Assessment of heavy metals accumulation in paddy rice (Oryza sativa)

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Heavy metals contamination in paddy field was conducted using Oryza sativa. Paddy soils and rice plants were sampled along seven transverse lines of the paddy field before the harvesting time. The paddy plants were separated into roots, stems, leaves and grains. Metal concentrations of the samples were carried out using ICP-OES Spectrometer machine OPTIMA 5300 DV. Metals enrichment factor and translocation were also measured in this study. The results showed that the plant roots of O. sativa accumulated relatively large amount of lead, cadmium, chromium and copper, while zinc highest in the stem. The correlation test showed that there was a strong correlation for zinc and cadmium concentrations in O. sativa with the paddy soil. Enrichment factor (EF) showed that lead, cadmium and copper were concentrated in the root of O. sativa, while chromium and zinc metals were more concentrated in the shoot part. Translocation factor (TF) showed that Oryza sativa was able to transfer zinc and chromium from root to the shoot part of O. sativa. O. sativa has the potential of being used for the phytoremediation of zinc and chromium from the paddy soil.

Key words: Heavy metals, Oryza sativa, enrichment factor (EF), translocation factor (TF).

INTRODUCTION

The accumulation of heavy metals in agricultural soils is a growing concern to the public as well as government agencies, due to the food safety issues and potential health risks as it has detrimental effects on soils ecosystems (McLaughin and Singh, 1999; Yanez et al., 2002). Heavy metals such as arsenic, cadmium, and mercury, are of primary concern in soil and food contamination, particularly in rice cropping system, because of their toxicity (Reeves and Chaney, 2001). These toxic elements accumulate in the soils, induce a potential contamination on food chain, and endanger the ecosystem safety and human health (Reynders et al., 2008).

Sources of heavy metals in soils are mainly from natural occurrence, derived from the parent materials and from human activities (anthropogenic sources). Anthropogenic inputs are associated with industrialization and agricultural activities such as atmospheric deposition, vehicle exhaust, waste incineration, waste disposal, urban effluent, fertilizer application and the traditional application of sewage sludge as the fertilizer in agricultural land (Bilos et al., 2001; Hlavay et al., 2001; Koch and Rotard, 2001).

Crops have different abilities to absorb and accumulate metals in their different part, and a wide variation in metal uptake and translocation between plant species and even...
between cultivars of the same species (Kurz et al., 1999; Arao and Ac, 2003; Liu et al., 2005; Yu et al., 2006). Plants absorb heavy metals from the subsurface of 25 cm depth zone of the soil where roots of most cereal crops are located (Ross, 1994; Mico et al., 2007). Once the adsorption of the heavy metals in the soil is saturated, more heavy metals would be distributed in the aqueous phase and the bioavailability of the heavy metals would subsequently be enhanced (Sridhara et al., 2008). Heavy metals in soil with high concentration will increase the potential of being taken up by plants. The pollutants will then be translocated from roots to shoots then to the grains, which is consumed by human population. Toxic metals are mostly geochemically mobile where they are readily taken up by plant roots and translocated to aerial parts (Satarug et al., 2003).

In Asia, rice is the most common crop grown on agricultural land. The total area of paddy fields has been estimated as over 1 million hectares and up to 50% of these are well cultured with high production of rice. According to Kitagishi and Yamane (1981), the dietary cereal intake, mainly rice by Asian is 574 g/day, which is about half of the total dietary intake. Thus, heavy metals existence in rice may have a large influence on metal intake by the human population. Therefore, this study was conducted to determine the extent of heavy metals (Pb, Cd, Cr, Cu and Zn) uptake by paddy plant (Oryza sativa) influenced by agricultural soils.

**MATERIALS AND METHODS**

**Study area**

The paddy fields were located at Kompipinan, Papar district, Sabah near quarries and main highway that link Papar district to Kota Kinabalu, within the area of latitude 05° 42' 08.7" N to 05° 42' 12.6" N and from longitude 115° 57' 15.6" E to 115° 57' 13.8" E. The sampling stations are shown in Figure 1. Seven sampling points within that area were chosen for this study. The exact position and coordinate for each sampling stations are shown in Table 1. Soil samples from the root zone were also taken.

