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Bioconcentration and Translocation Efficiency of Metals in Paddy (*Oryza sativa*): A Case Study from Alor Setar, Kedah, Malaysia

(Biopemekatan dan Kecekapan Translokasi Unsur Logam dalam Padi (*Oryza sativa*): Suatu Kajian Kes dari Alor Setar, Kedah, Malaysia)

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ABSTRACT

*This study aimed to measure and compares the concentration of metals accumulated in various parts (grains, stems and roots) of paddy (*Oryza sativa*). Thirty samples were collected from selected paddy field in Alor Setar, Kedah, Malaysia. Metals (^{75}As, ^{9}Be, ^{114}Cd, ^{59}Co, ^{52}Cr and ^{208}Pb) concentration in various parts of the paddy and soil were analysed by using the sensitive Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Bioconcentration factor (BCF) and translocation ratio were calculated based on the concentration of metals obtained. The mean concentration (mg/kg) of metals in grain samples were 0.06±0.12 for ^{75}As, 0.0038±0.0037 for ^{9}Be, 0.01±0.01 for ^{114}Cd, 0.14±0.19 for ^{59}Co and 0.21±0.15 for ^{208}Pb while ^{52}Cr concentration in all samples were below the ICP-MS detection limit. From the calculated translocation ratio, absorption of paddy had relation: root > stem >> grain. This study showed that measured concentration of metals in grain samples were all below the maximum permitted proportion (mg/kg) of Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation 1985.*

*Keywords: Bioconcentration factor (BCF); metals; paddy (*Oryza sativa*); translocation ratio*

ABSTRAK

*Kajian ini bertujuan untuk mengukur dan membandingkan kepekatan unsur logam yang terkumpul di dalam pelbagai bahagian (bijian, batang dan akar) padi (*Oryza sativa*). Tiga puluh sampel telah dikumpulkan dari sawah padi yang terpilih di Alor Setar, Kedah, Malaysia. Kepekatan unsur logam (^{75}As, ^{9}Be, ^{114}Cd, ^{59}Co, ^{52}Cr dan ^{208}Pb) dalam pelbagai bahagian padi dan tanah telah ditentukan dengan menggunakan peralatan sensitif Spektrometri Jisim-Plasma Gandingan Induktif (ICP-MS). Faktor biopemekatan (BCF) dan nisbah translokasi telah dikira berdasarkan kepekatan logam yang diperoleh. Purata kepekatan (mg/kg) unsur logam dalam sampel bijian adalah 0.06±0.12 untuk ^{75}As, 0.0038±0.0037 untuk ^{9}Be, 0.01±0.01 untuk ^{114}Cd, 0.14±0.19 untuk ^{59}Co dan 0.21±0.15 untuk ^{208}Pb manakala ^{52}Cr kepekatan dalam semua sampel adalah di bawah had pengesanan ICP-MS. Daripada nisbah translokasi yang dikira, hubungan penyerapan padi: akar > batang >> bijian. Kajian ini menunjukkan bahawa kepekatan unsur logam dalam sampel padi yang diukur adalah di bawah nisbah maksimum yang dibenarkan (mg/kg) oleh Jadual Keempat Belas (Peraturan 38) Peraturan-Peraturan Makanan Malaysia 1985.*

*Kata kunci: Faktor biopemekatan (BCF); nisbah translokasi; padi (*Oryza sativa*); unsur logam*

Introduction

Metal pollution is mainly cause by anthropogenic activities such as agriculture, mining, construction and industrial processes (Singh et al. 2004; Yap et al. 2009). Some metals such as copper and zinc are essential nutrients while others such as arsenic and lead possess harmful influences on ecosystem and human health if present in excessive amount. The presence of these metals are a health hazards to living organisms due to their persistence, non-biodegradable and non-thermodegradable characteristic in the environment (Sharma et al. 2007; Yap et al. 2009). Pollutants in the soil can be absorbed by the plants through their roots via vascular system. The uptake of metals in excessive amount may either cause harm to the plants itself or enter the food chain and accumulate when these plants are taken up. Metals accumulated in human body through the food chain can affect the central nervous system and cause many diseases (Coen et al. 2001; Zhao et al. 2009) potentially to infant and children.

