# Speciation of heavy metals in the sediments of former tin mining catchment

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**Abstract**

The chemical speciation of heavy metals (arsenic, chromium, copper, lead, zinc and tin) in the sediments of former tin mining catchment Bestari Jaya, Peninsular Malaysia was determined by using the latest version of the Community Bureau of Reference, usually called the BCR 3-step sequential extraction procedure. Furthermore, a fourth step was introduced for digestion and analysis of the residue. The analysis of total metal content was carried out by using microwave acid digestion. The percentage of each metal obtained from the 4 step extraction (acid-soluble + reducible + oxidizable + residual) is in good agreement with the percentage of total metal content obtained from microwave digestion, which implies the accuracy of the procedure. The degree of pollution in catchment sediments was assessed using geoaccumulation index $I_{geo}$ and pollution intensity $I_{POLL}$. The results indicate that (1) the sediments have been polluted with arsenic (8.8%), chromium (12.9%), copper (17.4%), lead (19.5%), zinc (14.9%) and tin (33.8%) and have high anthropogenic influences; (2) the calculation of geo-accumulation index suggests that catchment sediments have the background concentration for all studied metals (*Igeo* < 0); (3) High $I_{POLL}$ showed that all of these heavy metals pose high environmental risk. (4) the mobility order of metals in sediments at S1 and S2 was Sn>Pb>Zn>Cr >Cu >As, whereas at S3, S4 and S5, Cu>Pb>Zn>Cr>Sn>As; In conclusion, acidic pH, total organic carbon, scavenging ability and co-precipitation (inclusion, occlusion and adsorption) of studied metals with non-metals could account for change in the geo-chemistry of the catchment sediments.

*Keywords*: Enrichment factor; fractions; geoaccumulation index; $I_{POLL}$; particulate metals; sequential extraction

## 1. Introduction

Water bodies are extremely complex dynamic chemical systems consisting of different components, namely various solutes, organic matter and colloidal or particulate material. Hence, a number of chemical processes between dissolved metal pollutants and components are expected to take place in these water bodies [1] and it is the distribution of the heavy metal pollutants between the different chemical species and forms which determines their geochemical and biological reactivity [2-4]. Among the chemical reactions, association with colloids and particles forming suspended and bottom sediments are a key process in the cycling of heavy metals in natural waters on a wide range of timescales (chemical speciation of the second kind). In fact, it has been found that heavy metals occurring in water bodies are predominantly carried by suspended particles and only a small fraction is transported in the soluble form [5, 6]. The distribution of an element among

different inorganic compounds and organic complexes profoundly impacts its transport and bioavailability by determining its physical and chemical properties such as charge, solubility, and diffusion. In order to understand the environmental chemistry of an element, it is necessary to obtain completely the concentrations and chemistry of its various species under the different conditions possible in natural environments. Speciation science aims to characterize an element's forms for understanding the transformations between different forms, and to infer the environmental processes controlling these transformations [7]. According to International Union of Pure and Applied Chemistry (IUPAC) recommendation [8], chemical species are the chemical compounds that differ in isotopic composition, conformation, oxidation or electronic state, or in the nature of their complexes or covalently bound substances. There have been many studies [9-14] about adsorption, speciation distribution and bio-availability of heavy metals.

Natural sediments are complex mixtures of various phases, namely residues of weathering and

erosion such as clays and other alumosilicates, iron and aluminum oxyhydroxides, and sulfides, substances produced by biological activity, which are in the form of both organic (living microorganisms, biological detritus and humic substances) and inorganic (carbonates, phosphates and silica) [15]. Therefore, the following mechanisms of the association (generally termed "sorption") of metals with the specific phases of natural sediments can be considered to take place in oxic environments as opposed to anoxic environments where precipitation of trace metal sulfides may dominate:(1) coprecipitation with hydrous iron and manganese oxides and carbonates, (2) adsorption and cation exchange on/in clays, hydrous iron and manganese oxides and humic substances, (3) organometallic bonding with humic substances, and (4) incorporation in the crystal structure of clays and other alumosilicates [5, 16].

For analysis of chemical speciation, different methods have been employed by different researchers such as voltammetric method [17, 18], Liquid-liquid extraction method [19, 20], Ion exchange and adsorption columns/resins method [21, 22], Gas chromatography [23, 24], Liquid chromatography [25, 26], Capillary electrophoresis (CE) [27, 28] as well as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [29, 30]. ICP-MS could be good tool for sensitive speciation analyses of many environmentally important elements. Use of total concentration as a criterion to access the potential effects of the sediment contamination implies that all forms (phases) of a given metal have an equal impact on the environment. Such an assumption is untenable [31]. It is evident that just the speciation of metal pollutants with the various sediment phases determines their specific impact on the environment. Also, the type of phase specific bonding of metals in contaminated natural sediments specifies suitable methods of their potential cleaning and utilization [32]. A common but time consuming analytical method of evaluating particular metal-sediment phase associates is the method of sequential extractions (SE), adapted from the methods of soil and sediment chemical analysis [16]. The concept of the SE procedure is the partitioning of a solid material into specific phases or fractions that are selectively extracted, i.e. liberated and released into solution (leached) along with the associated trace metals, by using appropriate reagents arranged in increasing strength [15, 16, 31-33]. Speciation, using sequential extraction schemes, has been developed for assessing geochemical forms in soil and sediment [34-37]. Fractionation by selective chemical extraction removes or dissociates a specific phase with the associated metal bonded to it. The geochemical fractions most commonly analysed are: exchangeable, bound to carbonates, reducible, oxidisable and residual. Among the sequential extraction schemes proposed to investigate the distribution of heavy metals in soil and sediments, the five-step and six-step extraction schemes developed by Tessier et al. (1979)

and Kersten and Forstner (1986), respectively, were the most widely used [16, 38]. Following these two basic schemes, some modified procedures with different sequences of reagents or operational conditions have been developed [39-42].

Water bodies are important parts in the ecological system. In recent years, due to rapid industrialization and excessive mining activities, these water bodies have been contaminated by different forms of heavy metals. These contaminations pose severe eco-toxicological threats to aquatic wildlife and humans. The bio-geochemical behaviour, nutritional bioavailability and toxicity of metals are largely dependent on their chemical speciation. These studies have received extensive attention in recent years. In this study, sediments collected from Bestari Jaya catchment were analyzed using a modified BCR 3-step sequential extraction procedure [43]. A fourth step, i.e., digestion of the residue from the third step, was included using a microwave-assisted acid digestion procedure. The aims of this study were: (i) to determine the metal pollution levels in sediments at the former tin mining catchment (ii) to determine and compare the chemical speciation of heavy metals to evaluate relative mobility and bioavailability; and (iii) to evaluate the data on metal levels, mobility and bioavailability in the context of similar data reported from other countries. The purpose of this study was to examine the speciation of selected heavy metals (Cu, Zn, Pb, Cr, As and Sn) in the sediments of ex-tin mining catchment Bestari by using four step sequential extraction procedure, namely after Horowitz and Tessier et al. followed by ICP-OES detection [5, 16].

