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Bioavailability of Heavy Metal in Cooked Rice and Health Risk Assessment Using in Vitro Digestion Model

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Abstract

Rice ingestion is one of major pathway for heavy metal bioaccumulation in human. This study has employed an *in vitro* digestion model to determine non-carcinogenic health risk assessment (HRA) from bioavailability of heavy metal concentrations in cooked rice. Totally, 22 varieties of marketed rice samples were purchased to assess bioavailability of heavy metal (Cd, Cu, Cr, Fe and Zn) using *in vitro* digestion model. Bioavailability of heavy metal concentrations in were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Outputs showed bioavailability of heavy metal concentrations were decreased from Zn > Fe > Cu > Cr > Cd. Zinc was found as the most abundant bioavailability of heavy metal concentration in the cooked rice samples and Thai fragrant rice has the highest Zn content. Hazard Quotient (HQ) values for bioavailability of heavy metal studied were less than 1 for adult and children indicating that there were no any non-carcinogenic health risks present. The application of *in vitro* digestion model in determining bioavailability of heavy metal produces a more realistic estimation of human health risks exposure.

Keywords: Rice; Bioavailability; Heavy metal; *In vitro* digestion; Health risk assessment

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1. Introduction

Recently, heavy metal has attracted a great deal of attention worldwide and become main pollutant in areas with high anthropogenic pressure [1, 2]. Heavy metal is ubiquitous and persistent in environment, non biodegradable and easily exerts toxic effects to human compared to other pollutants such as pesticides or organic pollutants [3, 4, 5]. Rice consumption is one major pathway of heavy metal bioaccumulation in human [6]. Moreover, rice is the main staple food and third dominant agricultural food product in Malaysia [7, 8, 9]. Some researchers have revealed that rice contains higher heavy metal than other grains such as wheat and cereals [10].Heavy metal that commonly found in rice are such as iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr), cobalt (Co), copper (Cu), arsenic (As), and cadmium (Cd) [8, 9, 10, 11, 12, 13]. Heavy metal can enter human body through three routes namely ingestion, inhalation and dermal contact, with ingestion as the main route of exposure to human [2, 14].

There are thousand varieties of rice around the world [15]. Based on Bernas Malaysia, there are eight main varieties of rice marketed in Malaysia, namely parboiled rice, basmathi rice, glutinous rice, brown rice, local white rice, imported white rice, fragrant rice and broken rice. Assessment of heavy metal in rice can be conducted via two ways namely acid digestion method (aqua regia method, HNO3 combined with other types of acids such as H2SO4, H2O2 and HClO4) and *in vitro* digestion model [6]. Total heavy metal concentration always overestimates health risks posed by human through heavy metal exposure and thus, bioavailability concentration is always preferred [17]. Literature has shown that *in vitro* digestion model is preferred in bioavailability of heavy metal studies since it is simple, rapid, low in cost and may provide insights which not achievable in the in vivo studies [18, 19, 20]. Among all the *in vitro* digestion models, RIVM *in vitro* digestion model is the best model for bioavailability of heavy metal determination in rice [16]. This model involves three compartments which are oral cavity, stomach and small intestine [21].

In vitro digestion model is seen as an aid to a better and accurate Health Risk Assessment (HRA) of heavy metal in rice [16]. Previous studies in Malaysia such as done by the authors in [8, 9, 11] only focused on total heavy metal concentration studies. Few studies also have investigated bioavailability of heavy metal concentrations in varieties of rice available in local supermarket. Besides, previous studies commonly used raw rice rather than cooked rice [9, 18]. In comparison with raw rice, cooked rice is the best to be used as sample in HRA studies as the sample must be on the basis product it was ingested by customers so that risk evaluation will reflect real situation of human exposure [22]. Nevertheless, cooking was found to be a minor exposure route for heavy metal in rice [23]. Morgan [24] also stated that some rice can absorb heavy metal if contaminated water is used for cooking. However, cooking with deionised water produces no important modifications of heavy metal in rice [25]. Thus, cooking with deionized water is preferred in the study since no important effect on heavy metal concentration in rice produced.

