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SHORT COMMUNICATION

Distribution of Selected Heavy Metals Bioaccumulation in Various Parts of Indigenous Rice (*Bokilong*, *Ponsulak* and *Taragang*) in North Borneo

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ABSTRACT

The prospect of three native upland paddy landraces known as *Bokilong*, *Ponsulak* and *Taragang* as heavy metals accumulator for phytoremediation was determined. Bioaccumulation of heavy metals (As, Cd, Cr, Cu, Fe, Pb, and Zn) in various parts of paddy plants collected from Kiulu valley, North Borneo in the natural conditions during the vegetative phase and harvest season were analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). All selected heavy metals were traced in soil samples of all three paddy landraces rhizosphere where the most available heavy metals were Fe followed by Zn. Heavy metals bioavailability in soil seemed to be influenced by the local climate of the cultivation field. *Bokilong* landrace is an accumulator of As, Cd, Cu, Pb and Zn. *Ponsulak* paddy can help clean up the soil by phytoextraction of As, Cr, Cu, Fe and Zn. *Taragang* paddy has a prospect in phytoextraction of Cd and Pb to remediate excess amount of this element in the soil. Different heavy metals concentration trends were accumulated in these three paddy landraces in grain indicated different nutritional values. Heavy metal uptake characteristic differs between upland paddy landraces and there was also environmental influence affecting the mobility rate of these elements in paddy plant depending on the element type and paddy genotype.

Keywords: agrobiodiversity, hill paddy, phytoextraction, *Oryza sativa*, trace elements

Different regions had given birth to different rice landraces that have evolved to adapt with local climates, topography and mineral contents of soil originate from parents' material of the area (Hamdan *et al*., 2018; Marquez *et al.,* 2018). Taking advantages of the rich biodiversity of paddy landraces all over the world (Saito *et al*., 2018), recent studies had given a new direction of rice not just a source of food but rice could carry multifunction purposes (Hseu *et al*., 2009; Chen *et al.,* 2019). Phytoremediation technologies have designed paddy plants as a cost-effective green tools that can be used to remediate low and moderate heavy metal contaminated soil (Ibaraki *et al.,* 2014; Takahashi *et al*., 2016). Continuous accumulation of heavy metals in soil without soil remediation might inhibit plant growth and reduce grain yield which impacts food safety and food security (Fahr *et al*., 2013).

Paddy plants have high biomass to store heavy metals in the non-palatable areas when plants absorb these elements while growing from soil. Moreover, upland paddy landrace genetic makeup raises them as a tall paddy variety group as compared to wet field paddy variety which is distinctively shorter in stature. Subsequently, food safety and food security can be ensured with proper disposal management of paddy straws that have accumulated heavy metals in rice straws to decrease heavy metals concentration in soils. The measures to identify and to screen for paddy genotype that limit translocation of heavy metals from roots to rice grain have also been progressing which ensures food safety by preventing heavy metal risk in rice (Ishikawa *et al*., 2016; Kim *et al*., 2016). Moreover, one of the proposed countermeasures for alleviating malnutrition in third world countries which generally depended on rice as their staple food is by rice breeding program in producing iron and zinc biofortified rice (Slamet-Loedin *et al*., 2015).

Strategies to select suitable upland paddy landraces to be grown on land contaminated with specific heavy metals for upland paddy traits that limit non-essential heavy metal uptake in rice grain is restricted in Crocker Range, North Borneo as baseline data of local paddy landraces are limited (Hamdan *et al*., 2018). Although Crocker Range is rich with rice biodiversity, data on upland paddy heavy metal uptake characterization and heavy metals distribution in various organs according to specific landraces is limited in current literature. This study provides baseline data that can be used for selective breeding in the development on biofortified rice and biological tool in cleaning up heavy metal contamination in soil by assessment of selected heavy metal uptake characteristics of indigenous upland paddy landraces cultivated at Crocker Range, Sabah, North Borneo which are known as *Bokilong*, *Ponsulak* and *Taragang* by the natives.

