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IN VITRO STUDIES ON BIOACCESSIBILE HEAVY METALS IN RICE

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ABSTRACT
The tendency to accumulate in biological systems makes heavy metals a significant health hazard. Rice consumption is one major pathway of heavy metal bioaccumulation in human, because of its ability to absorb toxic element from soil and water more effectively than any other crops. Studies related to toxic elements in rice have attracted a great concern worldwide involving paddy field and marketed rice samples. However nowadays the fractions of metals are eventually absorbed into the systemic circulation *i.e.* bioavailability (*in vivo* studies) are gaining greater attention. A total of ten rice samples were collected randomly from the market, cooked and analysed for total heavy metal content. The cooked rice samples were subjected to *in vitro* digestion to find out the bioaccessible metal concentration using AAS. Based on the inherent and bioaccessible metal content total hazard quotient was worked out. *In vitro* digestion *r*esults indicated that bioaccessible Pb ranged between 0.07 to 0.76 mg kg^{-1} and the per cent of bioaccessibility was between 1.57 to 11.28. The bioaccessible Ni content was between 1.19 to 2.53mg kg^{-1} in digested extract and the percentage ranges from 11.63 to 32.86. There were no earlier studies on the bioaccessibility of Ni in rice. Bioaccessible Cd and Cr was 0.14 to 1.12 mg kg^{-1}0.96 to 4.21 mg kg^{-1} in digested extract respectively. The inherent metal content was above the permissible limit in most cases, the bioaccessibility of the analysed metals were independent of their total content. The total hazard quotient data analysis showed, there was no evident risk heavy metals consumed through rice.

KEY WORDS: Heavy metals –Bioaccessibile- *In Vitro* digestion - Rice

INTRODUCTION

The long-term excessive application of agricultural inputs like fertilizers, pesticides and mulch has resulted in the heavy metal contamination of soils (Boyd, 2010, Arao *et al.,* 2010, Zhang *et al*., 2013). Crops grown in soils contaminated with heavy metals have a greater accumulation of heavy metals and many researchers emphasized the toxic effects of heavy metals to the human body. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissue (Das *et al.,* 1997 and Melamed *et al.,* 2003).Consumption of food crops contaminated with heavy metals is a major food chain route for human exposure (Sharma, 2006, Khan *et al*., 2008). Rice is one of the three most important food crops in the world and it is the staple food for over 2.7 billion people. Among the thousand varieties of rice around the world, more than 140,000 varieties of cultivated rice (*Oryza sativa*) are thought to exist but the exact number is not known. Nearly 162 varieties of rice marketed in Tamil Nadu (TNAU AgriTech Portal, 2018). Many researchers have reported on the presence of heavy metals in rice and the metals that commonly found in rice are iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr), cobalt (Co), copper (Cu), arsenic (As), and cadmium (Cd) (Al-Saleh and Shinwari, 2001, Mohamed and Ahmed, 2006, Jorhem *et al*., 2008 , Zhang, 2009, Yap *et al*., 2009, Khairiah *et al.*, 2013). Some researchers have revealed that rice contains higher heavy metal than other grains such as wheat and cereals (Salim *et al.,* 2010, Liu *et al*. 2011, Fahim *et al*., 2013), which forces the researchers to look for heavy metals in rice.

Bioaccessibility of Heavy metals

Many studies on rice focused on total heavy metal content. But the total heavy metal content analysis overestimates the bio concentration factor, whereas *in vitro* simulation studies helps to know the accessible fraction rather than total content. The *in vitro* simulation of the human digestive tract including stomach and small intestine has been realized, and the physiologically based extraction test (PBET) is one of the most commonly applied methods (Ruby 1996). Sun *et al.,* 2012 used *in vitro* gastrointestinal methods to estimate the bioaccessibility of heavy metals in various food sources, such as rice, vegetables, and seafood. Besides, some factors affecting the bioaccessible metals in the gastric and small intestinal phases, vegetable species, cooking, and the difference in fresh and dry samples also studied (Intawongse and Dean 2008).There are only few studies focusing on risk assessment of human exposure to bioaccessible heavy metals in cereals (Meena Kumari, and Kalpana 2017). 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 (Devesa *et al*. 2008). Morgan (1999) also stated that some rice can absorb heavy metal if contaminated water is used for cooking. Nevertheless, cooking was found to be a minor exposure route for heavy metal in rice (Mondal *et al*., 2010). Previous studies in India such as done by Singh *et al.* 2011 only focused on total heavy metal concentration. 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 (Chandorkar and Prachi Deota 2013). Hence the present investigation was taken to assess the Bioaccessible heavy metals in cooked rice in and around Coimbatore region with cooked rice.

