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Assessment of Micronutrients and Toxic Elements in Rice Grains: Implications for Food Safety and Nutrition

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Abstract

The complete assessment of micronutrient and toxic elements (Cu, Mo, Sn, Cr, Ni, Zn and Pb) in rice grains and implications for food safety and nutrition is still not understood clearly. Pot experiments were designed to grow the two rice cultivars namely MH63 and LYMZ. For the accurate determination of the micronutrient elements and toxic element, here we used microwave assisted digestion for sample preparation and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the determination. Good linearity was maintained over the range studied with correlation coefficients (R^2) of 0.999-1.0. This work focused on evaluating these elements in two rice cultivars MH63 and LYMZ. The micronutrient elements and toxic element in the bran and kernel of both the cultivars were detected below the permitted level set by Food and Agriculture Organization of the United States/ World Health Organization(FAO/ WHO). Large portion of the micronutrient elements and toxic elements remain in the bran, very small amounts in the ppb levels remain in the kernel. The translocation of zinc into bran is highest among other nutrient elements. The load of nutrient element copper in the bran of both cultivars remains similar, i.e., 0.006g/kg whereas the toxic element lead in the bran of both cultivar lies below 0.008g/kg. The nutrient element zinc in the kernel of Lymz remains 0.066g/kg where as in MH63 is 0.025g/kg. Nutritionally essential trace element chromium in the kernel of both cultivars lies below 0.004g/kg (1mg/day tolerable limit). The concentration of nutrient elements in two rice cultivars were found to be very close to the minimum levels recommended by Food and agriculture organization (FAO). Thus, the nutrient quality of the rice grain depends on the status of soil, micronutrient element and toxic element concentrations in the permitted range of FAO/ WHO guide line.

Keywords Heavy metals, ICP-MS, microwave digestion, micronutrients, rice cultivars.

1. Introduction

Rice grain is staple diet for the majority of world's population. Asian countries like China, India, Indonesia and Bangladesh cultivates rice as their main food crop, which covers nearly 70% of the world rice production (FAO, 2011). The global demand of food is increasing day to day due to continuous increase of world population (God fray et al. 2010). Until 2030, 40% more rice should be produced to satisfy the demand of the rice consuming countries (Carlin et al., 2016). Rice plant is the potential accumulator of the nutrient elements necessary for the human health. The quality of the rice produced affects the health of the global rice consumers. Dietary minerals paid attention for the prevention of several diseases. Mineral nutrients such as Ca, Mg, K, Na, Cl, S and P are the components of the tissue structure and play significant role in cellular metabolism as well as in water and acid-base balance (Macrace et al., 1993). The trace minerals such as Cu, Cr, Zn, Mn and Si play crucial role for vitamin, hormone and enzyme action.

Rice is a complex matrix that mainly contains carbohydrates, proteins, water, essential amino-acids, fiber, vitamins and minerals (Verma and Srivastav, 2020). Contaminant elements like As, Pb, Hg, etc. are increased into the flooded paddy soil due to geogenic source, human activities, industrial waste, agricultural activities etc. Attention is needed to the micro element contents in the rice. Rice is the main source of these micro-elements intake into the human body, which may cause risk to the development of disease like tumor in various organs, may effect on the central nervous system as well as hair and teeth losses when exceeds the maximum tolerable daily intake (European Food Safety Authority, 2009; IARC, 2012; Pedrero and Madrid, 2009).

The nutritious and toxic properties of the plant are determined by the metal ions present in the chemical components (Tokalioglue, 2012). Heavy metals poisoning is the cause of toxic effects (Basgel and Erdemoglue, 2006). Lead and cadmium contamination is usually caused by the environmental pollution. The use of pesticides and fertilizers increases the levels of As in rice field (Giacomino et al., 2015). Copper (Cu), Zinc (Zn), Chromium (Cr), Nickel (Ni), Molybdenum (Mo), and Tin (Sn) play vital role in human biological processes and their deficiency and excess than the maximum tolerable limit have toxic effects on human health. (Bornhorst and McMillion, 2006; He, 2011).

