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DETERMINATION OF HEAVY METALS IN RICE AVAILABLE IN KANDY DISTRICT, SRI LANKA

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ABSTRACT

Rapid industrialization of the global economy has led to severe environmental pollution with chemicals resulting in contamination of most of the agricultural food products, especially rice, the staple food of South Asians. The contamination of heavy metals in rice, marketed in Kandy district was evaluated using inductively Coupled Plasma Mass-spectrophotometer (ICP-MS). Both locally cultivated and imported rice in Kandy district were analyzed for heavy metals. A total of 68 samples of branded, local, traditional and imported rice were investigated for selected heavy metals (As, Cd, Pb, Hg, and Se). The range of mean concentrations of different metals observed in different rice varieties were: 0.0106 – 0.1303 mg/kg, Pb: 0.150 – 0.2111 mg/kg, Cd: 0.0033 – 0.0480 mg/kg, Hg: 0.0056 – 0.0355 mg/kg and Se: <0.2 – 0.4706 mg/kg. Except mean concentration of Se in imported rice and Pb in traditional rice, none of the other mean concentrations of heavy metals exceeded the permissible level recommended by FAO/WHO Joint CODEX Alimentarius. It also reveals that 12% of total samples analyzed, including branded, imported and traditional rice were contaminated with Pb and estimated daily intakes(edis) of Pb, exceeded the safer value of tolerable daily intake(TDI) limit set by Joint FAO/WHO Expert Committee on Food Additives (JAFCA). Further 33% of imported rice samples were contaminated with As, Pb, Hg and Se and relevant edis exceeded the tdis of each element recommended by JAFCA. Out of total samples analyzed, 98% of samples were found not to exceed the Tolerable Daily Intake (TDI) with respect to heavy metals, namely, As, Cd, Se and Hg, while with regard to Pb, 88% of total samples were also under safer limits. The findings reveal that generally, rice available in Kandy district is quite safe with respect to the heavy metal contamination.

Key words: Daily intake, Heavy metals, Rice, Sri Lanka, CODEX.

INTRODUCTION

Rice (*Oryza sativa*) is the prioritized crop by most of the farmers throughout the year since it is the most widely consumed staple food and main carbohydrate source among Sri Lanka. Different natural and artificial sources of pollutants can contaminate the soil, plants and other environmental components with heavy metals (Voica *et al.,* 2012). Some of these agrochemicals contain trace heavy metals as impurities (Rezania *et al*., 1989). It was reported that chemical fertilizers, such as Super Phosphate contains considerable level of Cadmium as impurities (Pierzynski *et al*., 2000) and animal manure also contains some amount of trace metals (Wijewardena *et al*., 2004). Excessive addition of agrochemicals could lead to the contamination of agricultural soils. However, Rosemary *et al.,* 2014 has reported that both agricultural and non-agricultural soils were not contaminated to an environmental risk level of Cd, Pb and few other heavy metals. Currently, in Sri Lanka excessive use of agrochemicals; chemical pesticides and fertilizers has become a wrong but common practice. Many studies have shown that heavy metals in those fertilizers could accumulate in soils and become readily available to plants depending on the conditions prevailing (Sanjeewani *et al.,* 2013 and Chandrajith *et al*., 2012).

Heavy metals are potential environmental contaminants with the capability of causing human health problems; if it presents excess in the food we eat (Havanur *et al*., 2014). Heavy metals have long biological half-lives and they can be bio – accumulated through the food chains and finally to man and produce detrimental effects on human health. As a result, the presence of high amount of heavy metal in the environment represents a potential danger for human health. Elements such as Cd, Cr and As are considered carcinogenic (Edem *et al*., 2009). Jayasumana and co-workers have identified to have very large amounts of Arsenic in rice. The level of arsenic detected in rice was found to exceed the maximum tolerable limit in certain areas of Sri Lanka. (Jayasumana *et al*., 2015). Chandrajith *et al*., (2012) also reported that Cd levels in drinking water, surface water, rice and other geo-environmental media are within acceptable limits of Codex Alimentatius Commission allowable limit (0.2 mg/kg).