MATERIALS AND METHODS

Determination of heavy metals

Convenience sampling was chosen as a sampling method in this study due to easy accessibility and availability at a given time (Etikan *et al*., 2016). A total of 10 rice samples were randomly purchased from local consumer products superstores in and around Thondamuthur, Coimbatore. All the rice samples are boiled and polished, which denotes the dehusked rice from which the bran layer has been removed during milling. The origin of the rice samples were taken from the package label.All rice samples were cooked based on method suggested by Salim *et al*. (2010). A total of 50g of raw rice sample from each sample rice variety was weighed and washed three times with deionized water, then cooked with 100 ml deionized (1:2 ratio) water until there was no water left. As the rice samples were cooked, all the samples were dried and powdered. One gram of the powdered rice was taken for heavy metal analysis

Bioaccessible heavy metals

Bioaccessible form of heavy metal is the fraction of metal concentrations present in a within a time and being takenup by organisms from environment via ingestion (Peijnenburg & Jager, 2003, Versantvoort *et al.,* 2005). Determination of bioaccessible form of trace element and heavy metal can be done via *in vitro* digestion models. To analyze the bioaccessible fraction of the heavy metal, the methods applied by Versantvoort *et al*. (2004) and Yang *et al*. (2012) were adopted. The RIVM model includes simulated digestive processes starting from the mouth, stomach, and finally to the small intestine. The chemicals and reagents used for artificial saliva, Gastric juice, and duodenal juice were of ultra-pure quality and obtained from SIGMA, MERCK, and ACS reagents to minimize the contribution of trace heavy metals from these chemicals.

Pb, Ni, Cd and Cr heavy metals were analyzed by Atomic Absorption Spectrophotometer (M/s. VARIAN, AA240) in the Department of Environmental Sciences, TNAU by using appropriate instrumental parameters (table 1). The concentrations of metals were measured in with air acetylene flame using Nitrous oxide as flame support. The elements Pb, Ni, Cd and Cr were calibrated by standard solution using calibration points (0,2,4,6,8,10 ppm). Five concentration of the calibrant were analysed until a linear curve was achieved with an R^2 value of 0.9999. After calibration, sample of known concentration was checked to obtain reproducible results. All the statistical analysis was carried out using microsoft excel and SPSS 16 for windows statistical package. Arithmetical means and standard deviation were used to assess the contamination level in rice. The bio accessibility percentage was calculated by using the formula given by Meena Kumari and Kalpana (2017).

Bioaccessibility was calculated by using the formula

$$\text{Bioaccessibility (\%)} = 100 \times Y / Z$$

Where Y is the metal content in the bioaccessible fraction (mg of metal/100g sample) and Z is the total content of the particular metal (mg of metal/100g sample).

Total hazard quotient (THQ) was calculated using the formula given by USEPA (2007).

$$THQ = \frac{EF_r \times ED \times FI \times MC \times 10^{-3}}{R_f D_o \times BW \times AT}$$

THQ- Total hazard Quotient; Efr – Exposure Frequency (365 days/yr); ED- Exposure Duration – 65 years; FI- Food ingestion per day (0.208 kg/person/day); MC–Metal concentration in food (mg/kg of FW basis); BW- Average body weight (53 kg of Indian man) ; AT- Averaging time for carcinogens; R_fD_0 – Oral reference dose. Oral reference doses were 4E-03, 1E-03, 5E-03 for Pb, Cd,Cr and Ni(Dang *et al,* 1996, USEPA, 1997, US EPA 2011 and US EPA, 2015).