The efficacy of the determination technology has significant role for the quantification of the nutrient elements. The evaluation of nutrient elements in rice grain is usually carried out using ICP-MS because of its ability to ultra-trace multi-element analysis, requirement of small amount of analyte sample, high sensitivity and ability to measure a large range of concentrations (Bass et al., 2001; Montaser and Golightly, 1987). Sample preparation could be carried out using various digestion methods such as dry ash and microwave oven acid digestion although both of the methods have their own advantages and disadvantages (Puchyar et al., 1998). Microwave digestion is the most suitable method for standardization (Montaser and Golightly, 1987). The analysis of the rice samples by microwave oven digestion using 10mL 1% (v/v) HNO_3 is more appropriate, fast, safe and reduces the risk of contamination and volatilization (Pokhrel, et al., 2020).

As one of the most nutritious food used in the world major population, rice is the major sources for the transportation of trace elements from soil to human. The determination of nutrient and toxic elements, especially heavy metals, into the staple food rice is useful for nutritional assessment and occupational exposure as well as in the diagnosis and monitoring of disease. The purpose of this study is to evaluate the amount of nutrient elements & toxic element in the staple food rice. Amount of the contaminant elements present in the rice determines the quality of the rice. Hence it is necessary to quantify the trace elements in the rice for rating its quality.

2. Materials and methods

2.1 Rice cultivation

Pot experiments were carried out in the experimental field. Silty loam soil was used with available N 37.8±1.1 mg/kg, available P 21.9±1.9 mg/kg, available K 54.9±0.6 mg/kg, and pH 6.88±0.02. Four replicate pots (plastic buckets) having 0.26m, 0.30m and 0.23m height, top and bottom, respectively were designed for each cultivar of rice. The experimental field where pots were kept was covered by plastic tarpaulin to avoid unnecessary rain water contamination. Two different rice cultivars (Chinese cultivars) namely Minghu 63 (MH63) and Lu You Ming Zhan (LYMZ) were cultivated for this study. Four rice seedlings per pot at 4-leaf stage of two rice cultivars were transferred into plastic buckets. Fertilizer application and cultivation management throughout their growing period until maturing were based on the description of Pokhrel et al., (2020). The two different cultivars of rice have different maturing time. The Indica cultivar MH63 mature in 90 days where as LYMZ mature in 120 days. The indica cultivar MH63 was harvested after matured at 90 days whereas LYMZ was harvested at 120 days of maturation. In harvesting, paper pockets were used to collect the inflorescence of rice to avoid the contamination. After harvesting, grains were dried at room temperature (~25 °C) and stored in desiccators until further analysis.

2.2 Sample preparations

At tillering (27 day), booting (51 day), grouting (70 day) and maturing stage (90-120 day), rhizosphere water sample of both rice cultivar was collected using capillary and filtered using 0.22 µm polypropylene membrane filter and stored at -20°C for further determination of pH, total organic carbon (TOC), total Carbon (TC), inorganic carbon (IC) and total nitrogen (TN).

Rice grains were dehulled in a motorized de-husker, separated into whole kernel and bran. The separated whole kernel and bran were grinded to fine powder by tissuelyser-24 and stored in desiccators until analysis. About 0.1 g crushed rice samples (with the precision of 0.00001 g) were weighed into 100 mL polyteterfluoroethylene (PTFE) microwave tube and added with 10.0 mL of 1.0% (v/v) nitric acid. The Microwave vessels were capped and placed in the rotor of an ETHOS UP microwave apparatus (Milestone High Performance Microwave Digestion Technology, Italy) according to the report of Pokhrel et al. (2020). The operating program of the system was implemented as 120 ºC for 25 minutes (5 minute ramp time) and dropped to 60 ºC within 5 minutes. After the digestion was finished, the extract was diluted to constant volume with Milli-Q water, centrifuged (12000 rpm/min, 3min) and filtered through 0.22 µm polypropylene membranes into polyethylene centrifuge tubes for the nutrient element analysis.

