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TOTAL AND BIOAVAILABILITY CONCENTRATIONS OF HEAVY METALS IN VARIETIES OF COOKED RICE, AND HEALTH RISK ASSESSMENT

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By

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Faculty: Medicine and Health Science

Rice in the global market is increasing over the years similarly with the rice demand. Metal contamination in rice is a rising problem recently. However, limited information regarding the bioavailability of metals contamination and its health risks after rice ingestion was known. This study aimed to determine both total and bioavailability concentrations of metals (As, Cd, Cu, Cr, Co, Al, Fe, Zn and Pb) in varieties of cooked rice samples. Moreover, this study aimed to compare bioavailability concentrations of metals with the rice varieties, rice grain size and origin. This study also aimed to calculate Bioaccumulation Factor (BAF) of heavy metal in varieties of cooked rice samples and assess relationships between different bioavailability metals in varieties of cooked rice. In addition, this study also identified the similarity of chemical properties among the bioavailability metals using Cluster Analysis (CA). Lastly, this study has assessed human carcinogenic and non-carcinogenic health risks using Health Risk Assessment (HRA). About 1 kg of rice for 22 rice varieties were purchased from local groceries and supermarket based on the convenience sampling. Total metal digestion was determined by using nitric acid while bioavailability metal digestion was done using RIVM *in vitro* digestion model. The metal concentrations were then analysed by using Inductively Coupled Optical Emission Spectrometry Pelkin Elmer Optima 8300. Results found that Zn concentration was the highest while As was the lowest metals concentration in both total and bioavailability concentrations. All total and bioavailability concentrations of metals were below the maximum permitted levels stated by Malaysian Food Regulation (1985), FAO/WHO CAC (1984) and FAO/WHO CAC (1989). Mann-Whitney U test results (Z values) show that there was a significant difference between total and bioavailability of metals concentration (p <0.05). Kruskal walis tests results (X^2 values) also show that there was a significant differences between bioavailability concentrations of metals and rice varieties (p<0.05), except for As.

However, Kruskal Walis Test (X^2 values) shows no significant difference between bioavailability concentrations of metals with type of rice grains size ($p>0.05$). Nevertheless, Mann-Whitney U results (Z values) show no significant difference between bioavailability concentrations of metals with the rice origin ($p>0.05$). The bioaccumulation factor (BAF) values were found in the order of Fe>Cu>Al>Zn>Cd>Co>As>Pb>Cr with parboiled rice has the highest BAF values for Fe (BAF values>1). Spearman Correlation Coefficient results (r_s values) show strong positive relationships between the bioavailability metals; Fe_B and Al_B ($r_s=0.83$) and between As_B with Pb_B ($r_s=0.88$). From Cluster Analysis (CA) results, four clusters were identified, which were Cluster 1 (Pb, As, Co, Cd, and Cr), Cluster 2 (Cu and Al), Cluster 3 (Fe), and Cluster 4 (Zn). As for HRA determination, there was no non carcinogenic health risks found (Hazard Quotient, HQ<1) for adult and children through individual heavy metal exposure. However, there was non carcinogenic health risk present among adult via the combination of these heavy metal exposures (HI > 1). On the other hand, there were potential carcinogenic health risks present for adult and children via individual intake of As (Lifetime Cancer Risk > $1x10^{-4}$). Furthermore, the values for Total Cancer Risk (TCR) for Pb and As in both adult and children were above the acceptable range (TCR > $1 \text{ x } 10^{-4}$). As conclusion, this study shows that Zn was the highest metal found in 22 varieties of rice with a significant difference between total and bioavailability metal concentrations. There was also a significant difference between bioavailability metal concentration and the rice varieties. Besides, non-carcinogenic and carcinogenic health risks may posed by adult and children through their combined metal exposure in the rice.

Keywords*:* Rice, total, bioavailability, in vitro, health risk.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk Ijazah Master Sains

KONSENTRASI TOTAL DAN KEBOLEHDAPATAN BIOLOGI LOGAM DALAM PELBAGAI JENIS NASI, DAN PENILAIAN RISIKO KESIHATAN

Oleh

NOREEN ADILA BINTI OMAR

Mei 2015

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Beras dalam pasaran dunia semakin meningkat sejak beberapa tahun ini selari dengan permintaan beras. Pencemaran logam dalam beras merupakan masalah yang semakin meningkat baru-baru ini. Walau bagaimanapun, terdapat maklumat yang terhad mengenai pencemaran kebolehdapatan biologi logam dan risiko kesihatan selepas pengambilan beras. Kajian ini bertujuan untuk menentukan konsentrasi kedua-dua total dan kebolehdapatan biologi logam (As, Cd, Cu, Cr, Co, Al, Fe, Zn dan Pb) dalam pelbagai jenis sampel nasi. Selain itu, kajian ini bertujuan untuk membandingkan konsentrasi kebolehdapatan biologi logam dengan pelbagai jenis beras, saiz butiran beras dan asal beras. Kajian ini juga bertujuan untuk mengira Pengumpulan Faktor Biologi (BAF) logam dalam pelbagai jenis sampel nasi dan menilai hubungan antara kebolehdapatan biologi logam yang berbeza dalam jenis nasi. Di samping itu, kajian ini juga mengenalpasti persamaan sifat kimia antara kebolehdapatan biologi logam menggunakan Analisis Kelompok (CA). Akhir sekali, kajian ini telah menilai risiko kesihatan karsinogen dan bukan karsinogen manusia menggunakan Penilaian Risiko Kesihatan (HRA). Lebih kurang sebanyak 1 kg beras untuk setiap 22 jenis beras telah dibeli daripada kedai runcit tempatan dan pasar raya berasaskan persampelan mudah. Pencernaan total logam telah ditentukan dengan menggunakan asid nitrik manakala pencernaan kebolehdapatan biologi logam dilakukan menggunakan model *in vitro* RIVM. Kandungan logam telah dianalisis dengan menggunakan Induktif Bersama-Pelepasan Optik Spektrometri Pelkin Elmer Optima 8300. Hasil keputusan mendapati bahawa konsentrasi Zn merupakan yang tertinggi manakala kosentrasi As adalah yang paling rendah bagi kedua-dua konsentrasi total dan kebolehdapatan biologi logam. Semua konsentrasi total dan kebolehdapatan biologi logam adalah di bawah tahap maksimum dibenarkan yang dinyatakan di bawah Peraturan Makanan Malaysia (1985), FAO / WHO CAC (1984) dan FAO / WHO CAC (1989). Keputusan ujian Mann-Whitney U (nilai *Z*) menunjukkan terdapat perbezaan yang signifikan di antara konsentrasi total dan keblehdapatan biologi logam ($p < 0.05$). Keputusan ujian Kruskal Walis (nilai X^2) juga menunjukkan bahawa terdapat perbezaan yang signifikan di antara konsentrasi kebolehdapatan logam dan pelbagai jenis beras ($p < 0.05$), kecuali As. Walau bagaimanapun, keputusan ujian Kruskal Walis (nilai X^2) menunjukkan tiada perbezaan yang signifikan di antara konsentrasi kebolehdapatan biologi logam dengan saiz bijirin beras ($p > 0.05$). Di samping itu, keputusan ujian Mann-Whitney U (nilai Z) menunjukkan tiada perbezaan yang signifikan antara konsentrasi kebolehdapatan biologi logam dengan asal beras tersebut ($p > 0.05$). Nilai Pengumpulan Fakor Biologi (BAF) ditemui berada dalam turutan yang menurun daripada Fe> Cu> Al> Zn> Cd> Co> As> Pb> Cr dengan beras rebus mempunyai nilai BAF yang paling tinggi untuk Fe (nilai BAF> 1). Pekali Korelasi Spearman (nilai r_s) menunjukkan hubungan positif yang kuat wujud di antara kebolehdapatan biologi logam; Fe_B dan Al_B (rs = 0.83) dan antara As_B dengan Pb_B ($r_s = 0.88$). Dari keputusan Analisis Kelompok (CA), empat kelompok telah dikenal pasti, iaitu Kelompok 1 (Pb, As, Co, Cd, dan Cr), Kelompok 2 (Cu dan Al), Kelompok 3 (Fe), dan Kelompok 4 (Zn) . Bagi penentuan HRA, tiada risiko kesihatan bukan karsinogenik didapati (Hasil Bahagi Bahaya, HQ <1) untuk dewasa dan kanak-kanak melalui pendedahan logam berat secara individu. Namun, terdapat risiko kesihatan bukan karsinogen hadir di kalangan dewasa melalui pendedahan gabungan semua logam berat tersebut (Indeks Bahaya, HI> 1). Di samping itu, terdapat risiko kesihatan karsinogenik wujud bagi orang dewasa dan kanak-kanak melalui pengambilan As secara individu (Kanser Risiko Sepanjang Hayat > $1x10^{-4}$). Tambahan pula, nilai untuk Jumlah Risiko Kanser (TCR) untuk Pb dan As dalam dewasa dan kanak-kanak melebihi julat yang boleh diterima (TCR> $1 \text{ x } 10\text{-}^{4}$). Kesimpulannya, kajian ini menunjukkan bahawa Zn adalah logam yang paling tinggi terdapat dalam 22 jenis beras dengan perbezaan yang signifikan antara kepekatan total dan kebolehdapatan biologi logam. Satu perbezaan yang signifikan juga didapati antara kepekatan kebolehdapatan logam dan jenis beras. Selain itu, risiko kesihatan bukan karsinogen dan karsinogenik boleh dihadapi oleh orang dewasa dan kanak-kanak melalui pendedahan logam yang digabungkan dalam nasi. Hasil kajian ini boleh memberi panduan mengenai tahap kontaminasi logam semasa di dalam pelbagai jenis beras yang dipasarkan di Malaysia.

Kata kunci*:* Nasi, total, kebolehdapatan biologi, *in vitro*, risiko kesihatan.

