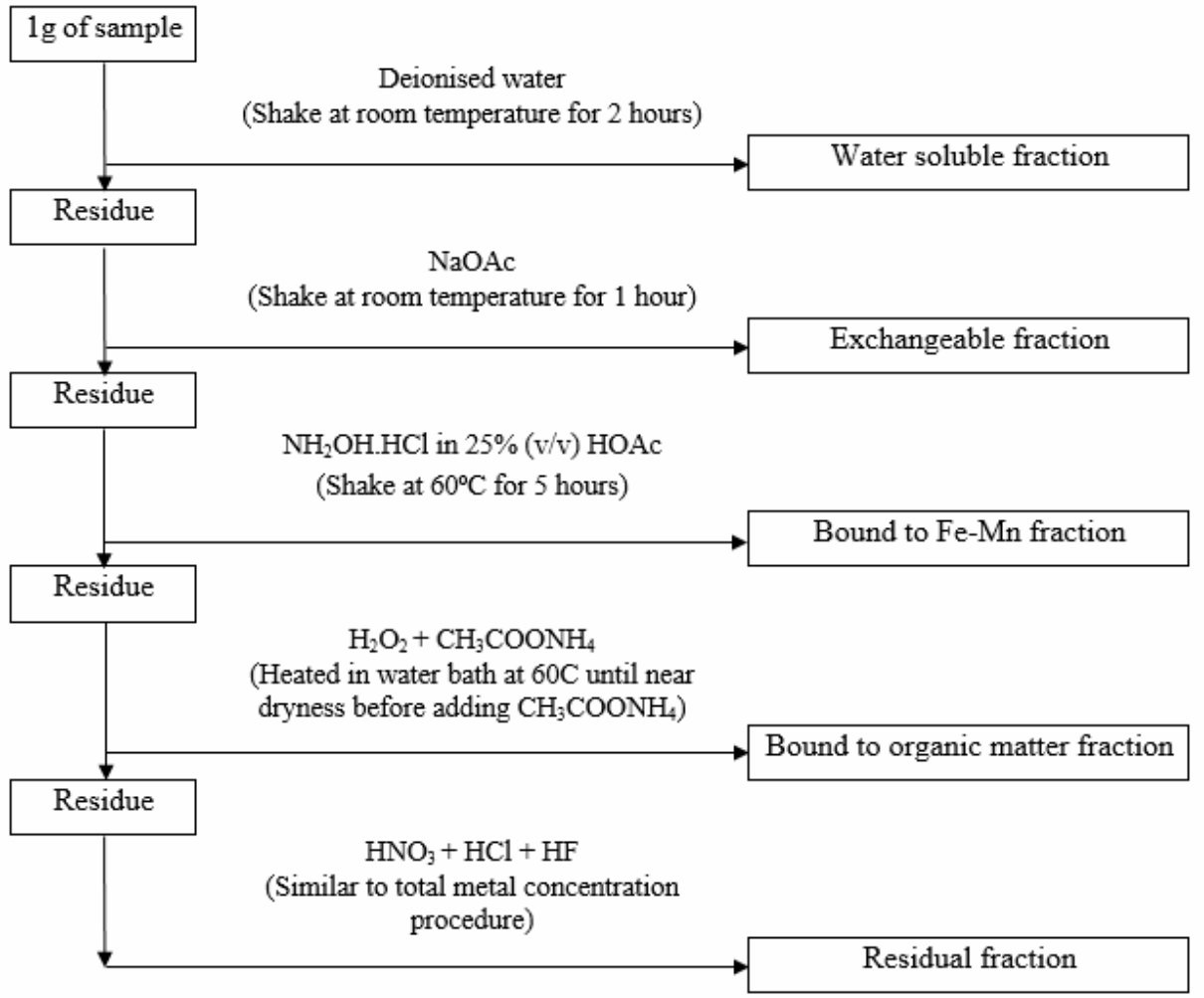

Figure 1. Flow diagram of sequential extraction procedure (modified after Tessier et al 1979).

The extractions were conducted in a centrifuge tube. At each step, the extracts were separated from the solid material by centrifugation at 3,800 rpm for 20 minutes. The supernatant liquid was transferred into another centrifuge tube and analyzed for heavy metals. Next, deionized water was added to the residue and centrifuged for 20 minutes. The second supernatant liquid was removed without any loss of the sediment residue.