2.3 Analytical method validation

The Limits of detection (LOD) and Limits of quantification (LOQ) were determined using the equation, LOD = 3 × SD/S and LOQ = 10 × SD/S. Where SD is the standard deviation and S is the slope of the calibration curve. Four replicate samples (n = 4) were used for the determination. Standard solution was used to study the linearity of the calibration curve. Calibration curve was plotted using the response value(Y) versus the concentration of analyte (X). Correlation coefficients, R^2 of the calibration curves shows linear relationships (0.999-1) over the selected ranges of heavy metal concentrations as indicated in Table 1

Table 1. Analytical characteristics of the method

Metals	Calibration curve of standard	R^2	LOD µg/L	LOQ µg/L
Cu	y = 2968.x + 1934	1	0.00618	0.02063
Mo	y = 2269.x + 1118	0.999	0.01089	0.03632
Sn	y = 1278.x + 843.3	0.999	0.01538	0.05129
Pb	y = 4396.x + 4580.	0.999	0.01965	0.06550
Cr	y = 1566.x + 1756	0.999	0.01332	0.04443
Ni	y = 1106.x + 1179	0.999	0.01768	0.05895
Zn	y = 374.0x + 941.5	0.999	0.02289	0.07631

The extract of kernel and bran was then subjected to ICP-MS (ICP-MS, NexION 300X, PerkinElmer, USA) for micro-nutrient elements & toxic element analyses. The total concentration of different nutrient elements present in bran and kernel was calculated on a dried weight (DW) basis. The efficiency of the method was evaluated by spiking the sample with known amount of metallic elements. The recovery was calculated using the following equation.

Table 2. Recovery of the nutrient and toxic element from the kernel of rice grain

Metal	Recovery %
Cu	95.23 ± 3.85
Mo	96.23± 3.68
Sn	93.00 ± 3.85
Pb	86.22 ± 2.3
Cr	97.04 ± 4.68
Ni	92.11 ± 4.75
Zn	101 ± 6.53

Percentage recovery of triplicate analytes, mean recovery ± SD

2.4 Statistical analysis

The four biological replicates and their average values were used for statistical analysis. Software DPS7.5 was used to conduct the significant test by least significant difference (LSD) among different treatments.

3 Results and discussion

Measurement of pH, TOC, TC, IC and TN

In both cultivars of rice, the pH of Soil pore water solution lies between 6.0 - 6.7 from first day of its plantation to its full grown, i.e. 70 days, which is the best pH value for the growth of rice plants (Pokhrel et al., 2020). The magnitude of pH is related with the amount of organic matter because organic matter induces the reduction of the oxidized forms of inorganic compounds. The organic matter which decomposes easily may retard this increase temporarily during its intensive decomposition. Paddy soil pore water solution pH is the key point to influence the nutrients available to plants, i.e. nitrogen, phosphorous, potassium and other nutrient elements (Pokhrel et al., 2020). The right soil pH range makes the plant easier for the uptake of nutrients. At low pH, nutrient elements are less available. Total carbon (TC) in the soil pore water solution of both cultivars is greater than total organic carbon (TOC) and Total nitrogen (TN) at all growth stages of rice. TOC in soil pore water solution at 70 days of MH63 is significantly higher where as in other stages there is no significant different in both cultivars. The paddy soils having higher content of total organic carbon exhibited higher bacterial biomass, nutrient holding capacity (cation and anion exchange capacity), nutrient turnover and stability, Nitrogen and phosphorous circulation activity which are sole responsible for the optimum uptake of nutrient elements by plants from paddy soil (Pokhrel et al., 2020). The pH of soil solution and the concentration of TOC, TC, inorganic carbon (IC) and TN are shown in the figure1a and 1b.

