Heavy Metals Bioavailability under Aerobic and Anaerobic Condition in Soil and Bubut Rice Plant Cultivated at Crocker Range, Borneo (Malaysia)

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***Abstract*—Bioaccumulation of heavy metals in rice that can pose health risk is a grave concern as more than half of world populations are rice consumers. Bioaccumulation of heavy metals in rice grain depends on the heavy metal bioavailability in the medium that provide nutrient sources to plant. Throughout paddy life-cycle from seed germination to development of mature seeds, paddy plants cultivated in flooded field are exposed to alternating anaerobic condition and aerobic condition. The aim of this study is to assess whether these two different paddy field conditions influence heavy metal bioavailability in soil and heavy metal accumulation in Bubut paddy plants cultivated at highland of West Coast Sabah, Malaysia. Heavy metal uptake characteristic of Bubut paddy in different part (root, straw and grain) were also determined for baseline data that can be used for selective breeding or phytomining. Bubut paddy plants were uprooted together with soil that were collected randomly at 3 months old age (reducing (anaerobic) condition) and harvest season (oxidizing (aerobic) condition) for heavy metal analysis by inductively coupled plasma optical emission spectrometry (ICP-OES). Higher bioavailability of heavy metal at oxidizing condition in soil and Bubut paddy plant were observed compared to reducing condition. Bubut paddy was found to be a potential candidate for Cr, Ni and Zn phytoextraction. Heavy metal concentration in Bubut rice grain is below the maximum permissible limit of Malaysia Food Regulation 1985 (MFR 1985). A comprehensive water irrigation management plan is required to strategically develop at West Coast Sabah to reduce bioavailability of toxic heavy metals and reduce heavy metals toxicity risk in rice consumption.**

***Index Terms*—bioavailability, trace elements, rainfed, *Oryza sativa*, highland, Borneo**

I. Introduction

Rice has been cultivated as an important food resource for many civilizations in the world for thousands of years [1]. Paddy plants are fierce survivors that have continue to evolve and adapt to various environment conditions, geographical features and climate change resulting in many new species which can able people to grow rice even in cold highland regions [2]. Borneo Island a hotspot of biodiversity also has given birth to diverse genotype of paddy species with unique characteristics to each different part of this third largest island in the world [3], [4]. The main staple food of indigenous people living in highland areas of Crocker range where Mount. Kinabalu is located at West Coast of Sabah is rice whom many still cultivated paddy following traditional custom. Their ancestors believe in paddy spirit which indicates how indispensable rice in their daily life [5].

Environmental concern such as soil erosion had prompted new land use policy from the government. Some traditional farmers now have changed the customary cultivation of rice field from shifting cultivation to permanent rice field [6]. Natural weathering processes, water irrigation and anthropogenic activities such as pesticide and fertilizer usage can contribute to accumulation of heavy metals in rice field [7], [8]. Consequently, heavy metals are elements that can be transferred to human through diet when heavy metals in soil were absorbed by plants. Bioaccumulation of heavy metals particularly non-essential heavy metals such as arsenic and lead to the rice grain is a major concern as it can be detrimental to human health like cancer [9], [10]. Phytoremediation is an *in situ* environmental friendly technology that is cost effective for farmers to clean up their soil from accumulation of heavy metals by using hyperaccumulator native plant [11]. Paddy plants have high biomass that can be used to store heavy metals in its straw part which is not use as food consumption. There are paddy plants that have evolved which restrict non-essential heavy metals from accumulating to rice grain which help reduce toxicity risk [12]. Different rice cultivators or genotypes grown in the same region have been observed to have different uptake characteristics of heavy metals [13], [14].

Rice is not only a source of energy to people living in developing countries or third world countries but also provide essential micronutrients such as iron, zinc and copper [15]. Understanding uptake characteristic of heavy metals in paddy plants is important for breeding programs in creating new breeds which not only produce high grain yield for food security. The quality of rice grain must also be taken in consideration for breeding new rice. Ideally the type of paddy which does not accumulate non-essential heavy metals at the same time rich with essential micronutrients which is good for human health.