Within the context of the perspectives mentioned above, main objective of present study was to determine the bioavailability of heavy metal concentrations found in cooked rice samples and compared with the permissible levels of heavy metals stated in Malaysian Food Regulation 1985, FAO/WHO Codex Alimentarius Commision, (CAC) and finding from previous bioavailability of heavy metal study. This present study also aimed to determine HRA derived from the bioavailability of heavy metal concentration in varieties of cooked rice marketed in Malaysia. HRA through ingestion pathway was determined in which non carcinogenic health risks on five heavy metals namely Cd, Cu, Cr, Fe, and Zn, were calculated.

2. Materials & Methods

Totally, twenty-two samples of raw rice were purchased from local supermarket and retail outlets in Selangor, Malaysia to represent major varieties of rice sold in Malaysia market. As per total examined rice varieties, 9 samples belonged to domestic products mainly from Peninsular Malaysia and 13 samples belonged to imported cultivars mainly from Thailand, Taiwan, Japan, Cambodia, India and Pakistan. Cooked rice samples were prepared based on method applied by the authors in [9, 26, 27]. The cooked rice samples were oven dried at 65 °C for 48 hours and grounded by using pestle and mortar. After the homogenization process, the cooked rice samples were passed through 0.25 mm mesh sieve (No 60 mesh sieve). Then, cooked rice samples were stored in polyethylene bags prior to bioavailability heavy metal digestion using RIVM *in vitro* digestion model. RIVM *in vitro* digestion method in this study was adopted from the authors in [18, 21]. All the chemicals used for enzymes preparation were prepared a day before and the enzymes were prepared on the day of analysis. All the apparatus were acid washed overnight and rinsed three times with deionized water before being used. Blank sample was run together with the standard solutions in every 10th samples for quality control. Lastly, Certified Reference Material (CRM) IRMM 804 was analyzed together with the rice samples and the recoveries were within 90.5%-102.4%.In addition, all rice samples were run in triplicate samples. Heavy metals in cooked rice samples were analyzed by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Descriptive statistics analyses were performed with Statistical Package for Social Science (SPSS) version 21.0. Health risks through ingestion pathway were calculated based on equations by the United States Environmental Protection Agency (USEPA, 2012). Average daily dose (ADD) and non-carcinogenic health risks(HQ) values for each heavy metal was calculated. Mean of heavy metal concentration for cooked rice samples were compared with Malaysian Food Regulation (1985), FAO/WHO and previous study.

3. Results

Bioavailability of heavy metal concentrations found in cooked rice samples (19 over 22 samples) were in the following order, Zn > Fe > Cu > Cr > Cd. Mean values of bioavailability heavy metal concentration from each cooked rice samples are given in Table 1. Comparisons with the Malaysian Food Regulation (1985)[28], FAO/WHO (1984) [29], FAO/WHO (1989) [30] and a previous study [17] also has been tabulated. From this study, it was found that Zn was the highest bioavailability heavy metal found in the cooked rice samples. Cadmium has the lowest bioavailability heavy metal concentration in all cooked rice samples. Bioavailability of Cu, Fe and Zn concentrations were the highest in Sample S (Calrose grain), Sample L (Organic black rice) and Sample U (Thai fragrant rice), respectively. Bioavailability of Cr concentration were the highest (0.13 mg/kg) in six cooked rice samples which were Sample B (Red rice), Sample J (Parboiled rice), Sample M (Local rice 5% roken rice), Sample O (Imported white rice), Sample S (Calrose grain rice) and Sample T (Steamed rice).