Rice is the predominant food in developing countries. According to Hossain and Narciso (2004), about 90% of rice is produced and consumed in Asia, and 96% in developing countries such as Malaysia. Kedah, Malaysia is the largest paddy plantation areas, which is under the Muda Agricultural Development Authority (MADA). Therefore, it plays an important role in supplying sufficient rice to the nation. Generally, paddy is grown twice a year during the wet and dry seasons (Khairiah et al. 2009). Its cultivation starts at the beginning of wet season and the seedling is transplant between July and September. Paddy is planted on cohesive black-grey colour clayey soil. This kind of clayey soil is very effective for paddy plantation because it can retain moisture for a long period (Kuchiba et al. 1951). There are various steps being taken in order to increase the productivity of paddy such as through the usage of new high yielding varieties, improved fertilizer and effective pest management activities (Khairiah et al. 2009). However, most of the farmers used chemical fertilizers in order to increase their productivity (Kuchiba et al. 1951). Heavily fertilization may results in metals uptake and accumulation at various parts of the plant.

Recently, several studies on paddy in relation to metal pollution have been carried out by a number of researchers in different regions of the world (Fu et al. 2008; Khairiah et al. 2009; Nawaz et al. 2006; Rahman et al. 2009; Rauf et al. 2011; Yang et al. 2004; Yap et al. 2009; Zazoli et al. 2006; Zwicker et al. 2010). In Malaysia, only a few notable studies on metal distribution of paddy was carried out by Khairiah et al. (2009) and Yap et al. (2009). Most of the studies done are focusing on total metal content in paddy. Current studies on bioaccumulation and translocation efficiency are lacking in Malaysia. Therefore, the objectives of this study were to determine the content of metals accumulated in various parts of paddy and to calculate bioconcentration factor (BCF) and translocation ratio of metals in paddy. The concentrations of metals were compared with permissible limit as stipulated by Malaysian Food Regulation (1985).

Study Areas and Methods

SITE DESCRIPTION

The study area is located in Kedah, which is known to be major paddy plantation areas in Malaysia, with approximately 106 632 ha under paddy cultivation. Kampung Jeruju which located in Mukim Jerlun, Kubang Pasu district, Alor Setar, Kedah, was chosen as sampling sites in this study (Figure 1).