Therefore, this study was undertaken to collect information on the extent of heavy metals in commercial rice in Kandy district. Furthermore, concentration of heavy metals in rice were compared with permissible level recommended by FAO/ WHO CODEX Alimentarious commission to understand whether there is a potential health hazard.

MATERIALS AND METHODS

Sampling of rice in Kandy district

Rice samples were collected throughout the Kandy district. According to the Department of Statistics highest population was found in Gangawata Korale, Kundasale, Yatinuwara, Udunuwara, Udaplalatha and Pathadumbara divisions. Thus rice samples were collected from the super market and local market in those regions. The rice samples were divided into five major groups of branded rice (Samba raw, Samba steamed, Nadu raw and Nadu steamed. Keeri samba), few traditional rice verities, two imported rice varieties of Basmathi and Ponni samba. Rice cultivated in Kandy district was also included in these samples. Sampling was done at random from different retailers and venders/super markets within these selected areas. All the samples were collected and stored in polythene bags and transported to the Laboratory for analysis. Considering the locally grown rice cultivation in Kandy district, highest rice cultivation was found in Minipe, Ududumbara, Medadumbara, Patha hewaheta, Udunuwara and Pathadumbara. Bg 403 variety is the newly improved and most popular variety grown in Kandy district.

Heavy metals Selected for study

Elements of Arsenic (As), Cadmium (Cd), Mercury (Hg), Lead (Pb) and Selenium (Se) were selected for the heavy metal analysis.

Maximum Permissible Level of heavy metal in rice

Maximum Permissible level (ML) means the maximum level of a specified contaminant, or specified natural toxicant, which is permitted to be present in a nominated food expressed, unless otherwise specified, in milligrams of the contaminant or the natural toxicant per kilogram of the food (mg/kg). According to the WHO/FAO Joint CODEX Alimentarius, maximum permissible levels of testing heavy metals are given in Table 1.

Table 1.Maximum Permissible Level (MPL) of heavy metal in rice.

Metal Type	As	Cd	Pb	Hg	Se
MPL (ppm)	0.2	0.2	0.2	0.1	0.3

Sample Analysis

Analyses were carried out at the Laboratory of the Office of the Registrar of Pesticides. Samples were cleaned with distilled water, dried and ground to yield powdered samples. Samples were stored in the cleaned polyethylene bags until further analysis. Rice sample was homogenized and 0.2 g of the sample was digested with 8.0 cm^3 of Concentrated Nitric Acid (69%, Analytical Grade), 0.5 cm^3 Conc. HCl and one millilitre of hydrogen peroxide (H_2O_2) (30%) in a microwave digester (CEM Gmbh-Jerman model: MARS 6; Serial No. MJ 2941). Elemental analysis was performed as per the laboratory validated method using FDA Elemental Analysis manual for Food and related products Analytical Methods (Ver. 1.0;2013), FDA U.S. Food and Drug Administration. The toxic five elements of arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) were determined by using Inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Fisher Scientific iCAP Q Model, SN-03380R). The equipment was calibrated by using Standard Reference Materials of elemental mixture.

In order to maintain the precision and accuracy of the sample analysis, method blank and Certified Reference Materials were analyzed with other unknown samples at each batch of rice sample analysis. All rice samples were analyzed in duplicates. As per the ISO/IEC 17025 Standards, two food samples of known concentrations of heavy metals were analyzed as Proficiency Testing in order to detect the accuracy of analysis.

RESULTS AND DISCUSSIONS

Nearly sixty-eight (68) samples were collected and analyzed for the selected heavy metals. Maximum, minimum and mean concentration of heavy metal contents available in the rice samples collected from the Kandy district is tabled below (Table 2).

Table 2. Minimum, Maximum and Mean Concentration of heavy metal contents available in rice samples collected from the Kandy district.