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I certify that a Thesis Examination Committee has met on 26 May 2015 to conduct the final examination of Noreen Adila Binti Omar on her thesis entitled “Total and Bioavailability Concentrations of Heavy Metals in Varieties of Cooked Rice, and Health Risk Assessment” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

<	Less than
>	More than
AAS	Atomic Absorption Spectroscopy
ADD	Average daily dose
ATSDR	Agency for Toxic Substances and Disease Registry
Al	Aluminium
As	Arsenic
AT	Averaging time
BAF	Bioaccumulation Factor
BW	Body weight
Fe	Iron
CA	Cluster Analysis
CAC	Codex Alimentarius Commission
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
ED	Exposure duration
FAO	Food and Agriculture Organization
GIT	Gastrointestinal tract
HQ	Hazard Quotient
$HQ_{\text{Sum Bioavailability}}$	Sum of Hazard Quotient for bioavailability concentrations
HRA	Health Risk Assessment
ICP- MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IR	Ingestion rate
IRIS	Integrated Risk Information System
IRRI	International Rice Research Institute
Kg	Kilogram
LADD	Lifetime average daily dose
LCR	Lifetime Cancer Risk
$LCR_{\text{Sum Bioavaiability}}$	Sum of Lifetime Cancer Risk for bioavailability concentrations
Mg	Miligram
NAS	National Academy of Sciences
NRC	National Research Council
Pb	Lead
PC	Principal Component
PCA	Principal Component Analysis
R_{fD}	Reference dose
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
SPSS	Statistical Packaging of Social Science
USEPA	United States of Environmental Protection Agency

WHO	World Health Organization
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Food security in Malaysia largely depends on achieving self-sufficiency in rice production at about 65-70% of local consumption (Arshad et al., 2011). Since paddy is a strategic crop in Malaysia, it is essential to maintain a domestic rice production level for food security purposes in tandem with the growing population (Najim et al., 2007). As paddy is categorized under food based agricultural sub-sector, Malaysians largely depend on paddy as rice is the main staple food in Malaysia (Fahmi et al., 2013; Ismail et al., 2013; Syahariza et al., 2013).

Even though rice is an essential staple food worldwide, environmental pollutants and bioaccumulation in rice are gaining attention. Polluted paddy soils increase the accumulation of environmental pollutants in rice, which is mostly impacted by anthropogenic activities (Cao et al., 2010; Hang et al., 2009). Some examples are metal mining (Smuc et al., 2012; Zhao et al., 2012; Nobuntou et al., 2010; Zhuang et al., 2009), electroplating and chemical activities (Ji et al., 2013; Liu et al., 2011), e-waste dismantling (Zheng et al., 2013; Fu et al., 2008), irrigation with heavy metal-contaminated water (Bhattacharya et al., 2010; Simmons et al., 2005), wastewater irrigation (Rhee et al., 2011; Singh et al., 2010), usages of fertilizers and pesticides (Khairiah et al., 2013; Zhang et al., 2011), and metal recycling (Minh et al., 2012). Besides, there are many environmental pollutants in rice, such as pesticides (Gao et al., 2013; Fuad et al., 2012), organic pollutants (Xu et al., 2013; Minomo et al., 2011), and heavy metal (Yin et al., 2012; Jamil et al., 2011). In Malaysia, these environmental pollutants have continued to concentrate in most paddy fields and have led to significant deterioration of both paddy soil and rice quality (Fuad et al., 2012; Yin et al., 2012; Jamil et al., 2011). Among all environmental pollutants stated, heavy metal is vital to be studied since it can bioaccumulate in the environment, as it is non biodegradable and toxic compared to other pollutants, such as pesticides and organic pollutants (Cao and Hu, 2000). Moreover, many studies related to heavy metal and rice have been conducted worldwide recently, such as in China (Hang et al., 2009; Rogan et al., 2009; Fu et al., 2008), India (Singh et al., 2010; Mondal and Polya, 2008), Korea (Chung et al., 2011), Saudi Arabia (Al-Saleh and Shinwari, 2001), Greece (Pasias et al., 2013), and Vietnam (Minh et al., 2012). On top of that, studies related to heavy metal and rice also have been done in Malaysia (Khairiah et al., 2013; Salim et al., 2010; Yap et al., 2009).

In Malaysia, a study has been done by Khairiah et al. (2013) and was carried out at Kampung Sungai Kedak, Mukim Mat Sirat, Langkawi Island, Kedah. Extraction of the five heavy metals (Fe, Zn, Cu, Pb and Cd) from the rice grain, leaf, stem and root has been carried out. According to the results, all the heavy metal were low and below the permissible level as stipulated in the Malaysian Food Act 1983, Food Regulations 1985 and Codex Alimentarius Commisssion (Khairiah et al. 2013). Thus, the low concentration of bioavailable Zn, Cu, Pb and Cd in the paddy soils were reflected in the low accumulation of those metals in the paddy plant parts (Khairiah et al. 2013). On the other hand, Yap et al. (2009) have studied about heavy metal in rice in Kota Marudu, Sabah to compare the content of heavy metals in various parts of the paddy plant. Heavy metals studied were Cd, Cr, Fe, Mn, Pb, and Zn. The results showed that Fe was the most predominant heavy metal in the rice grains and roots, while Mn was the most predominant metal in the rice husks, leaves and shoots (Yap et al. 2009). However, the concentrations of heavy metals in the rice grains were still below the maximum levels as stipulated by the Malaysian Food Act (1983) and Food Regulations (1985) (Yap et al. 2009). A study done by Salim et al. (2010) was to determine the concentration of 15 elements, including toxic and essential elements, in variety of marketed rice. All the elements were having low concentrations and also below the Malaysian Food Act (1983) and Food Regulations (1985) (Salim et al. 2010).

Heavy metal concentration in rice can be determined by using total and bioavailability concentrations of heavy metal (Omar et al., 2013). Total heavy metal concentration can be determined via acid digestion methods (Pasias et al., 2013; Singh et al., 2010). Acid digestion methods are mixture of acids, such as hydrochloric acid (HCl), nitric acid (HNO_3), sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), or perchloric acid ($HClO_4$). In fact, the total heavy metal concentrations do not consider the actual degree of ingestion exposure to heavy metal, and overestimates the heavy metal concentrations and human health risks (Versantvoort et al. 2005; Lee et al. 2006). Bioavailable fraction can be defined as the fraction of total heavy metal concentration present in a specific environmental compartment within a time and being uptaken by organisms or plants from direct environment, plant or via food ingestion (Peijnenburg and Jager, 2003). Versantvoort et al. (2005) stated that the bioavailability of heavy metal concentrations is preferable in the estimation of human health risks since it represents the proportion of ingested contaminants in the food that can reach the human systemic circulation. Moreover, the bioavailability of heavy metal concentrations can be determined by using an *in vitro* digestion model, which is fast, inexpensive and easy to use (Yang et al. 2012). *In vitro* digestion model is widely used to study the structural changes, digestibility, and the release of food components under the simulated gastrointestinal conditions (Hur et al., 2011). *In vitro* digestion (RIVM) Netherlands model is the best model for *in vitro* digestion model for rice (Omar et al., 2013; Verantvoort et al., 2005).

Furthermore, studies related to potential health risks due to heavy metal contamination in rice need better understanding on bioavailability of heavy metal in rice (Omar et al., 2013; Versantvoort et al., 2005). Moreover, it is crucial to carry out health risk assessment (HRA) to assess heavy metal health risks for rice consumption. Health risk assessment can be analyzed using the model developed by NRC (National Research Council) and NAS (National Academy of Sciences) to estimate the health risks caused by contaminants. Based on United States Environmental Protection Agency (USEPA, 2012), HRA consists of four main steps, namely hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification is examination of contaminant from the point of exposure, while dose-response assessment evaluates all the information obtained during the hazard identification. As for dose-response assessment, estimation on the person, when, where, and for how long the individual is exposed to the hazard, takes place. Exposure assessment is the third step in HRA that estimates the dose related to adverse effects to the exposed individual. Lastly, risk characterization represents the risks that are likely to be exposed to the populations; carcinogenic and non carcinogenic health risks (Lee et al., 2006; Versantvoort et al., 2005).

1.2 Problem statement

Local researchers like Yap et al., (2009), Salim et al., (2010), and Khairiah et al., (2013) have studied heavy metal contamination in rice in Malaysia. Most of the studies investigated the heavy metal concentration in field rice samples (Khairiah et al., 2013; Yap et al., 2009), while heavy metal contamination in marketed rice samples was not well documented (Salim et al., 2010). Studies using field rice samples were conducted in order to determine heavy metal concentration in different parts of paddy plants to look into the impacts of fertilizers usage and anthropogenic activities, such as industrialization (Khairiah et al., 2013; Yap et al., 2009). In this aspect, heavy metal intake via soil-crop system has been considered as the predominant pathway of heavy metal contamination in rice (Solidum et al., 2012). High heavy metal concentration in paddy soil increases the potential uptake of heavy metal around the root zone area, shoot, and lastly, to the rice grain (Khairiah et al., 2013; McLaughlin et al., 2000). Rice grain has been reported to accumulate the least heavy metal concentration compared to roots and shoots (Arunakumara et al., 2013), and heavy metal in rice grain represents the amount of heavy metal exposed to human.

On the other hand, studies done by Khairiah et al., (2013), Yap et al., (2009), and Salim et al., (2010) only focused on total heavy metal concentrations in rice that used acid digestion method. In fact, total heavy metal concentration does not identify the actual degree of heavy metal ingestion exposure since total heavy metal concentration only represents the sum of heavy metal concentration in the environment (Lee et al., 2006). Eventually, total heavy metal concentration does not represent heavy metal concentrations that being absorbed in human body and thus overestimates human health risks if being used in HRA (Lee et al. 2006; Versantvoort et al., 2005). Saleem et al., (2014) stated that a realistic evaluation of actual human health risks due to heavy metal exposure needs an evaluation of a fraction from the total heavy metal, namely bioavailability fraction.

However, there is limited quantitative data on HRA application in local studies in Malaysia (Khairiah et al., 2013; Salim et al., 2010; Yap et al., 2009) despite the unclear human health risk via rice intake. On top of that, daily rice intake by Malaysians is considerably high, which is about two and half plates per day (Norimah et al., 2008), and since Malaysians eat rice daily, they are exposed to long term health risks from heavy metal exposure through their daily rice consumption. Moreover, all the local studies in Malaysia used uncooked rice samples, which may overestimate heavy metal concentration in rice since cooking can reduce the concentration of heavy metal in rice grain (Naseri et al., 2014). Devesa et al., (2005) have mentioned that intake of heavy metal should be always evaluated on the basis of the product as ingested by the consumers so that the risk may reflect the real situation of human exposure (Devesa et al., 2005). Furthermore, there were limited studies have been done related to bioavailability of metals concentration from cooked rice to be used as a closer approximation of HRA determination (Torres-Escribano et al., 2008).

