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BIOAVAILABLE FORMS OF HEAVY METALS FROM RICE SAMPLES AND ITS POTENTIAL HEALTH RISK ASSESSMENT

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ABSTRACT

Food crops grown in contaminated soils have a greater accumulation of heavy metals and the consumption of food crops grown in the contaminated soils are the source of metals that enters into the human body. Rice being a major food crop, the presence of heavy metals should be monitored regularly for reducing health risk. The analysis of total heavy metal always overestimates the content which leads to misinterpretation of results; however, bioaccessible heavy metal analysis projects the actual health risk. Hence, the present study aims to assess the bioavailable form of heavy metals in rice. The rice samples were collected from 20 different places and used for the inherent and bioavailable metal estimation. *In vitro* simulated digestion method was applied for bioaccessible metal analysis. Metal concentration in polished rice ranged from 0.10 to 0.82, 0.10 to 1.07, 0.11 to 0.56 and 0.23 to 1.09 mg kg^{-1} for Lead (Pb), Nickel (Ni), Cadmium (Cd) and Chromium (Cr), respectively. Twenty five percent of the samples recorded less than 0.028, 0.01, 0.01, and 0.03 mg kg^{-1} of bioaccessible Pb, Ni, Cd, and Cr, respectively. A significant negative correlation was observed between total metal concentration and bioaccessibility percentage. Targeted Hazard Quotient (THQ) of all the metals were less than one for adults indicating that there were no health risks, which undoubtedly reveals the importance of bioaccessible metal analysis. Hence, regular monitoring of heavy metals is essential to reduce the intensive accumulation in the human food chain. Also, the present study has opened up a wide scope on human health risk assessment using an *in vitro* digestion model.

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1 Introduction

Heavy metals are the major contaminants causing serious health risks. There are many sources through which heavy metals are entering the human body and dietary accumulation via food crops often been reported by many researchers (Chaoua et al., 2019; Ghuniem et al., 2020). Investigation of metal accumulation in food grains is crucial, especially in rice. Since, rice can absorb metals from the soil than any other crops (Khairiah et al., 2013) due to its prevailing anaerobic conditions which favors the metals mobilization from roots to economic part (Huang et al., 2016).

The most preferable form by the people and easily available in the market is polished rice. for consumption which also contains heavy metals. Naseri et al. (2018) reported Ni concentrations of 0.49 ± 0.51 µg g^{-1} in the polished rice. Sen Gupta et al. (2016) observed polished Tamil Nadu Ponni rice, Karnataka Ponni, IR 20, Basmati rice, and reported purchased from the market showed 324.6, 245.3, 243.0, 309.5 ppm of Cr respectively. Similarly, many researchers reported on the presence of heavy metals in rice. Unlike raw rice, polished rice, polished rice is milled to remove the husk, bran, germ, and nutrients to some extent while processing, many researchers observed the presence of metals in the polished rice (Jorhem et al., 2008; Hang et al., 2009; Bhattacharya et al., 2010; Ihedioha et al., 2016; Cao et al., 2017).

The major drawback associated with total heavy metal estimation is an overestimation of the bioconcentration factor that leads to misinterpretation of actual human health risks. To fulfill the above lacuna, *in vitro* simulation studies used which estimates the fraction of metals that are available for absorption in the human gastrointestinal environment. The reliability of such studies was evident based on *In vivo-in vitro* correlation studies done by Juhasz et al. (2006) who confirmed the validity of *in vitro* methods for predicting relative bioavailability (RBA) of metals (arsenic and mercury) in foods. The validity of *in vitro* simulation of the human digestive tract including the stomach and small intestine were also confirmed by many researchers (Ruby et al., 1996; Sandberg, 2005; Intawongse & Dean, 2008; Sun et al., 2012).