Elements	LOQ mg/kg	Max. permissible level (MPL) (mg/kg)	Rice category	Samples tested	No. of positive samples (%) >LOQ	Mean concentration mg/kg	Mini. concentration (mg /kg)	Max. concentration (mg/kg)
As	0.010	0.2	Branded rice in the market	43	67%	0.0238 ±0.025	ND	0.0953
			Imported rice	09	56%	0.1303 ±0.227	ND	0.716
			Traditional rice	09	33%	0.0106 ±0.018	ND	0.0512
			Locally grown rice	07	100%	0.0392 ±0.020	0.0152	0.0818
Pb	0.150	0.2	Branded rice in the market	43	35%	0.139 ±0.283	ND	1.3
			Imported rice	09	44%	0.1289 ±0.177	ND	0.496
			Traditional rice	09	44%	0.2111 ±0.317	ND	0.8
			Locally grown rice	07	0	ND	ND	ND
Cd	0.020	0.2	Branded rice in the market	43	67%	0.0480 ±0.043	ND	0.2
			Imported rice	09	56%	0.0417 ±0.054	ND	0.153
			Traditional rice	09	11%	0.0033 ±0.01	ND	0.03
			Locally grown rice	07	43%	0.0208 ±0.031	ND	0.0823
Hg	0.020	0.1	Branded rice in the market	43	14%	0.0056 ±0.014	ND	0.0693
			Imported rice	09	11%	0.0223 ±0.067	ND	0.201
			Traditional rice	09	33%	0.0116 ±0.017	ND	0.0362
			Locally grown rice	07	86%	0.0355 ±0.019	ND	0.0651
Se	0.200	0.3	Branded rice in the market	43	2%	0.0046 ±0.03	ND	0.2
			Imported rice	09	33%	0.4706 ±0.912	ND	2.686
			Traditional rice	09	0	ND	ND	ND
			Locally grown rice	07	0	ND	ND	ND

Note: ND – Less than LOQ (<LOQ).

The Table 2 represents the mean, minimum and maximum concentrations of heavy metals of branded rice, locally cultivated, traditional and imported rice in Kandy district. The results of the analysis showed that Pb has the highest mean concentration in branded rice followed by Cd, As, Hg and Se with the distribution pattern of Pb> Cd> As> Hg> Se, while Se has the highest mean concentration in imported rice followed by As, Pb, Cd and Hg with the distribution pattern of Se> As> Pb> Cd> Hg. The results further indicated that the distribution pattern of heavy metals in traditional rice was Se> As> Pb> Cd> Hg and same for the rice grown in Kandy. It also revealed that highest mean concentration of As and Se was reported in imported rice while Pb in traditional rice, Cd in branded rice and Hg in rice grown in Kandy district.

The highest value of maximum concentrations of As, Hg and Se was reported in imported rice varieties while that of Pb and Cd, were reported in branded rice. The results revealed that only the mean concentration of lead (Pb) in traditional rice and mean concentration of selenium (Se) in imported rice exceed the Permissible Levels (WHO/FAO) of relevant elements in rice.