Three different indigenous upland paddy landraces which are *Bokilong* (IRGC 71510), *Ponsulak* and *Taragang* (IRGC 60495) were cultivated by traditional method in Kiulu sub-district (6°3’0”N, 116°25’58”E), the Sabah state of Malaysia. Samples were obtained from traditional farmers at the paddy vegetative phase of about three months old (early October 2016) and after plants have produced ripen seeds at the age of six months old (end January 2017). The cultivation area where samples were collected is about 55 km away from the capital city of Sabah, Kota Kinabalu. Plots of these three upland paddy landraces were in close vicinity next to each other. Paddy plants were cultivated on steep hill slopes by dry direct-seeded method on the rural agricultural field. Paddy seeds were sowed at the early start of the wet season of the area following the traditional practice of early settlers. The field has been prepared by the slash and burn activity after left fallow while waiting for the customary paddy planting season. No fertilizer or herbicide was applied on the cultivated field during the paddy cultivation season of collected paddy samples. Weeding was done manually by hand. There were no manual watering or proper irrigation system for the plants whereas water resources for the plant was only from raindrops throughout the paddy planting season. The cultivated area where these three indigenous paddy landraces had grown were on steep slopes with most soil were left uncovered, except for cultivated plants which can increase the rate of the leaching process. The soil pH is very acidic in the range of pH 4.2 - 4.8 and the color of the topsoil is red with clay texture that fits into the description of the United States Department of Agriculture (USDA) soil taxonomy of ultisols.

Paddy plants were randomly collected on cultivation area for each paddy landraces, ten paddy plants each at the age of three months old (vegetative phase) and, five paddy plants each during harvest season. Paddy plant roots were washed to remove soil that was still sticking on the root. Then paddy plant parts were segregated according to root, leaves and grain parts before the drying process.

Dried paddy samples (1.00 g) was digested with 10 ml of 65% HNO_3 overnight at room temperature and heated at 120 ˚C for two hours. Soil collected for heavy metal analysis were from paddy plants rhizosphere and dried in an oven at 105 ˚C until a constant dry weight was obtained. After soil was ground with a mortar and pestle, homogenized soil samples were obtained by sieving soil through a 63 µm size mesh. Soil samples (1.00 g) from each uprooted paddy plants was digested with 10 ml of *aqua regia* solution of HNO_3:HCl (3:1) at room temperature overnight and were heated at 70 ˚C for four hours (US EPA method 3050B). Then 10 ml of hydrogen peroxide (H_2O_2) was added on soil samples and heated at 70 ˚C until the digestive solution reduces to 5 ml. Complete digestion of paddy and soil samples solution was filtered with 0.45 µm pore size membrane filter paper after cooled and were diluted before heavy metal content of the samples was determined by ICP-OES (Perkin Elmer Optima 5300DV). Blank solutions were also prepared following the same procedures as control.

The majority of Malaysia’s agricultural highlands belong to the USDA soil taxonomy of ultisols in which one of its soil properties characteristic includes acidic soil (Shamshuddin *et al*., 2009; Hamdan *et al*., 2019). Heavy metal is very soluble in acidic soil that resulted in the occurrence of an increase in heavy metal accumulation in the Malaysia paddy field caused by impurities from products used in agriculture management practices (Juen *et al*., 2014). Amendments of soil pH have been intensively investigated to increase soil pH and for improving rice yields (Abdul Hamid *et al*., 2018). One of the main reasons is to reduce heavy metal bioavailability in soil because soil pH also affected soil heavy metal bioavailability (Kim *et* *al*., 2016; Kong *et al*., 2018). Many agricultural land in Malaysia also has a high concentration of Ferrum oxides formed by the natural weathering process. Naturally, the most available selected heavy metals in cultivation soils during sample collection for both periods were iron which was at least a hundred times greater than the other selected heavy metals in this study (Table 1). However, the bioavailability of Fe during both seasons seems stable compared to other selected heavy metals. Bioavailability of Fe at different paddy cultivation practices of the wet field and dry field conditions in the Crocker Range did not have significant changes (Hamdan *et al*., 2018). In comparison with other selected elements, only Zn bioavailability in the soil slightly had decreased at harvest season than growing season except for the *Ponsulak* cultivation area that had increased significantly compared to *Bokilong* and *Taragang* landraces. Bioavailability of As, Cd, Cr and Cu in soil was significantly higher at paddy harvest season compared to paddy growing season in the cultivation area (Figure 1). Although the bioavailability of Pb in soil taken from *Bokilong* and *Ponsulak* landraces showed similar consequential elevation during harvest season, only a slight elevation of Pb was determined for *Taragang* landrace.