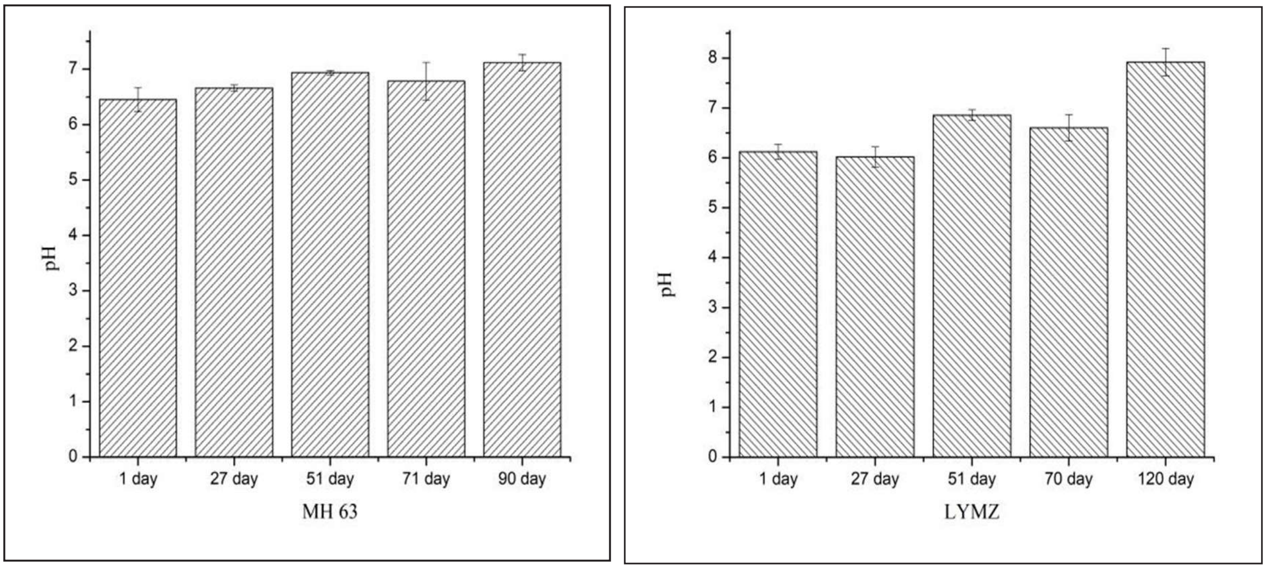


Figure 1a. pH of soil solution of 1, 27, 51, 71, 90 and 120 days of MH63 and LYMZ