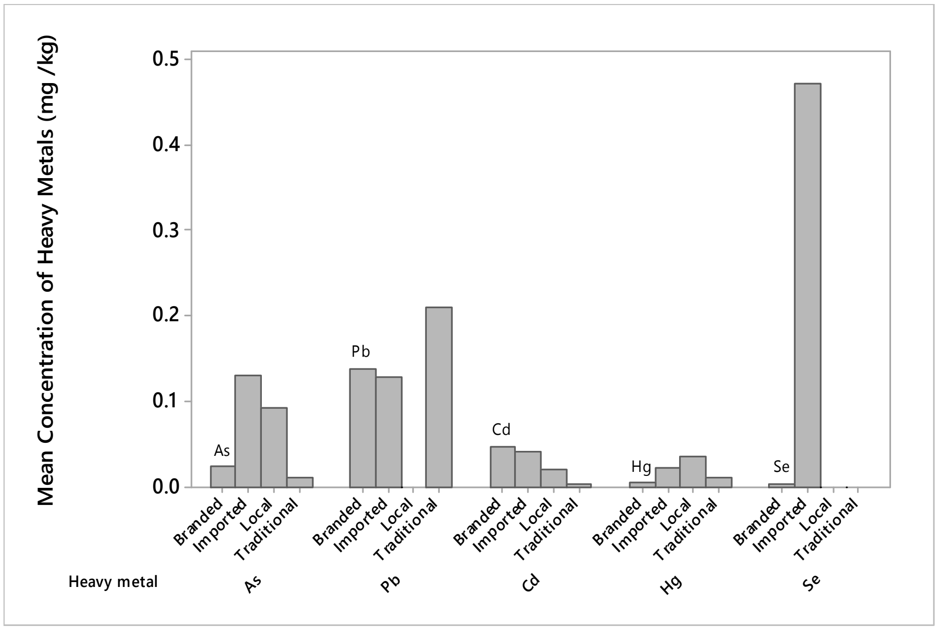

Figure 1. Comparison of Mean concentrations of heavy metals in rice varieties.

Table 3. Minimum, Maximum and Mean concentrations (mg/kg) of heavy metals in samba and Nadu varieties.

Element	Variety	Mean heavy metal concentration (mg/kg)			
		No. of samples	Mean concentration (mg/kg)	Minimum concentration (mg kg)	Maximum concentration (mg/kg)
Arsenic	Samba	17	0.034±0.026	<0.01	0.0738
	Nadu	26	0.017±0.022	<0.01	95.3
Lead	Samba	17	0.051±0.130	<0.15	0.5
	Nadu	26	0.196±0.339	<0.15	1.3
Cadmium	Samba	17	0.044±0.031	<0.02	99.5
	Nadu	26	0.05±0.050	<0.02	0.2
Mercury	Samba	17	0.00175±0.007	<0.02	29.75
	Nadu	26	0.0082±0.017	<0.02	69.3
Selenium	Samba	17	<0.2	<0.2	<0.2
	Nadu	26	0.007±0.039	<0.2	0.2

The results reveal that, arsenic content observed in samba is higher than that of Nadu, while cadmium, lead and mercury contents in Nadu were higher than that of samba varieties. It's clearly visible in Figure 2.

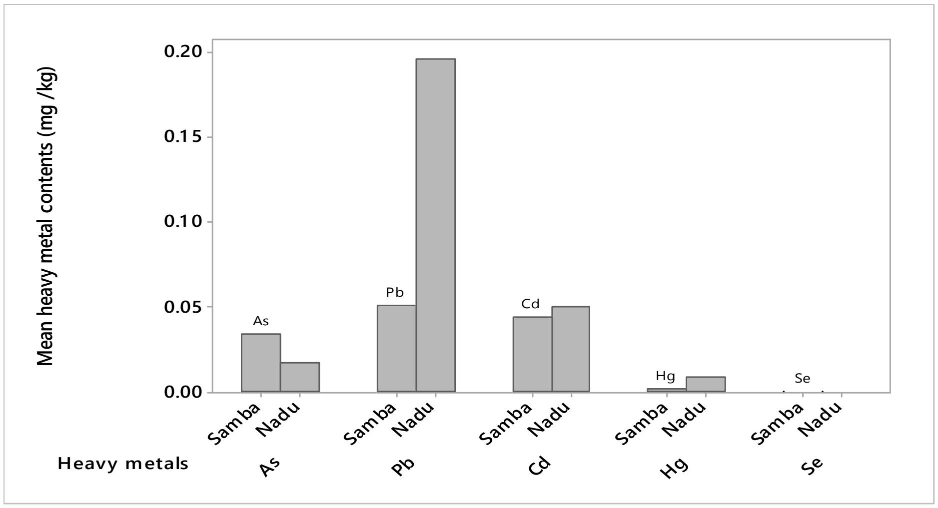

Figure 2. Mean concentrations (mg/kg) of heavy metals in samba and Nadu varieties

Heavy metal contents in raw and steamed rice categories

Concentration of selected heavy metals as means ± standard deviations in Nadu and samba varieties of samba raw, keeri samba, samba steamed, Nadu raw and Nadu steamed available in Kandy market are shown in Table 4 and Figure 3 below.