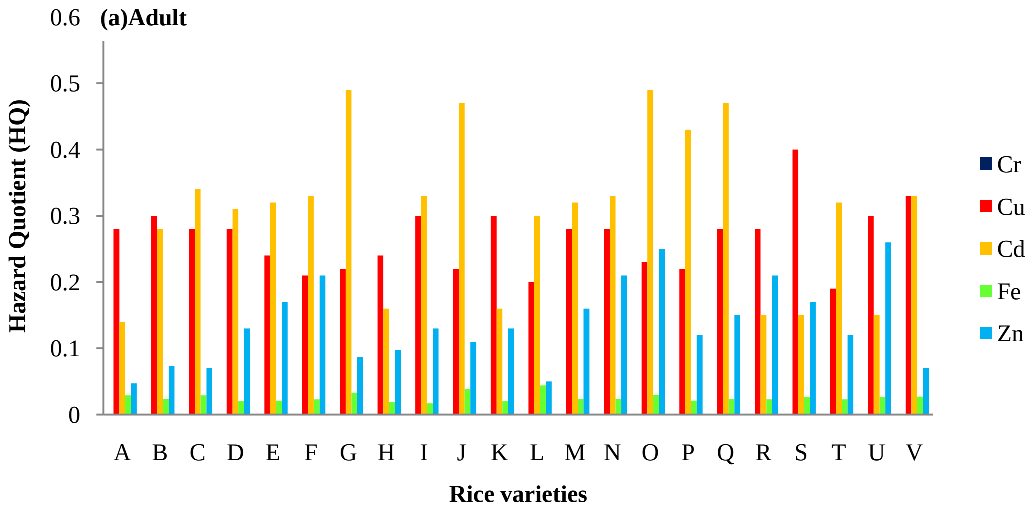
Table 1. Comparisons of bioavailability heavy metal concentration in cooked rice samples (mg/kg) with standards and previous study

Rice samples	Rice varieties	Origin	Mean concentration (mg/kg)				
			Cr	Cd	Cu	Fe	Zn
A	Local unpolished brown rice	Kedah, Malaysia	0.12	0.015	1.2	2.1	1.5
B	Red rice	Thailand	0.13	0.029	1.3	1.8	2.3
C	Eight grain rice	Mix	0.12	0.036	1.2	2.1	2.1
D	Ponni rice	India	0.096	0.032	1.1	1.5	4.1
E	Hill rice	Perak, Malaysia	0.098	0.033	1.0	1.6	5.2
F	Local fragrant rice	Malaysia	0.12	0.034	0.87	1.7	6.6
G	Siam rice	Thailand	0.10	0.049	0.92	2.4	2.6
H	Siam fragrant rice	Thailand	0.10	0.017	0.97	1.4	3.0
I	Basmathi rice	Pakistan	0.10	0.034	1.3	1.3	4.1
J	Parboiled rice	Malaysia	0.13	0.049	0.90	2.8	3.5
K	Special glutinous rice	Malaysia	0.10	0.017	1.3	1.5	4.1
L	Organic black rice	Taiwan	0.11	0.031	0.84	3.2	1.6
M	Local rice (5% broken)	Malaysia	0.13	0.033	1.2	1.8	4.9
N	Local white rice	Malaysia	0.12	0.034	1.1	1.8	6.6
O	Imported white rice	Thailand	0.13	0.049	0.96	2.2	7.9
P	Special Siam rice	Malaysia	0.11	0.045	0.92	1.6	3.7
Q	Milky glutinous rice	Malaysia	0.098	0.049	1.1	1.8	4.8
R	Thai glutinous rice	Thailand	0.12	0.017	1.2	1.7	6.7
S	Calrose grain rice	Japan	0.13	0.016	1.7	1.9	5.4
T	Steamed rice	India	0.13	0.033	0.79	1.7	3.9
U	Thai fragrant rice	Thailand	0.12	0.016	1.3	1.9	8.2
V	Imported brown rice	Cambodia	0.10	0.034	1.4	2.0	2.2
MFR			NP	1	30	NP	100
FAO/WHO 1984			1	0.4	10	NP	150
FAO/WHO 1989			NP	NP	NP	100	NP
[18]			ND	0.0097	ND	ND	ND

MFR is Malaysian Food Regulation; NP is No permissible limits; ND is Not detected