TABLE 1. Instrumental Parameters during elemental quntification in AAS

S.No	Name of the Metal	Wave Length (nm)	Slit Width (nm)	Flame System	Sensitivity (µg/mL)
1	Pb	217.0	0.5	Air-Acetylene	0.06
2	Cd	228.8	0.5	Air-Acetylene	0.01
3	Cr	357.9	0.2	Air-Acetylene	0.05
4	Ni	232.0	0.2	Air-Acetylene	0.01

RESULTS AND DISCUSSION

Rice grown in Asian countries constitutes more than 90 % of the global production. Many industrial process leads to increased heavy metal contamination such as cadmium (Cd), mercury (Hg) and lead (Pb) in soil, water and air. Fu *et al* 2008 reported the presence of Pb in polished rice to the tune of 0.69mg/kg, at the same time Huang *et al* 2009 reported 0.957 mg kg^{-1} in rice. Zhao *et al* 2010 also observed the heavy metals in rice. Undoubtedly, to the general population, the dietary intake is the main exposure pathway. So wherever rice is a staple food, therefore reasonable to hypothesize that rice have the potential health risk to consumers. The estimated rice intakes of adult (18 years old or over) and children (7–18 years old) were 342.90 g per day per person and 258.43 g/day per person (ZJFDA,2008).

TABLE 2: Bioaccessible Pb , Ni,Cd and Cr in rice grains

Sample No	Sample location	Lead(Pb)		Nickel(Ni)		Cadmium (Cd)		Chromium(Cr)	
		THQ (DE)	Bioaccessibility (%)	THQ (DE)	Bioaccessibility (%)	THQ (DE)	Bioaccessibility (%)	THQ (DE)	Bioaccessibility (%)
S1	Perurchettipalayam	7.46×10^{-4}	9.27	3.45×10^{-3}	20.04	1.256×10^{-3}	28.57	6.44×10^{-4}	30.71
S2	Pachapalayam	1.86×10^{-4}	4.87	2.33×10^{-3}	25.32	5.49×10^{-4}	12.07	9.73×10^{-4}	12.65
S3	Theetheepalayam	1.08×10^{-4}	1.57	2.17×10^{-3}	12.76	3.297×10^{-3}	35.59	1.287×10^{-3}	16.79
S4	Kalampalayam	2.65×10^{-4}	6.75	4.45×10^{-3}	21.22	1.648×10^{-3}	22.58	7.54×10^{-4}	15.84
S5	Madampatti	5.2×10^{-4}	2.50	4.96×10^{-3}	32.44	4.395×10^{-3}	50.91	2.104×10^{-3}	26.92
S6	Kuppanur	3.73×10^{-4}	3.76	2.70×10^{-3}	32.86	2.826×10^{-3}	27.48	2.111×10^{-3}	27.12
S7	Thenkarai	5.2×10^{-4}	11.28	4.96×10^{-3}	27.50	3.689×10^{-3}	45.85	2.684×10^{-3}	32.39
S8	Chennanurthenkarai	1.86×10^{-4}	3.86	2.33×10^{-3}	16.08	5.49×10^{-4}	18.67	1.35×10^{-3}	37.72
S18	Devarayapuram	6.87×10^{-5}	0.70	4.06×10^{-3}	11.63	1.491×10^{-3}	20.43	2.268×10^{-3}	77.69
S19	Parameshwaranpalayam	1.08×10^{-4}	0.90	4.19×10^{-3}	20.58	3.454×10^{-3}	43.78	3.304×10^{-3}	40.13

Bioaccessibile heavy metals in rice grains

In vitro digestion *r*esults indicated that bioaccessible Pb in rice ranged between 0.07 to 0.76 mg kg^{-1} and the per cent of bioaccessibility was between 1.57 to 11.28. Samples showed significant variations in the bioaccessible lead content among the samples, which was reflected in the accessible % as well as in the total hazard quotient. Physical and chemical state of the lead, age and physiological status of the person decides the absorption. Nearly 20-30% of Pb in adult is absorbed through gastro-intestinal tract. Organic lead absorbed more than inorganic lead (Jarup, 2003).The bioaccessible Ni content was between 1.19 to 2.53 mg kg^{-1} in digested extract and the percentage ranges from 11.63 to 32.86. There were no earlier studies on the bioaccessibility of Ni in rice. Bioaccessible Cd was 0.14 to 1.12 mg kg^{-1} in digested extract. Omar *et al* 2015 reported Bioavailable Cd concentration was the lowest in all cooked rice samples and showed bioaccessible heavy metal concentrations were decreased from Zn > Fe > Cu > Cr > Cd. Bioaccessible Cr was ranged between 13.20 to 58.27% and digested extract had 0.96 to 4.21 ppm. Omar *et al*., 2015 reported very low Cr concentration in cooked rice was 0.13 mg kg^{-1}unlikein the present study (table 2).