Table 4. Mean concentrations ± standard deviations (mg kg-1) of heavy metals in raw and steamed rice of branded varieties.

Category	No. of samples	Mean Concentration of Elements (mg/kg)				
		As	Pb	Cd	Hg	Se
Samba raw	07	0.036±0.024	0.026±0.069	0.050±0.028	0.004 ±0.011	<0.2
Keeri samba	03	<0.01	0.166±0.288	<0.02	<0.02	<0.2
Samba steamed	07	0.046±0.023	0.026±0.070	0.057±0.022	<0.02	<0.2
Nadu raw	10	0.024±0.027	0.18±0.059	0.063±.039	0.012 ±0.022	0.02 ±0.063
Nadu steamed	16	0.013±0.018	0.307±0.394	0.041±0.05	0.005 ±0.012	<0.2

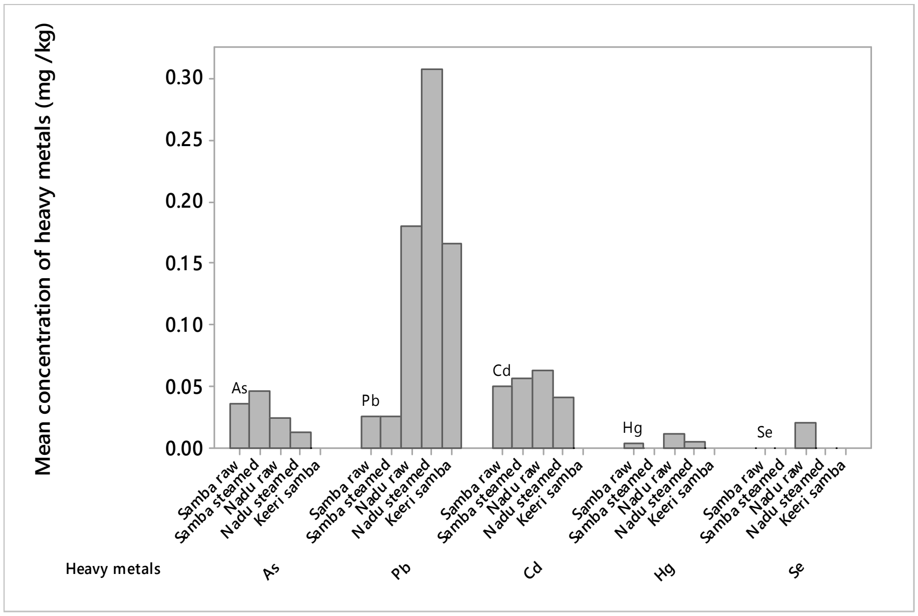

Figure 3. Comparison of heavy metals Content in raw and steamed rice types.

Keeri samba contains 0.166 mg/kg of lead while other elements of As, Cd, Hg and Se are below the detection limits. There is no significant difference of heavy metal contents in samba raw and samba steamed while heavy metal content in Nadu raw and Nadu steamed are significantly different.

Heavy metals in imported rice available in Kandy district

Concentration of selected heavy metals as means ± standard deviations in imported rice varieties of basmathi and ponni samba available in Kandy market are shown in Table 5 and Figure 4 given below.

Table 5. Mean Concentration ± standard deviations of Heavy metals in imported rice available in Kandy district.