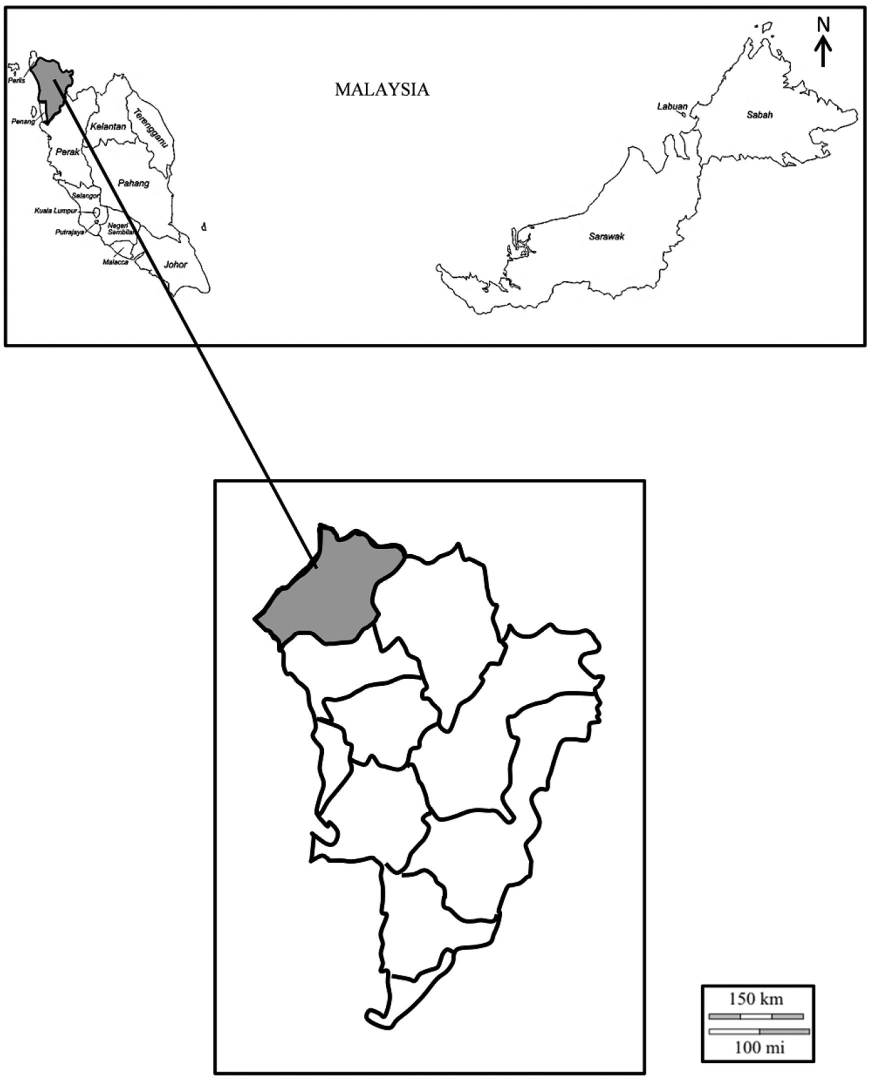

FIGURE 1. Kubang Pasu District, Alor Setar, Kedah

SAMPLES COLLECTION AND PRESERVATION

Ten sampling points were selected randomly where 3 clumps of paddy were collected as triplicated samples from each point. Top soils (0-15 cm) near the root zone were collected and used as background value of metals in the study area. The samples were kept in an acid washed zip-lock polyethylene bag and preserved at temperature less than 4°C before brought back to the laboratory for analyses. In the laboratory, paddy samples were washed with deionized water. Then, plants were divided into 3 parts which are root, stem and grain. The soil and paddy samples were oven-dried at 30°C to achieve complete dryness, followed by crushed using mortar and pestle. The soil samples were sieved through 2 mm mesh sieve, while paddy samples were sieved through 1 mm mesh sieve, respectively. The sieved samples were stored in acid washed polyethylene centrifuge tubes before proceed to digestion procedures.

SAMPLES DIGESTION AND ANALYSIS

The soil and paddy samples were digested using nitric-acid hydrogen peroxide (HNO_3-H_2O_2) digestion method (USEPA 1996). This method does not measure the true total metal concentration since metals bound up in crystal lattice of silicate minerals are not released. However, it gives a reasonable measurement of those metals that are available in the soil (USEPA 1996).

About 0.1 g of paddy sample was weighted and placed into a beaker with 10 mL of ratio 1:1 H_2O to HNO_3 (5 mL water + 5 mL concentrated HNO_3). The beaker was heated (95°C ± 5°C) on a hotplate for 15 min and covered with a petri dish in order to ensure heated sample does not evaporate away to the environment. The sample was cooled and 5 mL concentrated HNO_3 was added. It was heated for another 30 min. The step was repeated with an addition of 5 mL of HNO_3 until no brown fumes were given off by the sample in order to ensure complete digestion of the sample with HNO_3. The sample was left cooled and 2 mL water and 3 mL 30% H_2O_2 were added. The beaker was covered and heated gently. Repeatedly adding 1 mL aliquot of 30% H_2O_2 and heating until effervescence subsides. The sample was continued to be heated at 95°C ± 5°C without boiling for 2 h. A covering of solution was maintained over the bottom of the beaker at all times. After that, the sample was cooled and filtered through a cellulose acetate membrane filter paper (Whatman Milipores, Clifton, NJ) with pore size of 0.45 µm into a 100 mL volumetric flask. Finally make up to the mark with deionised water. The digested samples were analysed by using ICP-MS (Perkin Elmer ELAN DRC-e) to determine the concentration of metals.

QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

QA/QC procedures are essential in order to produce highly reliable and representative data (Praveena & Aris 2012). In the field, samples were collected in triplicate to estimate the variability resulted from the sampling activities. In addition, all containers used in the analysis were pre-washed with phosphate-free detergent solution before soaking in 5% HNO_3 for 24 h to ensure minimum mineral contamination. All reagents used in this study were of analytical reagent grade. Ultrapure water (water sensitivity >18.2 Mohms·cm at 25°C; Millipore, MA, USA) was used for laboratory applications includes reagents, blanks and standard preparation. Furthermore, in order to assess the accuracy of instrument performance, a series of standard solutions were prepared freshly by diluting stock standard solutions (ICP Multi-Element Mixed Standard III, Perkin Elmer) with ultrapure water. Apart from that, method detection limit was determined by calculate limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ are the concentration of the analyte corresponding to sample blank value plus three and ten standard deviation, respectively (Sanagi et al. 2009). The LOD and LOQ were calculated using the formulas below:

$$LOD = \frac{3 \times SD_{blank}}{b} \quad (1)$$

$$LOQ = \frac{10 \times SD_{blank}}{b} \quad (2)$$

where SD_{blank} is the standard deviation of blank samples and b is the slope of calibration curve.

In this study, the LOD obtained for As, Be, Cd, Cr, Co and Pb were 0.0005, 0.000002, 0.01, 0.0002, 0.000004 and 0.00003 µg/L, respectively, while LOQ calculated for As, Be, Cd, Cr, Co and Pb were 0.0016, 0.000008, 0.03, 0.0006, 0.000014 and 0.00009 µg/L for Pb, respectively.

RESULTS AND DISCUSSION

METAL ANALYSIS

Seven metals (^{75}As, ^{9}Be, ^{114}Cd, ^{59}Co, ^{52}Cr, ^{208}Pb and ^{80}Se) were selected for screening purpose in soil samples. Based on the screening result, 6 metals (As, Be, Cd, Co, Cr and Pb) were found to be in ICP-MS detectable concentration except for Se. Concentration of metals in soil samples collected from the study area is shown in Table 1 and the distribution of various metals in different parts of paddy (root, stem and grain) is shown in Figure 2.

Distribution of various metal concentration in the root, stem and grain of paddy was found to be uneven ($p < 0.05$). In general, higher concentrations of metals were found in the roots, compared to the stem and grain ($p < 0.05$). According to Liu et al. (2009), roots act as barrier for metal translocation thus protecting stem and grain parts from metal contamination. Among all the metals, the highest arsenic (As) concentration (4.62 mg/kg) was found in the root of paddy ($p < 0.05$). This is because the presence of iron plague on root surface is highly associated with accumulation of arsenic. The release of oxygen and oxidant in rhizosphere will induce

TABLE 1. Concentration of metals (mg/kg) in soil samples of the study area (n=10)

Metal/Soils	As	Be	Cd	Co	Cr	Pb	Se
Paddy Soil (mean ± SD)	0.60 ± 0.02	0.27 ± 0.02	0.20 ± 0.01	1.83 ± 0.03	2.30 ± 0.02	3.72 ± 0.02	BDL*