R. mucronata. *R. mucronata* samples were digested in a closed Teflon vessel following the procedure of Chojnacka et al (2005) and Al-Merey (2004). Aliquots of 0.05g of the sample were dissolved in 1.5 mL of HNO_3 and oven digested at 120ºC for 8 hours. After digestion and cooled down to room temperature, the digested sample was transferred into 15 mL centrifuge tube and adjusted to 10 mL with deionized water. For analytical accuracy, standard reference material NIST 1547 Peach Leaves were analyzed with each batch of samples.

Concentrations of As, Cr and Pb in sediments and *R. mucronata* samples were determined by Inductively Coupled Plasma Mass Spectrometry Perkin Elmer ELAN 9000 (ICPMS).

Statistical analysis. The means and standard deviations were calculated for all heavy metals. One way ANOVA and Tukey post hoc test was employed for assessment of significant differences observed in the concentration of bioavailable metals in sediment and the accumulation pattern in *R. mucronata*. Statistical significance difference was set at $p<0.05$. The relationship between bioavailable metals in sediment and uptake by *R. mucronata* was evaluated by hierarchical cluster analysis using PRIMER 6.

As only a single sampling was carried out for dry and wet season, the data does not adequately represent the variable dry and wet episodes of the seasons. Thus, the data from the two sampling periods were combined for the statistical analysis.

Results and Discussion. For method validation, certified reference material NIST 1646a and NIST 1547 were determined as a precision check (Table 1). Recovery of each element was calculated as the ratio (%) between measured and certified values. The recovery percentage varied from 93% to 122%.

Table 1

Analysis of certified reference materials NIST 1646a Estuarine Sediment and NIST 1547 Peach Leaves (µg/g dry wt.; min–max)

Element	*NIST 1646a*			*NIST 1547*		
	Measured value	*Certified value*	*Recovery (%)*	*Measured value*	*Certified value*	*Recovery (%)*
As	6.48±0.30	6.23±0.21	104	0.073±0.001	0.060±0.018	122
Cr	39.46±0.58	40.9±1.9	96		Not certified	
Pb	10.86±0.29	11.7±1.2	93	0.97±0.05	0.87±0.03	111

Values are expressed as mean ± SD.

Sequential extraction procedure validation was performed by comparing the sum of the five fractions with the total metal concentrations. There was a good agreement between the sums of the fractions and the total metal contents, with the recovery of 97% for As, 117% for Cr and 81% for Pb.

Sediment total metal concentration. The distribution of As, Cr, and Pb in sediment is shown in Figure 2. Metal concentration in Kelantan Delta was in decreasing order of Pb>Cr>As. Pb concentration ranged from 31.30-75.84 µg/g dry wt. and averaged 55.05 µg/g dry wt. Cr concentrations ranged from 29.90-92.46 µg/g dry wt. with an average of 52.77 µg/g dry wt. while As ranged from 18.51-35.06 µg/g dry wt. and the average concentration was 24.93 µg/g dry wt.

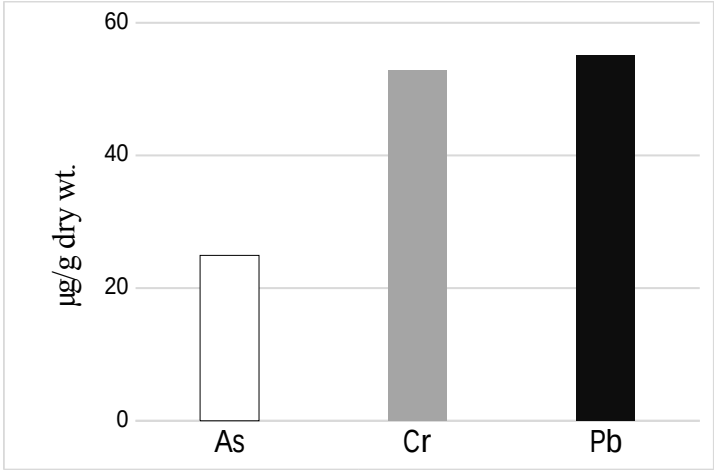

Figure 2. Total concentration of As, Cr, and Pb in sediment from Kelantan Delta.

As and Pb concentrations in sediment from Kelantan Delta were higher than mean Earth crust and east coast of Peninsular Malaysia (Table 2). The relatively high values of As and Pb is probably attributed to the result of a combination of multiple factors including industrial effluents to the river, inputs from weathering and the effects of local activities nearby such as fishing and seafood processing activities. The concentration of Cr was lower than the mean Earth crust values probably due to extreme weathering of rocks in tropical regions Pidwirny (2006) as they are exposed to abundant rainfall and hot temperatures weather.