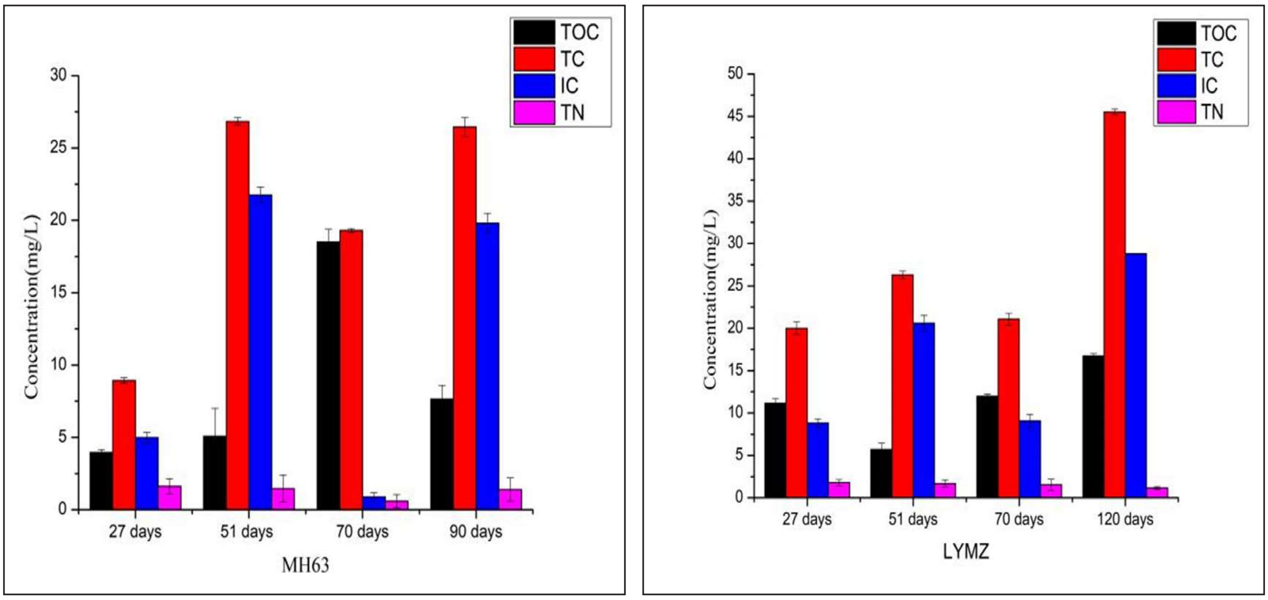


Figure1b. TOC, TC and TN of soil solution of 27, 51, 70, 90 and 120 days

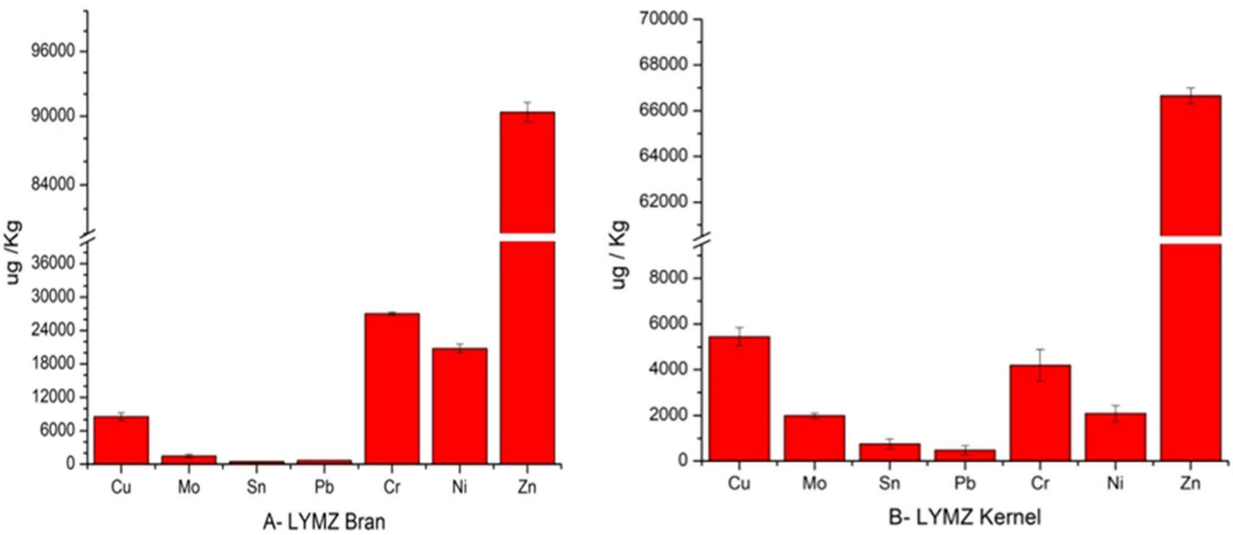
3.1 Micronutrient elements & toxic element in the rice grain

The amounts of Zn, Cr, Ni, Cu, Sn, Pb, and Mo present in the bran and kernel of both the rice cultivars are presented in figure below. The large concentrations of Zn, Cr, Ni and Cu are present in the bran samples. The amount of Zn is found to be the highest than other elements in the bran and kernel of both cultivars. The amount decreased in the order (Zn, Cr, Ni, Cu, Mo, Pb and Sn) in the bran of LYMZ where as in the bran of MH63, the order is (Zn, Cr, Ni, Cu, Sn, Pb, and Mo), respectively. This indicates that the distribution of the micro nutrient elements & toxic element is mainly in the external parts of the grain, i.e. bran. The amount of the micronutrient elements and toxic element in the bran of the LYMZ is higher than MH63. The distribution of the micronutrient elements & toxic element in the bran of two rice cultivars is different and it depends on the rice cultivars and maturation period of rice grains. The distribution of micronutrient elements Cu, Mo, Sn and toxic element, Pb in the bran of the LYMZ is six fold greater than in the bran of the MH63. The distribution of Cr in the bran remained double in LYMZ than in the bran of MH63 whereas the concentration of Ni in both rice cultivars remains similar. The translocation of Zinc (Zn) from shoot to bran of MH63 is higher than in the bran of LYMZ. The amount of different micronutrient elements and toxic element distribution in the rice determines the nutritional quality of rice.

The accumulation of Zn, Cr, Ni and Cu in the kernel of the LYMZ is lower than in its bran. The amount of Zn present in the kernel of MH63 is three times lower than in the kernel of LYMZ. The major distribution of Zn in the brans of each rice cultivar is greater than in the kernel. The proportion of Sn in MH63 kernel is ten times greater than in LYMZ kernel. The kernel of both the cultivars of rice contained similar amounts of Cu. The translocation of the toxic element lead (Pb) from bran to kernel is very low in both the species of rice. The distribution of micronutrient element in the bran is significantly higher than in the kernel of both the cultivars.

Both cultivars of rice contain significant amount of copper in the bran whose translocation from bran to kernel in both cultivars of rice is not significantly different. The distribution of Cu in Kernels of both cultivars of rice is lower than 5.50 mg/kg. A very small concentration of copper is essential for both the humans and animals. The dietary demand of Cu is supplemented by the rice because of its presence in the bran as well as in the kernel. The divalent cation of copper is essential for brain energy metabolism and antioxidant metabolism. The gastrointestinal copper deficiency affects the developing cerebellum on motor function, balance and coordination (Milne and Neilson, 1996).