Manuscript received April 11, 2019; revised August 20, 2019.

doi: 10.18178/joaat.6.3.187-193

Apart from genetic inheritance, environmental conditions also influence the heavy metal uptake in paddy plants [13], [16], [17]. Bioavailability of heavy metals in soil for plant uptake depends on the solubility of those heavy metals in soil. Different water management system in paddy cultivation areas exhibited significant behavior difference of heavy metals bioavailability between aerobic and anaerobic conditions [18]-[20]. At the highland of West Coast Sabah in Kiulu district where water irrigation facilities has not been provided for paddy farmers, rice are only cultivated following traditional system once a year as they have to rely on rainfall amount. Rice cultivation environmental condition of how long the duration of aerobic and anaerobic condition between seedling transplant and harvest season will depend very much on mother's nature temperament. Bubut paddy variety heavy metal uptake characteristic has not been reported and we try to provide baseline data from this research. We have assessed heavy metal bioavailability in soil when rice field were in reducing (anaerobic) condition and oxidizing (aerobic) condition, and whether bioavailability of heavy metals in soil will also influence the uptake of heavy metals in Bubut paddy plant which can be used for water irrigation management plan strategies.

II. METHOD

A. Paddy Cultivation Area

Bubut paddy samples were cultivated in rice field at Kampung Poturidong Lama (N 06º 03.030', E 116º 17.352') next to Kiulu Pukak Mantob road which is located in the Kiulu sub-district and Tuaran district of Sabah, Malaysia. This is a part of West Crocker Formation with soil parent material is from sedimentary rock [21]. The color of soil in the paddy field is red indicates ferrum oxides dominance. Bubut paddy is a long-grain rice and this trait belongs to the *Indica* species group. Bubut Paddy is a traditional cultivator which takes 6 months to complete its life-cycle and is usually grown in flooded field adapted on the mountainous terrain of the Crocker range, Borneo Island environment. The rice field is a dedicated agricultural field for growing rice for three generations already and cultivated each year if the traditional farmers are able. After rice harvesting, the rice field will be left to fallow until the next rice growing season. Generally, the month of October was among the highest amount of rainfall in this area which is close to the capital city of Sabah, Kota Kinabalu. In contrast, January occur as one of the month which received the lowest amount of rainfall. There is no irrigation facilities provided from the government. Therefore the paddy field were filled up by rain water and traditional farmers had grown this paddy according to the custom traditional growing paddy season. Seeds sowing were done in July 2017 and seedlings were transplanted a month later to the flooded rice field (August 2017). First sampling were conducted when Bubut paddy plant were in growing phase at about 3 months old (October 2017), whereas the second sampling were conducted during harvest season at 6 months old (January 2018). Five Bubut plants were taken randomly together with the soil where the plants were growing and five water samples were taken for each sampling time.

B. Heavy Metal Analysis Preparation

Prior to nitric acid digestion, water samples were filtered with 0.45 µm Whatman membrane pore size by syringe and preserved with HNO_3 to prevent any biological processes from occurring. Soil samples were air-dried before grinded and sieved through a 0.63 µm mesh size sieve. One gram of sieved soil samples were digested with 10ml HNO_3 and heated at 70 ℃ for 6 hours. Then 10ml H_2O_2 were added and heated until the volume solution reduced to 5ml. Soil sample solution were filtered with 0.45 µm Whatman membrane filter paper and diluted.

