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Research paper

Determination of Cd, Cu, Ni and Pb in Selected rice Grain Available in Malaysia

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Abstract

This study aims to determine the levels of heavy metals (cadmium, copper, nickel and lead) in imported and domestically produced rice grains sold in Malaysia market. A total of 10 brands of rice samples was purchased from the local market and analyzed using atomic absorption spectroscopy (AAS). Good linearity was observed over the range studied with a correlation coefficients (R^2) of 0.9884-0.9999. The range values of the metals detected in rice grain samples were 0.67-0.8 mg/kg for cadmium, 2.14-7.0 mg/kg for copper, 1.85-2.68 mg/kg for nickel and 0.94-3.28 mg/kg for lead. The mean value of rice obtained were above the permitted level set by the Malaysia Food Act 1983 (Act 281) and Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO) (2015). The Provisional Tolerance Weekly Intake (PTWI) calculated were also above the prescribed limit except for copper.

Keywords: *AAS, Heavy metals, Rice grain, Malaysia*

1. Introduction

Rice is a staple food to more than 40% of the world population with Asia dominating the consumer and rice supply to the world rice exports [1]. Rice consumption per capita in Malaysia in 2016 was 82.3 kg with gradual increase ≥ ±0.5 kg annually since 2013. In Malaysia, 65% of long grain rice are imported from Thailand and Vietnam whereas 20% of the Basmati rice are imported from Pakistan and India [2].

Implementation of modern agriculture due to the developing pace of industrialization movement has cause an increment of heavy metals existence in plants that is derived from extensive usage of inorganic pesticide, mineral fertilizers and disposal or leakage of industrial waste [3]. Effects of these treatment has raised the bioavailibility of several toxic element in soil including heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), mercury (Hg), and lead (Pb) [4, 5,6,7]. The inorganic toxic compounds that is leached into the soil system will be osmose by plant roots through passive and active transport along with other nutrients. Heavy mental upatake are indispensable through plant metabolism and respiration process [8]. Crops such as rice plant (*Oryza sativa L.*) will be more susceptible for heavy metal absorption as it is cultivated in flooded soil, where the toxic element will be introduced both from soil and the water irrigation [9]. Other metal elements that has been reported in rice grain includes antimony (Sb), copper (Cu), nickel (Ni), vanadium (V) and zinc (Zn) [10,11,12].

Heavy metals reliability to cause human toxicity are due to the fact that it is non-biodegradable, highly persistence in human body, have long biological half-lives and toxic even being available at low concentrations [13,14,15,16]. Consumption of food products tainted with heavy metal are a potential health risk as it can cause terminal and long term physiological effects to the human body. Toxic metals As, Cd and Pb has been reported as carcinogenic to humans by the International Agency for Research on Cancer in 2012. Exposure of Cu, Cd and Pb has known to caused an adverse effect to kidney, respiratorial tract, digestion system and bones through constant daily minimal exposure [15,17]. Ni on the other hand also induced similar health impact, including disruptive effect to neurological, cardiovascular and reproductive system [18]. Pb and Cd has been a major health concern through its entry in food chain as the pollutants concentration in cultivated area has increased continously [9,10,13]. Children with age less than 8 years has lower susceptibility compared to adults age16 year and above. Danger imposed by metal food contaminants are more prominent to have severe effects to children than adult.

Amount of pollution in the environment has shown strong correlation with the concentration of heavy metals in plants including rice grains [19]. Due to the toxicity of these heavy metals, it is important to monitor their levels to be within the established threshold. Therefore, this study aims to determine the concentration of selected heavy metals (Cd, Cu, Ni and Pb) in imported and domestically produced rice grains sold in Malaysia market using atomic absorption spectroscopy (AAS).

2. Materials and methods

2.1. Reagents and solutions

Stock solutions of Cd, Cu, Ni and Pb (1000 mg/L in 2% nitric acid), HNO_3, and H_2SO_4 were obtained from Merck, Germany. Standard solutions for the calibration curves were prepared using distilled water and HNO_3.

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2.2. Samples

A total of 10 white rice grain samples commercially available were purchased from local market in Malaysia in March 2018. Selection of rice samples includes 5 imported and 5 domestically produced rice grain. For each of the brand, 50 g of rice grain was weighed and pound to become a rice grain powder. Pounded rice grain powder are kept in plastic container, labeled and stored in room temperature for further analysis.