The concentration of chromium (Cr) in the bran of LYMZ is fivefold greater than its kernel. The accumulation of chromium in the kernel of LYMZ scales a little greater than in the kernel of MH63. Large amount of chromium remains in the bran and the edible portion (kernel) is loaded with extremely small amount. Data shows that the proportion of chromium translocation from bran to kernel is relatively very low as compared with its distribution in the bran of both the cultivars of rice. Chromium plays a significant role for carbohydrate, lipid and protein metabolism by enhancing the action of insulin (Europian Food and Safety Authority, 2009; Pokhrel and Pokhrel, 2022). A low molecular weight chromium binding substance, Chromodulin, is formed by binding chromium with an oligopeptide. Chromodulin binds with insulin receptor and promotes the activity of insulin (EFSA panel on contaminant in Food Chain, 2010). Antioxidant property of chromium also shows its nutritional importance in the food (EFSA, 2014). Therefore, it is necessary to have systematic investigation of its regulation, utilization and acquisition. The supplemental intake of chromium up to 1 mg/day has not shown any adverse effect (Cefalu et al., 2010).



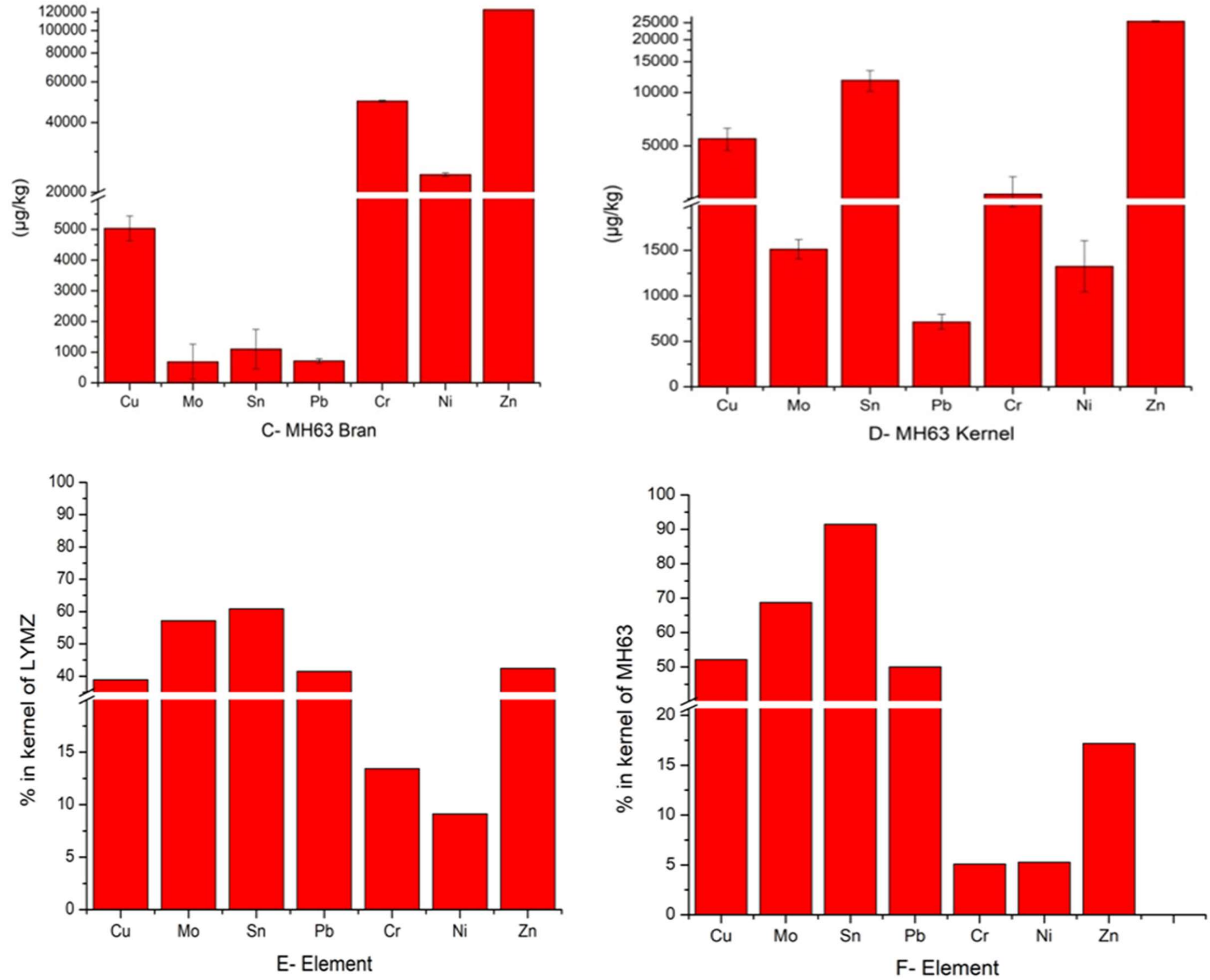


Figure 2. A- Nutrient element in the Bran of LYMZ; B- Nutrient element in the Kernel of LYMZ; C-Nutrient element in the Bran of MH-63; D- nutrient element in the Kenel of MH-63; E- % transfer of nutrient element from Bran to Kernel of LYMZ; F- % transfer of nutrient element from Bran to Kernel of MH-63.

The distribution of Cu, Mo, Sn, Pb and Cr are skewed from relatively high to low value whereas Cu and Cr has indispensable nutritional value at trace level. Therefore, it is essential for monitoring and further studies to assess the health risk and potential long term effects of these metals.

The concentration of tin (Sn) in the bran as well as in the kernel of both the cultivars of rice is very low relative to other micronutrient elements. Only 5% of the ingested amount is absorbed into the human body. There is no Sn in the body of new born baby; therefore, it is a non-essential element (Johnson et al., 2011).