Paddy plants were washed free from soil with tap water and rinsed with distilled water after plants part were separated by roots, straw and grain. Then plant samples were dried in oven at 60 ℃ for more than 48 hours until a constant dry weight were obtained. After dried samples were grinded into powder, 1 gram of powder samples was digested with 20ml HNO_3 which were left in room temperature for overnight. The next day, samples were heated at 120 ℃ for 4 hours. Samples were then filtered with 0.45 µm Whatman membrane filter paper after cooling. Solution extract of paddy plant and soil samples, and water for heavy metal analysis were stored at 4 ℃ prior to heavy metal concentration determination by ICP-OES Spectrometer machine Perkin Elmer Optima 5300DV.

C. Enrichment Factor and Translocation Factor

Enrichment Factor (EF) is to determine the accumulation of heavy metals in paddy plant relative to its growing environment such as soil or water. If the plants EF value is more than 1 suggests that the plants can be used as a bioindicator to monitor soil health and to assess whether the soil had accumulated excessive heavy metals that might cause toxicity risk. Heavy metals hyperaccumulator plant candidate for phytoremediation can also be assessed from Enrichment factor value. Enrichment factor is the ratio of mean concentration of heavy metals in paddy plant parts (root/straw/grain) to mean concentration of heavy metals in soil.

Translocation Factor (TF) indicates the efficiency of heavy metals translocation from root to other part of paddy plant such as the straw and grain part. Ratio of the heavy metal mean concentration in straw and grain to heavy metal mean concentration in roots is how translocation factor was calculated.

III. RESULTS & DISCUSSIONS

A. Bioavailabity of Heavy Metals in Bubut Paddy Cultivated Environment

Paddy plants were still growing in flooded conditions submerged (anaerobic) in shallow water when soil were first sampled. At this time the mean water pH was acidic at 5.5, whereas the mean soil pH was strongly acidic (<5).

In contrast, the second soil sampling was carried out during Bubut paddy harvesting season when the soils were less saturated with water and were in oxidizing condition. Mean concentration of heavy metals in Bubut paddy soils were significantly elevated between these two different growing phases (Table I). Similar observation were obtained from a field study of a different paddy variety growing in the same growing condition and in close proximity of Bubut paddy cultivation site [18]. Soil heavy metal bioavailability differences of the studied life-cycle phases of Bubut paddy might be due to the influence of the physical-chemical soil properties of two different soil conditions which is anaerobic condition and aerobic condition [7]. During anaerobic condition, non-essential heavy metals - arsenic, cadmium and lead, which do not involved in plant physiological processes were not detected in soil samples. However, all selected heavy metals presence were detected during aerobic conditions except for cadmium which was also not detected in water samples.

TABLE I. Bioavailability of Heavy Metals Mean Concentration and Standard Deviation in Water (mg/l) and Paddy Plant Soil (mg/kg) in Different Growing Seasons Where First Sampling Were Taken in Anaerobic Condition and Second Sampling Were Taken in Aerobic Condition

	Water (mg/l)	Soil (mg/kg)	
	Oct 2017	Oct 2017	Jan 2018
As	0.24±0.02	n.d.	0.23±0.00
Cd	n.d.	n.d.	n.d.
Cr	0.54±0.16	0.74±0.00	1.83±0.01
Cu	n.d.	0.20±0.00	0.53±0.00
Fe	8.75±1.44	350.38±0.05	646.81±3.26
Ni	0.07±0.05	0.11±0.00	0.60±0.00
Pb	0.08±0.04	n.d.	0.40±0.01
Zn	0.78±0.05	1.55±0.00	5.75±0.08