2.3. Sample preparation

Method on *aqua-regia* digestion from Praveena et al. was used with slight modification [12]. An amount of 2.5 g of rice grain powder are weighed and decomposed using 1:5 ratio (v/v) of H_2SO_4 and HNO_3. The digested solution was heated to 90 °C using a hotplate until the red fume has evaporated completely. A 10-mL distilled water was added to the mixture and filtered using Whatman 0.45 µm filter paper. The sample solution was diluted to a final volume of 25 mL with distilled water and then stored in a glass container to be analyzed on the same day.

2.4. Atomic absorption spectroscopy analysis

The digested sample was analyzed using atomic absorption spectroscopy (AAS) from Perkin Elmer (model Analyst 400) with hollow cathode lamp as spectral sources for Cd, Cu and Ni and Electrodeless Discharge Lamp for Pb. The wavelength was adjusted at 228.80 nm, 324.80 nm, 232.00 nm and 283.31 nm for Cd, Cu, Ni and Pb, respectively. The lamp current and slit width of each metal was adjusted accordingly for the determination of Cd, Cu, Ni and Pb. Washing step using distilled water was conducted between sample intervals to ensure no deposition of the previous metal in the instrument. Concentration of the metals was expressed as mg/kg.

3. Results and Discussion

3.1. Analytical method validation

The LOD and LOQ were determined using the following equation, LOD = 3 × SD/S and LOQ = 10 × SD/S, where, SD is the standard deviation of a response, and S is the slope of the corresponding calibration curve. Each sample were replicated in triplicates, n=3. Linearity of the calibration plots was studied using standard solutions. Calibration curve was constructed by plotting the response value (y) versus the concentration of analyte (x). Correlation coefficients, R^2 of the calibration curves shows linear relationships (0.9884-0.9999) over the selected ranges of heavy metal concentrations as indicated in Table 1.

Table 1: Analytical characteristics of the method

Metal	Calibration curve of standard	R^2	LOD (mg/kg)	LOQ (mg/kg)
Cd	y = 0.2509x + 0.0001	0.9999	0.08	0.33
Cu	y = 0.1113x + 0.0019	0.9967	0.21	0.67
Ni	y = 0.0551x + 0.00005	0.9987	0.3	1.0
Pb	y = 0.0116x - 0.0006	0.9884	0.2	0.67

3.2. Heavy metal in domestic and imported rice samples

Heavy metal composition in rice sample was determined in mg/kg and tabulated in Table 2. The concentration of metal elements acquired from this study were 0.67-0.8 mg/kg for Cd, 2.14-7.0 mg/kg for Cu, 1.85-2.68 mg/kg for Ni and 0.94-3.28 mg/kg for Pb. The data shows Cu has the highest concentration, followed by Pb, Ni and Cd.

The highest concentration of heavy metals detected in imported rice samples were 7.0 mg/kg for Cu (Import 1), 3.00 mg/kg for Pb (Import 5), 2.59 mg/kg for Ni (Import 5) and 0.8 mg/kg for Cd (Import 5). As for domestic rice samples, the highest concentration obtained were 4.44 mg/kg for Cu (Domestic 1), 3.28 mg/kg for Pb (Domestic 2), 2.68 mg/kg for Ni (Domestic 3), and 0.77 mg/kg for Cd (Domestic 3). Domestic rice samples dominated the highest concentration values for Ni and Pb whereas imported rice samples has highest concentration of Cd and Cu.

A study on Iranian grown and imported rice indicated the domestic rice sample has higher concentration of Cd, Ni and Pb compared to the imported rice grain with values of 0.16/0.13 mg/kg, 0.22/0.76 mg/kg and 0.196/0.55 mg/kg each, respectively [20]. Another similar study on Iranian rice grain indicated that imported sample has higher mean concentration of Cd, Pb, Cr, Ni and Co than domestically grown rice product [21].