BDL*= Below Detection Limit

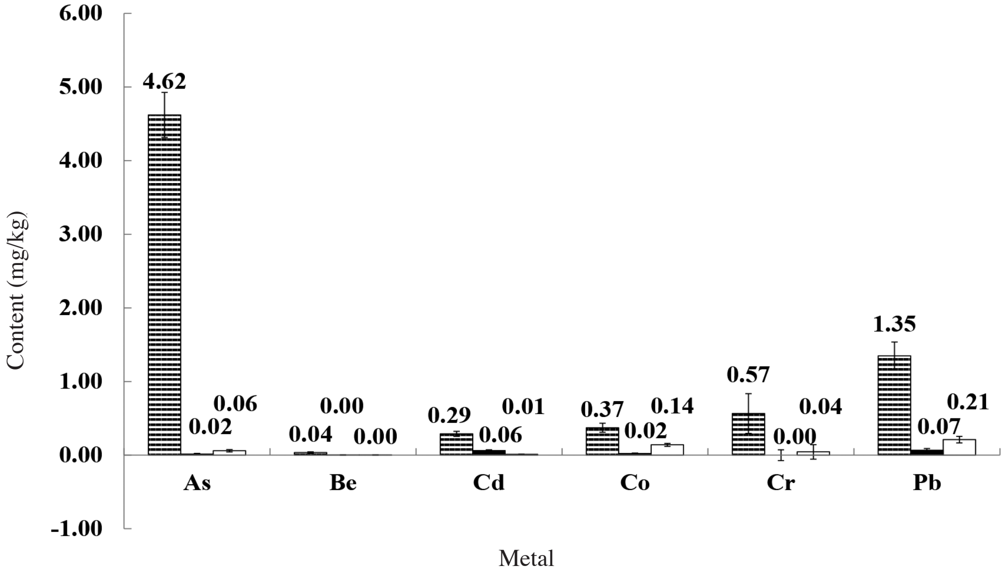

FIGURE 2. The distribution of various metals in the root, stem and grain of paddy (error bars are SD)

the formation of iron plague, which has high affinity to arsenate (Liu et al. 2006).

From Figure 2, beryllium (Be) has different distribution in root, stem and grain ($p< 0.05$). The highest concentration (0.04 mg/kg) of Be was found in the root part of paddy. Likewise for metals Cd, Co and Pb, significant differences were found between various parts of paddy ($p< 0.05$). However, Cr concentration was found in the root zone of paddy but in stem and grain, the Cr was below ICP-MS detectable limit. These suggest that each metal has different distribution in root, stem and grain parts of paddy. In general, the concentration of all metals were higher in root than in the stem and grain parts for paddy.

The content of all metals in grain samples analysed were below the maximum permitted proportion (mg/kg) of Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation 1985 (Table 2). This suggests that the rice planted on the study area is safe for consumption.

BIOCONCENTRATION FACTOR (BCF)

Biocencentration factor (BCF) is the ratio of the content of metals in various parts of paddy to that in the soil (Liu et al. 2009). Figure 3 shows the BCF values for As, Be, Cd, Co and Pb in paddy. If a BCF ≤ 1.00, it indicates that the plant can only absorb but not accumulate metal. On the other hand, if a BCF > 1.00, it means that the plant have the potential to accumulate metal (Liu et al. 2009).

Arsenic BCF value for various parts of paddy were 38.40 (root), 0.13 (stem) and 0.49 (grain). Based on the BCF, paddy can only accumulate As up the root zone (BCF>1). Since BCF value for stem and grain parts of paddy were less than 1.00, As can be absorbed from the soil but do not accumulate in these parts. Therefore it can be concluded that As bioavailability was very high in the root zone but low in stem and grain ($p< 0.05$). There are significant difference of Be and As uptake in various parts of paddy ($p< 0.05$). From Figure 3, BCF values of Be in root, stem and grain were less than 1.00. Bioavailability of Be was very low in the soil as well as in paddy. Therefore, paddy can absorb Be but does not accumulate it.