1.3 Study Justification

There are two major parts of heavy metal in rice studies, namely, field rice and marketed rice samples. Studies that have dealt with heavy metal in different parts of paddy plants preferred field rice samples, while heavy metal in rice studies involved consumers and thus, marketed rice is preferred (Arunakumara et al., 2013; Musa et al., 2011). A survey done by Musa et al., (2011) found that Malaysians prefer to buy marketed rice due to the rice availability and accessibility in the market. Rice attributes, such as flavor, taste of cooking, and well-cooked rice also affect Malaysians in choosing rice that is available in the market (Musa et al., 2011).

Total metal concentration represent the whole concentration of the heavy metal in rice while bioavailability of metal concentration represents the amount of heavy metal in rice that being absorbed in human body (Versantvoort et al. 2004). Total heavy metal concentration is commonly used for heavy metal determination in Malaysian rice studies (Khairiah et al., 2013; Salim et al., 2010; Yap et al., 2009). In order to assess the exposure of heavy metal in rice, bioavailability of heavy metal is considered. With the *in vitro* digestion model, the bioavailability of heavy metal concentration from rice to human in gastrointestinal tract (GIT) can be determined (Versantvoot et al., 2005). Among the *in vitro* digestion models, *in vitro* digestion (RIVM) Netherlands model is the best model for *in vitro* digestion model for rice, which involves three compartments; oral cavity, stomach, and small intestine (Yang et al. 2012; Versantvoort et al., 2004). Besides, the RIVM is the best *in vitro* model since it is easily done and need simple apparatus to be done (Wragg et al., 2002).

There is knowledge gap in understanding human health risk exposure due to heavy metal from rice intake (He et al., 2012). Thus, integration of HRA via bioavailability of heavy metal obtained provides a baseline data for future investigation related to heavy metal studies in rice. Studies in this nature, which incorporate bioavailability of heavy metal in HRA, are crucial in estimating exposure and in providing accurate estimation of health risk. The HRA via ingestion pathway is the main route for identification of many food contaminants in human (Intawongse et al., 2006). In addition, HRA is essential to determine the quality of human health and for prevention, cure, and control efforts towards heavy metal contamination sources (Omar et al., 2013). Nonetheless, previous studies in Malaysia had only focused on heavy metal concentration in uncooked rice (Yap et al., 2009; Salim et al., 2010). In fact, cooking can reduce heavy metal concentration in rice grain (Naseri et al., 2004). Therefore, cooked rice is preferred in identifying heavy metal in rice studies that involve consumers so that risk evaluation may reflect the real situation of human exposure.

1.4 Expected outcomes of the study:

1. The output of the study displayed variation, level, and distribution of total and bioavailability heavy metal concentrations in varieties of cooked rice consumed by Malaysians.
2. This study opened a wide field of estimation of human health risks from heavy metal contamination in cooked rice using *in vitro* digestion model.
3. This study provided evidence that the inclusion of bioavailability of heavy metal rather than total heavy metal concentration produces more realistic estimation in HRA of heavy metal.
4. This study depicts baseline information for the varieties of cooked rice quality in the Malaysian market.

1.5 Conceptual Framework

Figure 1.2 shows the conceptual framework of this study. There are many environmental pollutants whether organic and inorganic pollutants such as pesticides and metals (Fuad et al., 2012; Fu et al., 2008). However, metals are considered as the most essential to be studied because metal is always available in environment, persistent and non biodegradable (Cao et al., 2010).

Heavy metal due to environmental pollution absorbs and accumulates in soil and in irrigation water (Minh et al., 2012). Through the interaction between soil and plant root microbes, high concentration of heavy metal in soil enhances the potential uptake of the heavy metal by the paddy plants (Solidum et al., 2012). Hence, heavy metal is accumulated in other parts of paddy plants, as well as in the rice grain (Khairiah et al. 2013).

Metals can be absorbed into human based on three main routes which are ingestion, inhalation, and dermal contact (Intawongse et al., 2006). However, ingestion pathway has been considered as the main pathway for heavy metal through rice consumption

(Versantvoort et al. 2005). Health risk assessment through ingestion pathway portrays both carcinogenic and non carcinogenic health risks.

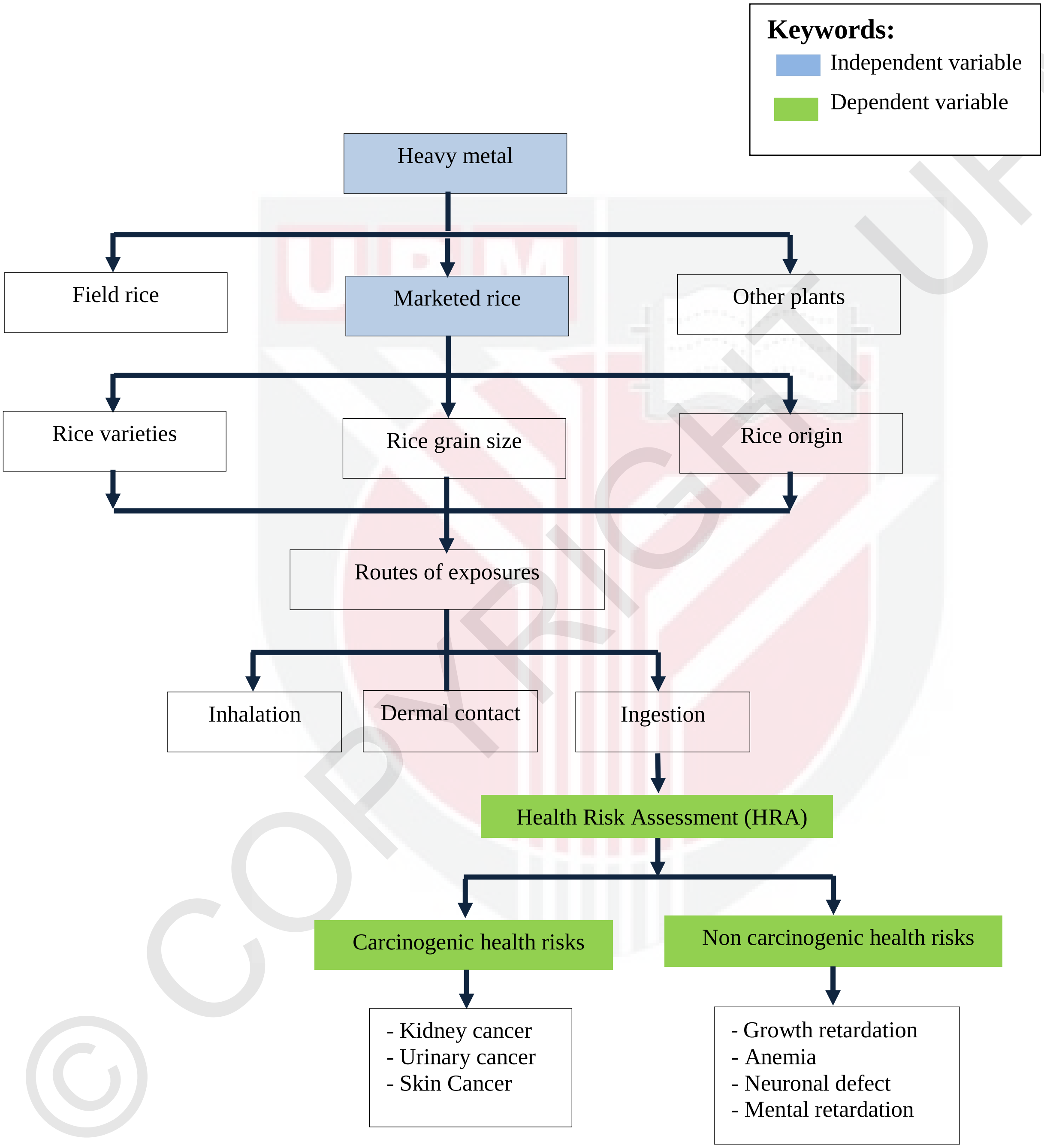

Figure 1.1 Conceptual framework of the study

1.6 Research Objectives and Hypotheses

1.6.1 General Objective

To determine the bioavailability of metals, such as zinc (Zn), iron (Fe), copper (Cu), cadmium (Cd), cobalt (Co), aluminium (Al), lead (Pb), arsenic (As), and chromium (Cr) concentrations in varieties of cooked rice samples and the health risks assessment among Malaysian.

1.6.2 Specific Objectives

1. To measure and compare both total and bioavailability concentrations of metals in varieties of cooked rice samples.
2. To compare the bioavailability concentrations of metals with the rice varieties, rice grain size and origin.
3. To calculate bioaccumulation factor of heavy metal in varieties of cooked rice samples.
4. To assess the relationships between bioavailability concentrations of different metals in varieties of cooked rice.
5. To measure the similarity of the chemical properties for each bioavailability concentrations of metal.
6. To assess human carcinogenic and non carcinogenic health risks of all metals exposure in varieties of cooked rice through ingestion pathway using Health Risk Assessment (HRA).

1.6.3 Research hypotheses

2. There is a significant difference between total and bioavailability metals concentrations.
3. There is a significant difference between the bioavailability concentrations of metals with the rice varieties, rice grain size and origin.
4. There is a significant relationship of bioavailability concentrations between different metals in varieties of cooked rice samples.
5. There are similar chemical properties of the bioavailability metals concentrations in varieties of cooked rice samples
6. There are carcinogenic and non carcinogenic health risks present for all metal exposure in varieties of cooked rice through ingestion pathway using Health Risk Assessment

1.7 CONCEPTUAL DEFINITION

1.7.1 Heavy metal

Heavy metal is a metal and metalloid with potential toxicity or ecotoxicity (Duffus et al., 2002).

1.7.2 Bioavailability

Bioavailability is the proportion of the ingested contaminant in food that reaches the systemic circulation (Versantvoort et al., 2005).