## 2. Study Area

Bestari Jaya (Batang Berjuntai) catchment is located at $3^0$, 24' 40.41" N and $101^0$ 24' 56.23" E. It is a part of the Kuala Selangor district in the Selangor state of West Malaysia. The district includes three main towns namely, Mukim Batang Berjuntai, Mukim Ulu Tinggi, Mukim Tanjung Karang. The study area, Bestari Jaya, is located in Mukim Batang Berjuntai. Bestari Jaya has a tropical, humid climate, with very little variation in temperature throughout the year. The average temperature of the area is 32 $^0$C with an average rainfall of 2500 mm and potential evaporation of 1600 mm per year. Tin mining activities have ceased from the last ten years, and is now sand mining. The catchment has a total of 442 small and big mining lakes and ponds. The whole catchment covers an area of 2656.31 hectors having Raja Muda reserve forest in the north, while Bestari Jaya Town and University Industry Selangor (UNISEL) are in the south. The catchment wastewater flow downstream to Sungai Ayer Hitam and Sungai Udang which ultimately end up with the River Selangor, the biggest river of the state [44].

## 3. Material and Method

### 3.1. Sampling and sample pre-treatment

Due to the large study area Global Positioning System (GPS) was used to determine the actual coordinates of the sampling sites and to reconfirm the location of the sampling site during subsequent sampling periods. The five sampling locations were finalized and the map of the Bestari Jaya catchment is shown in Fig. 1 and the geographic positions are listed in Table 1. The sampling site S5 is located at the junction of the two rivers Ayer Hitam River and Selangor River and close to the Bestari Jaya

catchment. S4 lies at the junction of the catchment to Ayer Hitam River, while S1, S2 and S3 are located at different altitude and latitude inside the ex-mining catchment. In total five sediment core samples were collected at different ex-mining lakes of the catchment in the month of December, 2010 (Fig. 1). The voyage was accomplished by using geological research vessel no. 1. The whole voyage took 7 days.
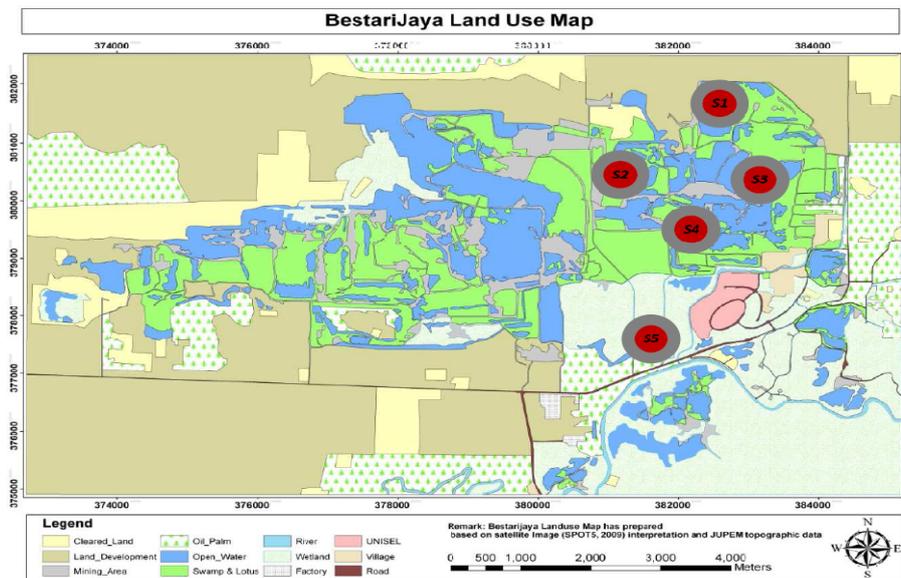


**Fig. 1.** Map of Bestari Jaya catchment

**Table 1.** Geology of sampling locations

| S1 | $3^0$ 26' 58.07" N $101^0$ 26' 09.96" E | 1.97 | 4.3 |
|----|------------------------------------------|------|-----|
| S2 | $3^0$ 26' 26.24" N $101^0$ 26' 42.56" E | 2.65 | 4.9 |
| S3 | $3^0$ 26' 10.20" N $101^0$ 25' 30.75" E | 3.42 | 3.8 |
| S4 | $3^0$ 25' 27.32" N $101^0$ 26' 01.48" E | 3.79 | 4.4 |
| S5 | $3^0$ 24' 36.98" N $101^0$ 26' 00.33" E | 3.78 | 5.8 |

Core sampling is the recommended method to be used when accurate surficial sediment sampling depths are important, vertical profiles are needed to assess quality of sediment at depth, and it is important to maintain an oxygen-free environment. For this purpose, the capability of known sediment corer such as the Russian type, KC sediment trap, Slide-hammer, Kajak-Brinkhurst, Phleger, Benthos, Alpine, Boomerang, and Ballchek were considered. These samplers are usually deployed using a winch that suspends the sampler about 5 meters above the sediment to be sampled and allowed to free fall,

penetrating the sediment and forcing the material into the sample liner. At the Bestari Jaya catchment, sediment samples were collected by using sediment core sampler (Kajak KC- Denmark Model 13.030). This sampler is designed for 2m undisturbed core sampling with high recovery percentage and non-compacted sediment column. The sampler can be used as line operated as well as hand-operated sampler. The Kajak comes as a quick-change model (13.030), speeds up the changing of corer tubes, which is very useful. All parts are made of AISI 316 stainless steel. An internal PP (polypropylene) liner is inserted into the stainless steel tube for easy (and cheap) storage of many samples. The stainless steel tube holds the orange peel closing system (core catcher) to prevent the sample from exiting. Five sediment cores extending into the soft sediment and underlying weathered bedrock were collected for investigation of their chemical properties (Core1, Core2, Core3, Core4 and Core5) from the main sediment entry point, middle or deep, and the departure point of open water (Fig. 1).

### 3.2. Sample preparation and analysis

Core samples were sealed and kept vertically in order to prevent mixing during transportation to lab. Before slicing all cores were preserved in a freezer at 4∘C and 2±0.2cm intervals. Slice samples were dried at 80-110 $^{o}$C and then ground with a pestle and mortar and sieved (<63 lm). The <63 lm fraction was used for analyses in this study due to strong association of metals with fine-grained sediments [33, 45-48]. Many previous studies have been completed on the sequential extraction of metals from sediments using this size fraction [49-53]. The moisture content of the dried sample was calculated by heating a portion of sediment in an oven at 105$^{o}$C to constant weight. Sediment data in this study are reported on a dry weight basis.

### 3.3. Reagents

Double de-ionized water (18 MX) supplied by an ELGA water purifier was used in this study. Ultra pure $HNO_3$ (Fluka), HF (Fluka), $H_2O_2$ (Merck), ammonium acetate (Merck) and analytical grade acetic acid (Sigma), hydroxylamine hydrochloride (Sigma) were used as the sequential extraction reagents in conjunction with microwave-assisted acid digestion. According to Method 3052 [54], selected samples (0.25g) were mixed with reagents, 9ml $HNO_3$, 2ml HCl, and 3ml HF acids. Digestion procedures were continued using a Multiwave 3000 Oven with 5 and 10 minute ramping and holding time, respectively. Semi digested samples were eventually fully digested by an additional 18ml saturated boric acid solution ($H_3BO_4$) during complexation, followed by 10 minutes holding time in a Multiwave3000 Oven.