Maximum bioavailability of Cu concentration was found in Calrose grain rice (1.7 mg/kg) while minimum bioavailable Cu concentration found in Steamed rice (0.79 mg/kg). On the other hand, Organic black rice (3.2 mg/kg) has the maximum bioavailability of Fe concentration among the cooked rice samples and the minimum bioavailability of Fe concentration found in Basmathi rice (1.3 mg/kg). Lastly, bioavailability of Zn concentration was the highest in Thai fragrant rice (8.2 mg/kg) while the lowest in local unpolished brown rice (1.5 mg/kg). Bioavailability of Cd concentration was the lowest in all cooked rice samples. The highest Cd bioavailability concentrations were found in four samples namely Sample G (Siam rice), Sample J (Parboiled rice), Sample O (Imported white rice) and Sample Q (Milky glutinous rice).

For the non carcinogenic health risks calculation, HQ values in adult for all heavy metal studied were less than 1 (HQ <1). Details for the non carcinogenic health risk (HQ) calculations for adult and children via cooked rice ingestion are presented in Figure 1. This study suggests that intake of single heavy metal through rice ingestion did not pose a significant non carcinogenic health risks to human. The highest HQ value was found for Cd while the lowest HQ value was for Cr.



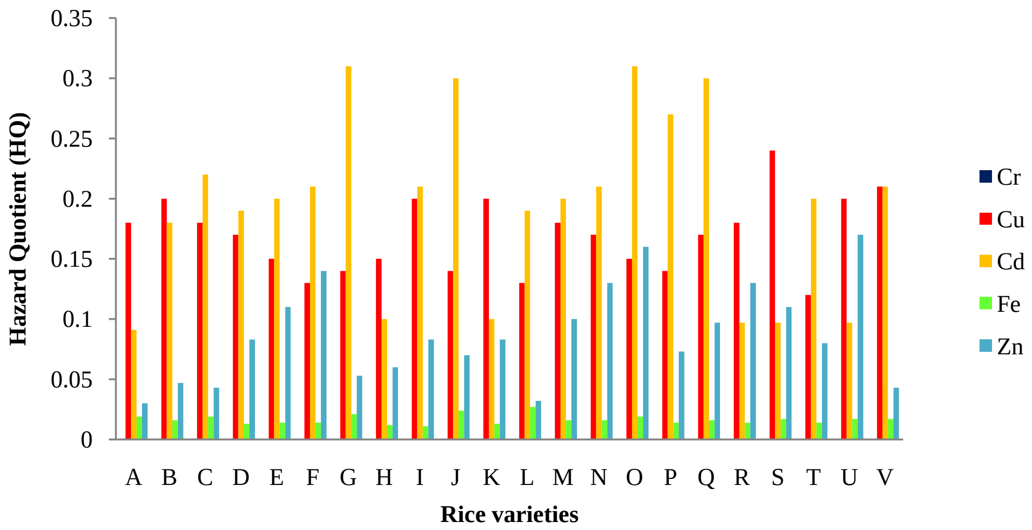


Fig. 1.non carcinogenic health risks (HQ) values for (a) adult and (b) children in 22 varieties of cooked rice samples