The amount of rainfall distribution was higher (inter-monsoon) during the paddy vegetative phase as compared to harvest season (Northeast monsoon) which falls into one of the driest months of amount rainfall distribution. Monsoon season affects average solar radiation and soil moisture in rainfed upland paddy cultivation area where the topography of Crocker Range was uneven than flat land (Teong *et al.,* 2017). Observation of different paddy cultivation practices by flooding method and non-flooding method had resulted in non-flooding soil had a higher concentration of heavy metal bioavailability even on non-polluted soil as compared to flooding method (Cattani *et al*., 2008; Slamet-Loedin *et al*., 2016; Hamdan *et al*., 2019). Local climate and soil physicochemical properties influence the heavy metal bioavailability in soil (Sanjeevani *et al*., 2013; Kong *et al*., 2018).

Excessive accumulation of heavy metals in the paddy field from anthropogenic activities like using contaminated irrigated water caused by mining activities had been the foundation of understanding how heavy metal can be transferred and accumulate in the human body by consuming contaminated food crops. High accumulation of heavy metals in cereals can be detrimental to human health (Tang *et al*., 2019). Cadmium was the first heavy metal that had been associated with heavy metal toxicity risk in the consumption of contaminated rice with diseased called Itai itai disease and causing cancer. Selected heavy metals mean concentration in harvested rice grains did not exceed the permissible limit of Malaysia Food Regulation 1985 (MFR, 1985) except for cadmium concentration in rice grain of *Ponsulak* and *Taragang* landraces (Table 1). Cadmium contaminated paddy land need to be remediated before it can be used to grow food crop that does not pose a high risk of cadmium toxicity to consumers (Hseu *et al*., 2009). Thus, many independent researchers from different countries have proposed to use paddy plants as a green tool to clean up agricultural soil by identifying paddy genotypes that have the high ability to extract cadmium from soil and store cadmium in its straw high biomass (Takahashi *et al*., 2016). At the same time translocation of cadmium from soil to its rice grain has to be restricted to avoid toxicity risk in case rice is also used for food resources (Hseu *et al*., 2009; Chen *et al*., 2019). Paddy plant can be a good specific heavy metal bioindicator if the concentration of heavy metal in paddy plant leaves is higher than the concentration of heavy metal in soil when the value of the bioaccumulation factor is more than 1, (BF)>1. Although, Cd accumulation in *Bokilong* landrace rice grain was lower than the other two landraces, *Bokilong* landrace accumulated more Cd in the leaves organ than *Ponsulak* and *Taragang* landraces (Table 1). Hence, *Bokilong* is a good phytoextraction candidate for Cd during the wet and dry season as it yielded a high value of bioaccumulation factor (Figure 2). Cadmium became more mobile from root to leaves in *Bokilong* landrace when paddy became less rainfed by nature (Figure 3). Water management system in paddy cultivation influence cadmium bioavailability in the soil where water saving condition increased Cd bioavailability than flooding condition (Cattani *et al*., 2008; Ishikawa *et al*., 2016). Cadmium mobility in the *Ponsulak*

Table 1. Mean and standard deviation concentration of heavy metals ($mgKg^{-1}$) in soil, paddy root, leaves and rice grain for each landrace according to the paddy age when samples were taken from the cultivation area