Category	No. of samples	Mean Concentration of Elements (mg/ kg)				
		As	Pb	Cd	Hg	Se
Basmathi	03	0.062±0.054	0.221±0.064	0.051±0.050	<0.02	0.373±0.646
Ponni samba	06	0.164±0.278	0.082±0.202	0.037±0.059	0.033±0.082	0.519±0.981

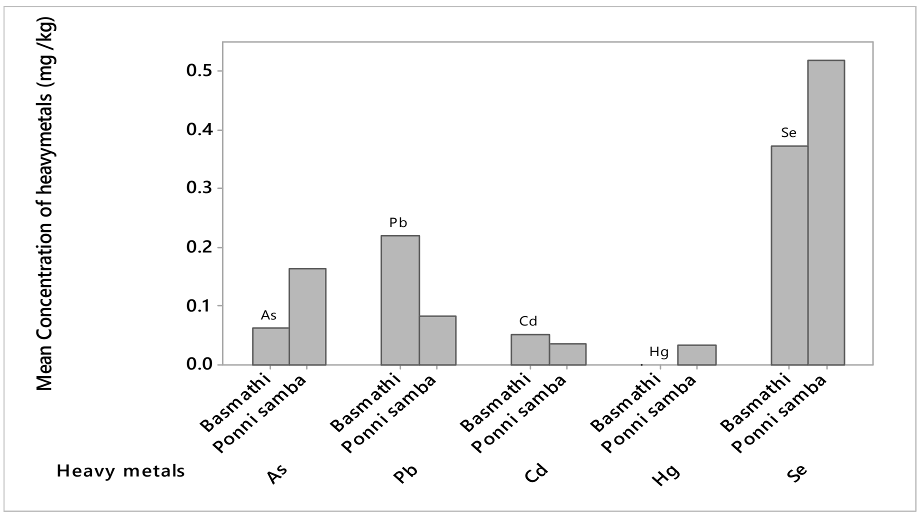

Figure 4. Comparison of heavy metal content in imported rice.

The results showed that Ponni sanba contains higher mean content of arsenic, mercury and selenium contents than that of Basmathi rice. Basmathi reported higher lead and cadmium contents than that was reported in Ponni samba.

Heavy metals in Traditional Rice and Rice Grown in Kandy District

Concentration of selected heavy metals as means ± standard deviations in varieties of traditional rice and locally grown rice varieties available in Kandy market /fields are shown in Table 6 and Figure 5 given below.

Table 6. Mean Concentration ± standard deviations of heavy metals in traditional rice and rice grown in Kandy district.

Category	No. of samples	Mean Concentration of Elements (mg /kg)				
		As	Pb	Cd	Hg	Se
Traditional rice						
Heenati	03	0.017 ±0.029	0.333 ±0.416	0.010 ±0.017	0.012 ±0.020	<0.2
Kuruluthuda	02	0.008 ±0.011	0.100 ±0.141	<0.02	0.016 ±0.022	<0.2
Suwendel	02	0.014 ±0.019	0.350 ±0.494	<0.02	0.018 ±0.025	<0.2
Rice grown in Kandy district (BG 403)	07	0.039 ±0.020	<0.15	0.020 ±0.031	0.035 ±0.019	<0.2