1.7.3 Bioaccumulation factor (BAF)

Bioaccumulation factor (BAF) was the concentration of heavy metal in rice grain from the concentration of heavy metal in soils (Liu et al. 2009).

1.7.4 Health Risk Assessment (HRA)

Health risk assessment is a scientific process by which quantification of potential environment hazards to human health is achieved. The HRA process utilizes tools of science and statistics to identify and measure the hazard, determine the possible routes of exposure, and finally, use that information to calculate a numerical value to represent the potential risk. A human HRA consists of four steps, namely, hazard identification, dose-response assessment, exposure assessment, and risk characterization (USEPA, 2012).

1.8 Operational Definition

1.8.1 Heavy Metal

Heavy metal such as Al, As, Cd, Co, Cr, Cr, Fe, Pb and Zn can be determined by using acid digestion method or *in vitro* method. Besides, Al, As, Cd, Co, Cr, Cr, Fe, Pb and Zn also can be analyzed by using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), and

Inductively Coupled Plasma Mass Spectrometry (ICP- MS) (Shakerian et al. 2012; Al-Saleh and Shinwari, 2010; Jorhem et al. 2008).

1.8.2 Bioavailability

The bioavailability of heavy metal concentration is determined via *in vitro* digestion model.

1.8.3 Bioaccumulation factor (BAF)

According to Satpathy et al., (2014), BAF < 1 or BAF = 1 denotes that the plant only absorbs the heavy metal, but does not accumulate, while when BAF > 1, this indicates that the plant accumulates the heavy metals. In this study, the BAF ratio was calculated for the determination of bioavailability concentration of heavy metal to the corresponding total heavy metal concentration in rice grain. Thus, the BAF was computed as follows:

$$BAF = \frac{C_B}{C_T}$$

where C_B represents the bioavailability of metals concentration in rice grain, while C_T represents the total metals concentration in rice grain.

1.8.4 Health Risk Assessment (HRA)

Based on USEPA (2012) and Saipan et al. (2009), average daily dose (ADD) (mg/kg/day) of a pollutant via rice consumption was applied in order to evaluate non carcinogenic HRA through ingestion exposure pathway on human. The equation below was used in the estimation of ADD via ingestion exposure pathways.

$$\text{ADD (mg/kg-day)} = \frac{C_{rice} \text{ X IR X EF X ED}}{\text{BW x AT}} \qquad \text{Equation 1}$$

Where:

ADD = Average Daily Dose (mg/kg-day)

C_{rice} = Average concentration in the rice (mg/g)
IR = Rice ingestion rate (g/day)
Ed = Exposure duration (years)
EF = Exposure frequency (day/year)
BW = Body weight (kg)
AT = Averaging time (ED x 365 days)

Then, the value of ADD was applied into the Hazard Quotient (HQ) calculation, as shown in Equation 2. The HQ is ratio of the dose divided by the heavy metal reference dose (R_{fD}).

$$\text{Hazard Quotient (HQ)} = \frac{ADD}{R_{fD}} \qquad \text{Equation 2}$$

Where;

HQ = Hazard Quotient
ADD = Average daily dose (mg/kg-day)
R_{fD} = Oral reference dose of heavy metal (mg/kg-day)

Then, the HQ was compared with the values of risk acceptability for non carcinogenic health risks. If the HQ does not exceed 1 (HQ < 1), it is assumed that no chronic non carcinogenic health risks are likely to occur. However, if the HQ exceeds 1 (HQ > 1), it is assumed that chronic non carcinogenic health risks are likely to occur.

As for the determination of carcinogenic health risks, the lifetime average daily dose (LADD) was calculated with Equation 3. Next, the Lifetime Cancer Risk (LCR) was quantified by applying the value of LADD in Equation 4.

$$\text{LADD (mg/kg-day)} = \frac{C_{rice} \times IR \times ED \times EF}{BW \times AT} \qquad \text{Equation 3}$$

Where;

LADD = Lifetime Average Daily Dose (mg/kg-day)
C_{rice} = Average concentration in the rice (mg/g)
IR = Rice ingestion rate (g/day)
Ed = Exposure duration (years)

EF = Exposure frequency (day/year)
BW = Body weight (kg)
AT = Averaging time (25550 days)

Carcinogenic risk is expressed as cancer potency (q*) value, and the following equation is used to quantify lifetime cancer risk:

$$\text{Lifetime cancer risk (LCR)} = \text{LADD} \times q^* \qquad \text{Equation 4}$$

Where:

LADD = Lifetime average daily dose (mg/kg-day)
q* = Cancer potency factor, also known as slope factor (mg/kg-day)

Slope factor was stated on USEPA's (2012) Integrated Risk Information System (IRIS). The USEPA guidelines specify that an acceptable risk is lifetime cancer risk of no greater than 1,000,000 (USEPA, 2012). Then, the LCR values are referred to the following table in order to access the risk acceptability for any carcinogenic health risks.

Table 1.1. Lifetime cancer risk (LCR) for carcinogenic health risk

Lifetime Cancer Risk (LCR)	
$> 10^{-4}$	Unacceptable/ Carcinogenic health risk present
$<10^{-4}$	Acceptable/ Carcinogenic health risk absent

(Source: USEPA, 2012)

REFERENCES

Abedin, M.D.J., Cresser, M.S., Meharg, A.A., Feldman, J. and Cotter-Howells, J. (2002). Arsenic accumulation and metabolism in rice (Oryza sativa L.). *Environmental Science and Technolology* 36: 962-968.

Agency for Toxic Substances and Disease Registry (ATSDR). (2011). Toxicological profile for Aluminium. ToxGuide. Atlanta, GA.

Agency for Toxic Substances and Disease Registry (ATSDR). (2008). Cadmium toxicity. Case Studies in Environmental Medicine (CSEM). Atlanta, GA.

Agency for Toxic Substances and Disease Registry (ATSDR). (2004). Toxicological profile for Cobalt. Public Comment. Atlanta, GA.

Alam, M.G.M., Snow, E.T. and Tanaka, A. (2003). Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. *Science of Total Environment* 308: 83–96.

Alloway, B.J. (2013). Heavy metal in soils: Trace metals and metalloids in soils and their bioavailability. *Springer* Third edition.

Alonso, E., Cambra, K. and Martinez, T. (2001). Lead and cadmium exposure from contaminated soil among residents of a farm area near an industrial site. *Achieve of Environmental Health* 56: 278–282.

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

Anjum, F.M., Pasha, I., Anwar, Bugti, M. and Butt, M.S. (2007). Mineral composition of different rice varieties and their milling fractions. *Pakistan Journal of Agricultural Science* 44(2): 332-336.

Ansari, M. and Raissy, M. (2011). Determination of Copper, Iron and Zinc in the Muscles of Freshwater Fish from Beheshtabad River. *Global Veterinaria* 7 (5): 502-505.

Arao, T., Kawasaki, A., Baba, K., Matsumoto, S. and Maejima Y. (2011). Arsenic contamination in soils and crops in Japan and various countermeasures. *Pedologist* 202-213.

Ariffin, T. (1998). Kegawatan ekonomi dan pengeluaran beras negara: Satu penilaian semula. *Agromedia* 4: 4-9.

Arredondo, M., Martinez, R., Nunez, M. T., Ruz, M. and Olivares, M. (2006). Inhibition of iron and copper uptake by iron, copper and zinc. *Biological Research* 39: 95-102.

Arshad, F.M., Alias, E.F., Noh, K.M. and Tasrif, M. (2011). Food security: self–sufficiency of rice in Malaysia. *International Journal of Molecular Sciences* 18(2): 83–100.

Arunakumara, K.K.I.U., Walpola, B.C. and Yoon, M.H. (2013). Current status of heavy metal contamination in Asia's rice lands. *Environmental Science and Biotechnology*. DOI 10.1007/s11157-013-9323-1.

Asghar, S., Anjum, F.M., Amir, R.M. and Khan, M.A. (2012). Cooking and eating characteristics of rice (Oryza sativa L.)-A review. *Pakistan Journal of Food Science* 22(3):128-132.

Azmi, M.Y., Junidah, R., Siti Mariam, A., Safiah, M.Y., Fatimah, S., Norimah, A.K., Poh, B.K., Kandiah, M., Zalilah, M.S., Wan, Abdul Manan, W.M., Siti Haslinda, M.D. and Tahir, A. (2009). Body mass index (BMI) of adults: Findings of the Malaysian Adult Nutrition Survey (MANS). *Malaysian Journal of Nutrition*. 15(2): 97-119.

Batista, B.L., Souza, J.M.O., Souza, S.S.D. and Fernando, B.J. (2011). Speciation of arsenic in rice and estimation of daily intake of different arsenic species by Brazilians through rice consumption. *Journal of Hazardous Material* 191: 342–348.

Batista, B.L., Nacano, L.R., Freitas, R.D., Oliveira-Souza, V.C.D., and Barbosa, F. (2012). Determination of Essential (Ca, Fe, I, K, Mo) and Toxic Elements (Hg, Pb) in Brazilian Rice Grains and Estimation of Reference Daily Intake. *Food and Nutrition Sciences* 3: 129-134.

Beard, J.L. (2001). Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. *Journal of Nutrition* 131: 568-580.

Bhattacharya, P., Samal, A.C., Majumdar, J. and Santra, S.C. (2010). Uptake of Arsenic in Rice Plant Varieties Cultivated with Arsenic Rich Groundwater. *Environment Asia 3(2):*34-37.

Bhattacharyya, P., Chakrabarti, K., Chakraborty, A., Tripathy, S., Kim, K. and Powell, M.A. (2008). Cobalt and nickel uptake by rice and accumulation in soil amended with municipal solid waste compost. *Ecotoxicology and Environmental Safety* 69 (3), 506–512.

Brammer, H. and Ravenscroft, P. (2009). Arsenic in groundwater: a threat to sustainable agriculture in South and South-east Asia. *Environment International* 35:647–654.

Brandon, E.F.A., Oomen, A.G., Rompelberg, C.J.M., Versantvoort, C.H.M., Engelen, J.G.M.V. and Sips, A.J.A.M. (2006). Cosumer product in vitro digestion model: bioaccessibility of contaminants and its application in risk assessment. *Regulatory Toxic and Pharmacology* 44: 161-171.