Calibration solutions were obtained by diluting stock solution in different solvents [51, 55] depending on the extracts analyzed (0.11 mol/L acetic acid solution for the extracts from Step 1, 0.5 mol/L hydroxylamine hydrochloride solution for the extracts from Step 2, 1.0 mol/L ammonium acetate solution for the extracts from Step 3 and 2% $HNO_3$ solution for the extracts from Step 4 and the digests from total digestion).

### 3.4. Apparatus

Finally, the sequential extracts samples were analysed by ICP-OES. The instrument parameters for metal analysis were recommended by Grasshoff [56]. Sediment extractions were conducted using a Balmar mechanical shaker (Model GFL 3005, USA) at 30±10 rpm for 16 h. A centrifuge (KUBOTA 5100, Japan) was used to obtain the supernatant extracts at 3000 rpm for 20 min. A microwave unit (CEM MARSX, USA) was used

for the total digestion of sediment samples. All lab-ware was made of polypropylene (PP), high density polyethylene (HDPE) or polytetrafluoroethylene (PTFE) which had been prepared for use by pre-washing in laboratory-grade detergent, rinsing with de-ionized water, and soaking in 2% $HNO_3$ (overnight), followed by thorough rinsing with deionized water.

### 3.5. PH measurement and TOC analysis

A total organic carbon analyzer (Apollo 9000, Tekmar-Dohrmann, USA) was used to determine the contents of the total organic carbon (TOC) in the sediment samples. An ultracentrifuge was used for the centrifugation of the extracts. A horizontal rotator shaker was used for the extraction and a pH value meter was used to detect the pH values of the sediment samples. The entire glass container used was soaked in 50% $HNO_3$ (V/V) and rinsed with deionised water. The extracts from the samples were stored in PTFE tubes and stored at 4$^{o}$C before analysis. The pH values and TOC contents are listed in (Table 1).

### 3.6. Sequential extraction method

Sequential extraction procedures available in the literature only bind to the analysis of soil and sediments samples [39-42]. A sequential leaching procedure was modified based on extraction schemes of Tessier et al. and Kersten and Forstner [16, 38]. The method identifies the metal among four operationally-defined host fractions, namely exchangeable, carbonate, reducible, organically bound and residual.

### 3.6.1. Step 1 (acid-soluble fraction-bound to carbonates)

For each sample, three 0.5g dry sediment replicates were placed into 50 mL of polypropylene centrifuge tubes. Twenty millilitres of 0.11 mol/L of acetic acid was added to the tube, which was then shaken for 16 h at a room temperature of 22 ± 5 $^{0}$C and a speed of 30±10 rpm. Separated from the solid phase by centrifugation at 3000 rpm for 20 min, the supernatant liquid was decanted into a 50mL polypropylene centrifuge tube and stored in a refrigerator at 4 $^{0}$C prior to analysis. The residue was washed with 10mL of de-ionized water, shaken again for 15 min and then centrifuged for 20 min at 3000 rpm. The supernatant was decanted and removed carefully to avoid loss of the solid residue.

### 3.6.2. Step 2 (reducible fraction–bound to Fe and Mn oxides)

Twenty millilitres of 0.5 mol/L hydroxylamine hydrochloride (adjusted to pH of around 1.5 by adding of 2mol/L $HNO_3$) was added to the residue from Step 1 in the centrifuge tube. Again, the tube was shaken for 16h at $22\pm5$ $^0$C and a speed of $30\pm10$ rpm. The extract was separated from the solid phase by centrifugation and decantation as described for Step 1 and stored at 4 $^0$C. The solid residue was washed as in Step 1 before proceeding to Step 3.

### 3.6.3. Step 3 (oxidizable fraction–bound to organic matter and sulfides)

Five millilitres of 8.8 mol/L $H_2O_2$ (pH of 2–3) was added carefully, in small aliquots, into the residue from Step 3 in the centrifuge tube. The tube was covered loosely and digested at room temperature for 1h with occasional shaking. The tube was then continuously digested for 1 h at $85\pm2$ $^0$C in a water bath with occasional shaking for the first 30 min, and the volume was then reduced to around 2–3mL by further heating of the uncovered tube. Another 5mL of 8.8 mol/L $H_2O_2$ (pH of 2–3) was added. Again, the covered tube was heated to $85\pm2$ $^0$C and digested for 1 h before the volume in the uncovered tube was reduced almost to dryness. After cooling, 25mL of 1.0 mol/L ammonium acetate (adjusted to pH 2 by adding of concentrated $HNO_3$) was added to the residue and the tube was shaken for 16 h at room temperature (overnight). The extract was separated from the solid phase by centrifugation and decantation as described above and stored at 4$^0$C.

### 3.6.4. Step 4 (residual fraction–strongly associated to the crystalline structures of the minerals)

The residue from Step 3 was digested in a mixture (3:1) of concentrated $HNO_3$ and HF. A detailed description of this procedure is given in the next section below.

### 3.7. Microwave-assisted acid digestion procedure

The metal content of the residue from Step 3 was determined using a microwave-assisted acid digestion procedure [57]. Three replicates of 0.5 g of samples were analyzed by digestion with a mixture of acid (9mL concentrated $HNO_3$ and 3mL concentrated HF). Samples were heated in the microwave unit. The temperature of samples rose to $180\pm5$ $^0$C in 10 min and remained at $180\pm5$ $^0$C for 9.5 min. Digests were centrifuged at 3000 rpm for 10 min to clear the supernatant which was then analyzed by inductively coupled plasma optical emission spectrometry ICP-OES (Perkin Elmer AA Analyst). In addition, the samples were directly digested using the microwave-assisted acid digestion procedure in order to quantify the total metal contents.

### 3.8. Statistical Analysis

To assess the intensity of metal contamination in Bestari Jaya sediments, the pollution index was calculated using [58]:

$I_{POLL} = Log_2 (C_n / B_n)$

Where, $C_n$ is the total elemental content in sediments and $B_n$ is the lithogenous portion of element.

Geo-accumulation index was used to assess heavy metal accumulation in sediments as introduced by Muller to measure the degree of metal pollution in aquatic sediments studies [59-62]

$I_{geo} = Log_2 (C_n / B_n \text{ x } 1.5)$

Where $C_n$ is the total elemental content in sediments, $B_n$ is the concentration of metals in sediments and 1.5 is a factor for normalization of background metals concentrations in sediments.

Statistical analysis software Multi Variable Statistical Program (MVSP) was used to understand the relationship amongst various metals and environmental indicators [58].

## 4. Results

The five sampling locations were finalized for sample collection at Bestari Jaya catchment as shown in Fig. 1 and the geographic positions of these sampling points are listed in Table 1. A total of five sediment cores were collected for investigation of their chemical properties (Core1, Core2, Core3, Core4 and Core5) from sediment entry point, middle or deep and departure point of open water (Fig. 1). During the samples analysis by ICP-OES, the accuracy of the sequential extraction method is verified by using standard sediment reference material (SRM-4354, National Institute of Standards & Technology NIST, USA). The extractable contents and recoveries in each extraction step of the standard reference material used are presented in Table 2. Total contents of Pb, Zn, Cu, As, Cr and Sn in the catchment sediments are presented in Table 3. To investigate variation of distribution patterns of metals in the non-residual fractions with depth, two sediment cores (with length of 40 and 50 cm, respectively) were collected at S2 and S4. The variation of element concentration in fraction A with depth at S2 and S5 is shown in Fig. 2. The concentration variation of elements in the fraction B with depth at S4 and S5 is shown in Fig. 3. The variation of element concentration in the fraction C with depth at S4 and S5 is shown in Fig. 4. The intensity of pollution in sediments of Bestari Jaya were measured by using $I_{geo}$ and $I_{POLL}$ indices. The results are given in Table 4. Fig. 5 shows relationships between metals and physical parameters in the sediments of Bestari Jaya.