Table 2

Comparison of metals abundance (µg/g dry wt.) in sediments of Kelantan Delta with the east coast of Peninsular Malaysia and mean Earth crust values

Element	*Kelantan Delta*	*East coast peninsular Malaysia*[1]	*Mean earth crust*[2]
As	18.28–34.95	-	1.8
Cr	29.90–92.46	59.8-74.0	100
Pb	31.30–75.84	13.4-19.3	15

[1] - According to Shazili et al (1998), [2] - Kabata-Pendias (2011).

A general assessment of the sediment quality was conducted by comparing the determined metal contents with the sediment quality guidelines (SQG) of the US EPA (Xiao et al 2013; Pradit et al 2013) (Table 3). The sediments of Kelantan Delta can be regarded as heavily polluted with As and Pb and moderately polluted with Cr.

Table 3

US EPA sediment quality guidelines (SQG) (µg/g dry wt.)

Element	*Non-polluted*	*Moderately polluted*	*Heavily polluted*
As	< 3	3–8	> 8
Cr	< 25	25–75	> 75
Pb	< 40	40-60	> 60

Sequential extractions. Most of the metals were bound to the residual fraction (Figure 3) with the bioavailable fractions (first four fractions) in the lower proposition.

Fraction 1: Water soluble. The water soluble fraction retained 0.17% As followed by 0.02% Pb and 0.02% Cr of the total metals respectively. This fraction consists of free ions and is considered to be the most available for plants uptake (Weis & Weis 2004).

Fraction 2: Exchangeable. This fraction consists of the exchangeable ions that can be passed easily into the water column when there are changes in water ionic strength and pH (Tokalioglu et al 2003). On average, Cr has the highest concentration of 0.93%, followed by As 0.14% and Pb 0.01%. of the total metal concentration. Metals in this fraction are easily available for biological uptake in the environment (Kim et al 2015; Okuku et al 2010).

Fraction 3: Bound to Fe-Mn oxide. Fe and Mn commonly occur as coatings on detrital particles and they are excellent scavengers for heavy metals (Jain 2004; Gleyzes et al 2002). This phase is readily available and may be released into the environment under unstable anoxic conditions. Pb was the most abundant metal in this fraction which comprised of 6.12%, followed by As (6.10%) and Cr (5.36%) of the total metal concentration.

Fraction 4: Bound to organic matter. 14.85% of As, 5.00% of Pb and 2.18% of Cr were found in binding to organic matter fraction. This fraction involves heavy metals that may be bound to various forms of organic matter such as living organisms, detritus, and coatings on mineral particles (Filgueiras et al 2004; Tessier et al 1979). Metals bound to organic matter is reasonably stable in nature but under strongly oxidizing conditions, organic matter can be degraded, hence leading to a release of heavy metals bound to

this component. This condition may occur when the sediment is re-suspended (by dredging, currents, flooding, and tides) and the sediment particles come into contact with oxygen-rich water (Sarkar et al 2014; MacFarlane & Burchett 2000).

Fraction 5: Residual. A significant proportion of Cr (91.52%), Pb (88.85%) and As (78.70%) was found in residual fraction. The residual fraction usually holds metals within their crystal structure and commonly contributed by natural sources. Metals in this fraction are expected to be chemically stable and unavailable for bioaccumulation (Abdallah 2017; Weis & Weis 2004). Therefore, the high percentage of residual fraction indicates that sediment in Kelantan Delta posed no environmental risk under natural conditions, and the metals were derived mainly from lithogenic origins.