Age		Samples	As	Cd	Cr	Cu	Fe	Pb	Zn
Bokilong	3 months old	Soil	0.30±0.20	0.18±0.01	0.86±0.02	1.12±0.02	1001.90±9.68	1.35±0.13	4.70±0.05
		Root	0.66±0.27	0.49±0.02	1.40±0.03	1.45±0.31	1344.45±7.83	4.66±0.12	170.44±1.96
		Leaves	0.58±0.15	0.57±0.01	1.54±0.02	21.26±0.14	1157.98±4.65	5.70±0.14	178.15±0.87
	6 months old	Soil	0.95±0.89	0.79±1.18	4.67±4.75	5.65±5.05	991.23±442.20	7.51±6.80	3.17±3.51
		Root	2.64±1.36	1.94±1.99	7.61±5.67	10.16±7.34	1309.51±712.72	3.97±2.05	204.63±83.87
		Leaves	5.59±8.25	4.07±5.26	0.64±0.72	14.70±17.23	964.08±168.67	5.27±1.74	204.95±68.13
		Grain	0.41±0.69	0.45±0.49	0.66±0.90	13.41±12.68	58.07±51.31	0.15±0.12	0.97±0.73
Ponsulak	3 months old	Soil	0.35±0.17	0.20±0.00	1.20±0.02	1.92±0.01	1735.39±13.75	1.86±0.12	6.02±0.02
		Root	0.27±0.12	0.52±0.01	0.79±0.02	8.03±0.06	445.13±4.29	4.07±0.07	129.39±0.88
		Leaves	0.20±0.14	0.33±0.01	0.89±0.02	10.86±0.17	450.00±4.80	2.38±0.24	143.85±1.63
	6 months old	Soil	4.72±6.73	5.10±4.48	1.82±0.82	4.89±5.32	1742.75±1092.55	12.29±11.88	56.75±82.28
		Root	0.56±0.84	4.23±5.13	2.56±2.29	4.68±3.20	431.06±299.22	13.06±11.44	162.03±14.85
		Leaves	5.91±10.08	2.61±3.98	5.23±4.75	8.82±5.20	462.30±277.23	n.d.	184.90±142.23
		Grain	0.59±1.02	2.21±2.59	2.08±2.66	0.84±0.82	181.83±64.27	n.d.	62.45±44.08
Taragang	3 months old	Soil	0.03±0.01	0.16±0.02	1.24±0.17	1.89±0.02	1422.51±16.25	1.36±0.07	7.39±0.07
		Root	0.52±0.12	0.36±0.02	3.36±0.02	12.46±0.15	3564.19±24.26	6.61±0.12	99.16±0.99
		Leaves	n.d.	0.36±0.02	1.13±0.02	8.85±0.09	325.04±0.24	2.87±0.07	99.08±0.84
	6 months old	Soil	0.75±0.77	6.16±7.84	4.52±4.92	5.29±5.90	1179.13±395.68	1.64±2.65	6.79±8.87
		Root	3.89±5.47	1.05±0.71	7.32±2.54	18.57±14.36	3364.17±1210.64	5.14±0.04	133.72±25.81
		Leaves	n.d.	1.12±0.87	4.03±6.58	2.22±2.57	650.29±316.07	5.38±5.32	35.86±57.49
		Grain	n.d.	1.21±0.90	9.06±6.67	0.20±0.13	339.76±165.86	1.24±0.26	54.55±84.28
MFR 1985		Grain	1.00	1.00	N.A.	30.00	N.A.	2.00	100.00

Notes: MFR 1985 – Malaysia Food Regulation 1985; n.d. – not detected; N.A. – Not Available.