**Table 2.** Results of analysis of standard reference materials (SRM) in comparison with certified values

| Standard Reference Material | Analysed SRM Value | Certified SRM Value | Recovery% |
|---|---|---|---|
| Sequential Extraction | | | |
| SRM-4354-Lake Sediment (n=3) | | | |
| Step 1 | | | |
| As | 4.89±0.40 | 5.0±0.38 | 91 |
| Cr | 3.58±0.10 | 3.67±0.13 | 87 |
| Zn | 4.48±0.79 | 4.14±0.39 | 102 |
| Cu | 36.44±1.6 | 49.0±1.8 | 70 |
| Pb | 78.81±2.00 | 74.30±2.00 | 107 |
| Sn | 187±5.00 | 205±2.00 | 108 |
| Step 2 | | | |
| As | 3.78±0.30 | 3.98±0.34 | 93 |
| Cr | 3.12±0.14 | 3.22±0.19 | 89 |
| Zn | 103.28±4.00 | 98.84±3.00 | 96 |
| Cu | 113.28±2.0 | 139.0±2.5 | 111 |
| Pb | 67.40±3.00 | 69.28±3.00 | 101 |
| Sn | 123±4.00 | 176±5.00 | 105 |
| Step 3 | | | |
| As | 1.48±0.23 | 1.62±0.32 | 97 |
| Cr | 105.12±4.00 | 109.00±5.00 | 108 |
| Zn | 56.28±2.00 | 68.74±3.00 | 100 |
| Cu | 30.28±2.0 | 39.32±2.5 | 103 |
| Pb | 11.10±2.00 | 9.20±3.00 | 105 |
| Sn | 73±2.00 | 76±3.00 | 110 |
| Step 4 | | | |
| As | 0.22±0.03 | 0.18±0.52 | 123 |
| Cr | 75.33±5.00 | 67.56±4.00 | 132 |
| Zn | 102.00±5.00 | 98.34±4.00 | 125 |
| Cu | 23.98±0.88 | 20.43±2.00 | 118 |
| Pb | 16.23±0.90 | 12.53±1.00 | 109 |
| Sn | 43±4.00 | 38±2.00 | 116 |
| Total Digestion | | | |
| SRM-4354-Lake Sediment (n=3) | | | |
| As | 10.46±1.00 | 10.78±1.00 | 103 |
| Cr | 187.15±4.00 | 183.45±4.00 | 118 |
| Zn | 265.76±4.00 | 270.06±5.00 | 104 |
| Cu | 203.98±4.00 | 247.75±3.00 | 119 |
| Pb | 173.54±2.00 | 165.31±2.00 | 127 |
| Sn | 426±5.00 | 495±4.00 | 138 |

**Table 3.** Heavy metals concentration of sediment samples at different locations using modified sequential extraction procedure

| Element | Fraction | Mean/ S.D[a] | S1 | | S2 | | S3 | | S4 | | S5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Concentration (µg/g) | Ratio% | Concentration (µg/g) | Ratio% | Concentration (µg/g) | Ratio % | Concentration (µg/g) | Ratio% | Concentration (µg/g) | Ratio% |
| As | 1 | Mean | 0.09 | 38.3 | 0.08 | 36.9 | 0.04 | 39.5 | 0.08 | 37.5 | 0.03 | 34.5 |
| | | SD | 0.07 | | 0.06 | | 0.02 | | 0.03 | | 0.01 | |
| | 2 | Mean | 0.05 | 19.3 | 0.07 | 29.5 | 0.06 | 26.4 | 0.06 | 27.8 | 0.06 | 29.6 |
| | | SD | 0.02 | | 0.04 | | 0.03 | | 0.04 | | 0.02 | |
| | 3 | Mean | 0.08 | 23.7 | 0.07 | 23.1 | 0.07 | 20.8 | 0.05 | 21.4 | 0.01 | 23.5 |
| | | SD | 0.04 | | 0.05 | | 0.02 | | 0.03 | | 0.008 | |
| | 4 | Mean | 0.03 | 18.8 | 0.04 | 20.5 | 0.05 | 13.3 | 0.07 | 13.3 | 0.03 | 12.4 |
| | | SD | 0.02 | | 0.03 | | 0.03 | | 0.03 | | 0.01 | |
| | Sum | | 0.25 | 100 | 0.26 | 100 | 0.22 | 100 | 0.26 | 100 | 0.13 | 100 |
| Total Content | | Mean | 0.19 | | 0.21 | | 0.21 | | 0.23 | | 0.11 | |
| | | SD | 0.03 | | 0.05 | | 0.05 | | 0.05 | | 0.07 | |
| Recovery (%) | | | 88% | | 91% | | 100% | | 98% | | 93% | |
| Cr | 1 | Mean | 0.57 | 0.4 | 0.48 | 0.58 | 0.7 | 0.72 | 0.18 | 0.5 | 0.24 | 0.78 |
| | | SD | 0.3 | | 0.25 | | 0.45 | | 0.05 | | 0.09 | |
| | 2 | Mean | 3.67 | 7.8 | 5.97 | 8.34 | 8.8 | 10.5 | 10.15 | 13.8 | 11.56 | 14.55 |
| | | SD | 0.85 | | 1 | | 0.5 | | 0.96 | | 1.24 | |
| | 3 | Mean | 8.29 | 16.5 | 11.25 | 18.4 | 15.65 | 18.24 | 19.45 | 21.56 | 19.67 | 18.34 |
| | | SD | 1.25 | | 0.55 | | 0.89 | | 0.88 | | 1.07 | |
| | 4 | Mean | 33.84 | 75.3 | 29.1 | 72.7 | 13.1 | 70.55 | 20.4 | 64.14 | 12.63 | 66.33 |
| | | SD | 2.15 | | 0.9 | | 0.45 | | 11.05 | | 1 | |