The risk evaluation study carried out from marketed consumable rice samples would depict precise results compared to rice obtained from the field (Devesa et al., 2008). Chandorkar & Deota (2013) investigated the bioavailability of heavy metals in rice varieties available in the local supermarket. As apparent from number of previous studies, it is clear that in rice grain, total heavy metal content was explored rather than a bioaccessible fraction. Kumari & Kalpana (2017) studied human health risk assessment by focusing on bioaccessible heavy metals in cereals. However the studies on the bioaccessible form of heavy metal are very limited, the present investigation was carried out to give a clear view of exposure risks to humans by determining the bioaccessible heavy metals in rice.

2 Materials and methods

Twenty rice samples (20 nos) were randomly purchased from local consumer superstores in and around Thondamuthur, Coimbatore (Table 1).

Table 1 Rice samples collected locations around Thondamuthur, Coimbatore

Sample no.	Name of the Place
S1	Perur Chettipalayam
S2	Pachapalayam
S3	Theetheepalayam
S4	Kalampalayam
S5	Madampatti
S6	Kuppanur
S7	Thenkarai
S8	Chennanur Thenkarai
S9	Ikkarai Poluvampatti
S10	Nathegoundenpalayam
S11	Alandurai
S12	Madwarayapuram
S13	Booluvampatti
S14	Jahirnaickenpalayam
S15	Narashipuram
S16	Vellimalaipattinam
S17	Pullagoundenpalayam
S18	Devarayapuram
S19	Parameswaranpalayam
S20	Thennamanallur

Tamil Nadu Pooni was chosen for the study based on consumer preference and the origin of the rice samples were taken from the package label. Among the samples, 35% were from Villupuram origin. A total of 50 g of polished rice from each sample was weighed and washed three times with deionized water, then cooked with 100 ml of deionized (1:2 ratio) water. The cooked samples were dried, powdered and five gram of powdered samples were taken for analysis. Convenience sampling was chosen as the sampling method for the present study for easy accessibility and availability (Etikan et al., 2016).

Determination of bioaccessible form of heavy metal was done via *in vitro* digestion using RIVM (Rijksinstituut voor

Volksgezondheid en Milieu) model (Versantvoort et al., 2005) as it mimics the human physiological condition includes simulated digestive processes starting from the mouth, stomach, and finally to the small intestine (Yang et al., 2012). The chemicals and reagents used for artificial saliva, gastric juice, and duodenal juice were obtained from SIGMA, MERCK, and ACS reagents to minimize the contribution of trace heavy metals from these chemicals. The simulated digestive process was initiated by adding 6.6 ml of artificial saliva to 5 g of cooked rice sample to represent the digestion process in the mouth. The mixture was then incubated for 5 min, and the pH was adjusted to 6.8. Afterward, 13.8 ml of artificial gastric juice was added, and the mixture was shaken for 2 h to represent the digestion process in the stomach. The pH of the gastric juice was adjusted between 2.0 and 3.0. Finally, 13.8 ml of duodenal juice, 6.7 ml of bile juice, 2.2 ml of $NaHCO_3$ solution (1 mol/L) were added simultaneously to represent the digestion process in the small intestine. Subsequently, the mixture was shaken for another 2 h and the pH was adjusted from 6.5 to 7.0 and incubated at 37±2 ^{0}C by using a continuous orbital shaker (55 rpm). In each step of the digestion processes in the mouth, stomach, and small intestine shaking was given for a certain period. The mixture was centrifuged at 2750 rpm for 10 min and the supernatant was collected and filtered through filter paper with a pores size of 0.45 µm and used for analysis (Versantvoort et al., 2005; Yang et al., 2012).

The heavy metals, Pb, Ni, Cd, and Cr were estimated using Atomic Absorption Spectrophotometer (Varian AA240). These heavy metals were calibrated by a standard solution using calibration points (0, 2, 4, 6, 8,10 ppm) and a linear curve was obtained with an R^2 value of 0.9999 (Sunitha et al., 2015). The bioaccessibility percentage was calculated by using the formula of Kumari & Kalpana, (2017) as mentioned below

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

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).