There was significant difference between BCF values of Cd in various parts of paddy ($p< 0.05$). In Figure 3, BCF value of Cd in the root and stem had exceeded value 1.00 thus indicating that the root and stem can absorb and accumulate Cd. Whereas BCF value of Cd in grain was less than 1.0 indicating grain in paddy can only absorb but not accumulate Cd. According to Liu et al. (2009), Cd uptake was strongly correlated with organic matter. From previous studies, researchers found that winter wheat and rice root accumulated high quantities of Cd^{2+} when grown in non-pollution area as in a medium containing Cd. Plants can response to Cd by synthesizing sulphur-rich peptides, phytochelatins (PCs). PC-heavy metal complexes were reported to accumulate in vacuoles of plants. The retention of Cd in the root cell vacuole might influence the

TABLE 2. Concentration of metals in paddy parts compared with maximum ermitted proportion of Malaysian Food Regulation 1985

Elements	Content of Heavy (mg kg^{-1}) (n = 30)			[(a)]Maximum permitted proportion (mg/kg)	[(b)]Minimal Risk Levels (mg/kg/day)
	Paddy parts	Range	Mean ± SD		
As	Roots	33.19	23.10 ± 12.67	1	***0.005
	Stems	0.50	0.08 ± 0.15		*0.0003
	Grains	1.91	0.29 ± 0.58		
Be	Roots	0.26	0.18 ± 0.11	NA	*0.002
	Stems	0.01	0.0038 ± 0.0037		
	Grains	0.01	0.0038 ± 0.0037		
Cd	Roots	2.16	1.45 ± 0.68	1	*0.0001
	Stems	0.27	0.31 ± 0.08		**0.0005
	Grains	0.15	0.06 ± 0.04		
Co	Roots	2.68	1.86 ± 1.06	NA	**0.01
	Stems	0.24	0.11 ± 0.08		
	Grains	2.51	0.70 ± 0.94		
Cr	Roots	9.57	3.64 ± 4.44	NA	#*0.001
	Stems	BDL	-		
	Grains	BDL	-		
Pb	Roots	8.63	6.74 ± 3.04	2	NA
	Stems	1.02	0.33 ± 0.32		
	Grains	2.29	1.05 ± 0.76		

(a) Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation1985
(b) ATSDR Minimal Risk Levels (MRLs) List
*** Acute exposure duration (1-14days) for oral route of exposure
** Intermediate exposure duration (>14-364 days) for oral route of exposure
* Chronic exposure duration (365 days and longer) for oral route of exposure
# Chromium (VI)
NA No available standards
BDL Below Detection Limit