**Table 3.** (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sum | | | 47 | 100 | 46.6 | 100 | 38.25 | 100 | 50.18 | 100 | 44.1 | 100 |
| Total Content | | Mean | 49.23 | | 47.55 | | 40.21 | | 48.81 | | 45.61 | |
| | | SD | 3.12 | | 3.4 | | 3.27 | | 3.19 | | 3.82 | |
| Recovery (%) | | | 89% | | 94% | | 91% | | 94% | | 99% | |
| Zn | 1 | Mean | 9.61 | 12.4 | 1.56 | 3.5 | 11.5 | 6.4 | 8.98 | 8.9 | 7.83 | 10.5 |
| | | SD | 5.79 | | 0.22 | | 2.1 | | 1.5 | | 1.1 | |
| | 2 | Mean | 23.78 | 30.7 | 9.46 | 21 | 18.23 | 17.4 | 16.77 | 14.78 | 16.66 | 12.67 |
| | | SD | 2.1 | | 2.65 | | 1.55 | | 0.98 | | 0.87 | |
| | 3 | Mean | 29.41 | 38 | 6.57 | 14.6 | 26.88 | 28.76 | 21.33 | 23.66 | 19.36 | 21.55 |
| | | SD | 7.1 | | 0.91 | | 2.1 | | 1.4 | | 0.46 | |
| | 4 | Mean | 14.65 | 18.2 | 27.4 | 60.9 | 13.29 | 47.44 | 11.88 | 52.66 | 23.14 | 55.28 |
| | | SD | 4.58 | | 19.34 | | 0.98 | | 0.78 | | 1.05 | |
| Sum | | | 77.45 | 100 | 44.97 | 100 | 69.9 | 100 | 58.96 | 100 | 66.99 | 100 |
| Total Content | | Mean | 68.12 | | 49.76 | | 71.35 | | 62.38 | | 72 | |
| | | SD | 2.85 | | 3.55 | | 2.1 | | 2 | | 2.34 | |
| Recovery (%) | | | 125% | | 90% | | 96% | | 87% | | 94% | |
| Cu | 1 | Mean | 0.56 | 4.5 | 0.48 | 5.3 | 0.32 | 4.2 | 0.83 | 4.8 | 0.98 | 5.5 |
| | | SD | 0.15 | | 0.18 | | 0.12 | | 0.5 | | 0.33 | |
| | 2 | Mean | 1.76 | 12.8 | 1.25 | 12 | 1.06 | 13.9 | 4.87 | 16.45 | 3.68 | 20.6 |
| | | SD | 0.45 | | 0.3 | | 0.27 | | 1 | | 0.7 | |
| | 3 | Mean | 2.78 | 29.5 | 4 | 33.2 | 2.32 | 30.3 | 8.59 | 38.5 | 7.43 | 41.5 |
| | | SD | 0.5 | | 0.8 | | 0.75 | | 0.98 | | 1.1 | |
| | 4 | Mean | 4.1 | 53.2 | 4.88 | 49.5 | 3.95 | 51.6 | 7.34 | 40.25 | 5.81 | 32.5 |
| | | SD | 0.8 | | 0.85 | | 0.92 | | 1.23 | | 1.94 | |

**Table 3.** (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sum | | | 9.2 | 100 | 10.61 | 100 | 7.65 | 100 | 21.63 | 100 | 17.9 | 100 |
| Total Content | | Mean | 8.9 | | 9.5 | | 7.71 | | 22 | | 17.27 | |
| | | SD | 1.2 | | 2 | | 1.34 | | 2.3 | | 3.22 | |
| Recovery (%) | | | 89% | | 91% | | 99% | | 105% | | 104% | |
| Pb | 1 | Mean | 1.1 | 2.5 | 0.85 | 2.9 | 0.75 | 2.4 | 0.95 | 3 | 0.91 | 4.6 |
| | | SD | 0.3 | | 0.26 | | 0.18 | | 0.2 | | 0.43 | |
| | 2 | Mean | 13.24 | 42.1 | 11.6 | 40 | 10.42 | 33.4 | 12.4 | 42.8 | 14.45 | 44.96 |
| | | SD | 0.87 | | 0.64 | | 0.75 | | 0.56 | | 0.88 | |
| | 3 | Mean | 0.66 | 1.8 | 0.35 | 1.2 | 0.64 | 2.1 | 0.7 | 2 | 0.95 | 6.14 |
| | | SD | 0.2 | | 0.21 | | 0.45 | | 0.2 | | 0.21 | |
| | 4 | Mean | 18.71 | 53.6 | 16.21 | 55.9 | 19.4 | 62.2 | 17.85 | 52.2 | 15.55 | 55.7 |
| | | SD | 0.98 | | 2.52 | | 3.76 | | 1.2 | | 0.9 | |

**Table 3.** (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sum | | | 33.71 | 100 | 29.01 | 100 | 31.21 | 100 | 31.9 | 100 | 31.86 | 100 |
| Total Content | | Mean | 32.45 | | 26.13 | | 29.8 | | 30.15 | | 30.4 | |
| | | SD | 2.1 | | 2.7 | | 6.22 | | 1.8 | | 2.5 | |
| Recovery (%) | | | 109% | | 111% | | 105% | | 115% | | 107% | |
| Sn | 1 | Mean | 6.77 | 3.51 | 8.45 | 8.92 | 8.1 | 6.53 | 7.81 | 8.23 | 6.75 | 6.4 |
| | | SD | 1.15 | | 0.98 | | 1.24 | | 0.96 | | 0.84 | |
| | 2 | Mean | 11.22 | 27.6 | 9.58 | 22.6 | 12.45 | 19.23 | 10.99 | 18.45 | 13.23 | 22.56 |
| | | SD | 1.8 | | 1.23 | | 1.35 | | 1.21 | | 0.97 | |
| | 3 | Mean | 24.58 | 15.9 | 19.4 | 18.3 | 29.44 | 21.43 | 28.77 | 22.35 | 26.73 | 19.78 |
| | | SD | 1.5 | | 1.22 | | 2.14 | | 1.35 | | 1.24 | |
| | 4 | Mean | 60.4 | 53.1 | 58.4 | 49 | 48.51 | 52.84 | 63.85 | 50.97 | 59.65 | 51.26 |
| | | SD | 2.1 | | 3.12 | | 2.75 | | 1.65 | | 2.32 | |
| Sum | | | 102.97 | 100 | 95.83 | 100 | 98.5 | 100 | 111.42 | 100 | 106.36 | 100 |
| Total Content | | Mean | 105 | | 100 | | 102 | | 112 | | 103 | |
| | | SD | 2.43 | | 2.8 | | 3.4 | | 2.95 | | 2.65 | |
| Recovery (%) | | | 103% | | 94% | | 104% | | 118% | | 105% | |