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

Where, THQ- Total hazard Quotient; EF_r – Exposure Frequency (365 days yr^{-1}); ED- Exposure Duration – 65 years; FI- Food ingestion per day (0.208 kg day^{-1} $person^{-1}$); MC – Metal concentration in food (mg kg^{-1} 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, respectively (Dang et al., 1996; USEPA 1997; USEPA 2020).

Pearson correlation analysis was carried out between the total metal content and the bioaccessible percentage. Principle component analysis (PCA) was performed using all observed variables. All the statistical analysis was carried out using SPSS 16 (version 16.0.0). *P*-value less than 0.05 ($P < 0.05$) is considered as significant. The graphs were plotted using OriginPro 2019 (version 9.6.5).

3 Results and discussion

Food crops contaminated with heavy metal via dietary intake undoubtedly are the entry route for human exposure. Among the food crops, rice constitutes more than 90% of the global cereal production and it supplies nearly half of the daily calories of the world population (Abbas et al., 2011; Ray et al., 2019). Heavy metal elements that commonly found in rice are iron (Fe), zinc (Zn), manganese (Mn), chromium (Cr), cobalt (Co), copper (Cu), arsenic (As), and cadmium (Cd) (Khairiah et al., 2013; Ghuniem et al., 2020). Praveena & Omar (2017) reported total concentration of Cr, Cd, and Pb in rice was 2.7, 0.16, and 0.11 mg kg^{-1} respectively. The presence of toxic elements in marketed rice is unavoidable due to its ability of accumulation and non-biodegradable nature (Morekian et al., 2013; Huang et al., 2016; Khanam et al., 2020). Several studies in India have reported that a high accumulation of heavy metals was noticed in cereal crops as compared to other crops (Bhattacharya et al., 2010; Singh et al., 2011; Satpathy et al., 2014).

3.1 Total heavy metals

In the present study, the inherent lead (Pb) content of the samples varied from 0.10 to 0.82 mg kg^1 and 50% of the samples had a metal concentration above the maximum permissible limit (0.2 mg kg^{-1}) given by WHO (2004). Lead content varied from sample to sample due to many factors and very low lead content (0.003 to 0.46 mg kg^{-1}) was reported by Singh et al. (2014); whereas Ihedioha et al. (2016) reported 3.99 ± 1.43 mg kg^{-1} of Pb in polished rice.

The studied sample contains Nickel (Ni) in the range of 0.10 to 1.07 mg kg^{-1} and about half of the samples showed metal content above the permissible limit. Ihedioha et al. (2016) reported a higher level of Ni (3.12 ± 1.49 mg kg^{-1}) in raw rice as compared to the present study. Long-term application of wastewater for irrigation has shown to increase heavy metals contamination in cultivated crops which would lead to several health hazards (Rai & Tripathi 2008). Cadmium (Cd) content varied from 0.11 to 0.56 mg kg^{-1} in polished rice and each sample differed significantly from each other and this may be due to the variations in the origin of the