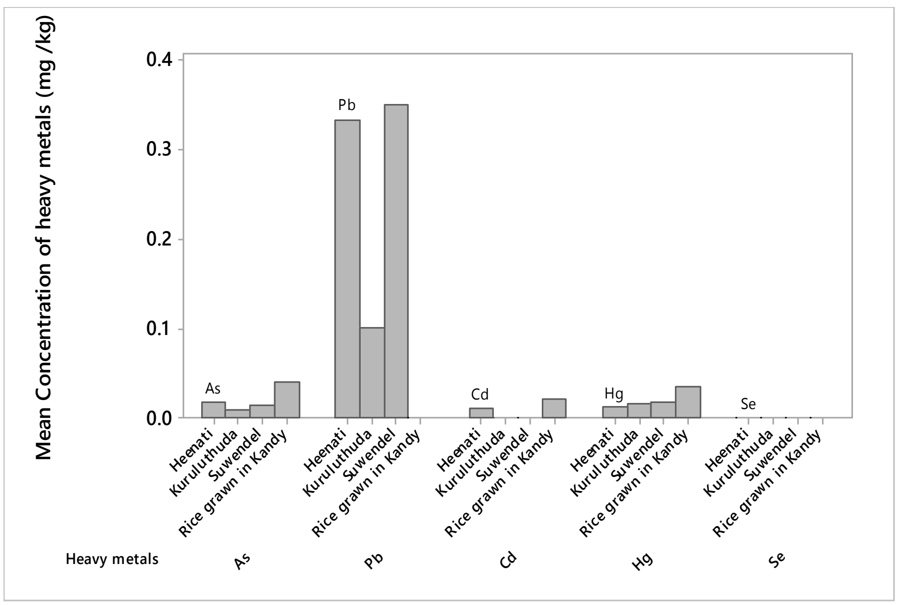

Figure 5. Comparison of mean concentrations of heavy metals in traditional and rice grown in Kandy district.

Though all the traditional rice samples contain higher amount of lead, the amount reported in locally grown rice samples are below the detection limits. Comparatively mean concentrations of Arsenic, Cadmium and mercury in rice grown in Kandy district, are higher than that of Traditional rice. It reveals that lead is freely available in the most of the rice varieties and highest amount of lead was reported in the traditional rice varieties of Suwendal rice collected from the Kandy district (0.350 mg /kg). In commercial rice varieties, higher content of lead was reported through Nadu varieties (0.196 mg /kg) and steamed Nadu contains higher amount of lead than the raw Nadu varieties. Maximum Permissible level (ML) of lead in rice is 0.2 mg /kg and lead concentration of traditional rice of Heenati and Suwedel exceed the ML of lead.

Safer values of Tolerable Daily Intake (TDI) for the selected heavy metals

Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended the provisional tolerable weekly intake (PTWI)/ provisional tolerable monthly intake (PTMI) of individual heavy metals to compare their pollution levels considering their toxicity accumulated in the human body. Accordingly Tolerable Daily Intake (TDI) of the relevant heavy metals were calculated and reported in Table 7.

Table 7. Safer values of Tolerable Daily Intake (TDI).

Metal Type	As	Cd	Pb	Hg	Se
TDI µg /kg bw /day	3.0[a]	0.8[b]	1.5[d]	0.57[c]	5
TDI Mg /kg bw /day * 10^{-3}	3.0	0.8	1.5	0.57	5

Note: a - The provisional tolerable weekly intake (PTWI) of 21 µg/kg bw (equivalent to 3 µg/kg bw/day) according to JEFCA (Joint FAO/WHO Expert Committee on Food Additives) (2010) (TRS 959); b-PTMI 0.025 mg/kg bw on a monthly basis according to JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2010) (TRS 960); c- 4 µg/kg bw per week, according to JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2010) (TRS 959); d-Based on cardio-vascular effects according to EFSA (European Food Safety Authority) (2010) FAO/WHO CODEX Alimentarius Commission and Environmental Protection Agency (EPA).

Calculation of Estimated Daily Intake of heavy metals observed in rice varieties

Mean estimated daily intake of heavy metals through different rice types were calculated by assuming daily intake of rice by a Sri Lankan healthy individual (60 kg of body weight) is 300 g. Safer values of Tolerable Daily Intakes of heavy metals and estimated daily intake of that for the rice samples analyzed are given in the Table 8.

$$EDI = (C_{metal}) \times DiR) / Bw$$

Where, C_{metal} = Concentration of metal in analyzed sample (µg kg-1), DiR= Daily intake of rice (kg day-1), Bw= Average body weight (kg).

Mean estimated daily intake of heavy metals through all the rice varieties:

Mean estimated daily intake of heavy metals through rice varieties were calculated and compared with the safe values of TDI (mg day-1kg-1bw).