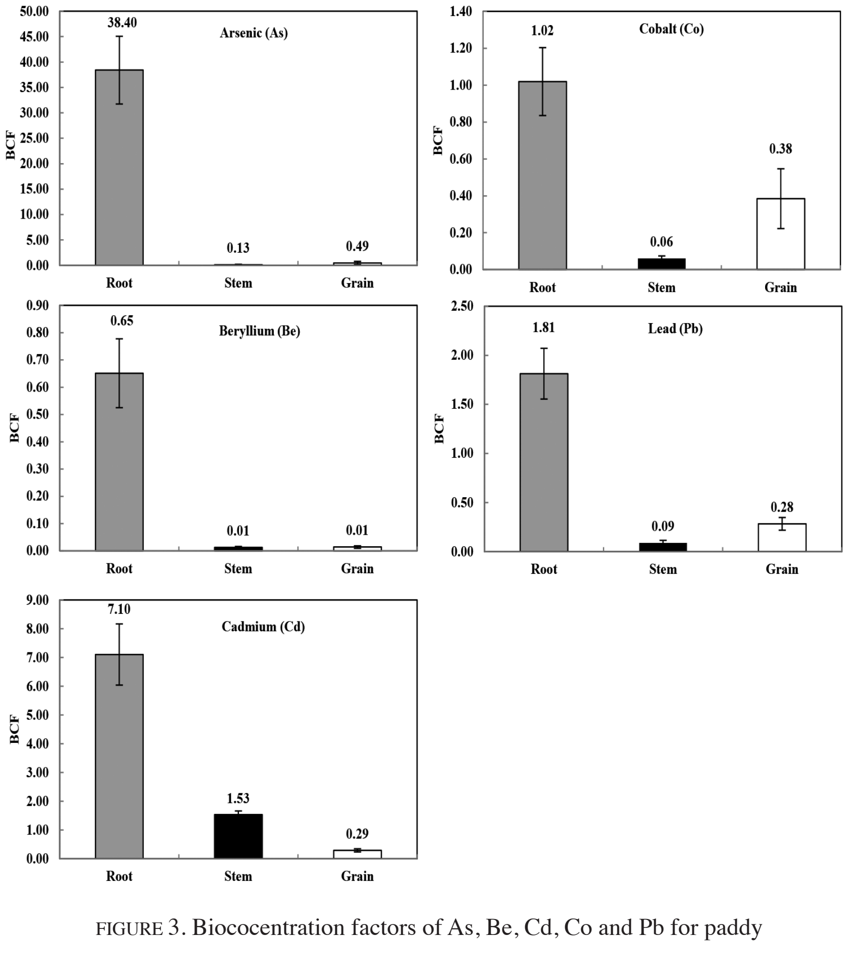

FIGURE 3. Biocentration factors of As, Be, Cd, Co and Pb for paddy

symplastic radial Cd transported to the xylem and shoot (Liu et al. 2009; Stolt et al. 2003).

BCF values of Co were found to have significant differences between various parts of paddy ($p < 0.05$). BCF value of Co for root was slightly greater than 1.00, meaning that root can absorb and bioaccumulate Co. However, stem and grain were all below 1.00. The stem and grain can therefore absorb Co from the soil but do not accumulate it. According to Zarcinas et al. (2004), Co was associated with Al and Fe which means this element was intrinsically associated with soil matrix.

The BCF value of Pb for paddy root was greater than 1.00 indicating the root can accumulate Pb. However, the BCF values for stem and grain were less than 1.00. The significant differences of Pb BCF values were found between root, stem and grain ($p < 0.05$). Therefore the stem and the grain can only absorb Pb but do not accumulate it. According to Khairiah et al. (2009), Pb was relatively stable in soils. The presence of Pb in soil is mostly contributed by natural soil deposits. Researches claimed that organic matter and clay are the dominant constituents contributing to Pb adsorption. Pb has a tendency to bind with oxides of Fe and Mn in soils. Therefore, Pb in the unavailable form might be caused by the clayey nature of soils and high amount of organic matter as well as Fe and Mn. The study site is located in rural areas, therefore the soil was quite unlikely to have received anthropogenic Pb which usually is originated from automobile exhausts and industrial activities (Khairiah et al. 2009).

TRANSLOCATION RATIO

Translocation ratios from root to stem and stem to grain, were calculated for selected metals under study. The translocation ratio was calculated based on (3) and (4) (Liu et al. 2009).

$$HM_{stem} / HM_{root} \quad (3)$$

$$HM_{grain} / HM_{stem} \quad (4)$$

where HM_{stem} represents metal concentration in stem; HM_{root} represents metal concentration in root; and HM_{grain} represents metals concentration in grain.

Figure 4 shows translocation ratios of As, Be, Cd, Co and Pb in paddy. All the values were below 1.00 from root to stem. However, the translocation ratios from stem to grain were all above 1.0 except for Cd. Generally, translocation ratio for As, Be and Pb has a significant difference between stem and grain of paddy ($p<0.05$). From Figure 4, translocation ratio of As, Be and Pb from root to stem was less than that from stem to grain. It indicates that these metals in the root zone transported weakly to the stem but somehow easily transported to grain when they are available in the stem of paddy.