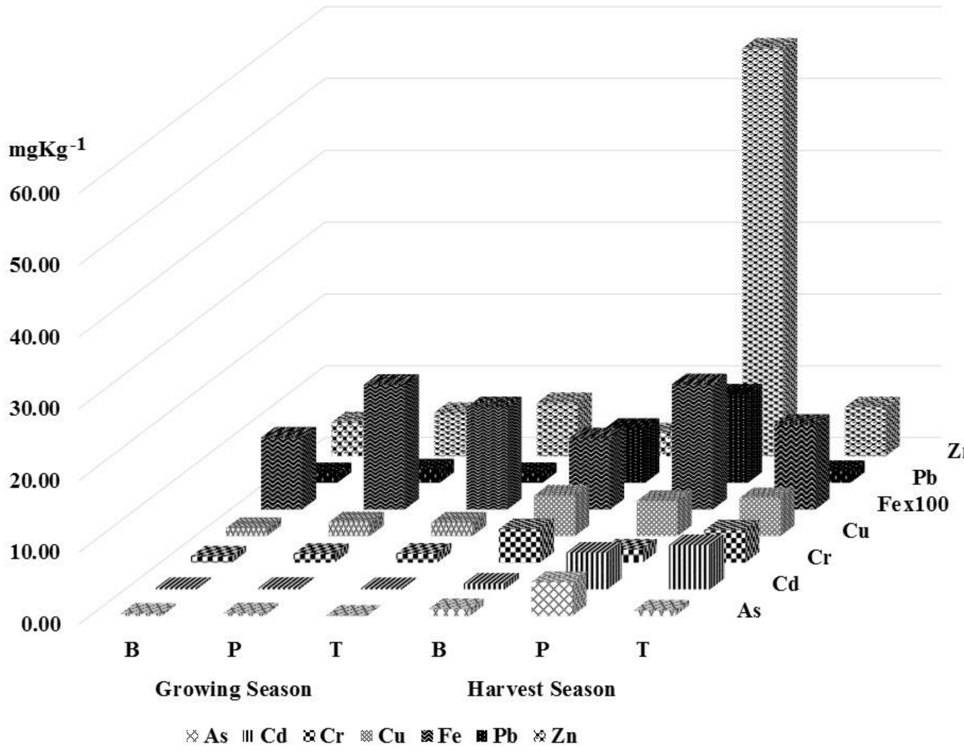

Figure 1. Bioavailability of heavy metals (mgKg^{-1}) in collected soil around paddy plant roots for each landrace (B: *Bokilong*, P: *Ponsulak*, T: *Taragang*) during the growing season and harvest season. The actual concentration of the ferrum element is 100 times greater than shown in the graph

paddy plant is the least efficient compared to the other two genotypes (Figure 3). Meanwhile, *Taragang* landrace is more efficient in transporting Cd from root to grain rather than root to leaves. Using a cadmium accumulator paddy genotype had been proven to help decrease cadmium accumulation in agricultural soil (Ibaraki *et al*., 2014). Native paddy landraces have been noted to adapt with their environment soil physicochemical properties or minerals available in the medium they are growing with by evolving independently as a form of survival in accordance on how rich genetic diversity is producing naturally in nature (Hamdan *et al*., 2018; Marquez *et al*., 2018).

Arsenic is a counterpart of cadmium in posing potential heavy metal toxicity risk for rice consumers as rice is the major cereal that is consumed globally. *Bokilong* and *Ponsulak* landraces significantly increase arsenic accumulation in its leaves between growing and harvest seasons whereas arsenic was not detected in the aerial part of the *Taragang* paddy plant (Table 1). Although, brown rice did not exceed the maximum permissible limit of MFR 1985, the high presence of As bioavailability in the soil had caused *Bokilong* and *Ponsulak* landraces to accumulate As in rice grain (Figure 1). Bioavailability of As in *Taragang* soil was lower than *Bokilong* and *Ponsulak* landraces might be the reason why As was not detected in *Taragang* rice grain (Table 1). The bioaccumalation factor of As for *Bokilong* and *Ponsulak* landraces was more than one indicates that these two landraces are a good phytoextraction candidate of arsenic in a field with similar environmental conditions of less rainfall amount (Figure 2). Arsenic translocation factor value significantly increased except for the *Taragang* landrace (Figure 3). Despite the fact that bioavailability of As in *Taragang* plot soil did increase during harvest season (Figure 1), As was only detected in *Taragang* paddy roots during both seasons (Table 1). As mobility in paddy *Ponsulak* landrace is more efficient compared to *Bokilong* landrace where translocation factor of *Ponsulak* landrace is higher compared to *Bokilong* landrace at least five times the ratios from root to leaves and grain to roots at harvest season. Environmental factors like the amount of water in soil easily influenced the mobility of As in *Ponsulak* genotype which is the most efficient among the other two genotypes.