Cao, H., Chen, J., Zhang, J., Zhang, H., Qiao, L. and Men, Y. (2010). 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 Sciences* 22 (11): 1792-1799.

Cao, Z.H. and Hu, Z.Y. (2000). Copper contamination in paddy soils irrigated with wastewater. *Chemosphere*. 41(1-2): 3-6.

Chakraborti, D., Rahman, M.M., Paul, K., Chowdhury, U.K., Sengupta, M.K., Lodh, D., Chanda, C.R., Saha, K.C. and Mukherjee, S.C. (2002). Arsenic calamity in the Indian subcontinent—what lessons have been learned? *Talanta* 58:3–22.

Chan, Y.H. (2003). Basic statistics for doctors. *Singapore Medical Journal* 44(12): 614-619.

Chen, J., Gaikwad, V., Holmes, M., Murray, B., Povey, M. and Wang, Y. (2011). Development of a simple model device for in vitro gastric digestion investigation. *Food and Function* 2(3-4): 174-182.

Chen, N.S.C., Tsai, A. and Dyer, La. (1973). Effect of chelating agents on chromium absorption. *Journal of Nutrition* 103: 1182-1186.

Chinoim N. and Sinbuathong S., (2010). Heavy metal contamination of soils from organic paddy fields in Thailand. 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1 – 6 August 2010, Brisbane, Australia.

Chu, K. W. and Chow, K. L. (2002). Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. *Aquatic Toxicology* 61: 53–64.

Chung, B.Y., Song, C.H., Park, B.J. and Cho, J.Y. (2011). Heavy Metals in Brown Rice (Oryza sativa L.) and Soil After Long-Term Irrigation of Wastewater Discharged from Domestic Sewage Treatment Plants. *Pedosphere* 21(5): 621-627.

Ciavatta, C., Manoli C., Cavani L., Franceschi C. and Sequi P. (2012). Chromium-Containing Organic Fertilizers from Tanned Hides and Skins: A Review on Chemical, Environmental, Agronomical and Legislative Aspects. *Journal of Environmental Protection* 3: 1532-1541.

Clemens, S., Aarts, M.G.M., Thomine, S. and Verbruggen, N. (2013). Plant science: the key to preventing slow cadmium poisoning. *Trends in plant science* 18(2): 92–9.

Coen, N., Mothersill, C., Kadhim, M. and Wright, E.G. (2001) Heavy metals of relevance to human health induce genomic instability. *Journal of Pathology* 195:293–299.

Coles, L.T., Moughan, P.J. and Darragh, A.J. (2005). *In vitro* digestion and fermentation methods, including gas production techniques, as applied to nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. *Animal Feed Science and Technology* 123-124 (1):421-444.

Department of Occupational Safety and Health (DOSH) (2008). Guidelines for Hazard Identification, Risk Assessment and Risk Control (HIRARC). Ministry of Human Resources Malaysia. ISBN 978-983-2014-62-1.

Department of Occupational Safety and Health (DOSH) (2000). Assessment of the health risks arising from the use of hazardous chemicals in the workplace. Ministry of Human Resources Malaysia.

Department of Statistics Malaysia. (2013). Retrieved 3rd July 2013 from http://www.statistics.gov.my

Devesa, V., Velez, D. and Montoro, R. (2008). Effect of thermal treatments on arsenic species contents in food. *Food and Chemical Toxicology* 46(1): 1-8.

Dubey, S., Misra, P., Dwivedi, S., Chatterjee, S., Bag, S.K., Mantri1, S. (2010). Transcriptomic and metabolomic shifts in rice roots in response to Cr (VI) stress. *BMC Genomics* 11: 648.

Duffus, J.H. (2002). "Heavy Metals"—A Meaningless Term? Pure Application. *Chemosphere* 74(5): 793–807.

El-Naggar, A.H., Osman, M.E.H., Dyab, M.A. and El Mohsenawy, E.A. (1999). Cobalt and lead toxicities on Calothrix fusca and Nostoc muscorum. *Journal of Union of Arab Biologists Cairo* 7(B): 421.

Erban, L. E. Gorelick, S. M. Zebker, H. A. and Fendorf, S. (2013). Release of arsenic to deep groundwater in the Mekong Delta, Vietnam, linked to pumping-induced land subsidence. *Proceedings of the National Academy of Sciences of the United States of America* 110 (34): 13751−13756.

European Food Safety Authority (EFSA). (2009). Scientific opinion of the panel on contaminants in the food chain. *The EFSA Journal* 980: 1-13.

European Food Safety Authority (EFSA). (2011). On the Evaluation of a new study related to the bioavailability of aluminium in food. *EFSA Journal* 9(5): 2157.

Everitt, B.S., Landau, S. and Leese, M. (2001), Cluster Analysis, Fourth edition, Arnold.

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

FAO/WHO. 1984. 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.

FAO/WHO. 1989. Evaluation of certain food additives and of the contaminants. 33rd Report of the Joint FAO/WHO Expert Committee on Food additives, Technical Report Series No. 776; 109:1265- 1270.

Fatmi, Z., Azam, I., Ahmed, F., Kazi, A., Gill, A. B., Kadir, M. M., Ahmed, M., Ara, N. and Janjua, N.Z. (2009). Health burden of skin lesions at low arsenic exposure through groundwater in Pakistan. Is river the source? *Environmental Research* 109(5): 575–581.

Figueiredo, D.D., Barros, P.M., Cordeiro, A.M., Serra, T.S., Lourenço, T., Chander, S., Oliveira, M.M. and Saibo, N.J. (2012). Seven zinc-finger transcription factors are novel regulators of the stress responsive gene OsDREB1B. *Journal of Experimental Botany* 63: 3643-3656.

Finnegan, P.M. and Weihua, C. (2012). Arsenic Toxicity: The Effects on Plant Metabolism. *Frontiers in Physiology* 3: 1-18.

Flora, G., Gupta, D. and Tiwari, A. (2012). Toxicity of lead: a review with recent updates. *Interdisciplinary Toxicology* 5: 47–58.

Fresco, L. (2005). Rice is life. *Journal of Food Composition and Analysis* 18(4): 249-253.

Fu, J., Zhou, Q., Liu, J., Liu, W., Wang, T., Zhang, Q. and Jiang, G. (2008). High levels of heavy metals in rice (Oryza sativa L.) from a typical E-waste recycling area in southeast China and its potential risk to human health. *Chemosphere* 71(7): 1269–75.

Fuad, M.J.M., Selvadurai, S. and Azima, A.M. (2012). The impact of pesticides on paddy farmers and ecosystem. *Advances in Natural Applied Science* 6(1): 65–70.

Fytianos, K., Katsianis, G., Triantafyllou, P. and Zachariadis, G. (2001). Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. *Bulletin of Environmental Contamination and Toxicology* 67: 423–430.

Gao, C.Y., Wang, D.S., Xiao, C.P., Qiu, D.G., Han, X.Z., Zhang, T. and Wu, Z.H. (2011). Comparison of cadmium-induced iron-deficiency responses and genuine iron-deficiency responses in Malus xiiaojinensis. *Plant Science* 181:269-274.

Gao, J., Zhou, H., Pan, G., Wang, J. and Chen, B. (2013). Factors Influencing the Persistence of Organochlorine Pesticides in Surface Soil from the Region around the Hongze Lake, China. *Science of the Total Environment* 443: 7–13.

Garg, P., Tripathi, R.D., Rai, U.N, Sinha, S. and Chandra, P. (1997). Cadmium accumulation and toxicity in submerged plant hydrilla verticillata (l.f.) royle. *Environmental Monitoring and Assessment* 47: 167–173.

Ghazanfarirad, N., Dehghan, K., Fakhernia, K., Rahmanpour, F., Bolouki, M., Zeynali, F., Asadzadeh, J. and Bahmani1, M. (2014). Determination of Lead, Cadmium and Arsenic Metals in Imported Rice into the West Azerbaijan Province, Northwest of Iran. *Journal of Novel Applied Sciences* 3 (5): 452-456.

Glahn, R.P., Lee, O.A. and Miller, D.D. (1999). In vitro digestion/Caco-2 cell Culture model to determine optimal ascorbic acid to Fe ratio in rice cereal. *Journal of Food Sciences* 64 (5): 925-928.

Glahn, R.P., Cheng, Z., Welch, R.M. and Gregorio, G.B. (2002). 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* 50(12): 3586-3591.

Grant, C.F. (2011). Heavy metals in the River Hamble: Sedimentary copper and other heavy metal concentrations and their sources within the River Hamble, UK, in the context of legislative requirements.

Gropper, S. S. and Smith, J. (2012). Advanced nutrition and human metabolism. Sixth edition. Yolanda Cossio.

Guerra, F., Trevizam, A. R., Muraoka, T., Chaves, N. and Canniatti-Brazaca, S.G. (2012). Heavy metals in vegetables and potential risk for human health. *Scientia Agricola* 69 (1): 54-60.

Habibah, J., Lee, P.T., Khairiah, J., Ahmad, M.R., Fouzi, B.A. and Ismail, B.S. (2011). Speciation of heavy metals in paddy soils from selected areas in Kedah and Penang, Malaysia. *African Journal of biotechnology* 10: 13505-13513.

Hang, X., Wang, H., Zhou, J., Ma, C., Du, C. and Chen, X. (2009). Risk assessment of potentially toxic element pollution in soils and rice (Oryza sativa) in a typical area of the Yangtze River Delta. *Environmental Pollution* 157(8-9): 2542-2549.

Hansch R., Mendel R.R., (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current Opinion in Plant Biology* 12:259–266.

He, Y., Pedigo, C.E., Lam, B., Cheng, Z. and Zheng, Y. (2012). Bioaccessibility of arsenic in various types of rice in an in vitro gastrointestinal fluid system.

Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes 47(2): 74-80.

Hicsonmez, U., Ozdemir, C., Cam, S., Ozdemir, A. and Erees, F.S. (2012). Major-minor element analysis in some plant seeds consumed as feed in Turkey. *Natural Science* 4(5): 298-303.