However, no significant difference were found in translocation ratio for Cd between stem and grain of paddy ($p>0.05$). This indicated that translocation ratio of Cd from root to stem was more or less the same compared to translocation ratio of Cd from stem to grain. Therefore, Cd available in the stem was more or less the same as those in the grain part of paddy. Besides, Co translocation ratio was found to have no significant differences from root to stem and stem to grain.

Therefore, for six metals, absorption order of paddy was root>stem>>grain. Additionally, the result showed that paddy transported all the metals very weakly into the stem (translocation ratio < 1.00) as shown in Figure 4. It is important to note that metals were transported weakly into the grain of paddy as root and stem act as barrier for the metal contamination. The results of this study agreed with the finding done by Liu et al. (2009) on the study of accumulation and translocation ratio of toxic metals in winter wheat. According to Liu et al. (2009), the winter wheat transported Cd, Cr, Pb, As and Hg very weakly from root to grain in various irrigation regions. This might be due to the fact that metals under studied have different chemical properties and thus each metal has peculiar accumulation and translocation capacity (Liu et al. 2009).

CONCLUSION

The findings of this study showed that As, Be, Cd, Co and Pb were present except Cr in various parts of paddy collected from a paddy field in Alor Setar, Kedah, Malaysia. Higher concentration of studied metals was found in the root, compared to the stem and grain ($p < 0.05$) in all samples. The concentration of metals (As, Cd and Pb) in grain samples were below the maximum permitted proportion (mg/kg) of Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation 1985. Based on BCFs values of As, Be, Cd, Co and Pb, it was found that root can absorb and accumulate higher amount of metals available in the soil sample. However, the grain only absorbs metals from the soil but not accumulate them. From the calculated translocation ratio, absorption of paddy had relation: root > stem >> grain. Overall, this study indicates that the vast majority of rice planted in the study area is safe according to Fourteenth Schedule (Regulation 38) of the Malaysian Food Regulation 1985. These results could provide valuable baseline data on food safety in Malaysia.

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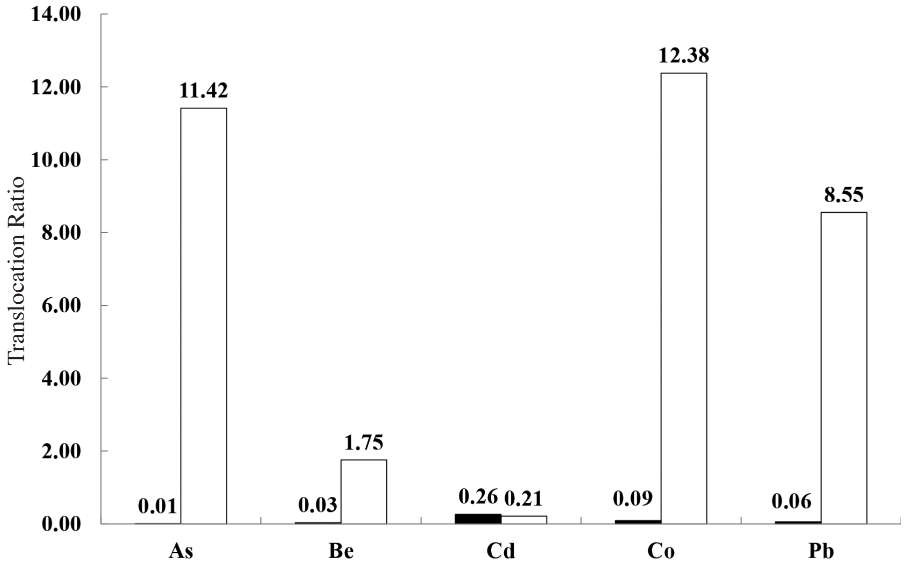

FIGURE 4. Translocation ratios of metals from root to stem and stem to grain of paddy

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