Fraction 1. Acid soluble; Fraction 2. Reducible; Fraction 3. Oxidisable; Fraction 4. Residual
Sum = Fraction 1 + Fraction 2 + Fraction 3 + Fraction 4
Total content: total acid digestion of samples using HF and $HNO_3$
Recovery = (sum/total content) 100.
Ratio = (Fraction/Sum) 100
a Standard deviation
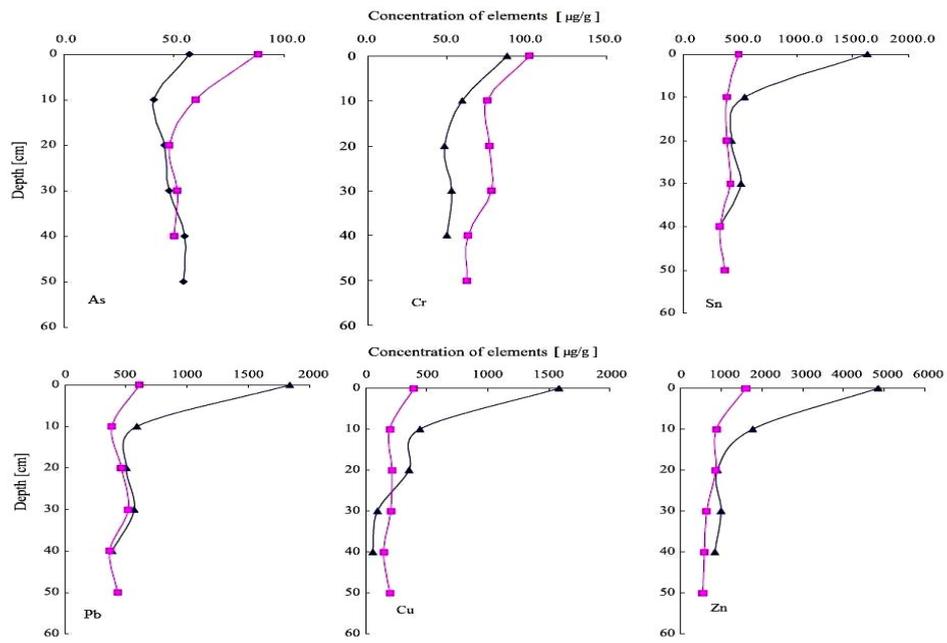
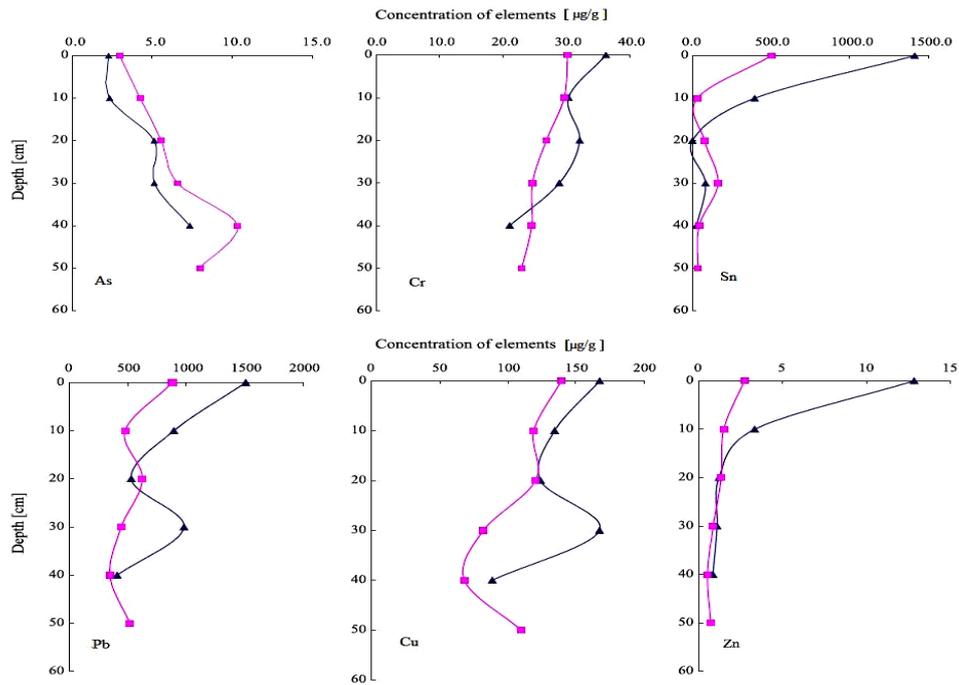**Fig. 2.** Concentration variation of elements in fraction A with depth at site S2 and S4



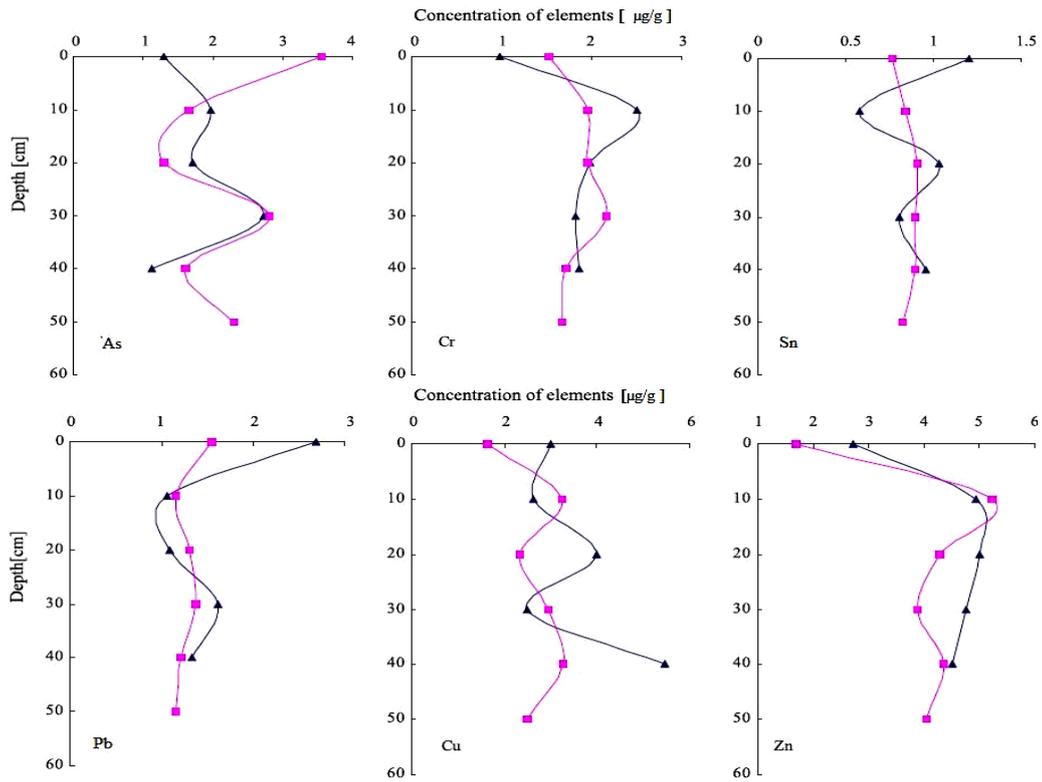**Fig. 3.** Concentration variation of elements in fraction B with depth at site S2 and S8

**Fig. 4.** Concentration variation of elements in fraction C with depth at site S2 and S8

**Table 4.** Comparison of different pollution indices in Bestari Jaya sediments

| Metals | As | Cr | Cu | Pb | Zn | Sn |
|---|---|---|---|---|---|---|
| $I_{geo}$ | 1.8 | 3.6 | 2.9 | 3.4 | 2.4 | 8.1 |
| $I_{POLL}$ | 1.1 | 2.6 | 2.8 | 3.9 | 2.7 | 7.7 |
| Pollution Index | Moderate | Moderate | Moderate | High | Moderate | High |
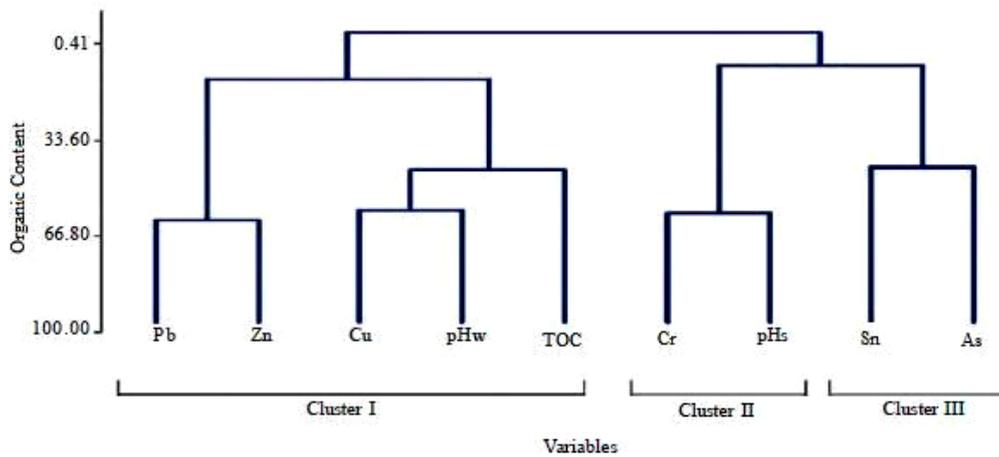