Table 2: Heavy metals contents in domestic and imported rice sample (means ± standard deviation) in mg/kg, n=3

Rice Type	Origin	Cd	Cu	Ni	Pb
Domestic 1	Malaysia	0.72±0.02	4.44±0.01	1.85±0.01	0.94±0.01
Domestic 2	Malaysia	0.72±0.01	2.39±0.01	2.00±0.01	3.28±0.09
Domestic 3	Malaysia	0.77±0.01	2.79±0.01	2.68±0.01	2.91±0.05
Domestic 4	Malaysia	0.70±0.01	3.68±0.01	2.32±0.02	1.97±0.04
Domestic 5	Malaysia	0.75±0.01	3.93±0.03	2.56±0.01	3.17±0.08
Import 1	Pakistan	0.68±0.01	7.00±0.02	1.98±0.01	2.05±0.04
Import 2	Thailand	0.67±0.01	3.01±0.02	2.08±0.01	1.81±0.05
Import 3	Thailand	0.71±0.01	2.97±0.01	1.98±0.06	1.72±0.07
Import 4	Thailand	0.65±0.01	2.14±0.01	1.97±0.02	1.04±0.09
Import 5	Thailand	0.80±0.01	2.78±0.02	2.59±0.01	3.00±0.05

3.3 Heavy metal assessment on permitted level of metal contaminants

Mean concentration of Cd, Cu, Ni and Pb are 0.72 mg/kg, 3.51 mg/kg, 2.20 mg/kg and 2.19 mg/kg, each respectively. Cd and Pb detected in rice samples in this study were higher than the permitted level of Cd and Pb contaminants in specified food in Malaysia Food Act 1983 (Act 281) & Regulations which are 0.4 mg/kg for Cd in rice and 2 mg/kg for Pb in any specified food. Permitted limit of Cu and Ni for rice or other specified food was not mentioned in Malaysia Food Act 1983 (Act 281) Regulations except for in drinking water [22].

Heavy metal and trace elements in rice grain can be reduced by up to 80 % through washing and soaking as demonstrated by Al Saleh et al. [23]. However, another study on heavy metal concentration in cooked rice sample using most preferred brands in Malaysia indicate the mean range of Cd detected were 0.090-0.63 mg/kg, Cu 1.10-3.30 mg/kg and Pb 0.096- 0.19 mg/kg [12]. Their maximum Cd range exceed the permissible limit by Malaysia Food Act 1983 (Act 281) & Regulations. The highest level they achieved is still lower than the mean value obtained in this study but it does show the value retained in the cooked rice sample can imposed serious effect due to daily and twice a day diet consumption.

Provisional Tolerance Weekly Intake (PTWI) was established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as a term used to express the accumulation of contaminant in the body through weekly intake of the specified food [24]. In this study, PTWI was calculated using the overall mean body weight for Malaysian population which are 62.65 kg and consumption of daily intake of cooked rice which are 2 and half plates per day (160 gm) [25,26,27]. Mean PTWI value calculated for this study was above the PTWI value set by FAO/WHO (2015) except for Cu, which can be triggered as a potential health hazard.

Table 3: Calculated PTWI (mg/kg bw/week) values for domestic and imported rice.

Metal	Min (mg/kg bw/week)	Max (mg/kg bw/week)	Mean (mg/kg bw/week)	PTWI** (mg/kg bw/week)
Cd	0.012	0.014	0.013	0.007
Cu	0.038	0.125	0.061	0.500
Ni	0.033	0.048	0.039	0.035
Pb	0.017	0.099	0.039	0.025

**PTWI stated in FAO/WHO (2015) [23, 24]

3.3.1. Cadmium

Cd detected in rice sample were above the permitted limit (0.72 mg/kg) that has been set by the Malaysia Food Act 1983 (Act 281) & Regulations and FAO/WHO (2015) [22,24]. China has reported a similar study where the range were between 0.128 mg/kg to 0.806 mg/kg [28]. Shiraz in Iran and Jamaica reported concentration of Cd for rice grain and imported rice grain at 0.27-0.48 mg/kg and 0.004-0.19 mg/kg, each respectively [21,29]. Variation on the amount of Cd between past studies indicate other factors should be considered as a parameter such as level of Cd in soil, water supply and fertilizers used on the rice crops [9,13,25]. Cd embedded in rice grain will not be fully released from the grain matrix during washing and it will become soluble during cooking process due to its acidity [30]. This will constitute major health effect to humans as cadmium will be filtered in kidney and can retain for 10-35 years in its active form due to its long biological half-life. The end results can cause irreversible kidney dysfunction, severe osteoporosis and cancer to multiple organs [31].