**Sample collection**

Random sampling was carried out at the study site and the sampling frequency is twice (2) at each sampling points. The sample collections were conducted before harvesting time in the paddy field. Each paddy plant was uprooted together with its root, stems, leaves and seed. The soil samples were collected from the surrounding roots of the paddy plant using Ponar Grab sampler at a depth of 0 to 25 cm from the soil surface (Machiwa, 2010). The paddy plants and soil samples were inserted into labeled plastic bags and placed on ice while transported to the laboratory, then kept in the refrigerator at 4°C for preservation before analysis.

**Figure 1.** Map of Kompipinan, Papar district, Sabah showing the sampling stations for paddy plants and soil.
Table 1. Location of sampling stations for paddy plants and soil sample.

| Station No. | Location               |
|-------------|------------------------|
| 1           | N 05° 42 08.7 E 115° 57 15.6' |
| 2           | N 05° 42 10.6 E 115° 57 15.0' |
| 3           | N 05° 42 12.6 E 115° 57 13.8' |
| 4           | N 05° 42 12.4 E 115° 57 13.1' |
| 5           | N 05° 42 10.6 E 115° 57 13.8' |
| 6           | N 05° 42 10.3 E 115° 57 14.5 |
| 7           | N 05° 42 08.2 E 115° 57 15.1' |

Sample preparation

The paddy plants were washed using deionized water to remove residues of impurities, then separated into the roots, stems, leaves and grains. Each part was cut to smaller sizes for easier digestion. The grains were finely ground using mortar and pestle to separate the white rice from its husk, and then dried in an oven at 65°C for 24 h. Soil samples were manually cleaned to remove remnants of roots, stones, twigs and other impurities. The soil samples were then homogenized and dried at room temperature. The dried soil samples were pulverized using mortar and pestle, and sieved to obtain <63 µm size.

Digestion

1 g dried samples of roots, stems, leaves, seeds from O. sativa and soil samples were placed into 50-ml conical flask. Each parts of the O. sativa were digested in 10 ml of 65% nitric acid solution and heated on a hot plate with temperature of 120°C for 2 h until it produce clear solution. 1 g of soil samples were digested with 10 ml of aqua-regia HNO₃: HCL solution with 3:1 ratio and then heated on a hot plate with 70°C temperature for 4 h. After cooling, the O. sativa and soil samples were then diluted by 50 ml of deionized water and filtered with 45 µm size Whatman filter paper. Samples that have been filtered were placed into the polyethylene bottles and kept in 4°C refrigerator until heavy metal analysis were done.

Quantification of heavy metals

Heavy metal concentrations were determined using ICP-OES Spectrometer machine OPTIMA 5300 DV for lead, cadmium, chromium, copper and zinc elements. A quality control program was applied which includes duplicate samples, in house reference materials, reagent blanks and certified international reference materials (Ramsey et al. 1987; APHA, 1995; Eaton, 2005). The precision and bias of the chemical analysis was less than 10%.

Enrichment factor (EF) and translocation factor (TF)

Quantification of the enrichment factor and translocation factor are shown in the Equation (1) from Lorestani et al. (2011) and Equation (2) from Singh et al. (2010). The value of enrichment factors indicated the mechanism of heavy metals absorption from soil to O. sativa; however the translocation factor is to examine the transport or transfer of the heavy metals from roots to shoots of O. sativa. The EF and TF values for heavy metals that were examined in this study are in mg/kg.

Enrichment Factor (EF) = \[
\frac{C_P}{C_S}
\]

Where

C_P = heavy metal concentration in plant
C_S = heavy metal concentration in soil

Translocation Factor (TF) = \[
\frac{C_S}{C_R}
\]

Where

C_S = heavy metal concentration in plants' shoot
C_R = heavy metal concentration in plants' root

RESULTS

Concentrations of heavy metals in Oryza sativa

The mean concentrations of heavy metals in O. sativa in this study showed that all metals were present in all parts of the O. sativa at different levels. Most of the metals concentrated in paddy roots, except for zinc which has the highest concentration in paddy stems as shown in Table 2. Concentrations of Cadmium, Copper and Zinc, except Lead, in paddy rice grains were within FAO/WHO (2002) permissible limits for human consumption.