4. Discussion

Table 1 shows that there are differences in bioavailability of heavy metal concentrations between the rice varieties. Zhang [10] stated that it is necessary to investigate heavy metal from varieties of rice in order to assess potential health risk. Each variety of rice demonstrated specific heavy metal characteristics due to differences in soil and climatic that affects rice samples original content [9]. Mean bioavailability of Fe concentration among each cooked white rice varieties was lower than unpolished or brown rice (Table 1). The authors in [31] stated that polishing removes about 60% of Fe from the rice grain and reversely causing a high Fe concentration in unpolished rice. Mean bioavailability of Zn concentration in cooked brown rice also was lower than cooked white rice. This difference happened as brown rice is richer in dietary fiber and phytate that may inhibit absorption of minerals such as Zn [32]. Heavy metal such as Fe, Zn and Cu are required in certain amounts for plants growth (enzymes activities, photosynthesis) [12]. However, these heavy metal at high doses can give significant negative impacts on human and bioaccumulation in food chain [2, 10]. Bioavailability of Cd concentration in all cooked rice samples was low but Cd is found to be easily taken up by rice plants even in low concentration [11]. Heavy metals such as Cd and Cr are much harmful even though in low concentration as they have a potential risk for renal impairment and bone diseases such as osteoporosis to male and female adult [10, 11]. In order to protect public health, a maximum permissible level of heavy metal in food has been established in Malaysia. Based on Malaysian Food Regulation 1985 (28), mean concentrations of bioavailability Cu, Cd and Zn in all cooked rice samples are below the maximum permissible level of heavy metal permitted and international standards stated (Table 1). This shows that all varieties of cooked rice samples studied are safe to be consumed by people as their daily staple food.

Even though health risks due to heavy metal contamination in rice is known worldwide, there is still limited application of HRA using *in vitro* digestion model output. Since public health risk associated with dietary intake of heavy metal increased, HRA is crucial to be done in order to detect health risks due to heavy metal contamination from food initially [16]. In this study, HRA was calculated using the average daily intake (ADD) and Hazard Quotient (HQ) through ingestion pathway. HQ values for all heavy metals studied were very low ($HQ < 1$). The HQ results suggest that single heavy metal exposure via rice ingestion did not pose a significant non carcinogenic health risks to adult and children. In fact, examples of non carcinogenic health risks are renal impairment, bone diseases, heart diseases and cardiovascular diseases [10]. Present study gives indication that application of *in vitro* digestion model for heavy metal in rice research contributes better health risks estimation to human.

There were a few limitations found in this study. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) gave lower sensitivity compared to Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Besides, both carcinogenic and non carcinogenic health risks values in this study need to be reconfirmed since Malaysian ingestion rate (IR) and body weight (BW) values used were based on previous surveys [33, 34].Thus, a set of questionnaire is needed in future to obtain the actual Malaysian IR and BW values to be used in HRA.

5. Conclusion

This study showed that bioavailability of heavy metal concentrations in cooked rice samples were decreased from Zn, Fe, Cu, Cr to Cd. Bioavailability of heavy metal concentrations were different between the varieties of cooked rice samples and each rice variety demonstrated specific heavy metal characteristics. Bioavailability of Cd concentration was the lowest in all cooked rice samples while Zn has the highest. Bioavailability of heavy metal concentrations in cooked rice samples from this study are also below the standards (Malaysian Food Regulation 1985, FAO/WHO 1984 and FAO/WHO 1989). All cooked rice samples were found to be safe for consumption while the HQ value for all heavy metal studied gives a value < 1 implicating no non-carcinogenic health risk present. Findings from present study are also important for future research to calculate HQ values through other pathways namely inhalation and dermal contact.

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References

[1] K. Zhao, A.W. Zhang and A.L. Zhou."Modeling transfer of heavy metals in soil – rice system and their risk assessment in paddy fields."*Environmental and Earth Science,* vol. 59(3), pp. 519–527, Feb. 2009.

[2] E.U. Islam, X.E. Yang, Z.L. Zhang and M. Qaisar."Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops."*Journal of Zhejiang University Science,* vol. 8, pp. 1-13, Jan. 2007.

[3] H. Cao, H. Zhu, Y. Jia, J. Chen, H. Zhang and L. Qiao."Heavy metals in food crops and the associated potential for combined health risk due to interactions between metals."*Human and Ecological Risk Assessment: An International Journal,* vol. 17 (3), pp. 700–711, June. 2011.

[4] B.D. Tembo, K. Sichilongo and J. Cernak."Distribution of copper, lead, cadmium and zinc concentrations in soils around Kabwe town in Zambia."*Chemosphere,* vol. 63 (3), pp. 497–501, Dec. 2006.

[5] D.C. Adriano. "Chapter 1: Introduction. In: Adriano DC (ed) Trace elements in terrestrial environment: biogeochemistry, bioavailability, and risks of metals,"in Springer, New York, 2001;pp 22–23.