The toxic element Pb in the bran of both cultivars of rice lies below 0.72mg/kg. Moreover it is 50% translocated from bran to grain in both cultivars. Paddy soil is the Primary source of lead in rice grain. The transport of Pb from soil to the grain is specially bound for human consumption. Lead contaminated paddy soil is the significant pathway for entering Pb into the human body. Different age groups have different tolerable limits of Pb in their body. The children under the age of seven have 6 µg per day and the age of seven and older have 15 µg per day of provisional total tolerable intake (PTTI). While for the adult and pregnant women, it is 75 µg and 25 µg, respectively (FAO/WHO, 1995). Provisional total tolerable intake (PTTI) level of Pb in the rice has not been ascertained yet. Pb enrichment in the rice of different cultivars is different. Even very low levels of Pb are carcinogenic and cause irreversible harmful effects to the immune system, reproductive system, kidney and neurotoxic effect as well (FAO/WHO, 1995). Hence, even a very low concentration of Pb in rice decreases its nutritional value. The consumption of rice polluted with Pb has potential health risk and can cause long term physiological effects to the human body.

The distribution of Molybdenum (Mo) in the kernels of LYMZ is not significantly different than its bran whereas the kernel of MH63 contained its amount higher than in bran. The amount of Mo in the kernel of both cultivars of rice lied in between 60-70% out of the total concentration in the grain. The load of Mo in the bran as well as in kernels of both cultivars lies below 2.00mg/ Kg rice. The amount of Mo present in the rice depends on the amount of Mo present in the soil and irrigation water. In humans, a limited number of enzymes use Mo as a cofactor. The Mo cofactor is essential micronutrient for humans. The deficiency of Mo cofactor biosynthesis in human causes the decrease of molybdoenzyme activity, which leads to neurological damage and even early childhood death (Johnson et al., 1980; Schwarz, 2005). The maximum tolerable limit is 2.00 mg/day based on intoxication in reproduction and growth in animals. Institute of medicine US panel on micronutrient (2001) set the standard dietary intake of Mo for adult men and women as 109.00 µg/day and 76.00 µg/day, respectively. Rice, as a staple food, acts as a continuous source of this precious micronutrient in trace amount for the humans.