The observed variation in metal concentrations for analyzed foodstuffs might be due to variable capabilities of absorption and accumulation of metals by the crops (MeenaKumari and Kalpana 2017). Bioaccessibility of Cr varied significantly among the rice grains. Cabrera-Vique and Bouzas (2009) study have reported that Cr absorption was higher for low levels of daily dietary intake (>40 µg) than for levels of 40–80 µg; for high levels (>80 µg) there was an increase in the dialyzable fraction. Anderson and Minerals (2000) have reported that iron deficiency stimulates the net absorption and bioavailability of Cr, which could be due to competition between both minerals at the same intestine absorption sites.

The minimum and maximum bioaccessibility in cadmium was in the range of 12.07 to 45.85%.These observations were similar to previous reports (Yang *et al*. 2012; Hu *et al.* 2013). Most of the Cd was accumulated in the cell vacuoles of the plants, hence, amounts of Cd are easily released from plant tissues during *in vitro* digestion and during the gastric phase, enzymes like pepsin and low pH releases most of the Cd. (Zhuang *et al*., 2016). However, increase in pH (from 1.5 in the gastric phase to 7.0 in the gastrointestinal phase) as well as addition of pancreatin and bile extracts might bring precipitation and/or restoration of portion of the solubilized Cd, thus reduces bioaccessible Cd during this phase. Heavy metals may accumulate in different sub-cellular components, which may affect their abilities of releasing from the organism in the gastric and intestinal solution (Fu and Cui 2013).

Target hazard Quotient (THQ)

A hazard quotient is a dimensionless index of risk associated with long term exposure to chemicals. It is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected. No adverse health effects are noticed as a result of exposure, if the Hazard Quotient is calculated to be less than 1. In the present study the Hazard Quotient (HQ) values for bioavailability of heavy metal studied were less than 1 for adult indicating that there were no any non-carcinogenic health risks present. Chandorkar and Prachi Deota 2013 reported the target hazard quotient for Cd was 15 and lead 78.6, but Omar et al, 2015 reported very low HQ value *i.e*. below one. Singh *et al* 2014 reported THQ of Cd (5.93 x 10^{-3}) , Pb (2.17 x 10^{-3}) , Cr (2.5 x 10^{-4}). He also reported that there was no evident risk heavy metals consumed through rice. In another study it was reported that 9.15 for Cd and 1.32 for Ni in rice (Sharma et al 2010). Liu et al 2011 reported the THQ less than 1 through rice consumption. Praveena and Omar, 2017 reported total concentration of Cr, Cd and Pb in rice was 2.7 mg/kg, 0.16mg/kg and 0.11mg/kg respectively, on the other hand, mean of bioaccessible heavy metal concentrations were decreased to 0.11 mg/kg, 0.027mg/kg and 0.022 mg/kg respectively. The highest HQ values for Cd was 0.47, 0.0008 was for Cr and 0.051 for lead (Praveena and Omar,2017).

Rice has ability to absorb toxic element from soil and water in paddy field more effectively than other crops. This is due to paddy plants is grown in water flooded conditions which permit toxic elements to be taken up by its root and accumulated in rice (Huang *et al*., 2016). Anaerobic condition in paddy field soil has resulted in higher mobilization rates and increase bioaccessible form of toxic elements in rice. Since toxic elements have the ability to accumulate and non biodegrable, the presence of toxic element concentrations in marketed rice is largely unavoidable (Morekian *et al.* 2013).

Total concentration and bioaccessible Cr were higher in rice grains compared than all other heavy metals. The bioaccessible of Cr may increase with acidic solutions in the stomach and absorbed throughout the small intestine. However, health risks should be further examined before a final decision can be made. This study has opened a wide field of estimation on human health risks from trace element and heavy metal contaminated in raw and cooked rice by using in vitro digestion model.Non intensive rice cultivation with small scale which can lead to discrepancy of quality of rice.

CONCLUSION

The present study has generated data on heavy metal concentration in rice samples that were collected in and around Coimbatore city. The study showed that the bio accessible heavy metals were within the maximum permissible limits in rice. Consumption of foodstuff with elevated levels of heavy metals for long term may lead to health disorders. It is therefore suggested that regular monitoring of heavy metals is essential in order to prevent excessive build-up of these metals in the human food chain and health risks should be further examined before a final decision can be made. This study has opened a view on human health risks from trace element and heavy metal contamination in rice by using an *in vitro* digestion model.

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