[6] J. Liu, X. Zhang, H. Tran, D. Wang and Y. Zhu."Heavy metal contamination and risk assessment in water, paddy soil, and rice around an electroplating plant."*Environmental Science and Pollution*, vol. 18 (9), pp. 1623-1632, May. 2011.

[7] Z. Fahmi, B.A. Samah and H. Abdullah."Paddy industry and paddy farmers well-being: A success recipe for agriculture industry in Malaysia."*Asian Social Science,* vol. 9 (3), pp. 177-181, Feb. 2013.

[8] J. Khairiah, A.R. Ramlee, H. Jamil, Z. Ismail and B.S. Ismail."Heavy Metal Content of Paddy Plants in

Langkawi , Kedah , Malaysia."*Australian Journal of Basic Applied Science*, vol. 7 (2), pp. 123–127, Feb.2013

[9] N.A.A. Salim, M.S. Elias, A.K. Wood, E. Sanuri, M.S. Hamzah and S.A. Rahman."Multi element analysis in rice grains by instrumental neutron activation analysis."*Journal of Nuclear Technology, vol*. 7 (2), pp. 1-11, Dec. 2010.

[10] J.Z. Zhang. "The toxicity assessment of heavy metals and their species in rice."University of Cincinnati, 2009.

[11] D.W. Yap, J. Adezrian, J. Khairiah, B.S. Ismail and R. Ahmad-Mahir."The uptake of heavy metals by paddy plants (Oryza sativa) in Kota Marudu, Sabah, Malaysia."*American-EurasianJournal of Agricultural and Environmental Science*, vol. 6 (1), pp. 16-19, Jul. 2009.

[12] L. Jorhem, C. Astrand, B. Sundstorm, M. Baxter, P. Stokes, J. Lewis and K.P. Grawe."Elements in rice on the swedish market: part 2. chromium, copper, iron, manganese, platinum, rubidium, selenium and zinc."*Food Additives and contaminants- Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*,vol. 25 (7), pp. 841-850, Jul. 2008.

[13] I. Al-Saleh and N. Shinwari."Report on the levels of cadmium, lead, and mercury in imported rice grain samples."*Biological Trace Element Research*, vol. 83 (1), pp. 91–6, Oct. 2001.

[14] H. Cao, J. Chen, J. Zhang, H. Zhang, L. Qiao and Y. Men."Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China."*Journal of Environmental Science*, vol. 22 (11), pp. 1792-1799, Sep. 2010.

[15] International Rice Research Institute (IRRI). (2013). Available www.irri.com.[January 21, 2014].

[16] N.A. Omar, S.P. Praveena, A.Z. Aris and Z. Hashim."Bioavailability of heavy metal in rice using in vitro digestion model: A Minireview."*International Food Research Journal*, vol. 20 (6), pp. 2979–2985, Jul.2013.

[17] S.W. Lee, B.T. Lee, J.Y. Kim, K.W. Kim and J.S. Lee. "Human risk assessment for heavy metals and as contamination in the abandoned metal mine areas, Korea."*Environmental Monitoring and Assessment*,vol. 119, pp. 233-244, Aug. 2006.

[18] L.S. Yang, X.W. Zhang, Y.H. Li, H.R. Li, Y. Wang and W.Y. Wang. "Bioaccessibility and Risk Assessment of Cadmium from Uncooked rice using an in vitro digestion model."*Biological Trace Element Research*, vol. 145 (1), pp.81-86, Jan. 2012.

[19] M. Intawongse and J.R. Dean."In-vitro testing for assessing oral bioaccessibility of trace metals in soil and food samples."*Trends in Analytical Chemistry,* vol. 25 (9), pp. 876-886, Oct. 2006.

[20] A.G. Oomen, C.J.M. Rompelberg, M.A. Bruil, C.J.G. Dobbe, D.P.K.H. Pereboom and A.J.A.M.