Maximum, Minimum and Mean Estimated Daily Intakes of heavy metals in rice varieties and safer values of TDI were given in the Table 8. Estimated daily intakes of the rice varieties were compared with the Tolerable Daily intakes (TDI) of the relevant elements. None of the Estimated Daily intakes calculated from the mean concentrations of the heavy metals reported from the rice varieties are exceeded the safer values of Tolerable Daily intakes (TDI).

Table 8. Estimated daily intake of heavy metals through commercial rice

Element	Safer values of TDI x 10^{-3} (mg day^{-1} kg^{-1}bw)	Rice varieties	MEDI x 10^{-3} (mg day^{-1} kg^{-1} bw)	MinI x 10^{-3} (mg day1kg1bw)	MaxI x 10^{-3} (mg$day^{-1}$$kg^{-1}$bw)	No. of samples (% exceeded the TDI
As	3.0	Branded rice	0.119	0.05	0.4765	
		Imported rice	0.6515	0.05	3.58	01(1.47%)
		Traditional	0.053	0.05	0.256	
		Locally grown	0.196	0.076	0.409	
Pb	1.5	Branded rice	0.695	0.75	6.5	05
		Imported rice	0.6445	0.75	2.48	01 (11.76%)
		Traditional	1.0555	0.75	4	02
		Locally grown	0.75	0.75	0.75	
Cd	0.8	Branded rice	0.24	0.1	1	01(1.47%)
		Imported rice	0.2085	0.1	0.765	
		Traditional	0.0165	0.1	0.15	
		Locally grown	0.104	0.1	0.4115	
Hg	0.57	Branded rice	0.028	0.1	0.3465	
		Imported rice	0.1115	0.1	1.005	01(1.47%)
		Traditional	0.058	0.1	0.181	
		Locally grown	0.1775	0.1	0.3255	
Se	5	Branded rice	0.02325	1	1	
		Imported rice	2.353	1	13.43	01(1.47%)
		Traditional	1	1	1	
		Locally grown	1	1	1	

Note: TDI- Tolerable daily intake (µg day-1 kg-1bw), MEDI- Mean estimated daily intake (µg day-1 kg-1bw), Min I- Minimum estimated daily intake (µg day-1 kg-1bw), Max I- Maximum estimated daily intake (mg day-1 kg-1bw).

CONCLUSION

Rice samples were analyzed under four categories of branded rice varieties, imported rice varieties, traditional rice varieties and locally grown rice varieties. All together 68 samples were analyzed in the project. Elements of As, Cd, Pb and Hg are more toxic elements than Se. Se is essential trace element for animals. It takes part in thyroxine conversion to tridethyronine in thyroid hormone biosynthesis and it is a serious factor of biological and antioxidant protection of DNA, chromosomes.

Considering the mean concentrations of the heavy metals contain in the rice samples, none of the concentration of heavy metals exceeded the relevant maximum permissible levels (MPLs) recommended by FAO/WHO, except selenium in imported rice and lead in traditional rice. To assess daily intake of heavy metals by rice, daily consumption of rice was calculated (EDI). The results showed that EDIs of Pb in five rice samples of branded rice, one of imported rice and two of traditional rice samples (11.76% of total samples) exceeded the Tolerable Daily Intake (TDI) of Pb reported by JAFCA. The results also indicate that 33% of imported rice was contaminated with AS, Hg, Pb and Se and relevant EDIs exceeded the relevant TDI limits of JAFCA. However none of the Mean Estimated Daily Intakes (MEDIs) exceeded the Tolerable Daily Intake of relevant heavy metals recommended by JEFCA and out of the total samples analysed, 98% of samples were found not to exceed the TDI with respect to heavy metals, namely, As, Cd, Se and Hg. But considering Pb, 88% of samples were under the safer tolerable limits. Therefore generally, rice in Kandy district is quite safe from heavy metals although further studies need to be carried out for the lead in rice and soil samples on the possible transfer of the lead from soil to rice. Selenium and mercury were rarely detected in the rice varieties. Locally grown rice varieties are much safer from the toxic elements than the imported rice.

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