3.3.2. Copper

Cu is an essential element to plant growth but could be toxic to plants in higher concentration. Permitted level for Cu and Ni were only set for drinking water and not in other food groups by the Malaysia Food Act 1983 [18,22]. Mean Cu detected in this study was 3.51 mg/kg, with the range of 2.14-7.0 mg/kg. Cu determination in rice crops has been reported between 3.095-4.54 mg/kg in Iran and China [12,29,30,32,33]. Rice grain in Jamaican market has Cu range of 0.77 to 2.72 mg/kg with mean of 1.65 mg/kg [4,25,29]. Different rice cultivars have showed great variation of Cu accumulation capacity, thus explains the significant variation range [4,26, 29,31,34]. Cu accumulation in rice grain are justified where Cu storage in roots can be translocated directly to the seed grain when movement of mass Cu flows during plant metabolism. However, seed grains contained the least Cu storage after roots, stems and leafs of rice plants [35].

3.3.3. Nickel

Ni concentration and PTWI value was above the permitted level as set by FAO/WHO (2015) [24]. Study on Ni concentration in rice crops are limited as Ni contamination are more prone in water and air. Ni induced toxicity are primarily through inhalation, causing effects that include skin allergies, lung fibrosis and cancer [36]. Ni dispensability and stability in plant are crucial for urease enzymatic reaction to occur where deficiency of nickel will result in lesions on plant [37]. Therefore, Ni uptake by plant are considered as an essential micronutrient which explains the value of its presence. Ni in soil are relatively soluble and derived from sedimentation from industrial waste and the atmosphere [38]. Crops such as coca, soya based, dry legumes are identified as foodstuff with high Ni content [39]. Studies have shown Ni excreted from the human body through urine, faeces and sweat [40]. Although ingestion of Ni can be flushed from the human system, health concern on Ni food contamination shall not be taken lightly as mixture of Ni with other toxic active compound in human system may result in severe toxicity.

3.3.4. Lead

Pb enrichment in rice from different cultivars and different countries has been demonstrated [19,20,23, 29,32,41-43]. Samples that are positive for Pb content are mostly with value higher than the stated by national standard limit and FAO/WHO (2015), including rice grain from Peninsular Malaysia and Thailand [24,33,43]. Mean value of Pb in this study were above the permitted limit by Malaysia Food Act 1983 (Act 281) & Regulations and FAO/WHO (2015) [22,24]. Plants cultivated in uncontaminated soil have Pb concentration of less than 1.00 mg/kg. Plants cultivated in contaminated soil due to mining and mineralization has reported range of 0.001 mg/kg to 3.34 mg/kg level of Pb [11,44,45]. Similar to Cd, Pb does not hold any biological role in plant physiology but it can be effectively absorbed by plant in an alarming level [46]. Rice status as a staple food in Asian dietary cause it to become the leading source of intake for Cd and Pb [11,12,19,20,23,42]. Pb has already been classified as carcinogenic by International Agency for Research on Cancer and continuous exposure to low level of Pb can cause irreversible harmful effect to the immune system, kidney, and reproductive system [24,47]. Children holds greater risk for neurotoxicity effects of Pb as it is able to pass through the blood-brain barrier and cause Pb-induced damage such as functional impairment, intellectual and behavioral deficits [48,49].

4. Conclusion

Mean values of Cd, Cu, Ni and Pb were determined using AAS in domestic and imported rice grain samples available in the Malaysian market. The values for heavy metals in all samples were higher than the permitted level of metal contaminants in specified food in Malaysia Food Act 1983 (Act 281) & Regulations [18]. PTWI (mg/kg bw/week) of the metals were also above the prescribed level by FAO/WHO (2015) except for Cu [23]. The values can be reduced through proper washing and soaking of the rice grain prior to cooking but level of the metals still persist in the rice after it is cooked [12, 40]. Source of the heavy metal in rice grain could be attributed primarily to soil content. Volume of industrial waste, inorganic pesticides and fertilizers usage in soil shall be controlled and improvised its formulation to reduce the heavy metals excess and accumulation in plant and soil. Aggressive steps to mitigate the heavy metal uptake in rice grain must be implemented effectively in order to avoid future health effect on epidemic level.

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