Sips."Development of an in vitro digestion model for estimation the bioaccessibility of soil contaminants."*Archieve of Environmental Contamination and Toxicology*, vol. 44 (3), pp. 281–287, Apr. 2003.

[21] C.H.M. Versantvoort, D.K.E. Van and C.J.M. Rompelberg."Development and applicability of an in vitro digestion model in assessing the bioaccessibility of contaminants from food." Report no. 320102002, Available from <http:/www.rivm.nl/en/>, National Institute for Public Health and the Environment, Bilthoven 2004;The Netherlands.

[22] V. Devesa, D. Velez and R. Montoro."Effect of thermal treatments on arsenic species contents in food."*Food Chemical and Toxicology*,vol 46 (1), pp.1-8, Jan. 2008.

[23] D. Mondal, M. Banerjee, M. Kundu, N. Banerjee, U. Bhattacharya, A.K. Giri, B. Ganguli, S.S. Roy and D.A.Polya"Comparison of drinking water, raw rice and cooking of rice as arsenic exposure routes in three contrasting areas of West Bengal, India."*Environmental and Geochemical Health,* vol. 32 (6), pp. 463-477, May 2010.

[24] J.N. Morgan."Effects of processing on heavy metal content of foods."*Advances in Experimental Medicine and Biology,* vol. 459, pp. 195–211, Jan. 1999.

[25] J.M. Laparra, D. Vaclez, R. Barbera, R. Farrac andR. Montoro."Bioavailability of inorganic arsenic in cooked rice: Practical aspects for human health risk assessments."*Journal of Agricultural and Food Chemistry*, vol. 53 (22), pp. 8829-8833, Nov. 2005.

[26] F. Kong, M.H. Oztop, R.P. Singh andM.J. McCarthy. "Physical changes in white and brown rice during simulated gastric digestion."*Journal of Food Science*, vol.76 (6), pp. E450-E457, Aug. 2001.

[27] A. Signes, K. Mitra, F. Burla and A.A. Carbonell-Barrachina."Contribution of water and cooked rice to an estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India."*Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 25 (1), pp. 41-50, Jan. 2008.

[28] Malaysian Food Regulations. Food Act 1983 (Act 281) & Regulations, International Law book Services, Kuala Lumpur, 1985.

[29] FAO/WHO. "Evaluation of certain food additives and of the contaminants mercury, lead and cadmium." FAO Nutrition Meetings Report Series 51, WHO Technical Report Series 505, Rome, 1984.

[30] FAO/WHO. "Evaluation of certain food additives and of the contaminants chromium, zinc and iron." FAO Nutrition Meetings Report Series 51, WHO Technical Report Series 505, Rome, 1989.

[31] R.P. Glahn, Z. Cheng, R.M. Welch andG.B. Gregorio."Comparison of iron bioavailability from 15 rice genotypes: Studies using an in vitro digestion/Caco-2 cell culture model."*Journal of Agricultural and Food*

Chemistry, vol. 50 (12), pp. 3586-3591, Jun. 2002.

[32] J.R. Hunt, L.K. Johnson and B.O. Juliano."Bioavailability of zinc from cooked philippine milled, undermilled, and brown rice, as assessed in rats by using growth, bone zinc, and zinc-65 retention."*Journal of Agricultural and Food Chemistry*,vol. 50 (18), pp. 5229-5235, Aug. 2002.

[33]A.K.Norimah, M.Safiah, K.Jamal, H.Siti, H. Zuhaidaand S. Rohida. "Food consumption patterns: Findings from the Malaysian Adult Nutrition Survey (MANS)."*Malaysian Journal of Nutrition*,vol. 14 (1),pp. 25-39, Mar. 2008.

[34]N. Zheng, Q.C. Wang, X.W. Zhang, D.M. Zheng, Z.S. Zhang and S.Q. Zhang."Population health risk due to dietary intake of heavy metals in the industrial area of Huludao City, China."*Science of Total Environment*, vol. 387 (1-3), 96–104, Nov. 2007.