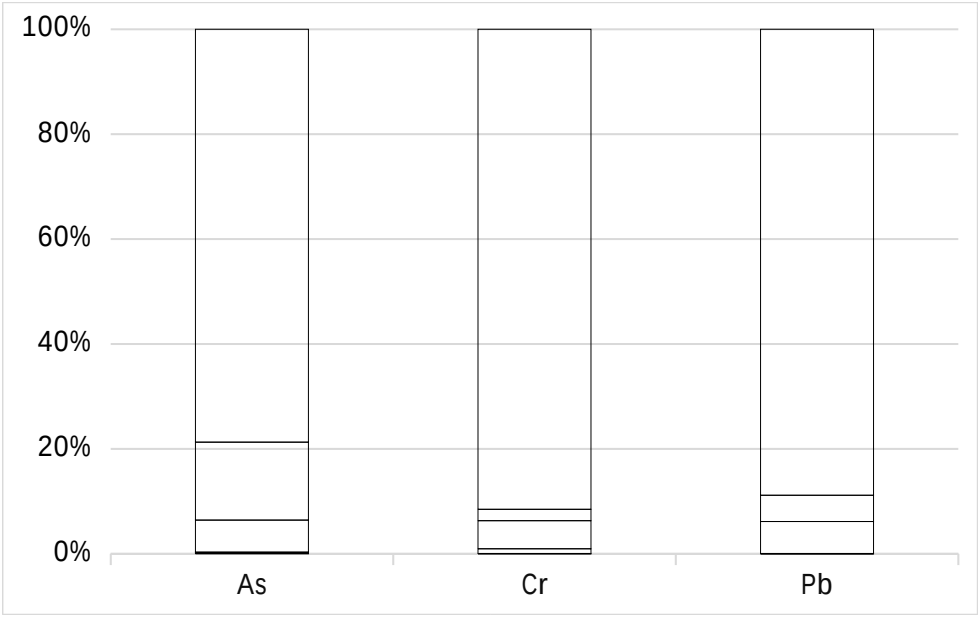

Figure 3. Fractionation of heavy metals in sediments from Kelantan Delta.

Bioavailable amount of heavy metals in sediment. The sequential extraction procedure employed in the present study was to assess the bioavailability of heavy metals in sediments. Bioavailable metals refer to the proportion of metals that can be released into the water (first four fractions). The highest concentration of available metal (Figure 4) in sediments was Pb (7.41 µg/g dry wt.) followed by Cr (5.35 µg/g dry wt.) and As (5.31 µg/g dry wt.). There was no statistically significant difference between the concentration of bioavailable metals in sediment as determined by one-way ANOVA ($F(2,51) = 0.795$, $p = 0.457$).

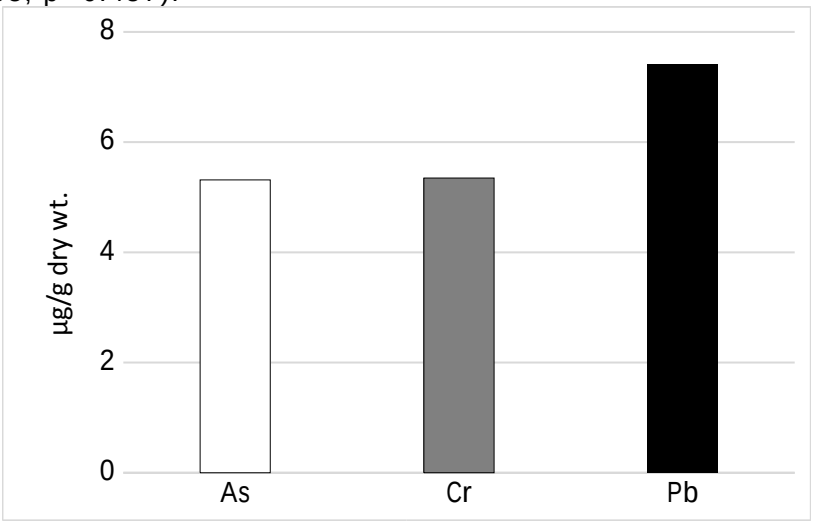

Figure 4. Concentration of available metals in sediment from Kelantan Delta.

Bioaccumulation of heavy metals in R. mucronata. Generally, heavy metals accumulated by *R. mucronata* was in decreasing order of Cr>Pb>As (Table 4). The highest concentration of metals was observed in root for all metals except As with an average of 9.37±3.33 µg/g dry wt. for Cr, 0.34±0.18 µg/g dry wt. for Pb, and 0.34±0.18 µg/g dry wt. for As. The accumulation by bark was minimal and lowest of all tissues with concentrations of As was 0.03±0.02 µg/g dry wt., Cr 7.28±2.13 µg/g dry wt., and Pb 0.26±0.11 µg/g dry wt. The accumulation of As in the leaves tissue was 0.05±0.03 µg/g dry wt., Cr 7.31±3.70 0.31 µg/g dry wt., and Pb 0.31±0.13 µg/g dry wt. Analysis of young, mature and senescent leaves of *R. mucronata* showed no significant difference. Hence, a composite of all type of leaves was used in the present study.

Table 4

Metals concentration in different parts of *Rhizophora mucronata* (µg/g dry wt.; min–max)

Specification	*No. of sample*	*As*	*Cr*	*Pb*
Root	15	0.01–0.08 (0.04±0.04)	2.97–13.34 (9.37±3.33)*	0.09–0.56 (0.34±0.18)
Bark	15	0.00–0.06 (0.03±0.02)	2.4–9.88 (7.28±2.13)*	0.14–0.54 (0.26±0.11)
Leaves	15	0.03–0.13 (0.05±0.03)	3.05–14.78 (7.31±3.70)*	0.14–0.63 (0.31±0.13)

*Significant at $p<0.01$. Values are expressed as mean ± SD.