n.d.-not detected

Water irrigation management system in Sabah for wetland paddy cultivation can be classified into three systems; (i) fully assisted irrigation facilities where water are always available (K1), (ii) equipped irrigation facilities and only available seasonally (K2), (iii) no irrigation facilities and rainfed (K3). Main irrigation system at highland areas for wetland paddy cultivation is still highly dependent on rainfall [22]. This prevents many paddy farmers from growing paddy more than one season per annum. Malaysia is a tropical country which have high amount of annual rainfall which should have no problem with water storage or irrigation if properly designed. Development of efficient irrigation management strategies must be taken in consideration since heavy metals bioavailability is also influenced by the environmental conditions [23]. Thus not just grain yield is a concern but the quality of rice that is produced also will be affected since plants not only absorb essential nutrient in soil but also absorb non-essential trace elements [20]. The uptake of heavy metals by rice plants is largely determined by the bioavailability of heavy metals in soil which, in turn, is determined by the solubility of trace metals that can enter the plants [19]. Bubut paddy is an *Indica* species which is traditionally cultivated in flooded field and were grown in areas which had relied on rainfall. Seeds sowing phase are done in aerobic condition and seedlings are usually transferred at paddy field to be grown in anaerobic condition after a month old. Closer to harvest season, soil will start to dry naturally depending on nature where plant will be gradually exposed to aerobic condition. The alternating wet and dry condition seemed to influence the bioavailability of heavy metals in Bubut paddy cultivated field. Understanding the local soil properties, environment and uptake characteristic of rice genotype is important for rice production performance in yield and quality of rice grain that will not pose any harm to human health and ensure livelihood sustainability.

B. Bioaccumulation of Heavy Metals in Bubut Paddy

Rice is reported to accumulate more arsenic compared to other major cereal grain production in the world [9]. In addition rice is the main staple food of the people living in the highland of the Crocker Range. Arsenic toxicity can be reduced by polished rice consumption compared to brown rice consumption [12]. However, not only arsenic content was reduced by polishing rice, other essential nutrients content tended to reduce as well. Among the essential selected micronutrients in this study, iron is the richest micronutrients in Bubut paddy grain brown rice (Table II). If toxicity is a concern and rice is polished, there will be a possibility that iron is one of the micronutrient that will be significantly reduced in comparison to Zn [4], [12], [24]. Rice which is rich with nutrient content like brown rice are becoming more in demand these days due to more awareness among health conscious rice consumers [25]. Thus this desirable nutrients needs to be retained so that farmer can have wider market and earn more value from their brown rice product [26]. Increasing demand for local brown rice will motivate the government to initiate more research on highland Borneo paddy which is currently lacking due to its characteristic had been described as low yield grain [27]. Nevertheless, agricultural soil must be cultivated in a sustainable manner which does not pose any risk to human health to ensure food safety and food security. Demand of highland local rice also indirectly aid biodiversity conservation and prevent the disappearance of genetic resources for breeding programs [15], [16], [28].

Globally rice as a major staple food seems to be a major concern as having the main role as main producers that transfer As through the food chain linking directly to human. Nevertheless, there are varieties which As were not detected in As grain [12]. Genotypes and environment interactions had steered As uptake in paddy plants according to seasonal variation whereas higher uptake of As in plants cultivated during dry season compared to grown in wet season [13], [17]. All selected heavy metals were already detected in the root part of three months old of Bubut paddy plants (Table II). Although As and Pb were not detected in soil when paddy plants were submerged in shallow water, the presence of As and Pb were detected in the water during anaerobic condition. Water irrigation management is one of the factors causing As contamination in agricultural fields [20], [29], [30].

TABLE II. MEAN CONCENTRATION OF HEAVY METALS (MG/KG) AND STANDARD DEVIATION IN DIFFERENT PART OF BUBUT PADDY PLANTS BY GROWING SEASONS AND MAXIMUM PERMISSIBLE LIMIT OF GRAIN HEAVY METALS CONCENTRATION CONTENT ACCORDING TO MALAYSIA FOOD REGULATION 1985 (MFR 1985)