Hossain, S.M. and Mohiuddin, A.K.M. (2012). Study on biofortification of rice by targeted genetic engineering. *International Journal of Agricultural Research, Innovation and Technology* 2 (2): 25-35.

Hunt, J.R., Johnson, L.K. and Juliano, B.O. (2002). 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* 50(18): 5229-5235.

Hur, S.J., Lim, B.O., Decker, E.A. and McClements, D.J. (2011). In vitro human digestion models for food applications. *Food Chemistry* 125: 11–12.

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

International Agency for Research on Cancer (IARC) (2012). Monograph on Cadmium, Chromium, Copper, Iron, Plumbum and Zinc. International Agency for Research on Cancer, Lyon, France.

International Rice Research Institute (IRRI). (2013). Retrieved 17th May 2013 from www.irri.com.

Ismail, M.M., Idris, N. and Hassanpour, B. (2013). Technical efficiency estimates of paddy farming in peninsular Malaysia: A comparative analysis. *Scholars Research Library, annals of Biological Research* 4(5): 114-118.

Jamil, H., Theng, L.P., Jusoh, K., Razali, A.M., Ali, F.B. and Ismail, B.S. (2011). Speciation of heavy metals in paddy soils from selected areas in Kedah and Penang, Malaysia. *Africa Journal of Biotechnology 10*(62): 13505–13513.

Janati, S.S.F., Beheshti, H.R., Feizy, J. and Fahim, N.K. (2011). Determinations of Cadmium, Lead, Arsenic and Mercury in Rice from Iran. *International Journal of Industrial Chemistry* ISSN (online): 2228-5547.

Jarapala, S.R., Kandlakunta, B. and Thingnganing, L. (2014). Evaluation of trace metal content by ICP-MS using closed vessel microwave digestion in fresh water fish. *Journal of Environmental and Public Health* http://dx.doi.org/10.1155/2014/201506

Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin* 68: 167–182.

Ji, K., Kim, J., Lee, M., Park, S., Kwon, H.J., Cheong, H.K., Jangf, J.Y., Kimc, D.S., Yuc, S., Kimg, Y.W., Leeh, K.W., Yangi, S.O., Jhungj, I.J., Yangk, W.H., Paekl, D.H., Hongm, Y.C. and Cho, K. (2013). Assessment of exposure to heavy metals and health risks among residents near abandoned metal mines in Goseong, Korea. *Environmental Pollution* 178:322–8.

John, W.H., Yasumatsu, K. and Moritaka, S. (1985). Iron fortification of rice. *United Kingdon edition* Academic Press.

Jorhem, L.A., Strand, C., Sundstram, B., Baxter, M., Stokes, P. and Lewis, J. (2008). Elements in rice on the Swedish market: Part 2. Chromium, copper, iron, manganese, platinum, rubidium, selenium and zinc. *Food Additives and Contaminant - Part A Chemical, Analysis, Control, Exposure and Risk Assessment* 25(7): 841-850.

Juhasz, A.L., Smith, A., Weber, J., Gancarz, D., Rees, M., Rofe, A., Kuchel, T., Sansom, L. and Naidu, R. (2010). Predicting the relative bioavailability of arsenic, cadmium, and lead via the incident soil ingestion pathway using in vitro techniques. 19th World Congres of Soil Science, Soil Solution for a Changing World. 1 – 6 August 2010, Brisbane, Australia.

Kabata-Pendias, A. and Pendias, H. (1992). Trace elements in soils and plants. Boca Raton, FL: CRC

Kanchana, S., Lakshmi, B.S., Ilamaran, M. and Singaravadive, K. (2012). Physical Quality of Selected Rice Varieties. *World Journal of Agricultural Sciences* 8 (5): 468-472.

Kennedy, G. and Burlingame, B. (2003). Analysis of food composition data on rice from a plant genetic resources perspective. *Food Chemistry* 80(4): 589-596.

Khairiah, J., Ramlee, A.R., Jamil, H., Ismail, Z. and Ismail, B.S. (2013). Heavy Metal Content of Paddy Plants in Langkawi , Kedah , Malaysia School of Environmental and Natural Resource Sciences , Faculty of Science and Technology. *Australian Journal of Basic and Applied Sciences* 7(2): 123–127.

Kim, Y.Y., Yang, Y.Y. and Lee, Y. (2002). Pb and Cd uptake in rice roots. *Physiologia Plantarum* 116: 368–372.

Kong, F., Oztop, M.H., Singh, R.P. and McCarthy, M.J. (2011). Physical changes in white and brown rice during simulated gastric digestion. *Journal of Food Science* 76(6): E450-E457.

Krewski, D., Yokel, R.A., Nieboer, E., Borchelt, D., Cohen, J., Harry, J., Kacew, S., Lindsay, J., Mahfouz, A.M. and Rondeau, V. (2007). Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. *Journal of Toxicology and Environment Health: Part B-Critical Reviews* 10: 1-269.

Lamm, S.H., Engel, A., Penn, C. A., Chen, R. and Feinleib, M. (2006). Arsenic cancer risk confounder in southwest Taiwan data set. *Environmental Health Perspectives* 114(7): 1077–1082.

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

Latunde-Dada, G. O. (1998). Gastrointestinal tract and iron absorption: A review. *Brazilian Journal of Food and Nutrition* 9: 103-125.

Lee, S.W., Lee, B.T., Kim, J.Y., Kim, K.W. and Lee, J.S. (2006). Human risk assessment for heavy metals and as contamination in the abandoned metal mine areas, Korea. *Environmental Monitoring and Assessment* 119(1-3): 233-244.

Liu, W.X., Liu, J.W., Wu, M.Z., Li, Y., Zhao, Y. and Li, S.R. (2009). Accumulation and translocation of toxic heavy metals in winter wheat (Triticum aestivum L.) growing in agricultural soil of Zhengzhou, China. *Bulletin of Environmental Contamination and Toxicology* 82(3): 343-347.

Liu, J., Zhang, X., Tran, H., Wang, D. and Zhu, Y. (2011). Heavy metal contamination and risk assessment in water, paddy soil, and rice around an electroplating plant. *Environmental Sciences Pollution* 18: 1623-1632.

Liu, X., Song, Q., Tang, Y., Li, W., Xu, J., Wu, J., Wang, F. and Brookes, P. C, (2013)Human health risk assessment of heavy metals in soil–vegetable system: A multi-medium analysis. *Science of the Total Environment* 463–464: 530-540.

Lin, H.T.,Wong, S.S. and Li, G.C. (2004). Heavy metal content of rice and shellfish in Taiwan. *Journal of Food and Drug Analysis* 12 (2): 167-174.

Makino T., Kamiya T., Sekiya N., Maejima Y., Akahane I. and Takano H. (2010). Chemical remediation of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and on-site verification. 2010 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1 – 6 August 2010, Brisbane, Australia.

Malaysian Food Act. (2004). Food Act 1983 (Act 281) & Regulations, International Law book Services, Kuala Lumpur.

Maleki, A., Zazoli, M. A. and Shokhrzadeh, M. (2007). Investigation of cadmium content in Iranian rice (Oryza Sativa) :Its weekly intake. *Journal of Applied Sciences and Environmental Management* 11 (1):101-105.

Mandak, E. and Nystram, L. (2012). The effect of in vitro digestion on steryl ferulates from rice (Oryza sativa L) and other grains. *Journal of Agricultural and Food Chemistry* 60(24): 6123-6130.

Marschnar, H. (1995). Mineral nutrition of higher plants (2nd ed.). London: Academic.

Masud, M.M., Rahman, M.S., Al-Amin, A.Q., Kari,F. and Filho, W.L. (2012). Impact of climate change: an empirical investigation of Malaysian rice production. *Mitigation and Adaptation Strategies for Global Change* 19(4): 431-444.

McClements, D.J. and Li, Y. (2009). Review of in vitro digestion models for rapid screening of emulsion-based systems. *Food and Function* 1(1): 32-59.

McLaughlin, M.J., Hamon, R.E., McLaren, R.G., Speir, T.W. and Rogers, S.L. (2000): Review: a bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research* 38: 1037–1086.

Meier, P.G., Fook, D.C. and Lagler, K.F. (1983). Organochlorine pesticide residues in rice paddies in Malaysia, 1981. *Bulletin of Environmental Contamination And Toxicology* 30(3): 351–7.

Meral, D.A. and Umran, D. (2007). Arsenic mineralization, source, distribution, and abundance in the Kutahya region of the western Anatolia, Turkey. *Environmental Geochemistry and Health* 29: 119–129.

Minh, N.D., Hough, R.L., Le, T.T., Nyberg, Y., Le, B.M., Nguyen, C.V. and Oborn, I. (2012). Assessing dietary exposure to cadmium in a metal recycling community in Vietnam: age and gender aspects. *Science of Total Environment* 416: 164–71.

Minomo, K., Ohtsuka, N., Nojiri, K., Hosono, S. and Kawamura, K. (2011). Chemosphere polychlorinated biphenyls in rice straw smoke and their origins in Japan. *Chemosphere* 84: 950–956.

Mondal, D. and Polya, D.A. (2008). Rice is a major exposure route for arsenic in Chakdaha block, Nadia district, West Bengal, India: a probabilistic risk assessment. *Applied Geochemistry* 23(11): 2987–2998.

Mooi, E. and Sarstedt, M. A. (2011). Concise Guide to Market Research. DOI 10.1007/978-3-642-12541-6_9, Springer-Verlag Berlin Heidelberg. Available online http://www.springer.com/978-3-642-12540-9

Morekian, R., Mirlohi, M., Azadbakht, L. and Maracy, M.R. (2012). Heavy metal distribution frequency in Iranian and imported rice varieties marketed in central Iran, Yazd, 2012. *International Journal of Environmental Health Engineering* 2(2): 1-5.

Morgan, A. (2001). Exposure and health effects. Office of Water, Office of Science and Technology Health and Ecological Criteria Division United States Environmental Protection Agency, Washington, DC, USA.

Moura, M. C. S. D., Moita, G. C. and Neto, J. M. M. (2010). Analysis and assessment of heavy metals in urban surface soils of Teresina, Piauí State, Brazil: a study based on multivariate analysis. *Comunicata Scientiae* 1(2): 120-127.