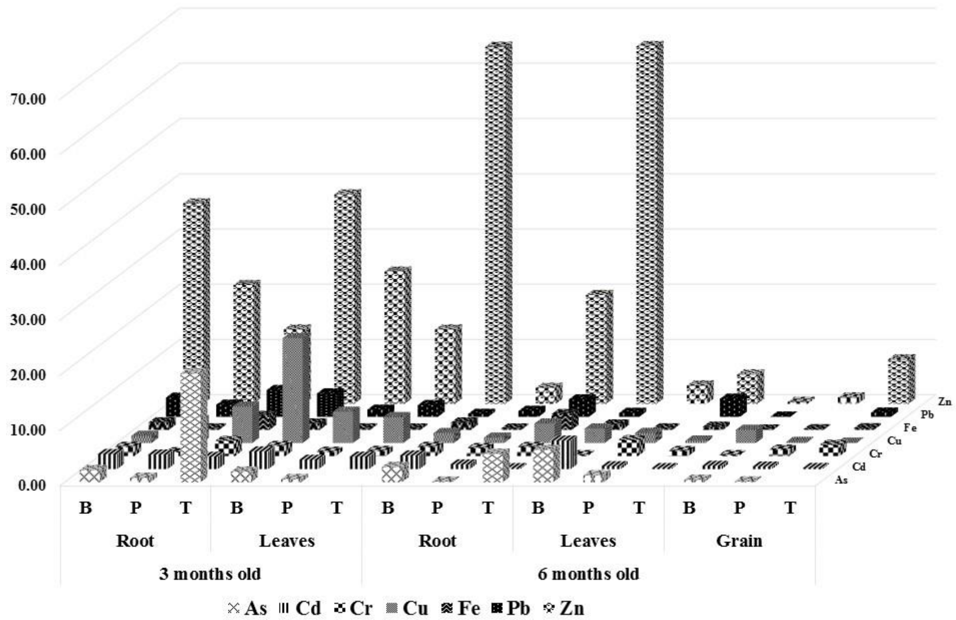

Figure 2. Bioacculamation of heavy metals in each landrace of rice plants (B: *Bokilong*, P: *Ponsulak*, T: *Taragang*) in different organs according to different paddy life cycle phases of the growing season (3 months old) and harvest season (6 months old)

Different genotypes have different efficiency in arsenic translocation from root to soil and there are also environmental factors influencing arsenic uptake in paddy plants (Ahmed *et al*., 2011). Bioavailability of arsenic in soil has been shown to be influenced by the environmental factor and water management practices in paddy cultivation. Dry season and non-flooding conditions made higher As bioavailability in soil compared to the wet season and flooding or submerge conditions (Hamdan *et al*., 2018).

Lead had also become an emerging concern posing health hazards in rice consumption in different regions of the world. In previous literature, lead concentration in rice grain had exceeded the permissible statutory standard guidelines (Ihedioha *et al*., 2016; Rahimi *et al*., 2017; Hamdan *et al*., 2018). A non-essential element for plant health, phytotoxicity of lead affect plant root growth that can reduce rice grain yield subsequently affect food security (Fahr *et al*., 2013). Thus, there is also a need to search for paddy genotype that not only reduce cadmium and arsenic accumulation in the agriculture field but can also restrict translocation into rice grain (Ishikawa *et al*., 2016) as well for the lead. *Taragang* is the only good candidate among three studied landraces (Figure 2) that can become a green tool to remediate Pb accumulation in soil. Although *Bokilong* and *Ponsulak* soil have higher Pb bioavailability concentration than *Taragang*, *Taragang* rice grain was traced having higher Pb concentration and mobility of Pb from root to grain is low (Figure 3). Pb was not detected in the leaves and grain of *Ponsulak* landrace during harvest season indicates low toxicity risk of Pb in *Ponsulak* rice consumption (Table 1).

Copper is one of the most mobile heavy metals in *Bokilong* paddy plant moving to leaves and grain. Thus, among these three genotypes, *Bokilong* is the richest grain with Cu nutrient (Table 1). *Bokilong* and *Ponsulak* landraces is a strong candidate for Cu phytoextraction for both seasons (Figure 2). Only *Ponsulak* landrace is a good candidate for chromium phytoextraction at harvest season. Similarly, like As which was determined in *Ponsulak* landrace, environmental factors easily influence the mobility rate of chromium from root to leaves when rainfall amount reduced during harvest season (Hamdan *et al*., 2019). Cr translocates more efficiently from root to grain rather than root to leaves for *Taragang* landrace.