Distribution of Chromium in O. sativa varied, and was in the order of root > leaf > grain > stem (Figure 2). Bhattacharyya et al. (2005) reported a similar result. Chromium concentration was higher in root than the shoot part due to redox reaction that occurs in the plants which causes the movement of Chromium from root to the shoot part. In addition, Chromium(III) can also react with carboxylic functional groups (COOH) in plants which prevents the translocation of the metals from root to the shoot, thus the Chromium concentration in the shoot part is low (Bhattacharyya et al., 2005).

Copper concentrations varied in descending order from root > grain > leaf > stem (Figure 3). Results obtained in this study were similar to the study conducted by Yap et al. (2009) and Fu et al. (2008). Copper is one of the nutrients needed to process a variety of enzyme activity in plants (Hang et al., 2009). However, the presence of copper metal that exceeded the permissible limits or standards may cause stunted growth of roots and plants (Jiang et al., 2007).

For distribution of Zinc concentration, accumulation occurred in descending order of stem > root > grain > leaf (Figure 4). Zinc accumulated in the plant was due to the absorption of the metal by roots from the plants surrounding soil (Jiang et al., 2007). However Zinc is an important nutrient required by plants to synthesize proteins, for hormones growth and reproductive processes of plants. Adequate Zinc concentration in the leaves of plant is 15 to 50 mg/kg. Nevertheless, at levels above 200 mg/kg of Zinc will cause toxicity to the plants which can cause the stunted root growth and leaf undersized (Jiang et al., 2007).
Table 2. Mean concentrations (+SD) of metals (mg/kg) in paddy plants and paddy soils.

| Parts   | Lead (Pb)    | Cadmium (Cd) | Chromium (Cr) | Copper (Cu) | Zinc (Zn)   |
|---------|--------------|--------------|---------------|-------------|-------------|
| Root    | 7.70 ± 1.27  | 0.38 ± 0.09  | 5.46 ± 2.26   | 4.94 ± 1.03 | 16.08 ± 1.67|
| Stem    | 0.04 ± 0.02  | 0.11 ± 0.06  | 3.26 ± 1.87   | 0.38 ± 0.06 | 29.60 ± 7.04|
| Leaf    | 0.26 ± 0.14  | 0.11 ± 0.05  | 4.34 ± 2.01   | 0.71 ± 0.18 | 12.40 ± 2.72|
| Grain   | 2.06 ± 2.42  | 0.13 ± 0.08  | 4.12 ± 3.17   | 0.74 ± 0.32 | 12.75 ± 4.42|
| FAO/WHO Standards | 0.2 | 0.2 | - | 20 | 50 |
| Paddy soils | 8.03 ± 1.07 | 0.32 ± 0.40 | 4.16 ± 0.68 | 6.62 ± 0.72 | 13.89 ± 1.63 |

Figure 2. Distribution of chromium (Cr) in Oryza sativa and paddy soil.

Figure 3. Distribution of copper (Cu) in Oryza sativa and paddy soil.
Cadmium concentration showed a decreasing pattern from root > grain > leaf > stem (Figure 5). Cadmium is one of the major pollutants produced in the industrial area (Horng et al., 2013). It is easily absorbed and spread to the whole paddy plant and its bioavailability in the soil. Cadmium metal uniformly dispersed in small
concentrations due to the nature of Cadmium that is readily absorbed by the plant and dispersed to the other plant parts in spite of its properties that does not carry vital nutrients to animals and humans (Yap et al., 2009). Lead concentrations varied in descending order of root > grain > leaf > stem (Figure 6). According to Li et al. (2007), most of the Lead that was absorbed in paddy plant is accumulated highly in roots compared to other parts of the plants. The possibility of Lead presence in O. sativa is believed to be due from the remnants of quarries and mining activities near the agricultural fields (Bliznakovska et al., 2013). Content of heavy metals in the soil also affects the intake of Lead in plants (Kachenko and Singh, 2004). Runoff from the road might also be a major source of Lead, which diffused into the soil and subsequently absorbed by plants that grow at the edge of the road (Xuedong et al., 2012).