Musa, M., Othman, N. and Fatah, F.A. (2011). Determinants of consumers purchasing behavior for rice in Malaysia. *American International Journal of Contemporary Research* 1:159-163.

Nagpal, N.K. (2004). Water quality guidelines for cobalt. Ministry of water, land and air protection, water protection section, water, air and climate change brand victoria.

Najib, N.W.A.Z., Mohammed, S.A., Ismail, S.H., Ahmad, A.W. and Amiza, W. (2012). Assessment of heavy metal in soil due to human activities in Kangar, Perlis, Malaysia. *International Journal of Civil and Environmental Engineering* 12: 28-33.

Najim, M.M.M., Lee, T.S., Haque, M.A. and Esham, M. (2007). Sustainability Of Rice Production: A Malaysian Perspective M.M.M. *The Journal of Agricultural Sciences* 130: 1–12.

Naseri, M., Rahmanikhah, Z., Beiygloo, V. and Ranjbar, S. (2014). Effects of two cooking methods on the concentrations of some heavy metals (cadmium, lead, chromium, nickel and cobalt) in some rice brands available in Iranian Market. *Journal of Chemical Health Risks* 4(2): 65–72.

Nassef', M., Hannigan, R. K., Sayed, A. E. L. and Tahawv, M. S. E. L. (2006). Determination of some heavy metals in the environment of Sadat industrial city. Proceedings of the 2"d Environmental Physics Conference. 18-22 Feb. 2006, Alexandria.

Nazif, W., Perveen, S. and Saleem, I. (2006). Status of micronutrients in soils of district bhimber (Azad Jammu And Kashmir). *Journal of Agricultural and Biological Science* 1(2): 35-40.

Nobuntou, W., Parkpian, P., Oanh, N.T.K., Noomhorm, A., Delaune, R.D. and Jugsujinda, A. (2010). Lead distribution and its potential risk to the environment: lesson learned from environmental monitoring of abandon mine. *Journal of Environmental Sciences and Health- Part A Toxic/Hazardous Substances and Environmental Engineering* 45(13): 1702-1714.

Norimah, A.K., Safiah, M., Jamal, K., Siti, H., Zuhaida, H. and Rohida, S. (2008). Food consumption patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). *Malaysian Journal of Nutrition* 14(1): 25-39.

NRCC (National Research Council of Canada). NRCCNo. 15391; NRCC: Ottawa, Canada, 1978.

Okoro, H. K., Fatoki, O. S., Adekola, F. A., Ximba, B. J. and Snyman, R. G. (2012). A review of sequential extraction procedures for heavy metals speciation in soil and sediments. Open Access Scientific Report.

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

Oomen, A.G., Rompelberg, C.J.M., Bruil, M.A., Dobbe, C.J.G., Pereboom, D.P.K.H. and Sips, A.J.A.M. (2003). Development of an in vitro digestion model for estimation the bioaccessibility of soil contaminants. *Archives of Environmental Contamination and Toxicology* 44: 281–287.

Owens, G. (2001). Cereals Processing Technology. Woodhead Publishing Limited, Cambridge, England

Paddy Statistics in Malaysia. Department of Agricultural Malaysia. (2013). Retrieved 20th October 2013 from http://www.agricultural.gov.my

Pallant, J. (2010). Spss Survival Analysis. 4th edition. Allen & Unwin Book Publishers, Australia.

Panda, S.K., Baluska, F. and Matsumoto, H. (2009). Aluminum stress signaling in plants. *Plant Signalling Behaviour* 4(7): 592-597.

Pasias, I.N., Thomaidis, N.S. and Piperaki, E.A. (2013). Determination of total arsenic, total inorganic arsenic and inorganic arsenic species in rice and rice flour by electrothermal atomic absorption spectrometry. *Microchemical Journal* 108: 1–6.

Pathirana, R., Chandrasiri, P.A.N. and Sirisena, S.G. (1995). Response of rice cultivars to increased iron and aluminium concentrations. In: Date RA (ed) Plant Soil Interaction at Low pH. Kluwer Academic Publishers, Dordrecht. pp 413–417.

Peijnenburg, W.J.G.M. and Jager, T. (2003). Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. *Ecotoxicology and Environmental Safety* 56: 63–77.

Peraza, M. A., Ayala-Fierro, F., Barber, D. S., Casarez, E. and Rael, L. T. (1998). *Environmental Health Perspectives* 106 (1): 203-216.

Phan, K., Sthiannopkao, S., Heng, S., Phan, S., Huoy, L., Wong, M.H. and Kim, K.W. (2013). Arsenic contamination in the food chain and its risk assessment of populations residing in the Mekong River basin of Cambodia. *Journal of Hazardous Material* 262: 1064−71.

Plum, L. M., Rink, L. and Haase, H. (2010). The Essential Toxin: Impact of Zinc on Human Health. *International Journal of Environmental Research and Public Health* 7(4): 1342–1365.

Praveena, S. M., Ahmed, A., Radojevic, M., Abdullah, M. H. and Aris, A. Z. (2008). Multivariate and Geoaccumulation Index Evaluation in Mangrove Surface Sediment of Mengkabong Lagoon, Sabah. *Bulletin of Environmental Contamination and Toxicology* 81:52–56.

Qaiyum, M.S., Shaharudin, M.S., Syazwan, A.I. and Muhaimin, A. (2011). Health risk assessment after exposure to aluminium in drinking water between two different villages. *Journal of Water Resource and Protection* 3: 268-274.

Qian, Y., Chen, C., Zhang, Q., Li, Y., Chen, Z. and Li, M. (2010). Concentrations of cadmium, lead, mercury and arsenic in Chinese market milled rice and associated population health risk. *Food Control* 21: 1757-1763.

Rahman, M.A., Hasegawa, H., Rahman, M.M., Rahman, M.A. and Miah, M.A.M. (2007). Accumulation of arsenic in tissues of rice plant (Oryza sativa L.) and its distribution in fractions of rice grain. *Chemosphere* 69:942–948.

Ramachandaran, V., Bhujbal, B.M. and Souza, T.J.D. (1998). Influence of rock phosphate with and without vegetable composition on the yield, phosphate and cadmium contents of rice (Oryza sativa L.) grown on an ultisol. *Fresenius Environmental Bulletin* 7: 551-556.

Reeves, P.G. and Chaney, R.L. (2008). Bioavailability as an issue in risk assessment and management of food cadmium: A review. *Science of the Total Environment* 398 (1-3), 13-19.

Rhee H. P., Yoon C. G., Son Y. K. and Jang J. H. (2011). Quantitative risk assessment for reclaimed wastewater irrigation on paddy rice field in Korea. *Paddy and Water Environment* 9:183–191.

RICEpedia. Rice types in Malaysia. BERNAS Official page. Can be accessed at www.bernas.com.my. Accesed on 23 July 2013.

Robert, B. and Richard, B. (2008). Chapter 23. Business research methods and statistics using SPSS. Sage Publications LTD.

Rogan, N., Serafimovski, T., Dolenec, M., Tasev, G. and Dolenec, T. (2009). Heavy metal contamination of paddy soils and rice (Oryza sativa L.) from Kocani Field (Macedonia). *Environmental Geochemistry and Health* 31(4): 439-451.

Rossman, T.G. (2003). Mechanism of arsenic carcinogenesis: an integrated approach. *Mutation Research* 533: 37–65.

Roychowdhury, T., Tokunaga, H. and Ando, M. (2003). Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic-affected area of West Bengal, India. *Science of the Total Environment* 308(1–3): 15–35.

Roy, P., Orikasa, T., Okadome, H., Nakamura, N. and Shiina, T. (2011). Processing conditions, rice properties, health and environment. *International Journal of Environmental Research and Public Health* 8: 1957-1976.

Ruby, M.V., Schoof, W.B., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D.E., Casteel, S.W., Berti, W., Carpenter, M., Edwards, D., Cragin, D. and Chappel, W. (1999). Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environmental Science and Technology* 33: 3697–3705.

Sadeghzadeh, B. (2013). A review of zinc nutrition and plant breeding. *Journal of Soil Science Plant Nutrition* 13 (4): 905-927.

Saipan, P. (2009). Health Risk Assessment of inorganic arsenic intake of Ron Phibun residents via duplicate diet study. *Journal of the Medical Association of Thailand* 92(6): 849-856.

Saleem, K.S., Miller, B. and Price, J.L. (2014). Subdivisions and connectional networks of the lateral prefrontal cortex in the macaque monkey. *Journal of Comparative Neurology* 522(7):1641-90. doi: 10.1002/cne.23498.

Salim, N.A.A., Elias, M.S., Wood, A.K., Sanuri, E., Hamzah, M.S. and Rahman, S.A. (2010). Multielement analysis in rice grains by instrumental neutron activation analysis. *Journal of Nuclear Science and Technology* 7(2): 1-11.

Satpathy, D., Reddy, M.V. and Dhal, S.P. (2014). Risk Assessment of Heavy Metals Contamination in Paddy Soil, Plants, and Grains (Oryza sativa L.) at the East Coast of India. *BioMed Research International*. [http://dx.doi.org/10.1155/2014/545473].

Schumann, K., Classen, H.G., Hages, M., Prinz-Langenhol, R., Pietrzik, K. and Biesalski, H.K. (1997). Bioavailability of oral vitamins, minerals and trace elements in perspective. *Drug Research* 47: 369-80

Schuenemeyer, J. H. and Drew, L.J. 2011. Statistics for earth and environmental scientists, pp. 246-248. A John Wiley & Sons, INC., Publication.

Seyfferth, A.L., McCurdy, S., Schaefer, M.V. and Fendorf, S. (2014). Arsenic concentrations in paddy soil and rice and health implication for major rice-growing regions of Cambodia. *Environmental Science and Technology* 48: 4699-4706.

Shabbir, M.A., Anjum, F.M., Khan, M.R., Nadeem, M. and Saeed, M. (2013). Assessment of heavy metals and aflatoxin levels in export quality Indica rice cultivars with different milling fractions. *African Journal of Agricultural Research* 8 (25): 3236-3244.

Shakerian, A., Rahimi, E. and Ahmadi, M. (2012). Cadmium and lead content in several brands of rice grains (Oryza sativa) in central Iran. *Toxicology and Industrial Health* 28(10) 955–960.