All three paddy genotypes are a good zinc phytoremediation tool candidate throughout the

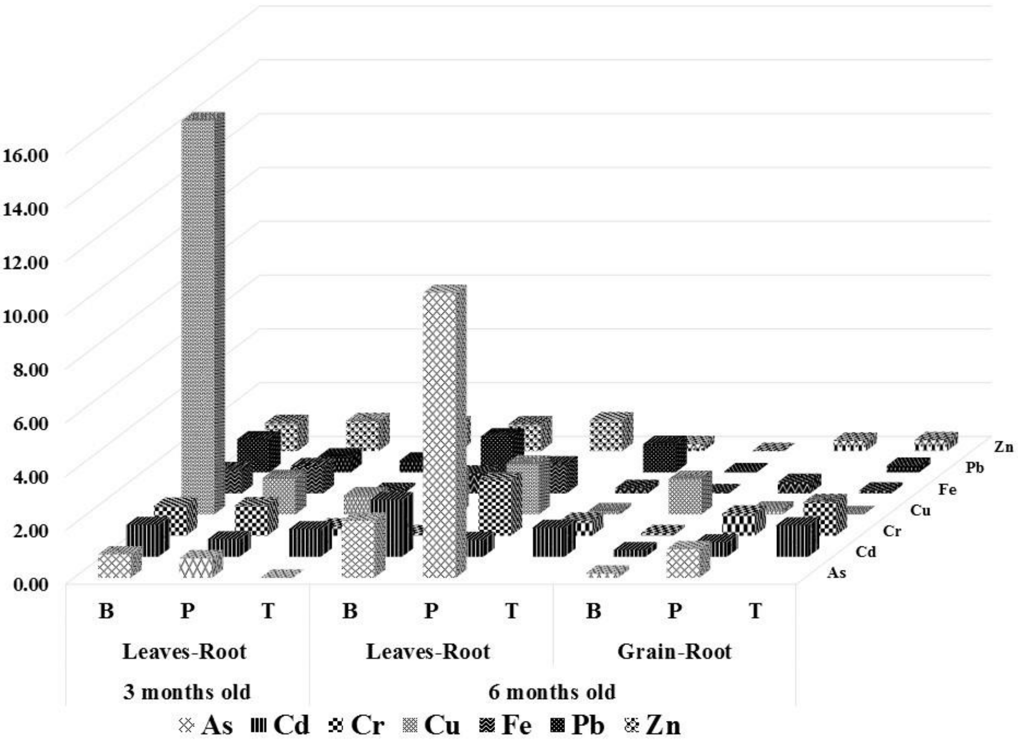

Figure 3. Translocation factor of heavy metals from root to leaves or root to grain according to paddy age when collected from cultivation areas for each paddy landraces (B: *Bokilong*, P: *Ponsulak*, T: *Taragang*)

paddy plant life cycle because the accumulation of Zn tends to keep on increasing in the paddy organs.

Although Fe and Zn were more bioavailable in the soil, translocation of these micronutrients from root to grain was not so much transferred compared to leaves. Moreover, uptake of Fe is more restricted in the roots compared to the aerial part of paddy plants (Table 1). Nevertheless, among selected heavy metals, the *Taragang* rice grain is the richest with iron micronutrient for all paddy landraces in this study. These three landraces are not suitable for Fe phytoextraction as the bioaccumulation factor for Fe is not more than one (Figure 2). Phytoavailability of Fe was not affected by the amount of rainfall as there are no significant changes of Fe accumulation in each part of the paddy plant that were examined at both seasons (Table 1).

Decades of fertilizers and herbicides usage had elevated heavy metal soil bioavailability in the paddy environmental background. This is one of the main contributions to heavy metal toxicity in rice that resulted in a high accumulation of non-essential heavy metals in rice grain and not because of heavy metals mobility efficiency in paddy plants (Ihedioha *et al*., 2016). Upland paddy landraces are useful phytoextraction candidates as it can also be useful not only for upland paddy cultivation field but other food crop drylands. Heavy metal uptake characteristic differs between upland paddy landraces and there is also environmental influence affecting the mobility rate of these elements in paddy plant depending on the element type and paddy genotype. Upland paddy agrobiodiversity cultivation needed to be strongly supported and promoted. This is because baseline data on heavy metal uptake characteristics in different paddy landraces are still limited in the Crocker Range as strategies to reduce toxicity risk and to increase multi-nutrients enrichment in rice diets.

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