	3 months old		6 months old			
	Root	Straw	Root	Straw	Grain	MFR 1985
As	1.69±0.04	0.06±0.00	0.51±0.00	0.22±0.00	0.07±0.00	1
Cd	n.d.	n.d.	n.d.	n.d.	n.d.	1
Cr	0.87±0.02	0.41±0.01	1.20±0.00	2.06±0.00	0.32±0.00	N.A.
Cu	0.62±0.01	0.30±0.01	0.42±0.00	0.36±0.00	0.28±0.01	30
Fe	3469.90±51.76	220.50±2.98	1382.24±0.67	220.24±0.21	5.91±0.17	N.A.
Ni	1.30±0.04	0.24±0.01	0.49±0.00	0.73±0.00	0.26±0.00	N.A.
Pb	1.09±0.03	n.d.	0.23±0.01	0.06±0.00	0.13±0.00	2
Zn	9.05±0.19	10.25±0.11	8.57±0.02	9.65±0.02	4.30±0.04	100

Note: n.d. – not detected, N.A.- Not Available.

TABLE III. ENRICHMENT FACTOR (EF) OF HEAVY METALS DURING DIFFERENT LIFE-CYCLE OF BUBUT PADDY PLANTS

	3 months old		6 months old		
	Root	Straw	Root	Straw	Grain
As	-	-	2.22	0.96	0.30
Cd	-	-	-	-	-
Cr	1.18	0.55	0.66	1.13	0.17
Cu	3.10	1.50	0.79	0.68	0.53
Fe	9.90	0.63	2.14	0.34	0.01
Ni	11.82	2.18	0.82	1.22	0.43
Pb	-	-	0.58	0.15	0.33
Zn	5.84	6.61	1.49	1.68	0.75

Lead is becoming an emerging concern in rice consumption apart from arsenic, as lead also can cause carcinogenic risk [10]. All selected heavy metals concentration in grain for this study did not exceeded the maximum permissible limit of Malaysia Food Regulation 1985 (Table II). In Bubut paddy plant variety, lead accumulation was mainly in the root compared to other part of the plant investigated (Table III). Lead can contaminate agriculture site in rural areas by open burning using petrol. In highland Sabah, common practice in paddy field preparation before seed sowings are usually clearing the land from weeds by slashing and burning by using petrol [30]. Shifting cultivation were a common traditional practice where lands are left fallow for many years to increase the soil fertility again after several years of cultivation. Flat land are limited in highland areas for wet field rice cultivation. Therefore, in current condition many villagers had switch to permanent cultivation of paddy field due to changes in agriculture land usage policy especially for growing wetland paddy [6]. Continuous cultivation on the same field for many years can cause lead accumulation in soil and plant phytotoxicity which impact farmer's livelihood sustainability if Pb concentration in farming land is not monitored and manage in a sustainable way.

C. *Bubut Paddy as Heavy Metals Hyperaccumulator*

At the stage where Bubut paddy plants were growing in aerobic condition, translocation of heavy metal from root to straw were significantly more efficient when compared to anaerobic conditions (Table IV). The uptakes and enrichment of heavy metals by paddy plants were in the same shift by the bioavailability of heavy metals in soil as affected by the reducing condition and oxidizing condition. Environmental conditions influenced the mobility efficiency of selected heavy metals from paddy root to paddy leaves except for Zn which translocation factor remained similar for both seasons. Likewise Zn mobility from root to leaves also showed no significant difference in different seasonal variation in Pandasan paddy grown in similar environmental conditions in close proximity with Bubut paddy field site [18]. Zn is an important micronutrients in living things where in plants it helps to maintain healthy root development in the beginning of paddy seedlings lifecycles [31]. Roots are an important organ for plants as it absorb water and nutrient from soil for plants growth and well-being. As an essential micronutrients, Zn had played it roles efficiently in Bubut paddy plant even at three months old where no morphophytoxicity symptoms were observed. Bioaccumulation of selected micronutrients in the leaves at three months old of growing Bubut paddy plants yielded Zn as the most highly efficient. Moreover, Zn has the highest value of Enrichment Factor in grain (Table III) and one of the selected heavy metals that also translocate efficiently to the grain and not just to the leaves which indicates Zn is very mobile in Bubut paddy variety (Table IV). Therefore, Bubut paddy can also be a good candidate for phytoextraction in reducing Zn in contaminated soil at West Crocker Formation.