**Fig. 5.** Dendrogram of cluster analysis amongst heavy metals bioavailability in sediments

## 5. Discussion

### 5.1. Quality assurance

In order to verify the accuracy of the sequential extraction method, a standard sediment reference material (SRM-4354, National Institute of Standards & Technology NIST, USA) was analyzed using 4 replicates. The extractable contents and recoveries in each extraction step of the standard reference material used are presented in Table 2. Recoveries were good, averaging 88±12% for Step 1, 98±10% for Step 2, 106±13% for Step 3, and 123±10% for Step 4. The recoveries of all metals from Step 1 were greater than 89%, ranging from 89% to 115%. The recoveries for metals from Steps 2 and 3 were between 84% and 124%. For Step 3, the recoveries for all metals were slightly higher than 100%, ranging from 112% to 139%, indicating that organic matter is more likely to cause interference in sediment extracts. This is logical since the high concentration of organic matter observed in most sediments is likely to complex metals and render them unavailable for competition with the extracts. The accuracy of the microwave-assisted acid digestion procedure for total metal determinations was checked by using the SRM-4354 standard reference material. Analysis of these reference materials in 4 replicates showed satisfactory accuracy, with the recoveries for all metals in SRM-4354 between 80% and 124%. An internal check on the results of the microwave extraction procedure, and the sequential extraction procedure was performed by comparing the sum of the 4 steps (acid-soluble + reducible + oxidizable+residual) from the sequential extraction procedure with the total metal content from the microwave-assisted acid digestion procedure. The recovery of the sequential extraction procedure was calculated as follows:

$$Recovery = \frac{Fraction^1 + Fraction^2 + Fraction^3 + Residual\ fraction}{Total\ digestion} \times 100$$

With respect to the standard sediment reference material SRM-4354, the results shown in Table 3 indicate that the sums of the 4 fractions are in agreement with the total metal contents with satisfactory recoveries (80–126%). As shown in Table 3, results for the analysed sediments indicate that the sums of the 4 fractions are in good agreement with the total metal content.

### 5.2. Total metal content

Total contents of Pb, Zn, Cu, As, Cr and Sn in the catchment sediments are presented in (Table 3). The sediments of the present study were acidic to about neutral in nature with the pH ranging from 3.8 to 5.8 (Table 1). The decrease in pH from catchment to River Ayer Hitam is due to admixture of a wide variety of sediments during flooding from the catchment area. The pH during raining periods might be related to the normal soil pH of the region. Increase in organic matter content in (from 1.97 to 3.79mg/g) and corresponding increase in downstream direction clearly indicates its influx during the washout from the surrounding areas (Table 1). Table 3 shows mean metal concentrations in the sediments in each of the five sampling locations. The greatest concentration levels of most of the metals analysed are found in the sediments from the S2 and S3, which are found near the so-called 'Main Dredging Belt'. Tin is the most abundant metal in all of the studied locations because area catchment is the former tin mining area. High concentrations of As, Zn, Pb, Cr and Cu are found in the sediments of the S2-S4. We should point out that the Pb content in the S1 (33.5μg/g) is notably higher than that of S2-S5. This is because the S1 is near a heavily travelled highway. The levels of Cr (50.18 μg/g, 44.1 μg/g) and As (0.26 μg/g, 0.13 μg/g) found in the sediments of the two sampling locations S4-S5 are similar to those reported by Salomons and Forstner, in unpolluted sediments [63].

### 5.3. Metal speciation

The extractable contents of As, Cr, Zn, Cu, Pb and Sn, and the extracted percentages of these metals with respect to the sums of 4 fractions in the catchment sediments from each extraction step are shown in Table 2 and 3. All metals except for As were present at higher percentages in the acid-soluble fractions (the most labile fraction) in S2 relative to those in S1,S3,S4,S5 reflecting that Cr, Cu, Ni, Pb and Zn were more mobile and potentially more bio-available in sediments from S2. The non-residual fractions (acid-soluble+ reducible+oxidizable) of all metals in sediments were greater in S1, S2 (89% for Cr; 94% for As; 125% for Zn; 91% for Cu; 111% for Pb and 103% for Sn) than in S3, S4, S5 (100% for As; 99% for Cr; 96% for Zn; 105% for Cu; 115% for Pb and 118% for Sn). Therefore, the results indicate that heavy metals in sediments from S1, S2 and S3 are potentially more available for exchange and/or release into the aquatic environment relative to those from S4 and S5. In sediments from S2, S3, S4, the mobility of heavy metals studied decreased in the order As > Cr > Cu > Zn > Pb > Sn. Meanwhile, sediments from S4 and S5 had the same order for As, Cr, Pb and Sn, and a reverse order for Cu and Zn (Cu > Zn). Of the elements

studied, As was present at the highest relative level in the acid-soluble faction (31% and 33% for samples from S1and S2). However, As was hardly detected in the reducible fraction, inferring that As bound to Cr and Cu oxides in the study area was very low. Comparing the results obtained from the two sampling sites S1 and S7, data for As distribution patterns in sediments differed. The dominant proportion of As was found in the oxidizable fraction (50%) in the S1 and S2, likely due to the higher content of organic matter and sulfides which are substances that can be easily oxidized. In contrast, at S3, S4, S5, the highest concentration (50%) was found in the residual fraction – the most chemically recalcitrant and least bioavailable in sediments. Unlike As, for the heavy metals analyzed, Cr was the least mobile in sediments from both sampling sites with the highest percentage in the residual fraction (79% and 83% for S1 and S2). Again, these results are in agreement with the findings from Barcelona, Spain [52]. Other studies carried out in Spain and in China also show that Cr was found mainly in the residual fraction in all samples [49-53]. These results indicate that Cr has the strongest association to the crystalline structures of sediments. In the present study, similar results for the speciation of Cr were observed for both sampling sites S1 and S2. Copper was mainly bound to organic matter and sulfides, particularly in sediments from S1 and S2, where the highest percentage was found in the oxidizable fraction. The high stability constant of organic Cu compounds results in stable complex formation between Cu and organic matter [51]. It has been shown in other studies that under oxidizing conditions, the solubility of Cu is increased, as it is a chalcophile element that is mainly bound to sulfides in nature [64, 65].

The results agree with the results of other studies [52, 66], which found that a large proportion of Cu in sediments is associated with the organic fraction.