Shanker, A. K., Cervantes, C., Loza-Taverac, H. and Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International* 31: 739– 753.

Sharma, P. and Dubey, R.S. (2005). Lead toxicity in plants. *Brazil Journal of Plant Physiology* 17 (1): 35-52.

Sharp, P. and Srai, S. J. (2007). Molecular mechanisms involved in intestinal iron absorption. *World Journal of Gastroenterology* 13(35): 4716-4724.

Shimbo, S., Zhang, Z.W. and Watanabe, T. (2001). Cadmium and lead contents in rice and other cereal products in Japan in 1998-2000. *Science of the Total Environment* 281 (1-3):165-175.

Signes, A., Mitra, K., Burla, F. and Carbonell-Barrachina, A.A. (2008). 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* 25(1): 41-50.

Signes-Pastor, A.J., Al-Rmalli, S.W., Jenkins, R.O., Carbonell-Barrachina, A.A. and Haris, P.I. (2012). Arsenic bioaccessibility in cooked rice as affected by arsenic in cooking water. *Journal of Food Science* 77 (11): T201-T206.

Silins, I. and Hogberg, J. (2011). Combined Toxic Exposures and Human Health: Biomarkers of Exposure and Effect. *International Journal of Environmental Research and Public Health* 8: 629-647.

Simmons, R.W., Pongsakul, P., Saiyasitpanich, D. and Klinphoklap, S. (2005). Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: Implications for public health. *Environmental Geochemistry and Health* 27: 501–511.

Singh, A., Sharma, R.K., Agrawal, M. and Marshall, F.M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology* 48(2): 611–9.

Smuc, N.R., Dolenec, T., Serafimovski, T., Tasey, G., Dolenec, M. and Vrhovnik, P., (2012). Heavy metal characteristics in Kocani Field plant system (Republic of Macedonia). *Environmental Geochemistry and Health* 34:513–526.

Solidum, J., Dykimching, E., Agaceta, C. and Cayco, A. (2012). Assessment and Identification of Heavy Metals in Different Types of Cooked Rice Available in the Philippine Market. *2nd International Conference on Environmental and Agriculture Engineering* 37: 35-39.

Syahariza, Z.A., Sar, S., Hasjim, J., Tizzotti, M.J. and Gilbert, R.G. (2013). The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains. *Food Chemistry* 136(2): 742-749.

Tabachnick, B.G. and Fidell, L. S. (2007). Using multivariate statistics (5th edn). Boston Pearsom Education.

Tanaka, K., Fujimaki, S., Fujiwara, T., Yoneyama, T. and Hayashi, H. (2007). Quantitative estimation of the contribution of the phloem in cadmium transport to grains in rice plants (Oryza sativa L.). *Soil Science Plant Nutrition* 53: 72–77.

Tarley, C.R.T. and Arruda, M.A.E.Z. (2004). Biosorption of heavy metals using rice milling by-products. Characterisation and application for removal of metals from aqueous effluents. *Chemosphere* 54: 987–995.

Tey, J.Y.S. and Radam, A. (2011). Demand patterns of rice imports in Malaysia: Implications for food security. *Food Security* 3(2): 253–261.

Thomas, R., Wan-Nadiah, W.A. and Bhat, R. (2013). Physiochemical properties, proximate composition, and cooking qualities of locally grown and imported rice varieties marketed in Penang, Malaysia. *International Food of Research Journal* 20(3): 1345-1351.

Torres-Escribano, S., Leal, M., Vaclez, D. and Montoro, R. (2008). Total and inorganic arsenic concentrations in rice sold in Spain, effect of cooking, and risk assessments. *Environmental Science and Technology* 42(10): 3867-3872.

Tran, T.A. and Popova, L.P. (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turkish Journal of Botany* 37: 1-13.

Tripathi, R.M., Raghunath, R. and Krishnamoorthy, T.M. (1997). Dietary intake of heavy metals in Bombay city, India. *Science of the Total Environment* 208(3): 149-159.

Uraguchi, S. and Fujiwara, T. (2012). Cadmium transport and tolerance in rice: perspectives for reducing grain cadmium accumulation. *Rice* 5(1): 5.

USEPA. 2012. Integrated Risk Information System (IRIS). Available at: www.epa.gov/IRIS.

USEPA. 2014. Integrated Risk Information System (IRIS). Available at: http://www2.epa.gov/lead/learn-about-lead

USEPA. 1994. Integrated Risk Information System (IRIS). Available at: www.epa.gov/superfund/lead/products/ch04.pdf

Vassilev, A., Vangronsveld, J. and Yordanov, I. (2002). Cadmium phytoextraction: present state, biological backgrounds and research needs. *Bulgarian Journal of Plant Physiology* 28(3–4): 68–95.

Verma, T.S. and Tripathi, B.R. (1983). Zinc and iron interaction in submerged paddy. *Plant Soil* 72:107–116.

Versantvoort, C.H.M., Oomen, A.G., Kamp, E.V.D., Rompelberg, C.J.M. and Sips, J.A.M. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology* 43: 31-40.

Versantvoort, C.H.M., Van, D.K.E. and Rompelberg, C.J.M. (2004). 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, The Netherlands.

Wang, H. S., Sthiannopkao, S., Chen, Z. J., Man, Y. B., Du, J., Xing, G. H., Kim, K. W., Yasin, M. S. M., Hashim, J. H., and Wong, M. H. (2013). Arsenic concentration in rice, fish, meat and vegetables in Cambodia: a preliminary risk assessment. *Environmental Geochemistry and Health* 35 (6): 745−755.

Wapnir, R. A. (1998). Copper absorption and bioavailability. *American Society for Clinical Nutrition* 67(suppl): 1054S–60S.

Welch, R.M., Graham, R.D., 2004. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany* 55: 353-364.

Williams PN, Villada A, Deacon C, Raab A, Figuerola J, Green AJ, Meharg AA (2007) Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environmental Science and Technology* 41:6854–6859.

World Health Organization (WHO). (2001). Environmental Health Criteria 224: Arsenic and Arsenic Compounds. Report number: Environmental Health Criteria 224.

Wragg, J. and Cave, M.R. (2002). In-vitro methods for the measurement of the oral bioaccessibility of selected metals and metalloids in soils: A Critical Review. R&D Technical Report, P5-062/TR/01.

Xu, J., Yang, L., Wang, Z., Dong, G., Huang, J. and Wang, Y. (2006). Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil. *Chemosphere* 62: 602-607.

Xu, X.Y., McGrath, S.P., Meharg, A. and Zhao, F.J. (2008). Growing rice aerobically markedly decreases arsenic accumulation. *Envrionmental Science and Technology* 42: 5574–5579.

Xu, J., Yang, S., Peng, S., Wei, Q. and Gao, X. (2013). Solubility and leaching risks of organic carbon in paddy soils as affected by irrigation managements. *The Scientific World Journal*. [http://dx.doi.org/10.1155/2013/546750].

Yafa, C. and Farmer, J.G. (2006). A comparative study of acid-extractable and total digestion methods for the determination of inorganic elements in peat material by inductively coupled plasma-optical emission spectrometry. *Analytica Chimica Acta* 557: 296–303.

Yamasaki, H., Pilon, M. and Shhikanai, T. (2008) How do plants respond to copper deficiency? *Plant Signaling and Behavior* 4:231–232.

Yang, J., Monine, M.I., Faeder, J.R. and Hlavacek, W.S. (2008). Kinetic Monte Carlo method for rule-based modeling of biochemical networks. *Physical Review E* 78: 1-7.

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

Yap, C.K., Krishnan, T. and Chew, W. (2011). Heavy Metal Concentration in Ceiling Fan Dusts Sampled at Schools Around Serdang Area, Selangor. *Sains Malaysiana* 40: 569-575.

Yap, D.W., Adezrian, J., Khairiah, J., Ismail, B.S. and Ahmad-Mahir. R. (2009). The uptake of heavy metals by paddy plants (Oryza sativa) in Kota Marudu, Sabah, Malaysia. *Journal of Agriculture and Environment Science* 6(1): 16-19.

Yin, S.A., Ismail, A. and Zulkifli, S.Z. (2012). Copper and zinc speciation in soils from paddy cultivation areas in Kelantan, Malaysia. *Acta Biologica Malaysiana* 1(1): 26-35.

Yongming, H., Peixuan, D., Junji, C. and Posmentier, E. S. (2006). Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Science of the Total Environment* 355: 176– 186.

Zarcinas, B.A., Ishak, C.H., McLaughlin, M.J. and Cozens, G. (2004a). Heavy metals in soils and crops in Southeast Asia. 1. Peninsular Malaysia. *Environmental Geochemistry and Health* 26: 343-357.

Zarcinas, B.A., Ishak, C.H., McLaughlin, M.J. and Cozens, G. (2004b). Heavy metals in soils and crops in Southeast Asia. 2. Thailand. *Environmental Geochemistry and Health* 26: 343-357.

Zhang, J.Z. (2009). The toxicity assessment of heavy metals and their species in rice. *University of Cincinnati.*

Zhang, W.J., Jiang, F.B. and Ou, J.F. (2011). Global pesticide consumption and pollution: with China as a focus. *Proceedings of the International Academy of Ecology and Environmental Sciences* 1:125–144.

Zhao, H., Xia, B., Fan, C., Zhao, P. and Shen, S. (2012). Human health risk from soil heavy metal contamination under different land uses. *Science of the Total Environmental* 418: 45–54.

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

Zheng, J., Chen, K., Yan, X., Chen, S.J., Hu, G.C., Peng, X.W., Yuan, J.G., Mai, B.X. and Yang, Z.Y. (2013). Heavy metals in food, house dust, and water from an e-waste recycling area in South China and the potential risk to human health. *Ecotoxicology and Environmental Safety* 96: 205–212.

Zhuang, P., McBride, M.B., Xia, H., Li, N. and Li, Z. (2009). Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of the Total Environment* 407(5): 1551-1561.

Zimmerman, A. J. and Weindorf, D. C. (2010). Heavy metal and trace metal analysis in soil by sequential extraction: A review of procedures. *International Journal of Analytical Chemistry* Article ID 387803: p 7.