TABLE IV. TRANSLOCATION FACTOR OF HEAVY METALS FROM ROOT TO STRAW AND ROOT TO GRAIN ACCORDING TO DIFFERENT GROWING SEASONS

	3 month old	6 months old	
	Root to Straw	Root to Straw	Root to Grain
As	0.04	0.43	0.14
Cd	-	-	-
Cr	0.47	1.72	0.27
Cu	0.48	0.86	0.67
Fe	0.06	0.16	0.00
Ni	0.18	1.49	0.53
Pb	-	0.26	0.57
Zn	1.13	1.13	0.50

Soil developed from Crocker Formation in Ranau area was bordering with ultrabasic soils which is known to contain high concentration of Ni, Cr and Co. In this area the ultrabasic soils probably has intruded into the Crocker Formation due to tectonic processes in the past and due to erosion and deposition in the recent time. High concentration of nickel and chromium in soil might be too toxic and affect vegetation wellbeing [8]. In Kiulu paddy growing area, the occurrences of high Ni and Cr is low due to its far proximity to ultrabasic soil occurrences. This is proved by the low amount of Ni and Cr content in Bubut paddy growing area in Kiulu (Table III). In the growing phase of Bubut Paddy plant lifecycle, the plant root part is most enriched with Ni among the selected heavy metals, although Fe was most bioavailable in the soil during that time (Table I). Ni was translocated more to the straw of Bubut paddy plants compared to other plant part which reduced toxicity risk through rice consumption (Table IV). High concentration of Ni in the leaves also in an evolutionary aspect as a defense mechanism against pest attacks on the leaf which can affect grain yield [32]. Many indigenous people living at the highland of the Crocker range relies on agriculture for their main source of livelihood. Thus, not only Bubut paddy is a food resource but can also hold many multifunction purpose such as assist in cleaning up the soil from excessive accumulation of trace elements.

Different climate and soil type also have different microorganism species which form a complex interaction with the plant rhizosphere influencing the uptake of trace elements with its different form and speciation from the soil [33]. Two species of common chromium in the environment are Cr(III) which is less toxic, less soluble and has limited mobility, while the hexavalent form Cr(V1) which is more toxic, more water-soluble and more mobile. Distribution of these two different Cr species in rice plants varies which can affect plant growth [34]. Alternating wet and dry soil condition effect the bioavailability of these elements [35]. During harvest season, translocation factor from root to straw showed Cr mobility is the most efficient among other selected heavy metals [Table IV]. At study site, bioavailability of Cr in water and soil is much higher compared to Ni [Table I]. Cr is more enriched in all parts of paddy plant studied during harvest season compared to Ni [Table II] indicates oxidizing condition considerably impact Cr mobility rate. Selected heavy metals translocation during harvest season from Bubut paddy root to paddy straw in the order from efficient to slow Cr>Ni>Zn>Cu>As>Pb>Fe. Bubut paddy genotype accumulate Cr in its straw to clean up soil at the same time restrict translocation to the consume part which is the grain.

IV. CONCLUSION

Under field conditions, higher heavy metals bioavailability occurred under oxidizing condition than reducing condition in soil and bubut paddy plant .Water management strategies of growing wetland paddies needs to be developed at rain fed rice fields as soil bioavailability interacts with environment condition. Concurrently also affect the both essential and non-essential heavy metal phytoavailability uptake into plant and rice grain.

ACKNOWLEDGMENT

The authors wish to thank Janet Rumpud and Freddy Sikin for sharing Bubut paddy samples cultivated from their agriculture field. All the technical assistance received from laboratory of Faculty of Science and Natural Resources, Universiti Malaysia Sabah were most appreciated for conducting heavy metal analysis experiments. This work was supported in part by a grant from Universiti Malaysia Sabah (SLB0153-2017).

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