The highest percentages of Ni and Pb were found in the residual fractions in both S1 and S2 (55% and 56%, respectively) and S3, S4, S5 (77% and 62%, respectively), indicating that these metals were strongly bound to sediments. Yuan in (2004) reported that Pb was dominant in the residual fraction of marine sediments from the East China Sea [53]. With regard to Pb, all sampling sites have a similar distribution pattern. Indeed, most of the Pb is present in the residual fraction, followed by the reducible fraction (bound to Fe and Mn oxides), at values of 40% and 33% for sediments from S3 and S4 respectively. This is in agreement with results reported by Morillo (2004) which showed that Cr and Cu hydrous oxides are important scavengers of Pb in sediments [51]. With respect to Zn, for sediments in S3, 81% of the total content was in the

non-residual fractions, while most of Zn was found in the residual fraction (61%) in sediments from S4 and S5, indicating that under changing environmental conditions Zn in sediments from S1 and S2 is potentially more available compared to S3 and S4. The highest value for Zn was observed in the oxidizable fraction (bound to organic matter and sulfides) in sediments from S1 and S2. This may result from the input of organic matter from the nearby palm oil tress plantation and Raja Muda reserve forest.

*5.4. Variation of metal distribution patterns with depth*

To investigate variation of distribution patterns of metals in the non-residual fractions with depth, two sediment cores (with lengths of 40 and 50 cm, respectively) were collected at S2 and S4. The reason for choosing these two sites for this study is that the S2 site is the middle of the catchment where most of the mining activities have been carried out, while S4 is at the junction of the catchment to Ayer Hitam River (recipient of catchment water) and is more polluted than the others [67].

*5.4.1. Fraction A*

The variation of element concentration in fraction A with depth at S2 and S5 is shown in Fig. 2. The concentrations of metals in the top sediments are the highest compared to other depth subsamples for most of the elements at the two sampling sites except for As at S4 and Cr at S5. Variation trends of concentration for each element in the two sample cores were similar. The surface enrichment may be due to contamination deposited from the surface waters, which also indicates that the pollutions have been posed in recent years. This is because the pollution is always absorbed into top sediment at first, and then sinks into deeper positions by chemical exchange. At the same time, reductive dissolution of Cu–Cr oxides at depth may also result in the release of any sorbed heavy metals. The fact that much higher concentrations of As, Pb, Zn and Sn in the fraction A in the top sediments were found at S4 than that at S5 indicates that S4 suffered much heavier pollution from these elements than S5. The discrimination became small with the deepening of depth, especially when the depth was deeper than 20 cm. This phenomenon can be interpreted by the fact that the elements in the fraction A were mainly caused by pollution in the top sediments. With the deepening of depth, the effects of pollution become slighter and the distribution of elements mainly depends on the sediments themselves geographically.

### 5.4.2. Fraction B

The concentration variation of elements in the fraction B with depth at S4 and S5 is shown in Fig. 3. Higher concentrations were found in the top sediments for most of the elements except for As and Cr. It should be noted that a much higher concentration of Pb, Cu, Zn and Sn was found in the top sediments at S4 than that at S5. Only 128.2 and 114.2 µg/g Cu were detected in this fraction in the top sediments at S4 and S5, respectively. At deeper positions, Cu in the fraction B could not be detected. The results show that a larger amount of elements may be sorbed by more Cu–Pb oxides in the top sediments than at the deeper positions. That could be interpreted by the fact that the reductive dissolution of Cu–Pb oxides will occur at a deeper position in the absence of significant sulphide, which could fix the Cu and Pb as sulphide phase followed by precipitation upon crossing the oxic/anoxic boundary. The freshly formed Cu–Pb oxides may also scavenge a significant amount of upwards diffusing heavy metals. For Zn and As, the variation trends were not similar to those of the other elements. Zn was not detected in this fraction in the top sediments at both sampling sites S4 and S5. Only a small amount of As (3.0 and 2.3 µg/g for S4 and S5, respectively) was detected in the top sediments. The concentration of Zn increased with depth from the top to the 20-cm position and kept good linearity at S8. A similar result as that for Zn is concluded for As at S5 and S4.

### 5.4.3. Fraction C

The variation of element concentration in the fraction C with depth at S4 and S5 is shown in Fig. 4. The variation of the concentrations of elements in this fraction was more complex than the other two fractions. There was no evident rule that could be concluded from the results, but some useful information could be obtained. In both sampling sites, the concentration of Zn increased immediately from the top to the 10-cm depth and then decreased gently. The variation trends of Cu and Cr at site S4 were similar to that at S5. The concentrations of Pb, Zn and As decreased with the depth. As for Pb and Sn, the variation was similar at S4 and S5. Elements in the fraction C mainly bound to various forms of organic matter by complexation and peptization properties of natural organic matter or bioaccumulation in certain living organisms through different ways. So the variation of the elements in this fraction became more complex and irregular than that in the fractions A and B.

### 5.5. Heavy Metals Pollution

To determine the intensity of pollution in sediments of Bestari Jaya, $I_{geo}$ and $I_{POLL}$ indices are calculated. The results which are given in Table 4, clearly indicate that $I_{POLL}$ shows pollution intensity of moderate to very high that are not only compatible with the anthropogenic portion of the elements but also in accordance with the macro benthic studies. It should be pointed out that the very high pollution intensity ($I_{POLL} = 7.7$) for Sn could be due to the mobile nature of Sn in sediments. The chemical partitioning studies may show higher values for Sn and therefore higher pollution intensity is found by $I_{POLL}$. Fig. 5 shows relationships between metals and physical parameters in the sediments of Bestari Jaya. It is clear that all studied metals are strongly related to pH and organic contents in sediments. Based on bioavailability cluster analysis, all studied metals have the same behaviour and positive strong relation. It reveals that increase in pollution discharge in the study area can enhance adsorption of heavy metals in organisms.

Finally, we concluded that the $I_{POLL}$ index can be effectively used to show environmental pollution more meaningfully. Since $I_{POLL}$ uses background concentrations of metals within the area of study, it provides better results than other pollution indices. Though cluster analysis is an efficient tool to know the inter relationship amongst various parameters, it fails to provide quantitative information.

### 6. Conclusions

The sequential extraction (SE) procedure is advantageous, as it enables us to evaluate a potential environmental availability of heavy metals associated with specific sediment phases under various environmental conditions. In particular, a major proportion of heavy metals studied (25 to 50 %), being associated with mineral lattices is essentially unavailable and it is not expected to be released into solution over a reasonable time span under the condition normally encountered in mining wastewaters. On the other hand, the next considerable proportion of the heavy elements (27 to 44 %), associated with Cr-Pb coatings, is probably available as Cr-Pb oxides, thermo-dynamically unstable under anoxic conditions. The rest of the metals, i.e. organics-associated (10 to 20 %) may also be available. Exchanged and adsorbed metals, although readily available, represent a relatively minor percentage (< 1 %). Finally, most As can be easily available, as it was identified in the exchangeable and carbonate forms only. The SE procedure provides useful technological information about separation methods (e.g. flotation) that could be used in cleaning the sediments. Moreover, in order to identify the

storage of heavy metals in ex-mining sediments, total metal contents have been obtained. This represents the first such study of its kind in Malaysia. Overall, the total metal contents in the sediments in Malaysia are lower. However, since sediments can act as a reservoir for heavy metals in the aquatic system, their potential risks to the environment must be considered.

## Acknowledgment

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