DISCUSSION

Concentration of heavy metals in paddy soils

In this study, Chromium concentrations ranged in soil from 3.44 to 5.11 mg/kg. The use of phosphate fertilizers in agricultural activities will increase the Chromium metal content in crops as the fertilizer is manufactured from phosphate ore with high content of heavy metals such as Chromium, Lead and Iron (Saaedia and Nabila, 2013). Copper concentration in O. sativa ranged from 5.54 to 7.86 mg/kg. Low level of copper in shoots part of paddy plants might be attributed to low dispersion and bioavailability of this metal in the soil (Fernandes and Fernando, 1990). Zinc concentrations in the soil ranged from 11.36 to 15.82 mg/kg. Zinc is produced in many environments through natural process and released from the earth's crust (Kennedy and Burlingame, 2003). Cadmium concentrations in the soil ranged from 0.13 to 1.23 mg/kg, while the range for Lead concentrations was between 6.37 to 9.35 mg/kg. Based on the results obtained, metal levels in the soils did not exceed the FAO/WHO (2002) permissible limits, except for Cadmium and Lead.

Enrichment factor (EF) and translocation factor (TF)

The absorption mechanism of heavy metals by O. sativa can be analyzed using enrichment and translocation factor analysis. Figure 7 shows the enrichment factor of metals from the soil to other parts of O. sativa which was in decreasing order of Zn (5.16) > Cd (4.12) > Cr (4.00) > Pb (1.28) > Cu (1.03). Based on the results obtained, the enrichment value for all studied metals were greater than 1, which indicates that the O. sativa is a hyper accumulator plant with high potential to absorb metals from the soil (Lorestani et al., 2011). Translocation factor for selected metals from the roots to the shoots of O. sativa is indicated in Figure 8, and the trend showed that metal levels varied in descending order of Zinc (3.43) > Chromium (1.97) > Cadmium (0.94) >
Copper (0.37) > Lead (0.29). The results showed that only Zinc and Chromium have the translocation factor value greater than 1, which means that O. sativa was able to hyper accumulate only Zinc and Chromium from

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**Figure 7.** Enrichment factors of heavy metals from paddy soil to Oryza sativa.

**Figure 8.** Translocation factor of heavy metals from the roots to shoots of Oryza sativa.
roots to the shoots (Resvani and Zaefarian, 2011).

Statistical analysis

Correlation analysis (P < 0.05) was carried out to study the correlation of metals from *O. sativa* parts with the paddy soil. The results obtained show that there were strong correlations for Chromium in roots with stems (r = 0.87), leaves (r = 0.90) and grains (r = 0.80). Similarly, there was strong correlation for Chromium in the stems with leaves (r = 0.85) and grains (r = 0.93). However, for Copper, there was no significant correlation between all parts, except between roots and stems (r=0.81). There was a negative correlation between leaves and stems for Zinc metal with (r=0.07). Cadmium showed a moderate correlation for roots and grains (r=0.67). Correlation analysis showed that Chromium, Lead and Copper in *O. sativa* did not correlate significantly with the paddy soil. Studies conducted by Horng et al. (2013) indicated that Chromium showed no significant correlation between roots and other parts of *O. sativa*. However, the Chromium concentration showed a significant correlation between the stems, leaves and grains with each other. Miclean et al. (2013) reported a significant correlation for Lead, Cadmium and Copper in plants and its surrounding soil.

According to Horng et al. (2013), the significant relationship between parts of *O. sativa* and paddy soil was due to the presence of some residual soil particles in the plants during the analysis, and most of the heavy metals absorbed by plants were derived from the soil.

Conclusion

Heavy metals in this study accumulated abundantly in the root compared to other parts of *O. sativa*, except for Zinc which showed the highest concentration in the stem. The analysis of enrichment factor showed that Lead, Cadmium and Copper were accumulated more in the root part while Chromium and Zinc were accumulated more in the shoot part. Each heavy metal was present in a different concentration in every part of the plant. The metal content in the *O. sativa* and paddy soil in this study does not exceed the permissible standard set by the FAO/WHO except for Cadmium and Lead. The translocation factor analysis showed that *O. sativa* was able to transfer only Zinc and Chromium metals from the root to the shoot, which implies that *O. sativa* has the potential to be used for the phytoremediation of Zinc and Chromium in paddy soil.

Conflict of Interest

The authors have not declared any conflict of interest.

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