R. mucronata from Kelantan Delta show the ability to accumulate heavy metals and tolerance to relatively high levels of heavy metal in sediment. This tolerance appears to be the result of metals in sediment predominantly being in unavailable form in relation to exclusion process in root tissue (MacFarlane & Burchett 1999; Weis & Weis 2004). To illustrate this point, *R. mucronata* showed limited uptake of these metals. As and Pb accumulated in root tissue of *R. mucronata* were less than 1% of sediment total metal concentrations and both with an average BCF of <0.01. However Cr accumulated in root tissue was around 20% of sediment total metal concentration, with an average BAF of 0.20±0.09 indicating the statistically significantly higher capacity of the roots ($p<0.01$) to bioaccumulate Cr compared to As and Pb (Table 4).
Interestingly, this species exhibit effective translocation of heavy metals to the aerial parts. *R. mucronata* efficiently translocate As (TF=1.69±1.38) and Pb (TF=1.14±0.77) and moderately translocate Cr (TF=0.66±0.33) to the leaves tissue.

Cluster analysis. Based on the hierarchical cluster analysis (Figure 5) there were two main cluster: the first represent a residual fraction, and the second constituted by all the remainder fractions and plant tissues. The first cluster is characterized by the metals bound to residue which was not associated with roots uptake as they are unavailable. The second cluster consists of fractions that correspond with accumulation in the plant. Accumulation in the plant was highly correlated with the easily available fraction (water soluble and exchangeable) and weakly associated the readily available fraction (bound to Fe-Mn oxide and bound to organic matters).

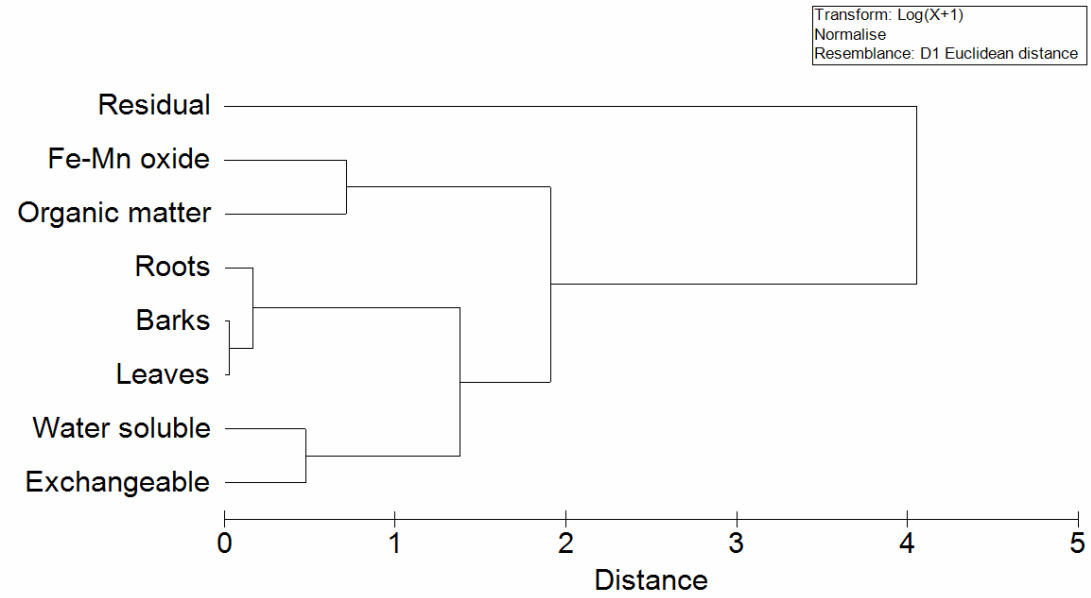

Figure 5. Cluster analysis based on group average Euclidean distance, on the fractionation of heavy metals in sediment and their concentration in the different parts of *Rhizophora mucronata* from Kelantan Delta, based on log(mean+1) transformed data.

Conclusions. Kelantan Delta is heavily polluted with As and Pb and moderately polluted with Cr. However, according to sequential extraction analysis of the sediment, the metals were predominantly found in residual fraction of lithogenic origins and thus posed a low hazard to the environment. In general, *R. mucronata* from Kelantan Delta exhibit a restricted uptake of heavy metals into their root, BAF<1. However, this species effectively translocate As and Pb to the aerial portions of the plant (TF>1), with Cr only moderately translocated (TF<1). Cluster analysis showed that accumulation of heavy metals in *R. mucronata* is correlated with bioavailable metals in sediment. Principally, water soluble and exchangeable fractions were the direct threats to the environment (plant).

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