The accumulation of Ni in bran and kernel of both the cultivars of rice was less than Cr. Its distribution in the bran of both the cultivars lied below 23.84 mg/kg where as in the kernel remained in the range 1.32 - 2.08 mg/kg. The amount retained in the kernels of LYMZ and MH63 is 10 and 20 times less than in bran of both cultivars respectively. The concentration of 0.05 to 5.00 mg/kg wet weight nickel is present in most plant tissues (Fabino et al., 2015). The normal concentration of nickel in plant tissue ranges from 0.1 to 1.0 mg/kg (Marschner, 1995). Content of nickel in the kernel of both the cultivars of rice is within the normal range. Rice kernel contained lower level of Ni than in bran. The uptake of Ni from soil and its translocation in the edible portion is positively correlated with soil Ni content. Soil pH controls the accumulation of Ni in rice grain (Wang et al., 2020). Rice is the major source of Ni exposure to human. The average daily intake of Ni for adults is estimated to be 100-300 µg/day based on standard body weight of 60 kg (USEPA, 2000).

Zinc is mainly stored in the bran. The distribution of Zinc in the bran and kernel of LYMZ is 90.36 mg/kg and 66.65mg/kg whereas in MH63 is 123.18 mg/kg and 25.45 mg/kg, respectively. The amount present in the bran was higher than in the kernel. Data shows that the translocation of Zn from bran to kernel is higher in LYMZ than in MH63. Moreover, among the above mentioned mineral micronutrients, transport of zinc from soil to grain is relatively very high. The concentration of Zn in rice grain increases its nutritional value for humans. When rice is dehulled, vitamins, minerals and zinc content are reduced in the kernel (edible portion). Zinc plays vital role to strengthen the immune system. Institute of Medicine US panel on Micronutrient established (2001) a fact that immune system is sensitive to zinc status in the body. Body cannot store the Zinc; hence, it should be supplied regularly. The deficiency of zinc in the body causes poor growth and increases the risk of infections (IOM US Panel on Micronutrient, 2001). Zinc containing rice furnishes regular admittance of this micronutrient and maintains the good health, optimizes immune system and reduces the severity of illness. The recommended daily average intake to meet the sufficient nutrient requirement for the healthy male and female above 19 years of age is 10mg and 8mg, respectively whereas the maximum tolerable limit is 40mg for both male and female(IOM US Panel on Micronutrient, 2001). Hence, it is an attractive nutrient for consumers.
The percentage translocation of micronutrient elements Cu, Mo, Sn and toxic element Pb from bran to kernel of MH63 is higher than in LYMZ whereas the translocation of Cr, Ni and Zn from bran to grain is higher in LYMZ. The percentage translocation of Mo, Sn and Pb from bran to kernel is greater than the other nutrient elements in both the cultivars of rice. In both the cultivars of rice, the percentage translocation of the micronutrient element Zn from bran to kernel is less than 50% whereas Cr and Ni remains below 15% in LYMZ and 5% in MH63.
Rice is a potential accumulator of essential micronutrient elements and toxic elements. Hence, it is necessary to evaluate the micronutrient elements and toxic element in the rice. The content of micronutrient elements and toxic elements in the rice determines its nutritional value. Different rice cultivars have different capacity to assimilate the nutrient elements and toxic elements from underground organs to above ground organs, i.e., bran and kernel. The amount of micronutrient elements and toxic elements present in the bran and kernel depends on the quality of paddy soil and rice cultivars. Hence, it is necessary to determine the micronutrient elements and toxic elements present in all the cultivars of rice to monitor the nutritional value among different rice species.

4. Conclusion

Study shows that rice contains almost the entire micronutrient elements necessary for maintaining the healthy immune system of our body. It acts as the substantial source for the potential micronutrient elements. Almost all micronutrient elements are concentrated in the bran and very small portion is translocated into the kernel.

Chromium, copper, nickel and zinc are present in significant amounts in the bran. When rice is dehulled, large portion of micronutrient elements are removed from the bran and ppb level remains in the kernel. As the staple food globally for maintaining good health, it acts as the continuous source of micronutrient elements for the human body. It is also a rich source of vitamin A and vitamin K necessary for the growth of body. Rice plant assimilates the micronutrient elements and toxic elements present in the paddy soil through underground organs (roots) to above ground organs, i.e. shoot, leaf, bran and kernel. Therefore, it is necessary to measure concentration of the micronutrient elements and toxic elements in the paddy soil before to cultivate the rice plant because soil pollution decreases the quality of the rice grain.

Conflicts of interest : The authors have declared no conflict of interest.

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