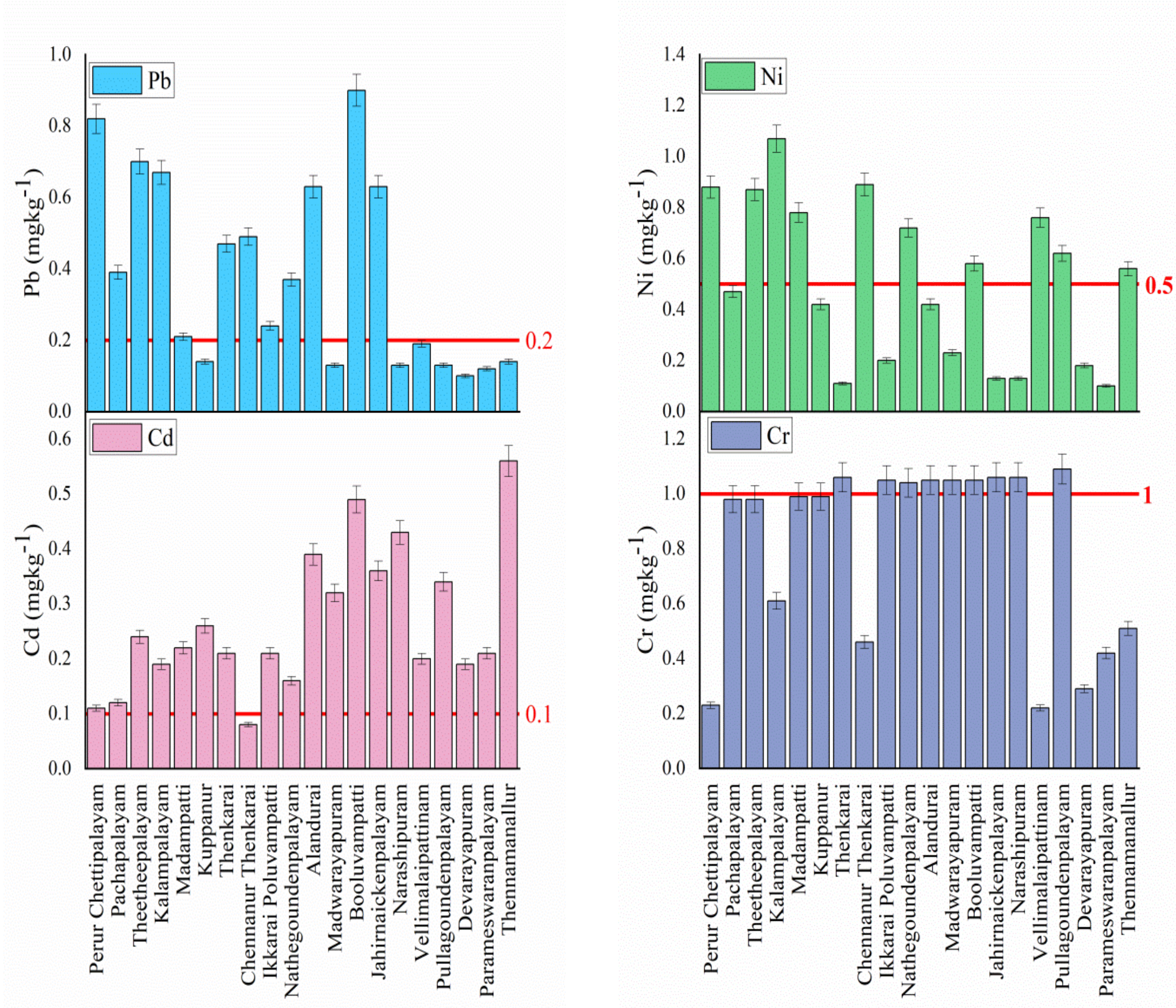

Figure 1 Total metal concentration in rice samples (Straight red line indicates WHO(2004) standards for respective heavy metals)

samples. Chandorkar & Deota (2013) (1.67 mg kg^{-1}), Singh et al. (2014) (0.25 mg kg^{-1}) and Ihedioha et al. (2016) (1.10 ± 0.53 mg kg^{-1}) also reported Cd in polished rice.

Chromium (Cr) content varied from 0.23 to 1.09 mg kg^{-1}, in polished rice. Singh et al. (2014) (0.07 mg kg^{-1}) and Solidum et al. (2012) (6.3 x 10^{-6} to 6.5 x10^{-6} mg kg^{-1}) also reported Cr in rice which was quite low when compared to the present study. Omar et al. (2015) reported very low Cr concentration in cooked rice was 0.13 mg kg^{-1} unlike in the present study. Sen Gupta et al. (2016) observed 324.6 mg kg^{-1} of Cr in Tamil Nadu Ponni rice purchased from the market. Praveena & Omar (2017) reported total concentration of Cr, Cd, and Pb in rice was 2.7, 0.16, and 0.11 mg kg^{-1}, respectively. The total metal content of rice samples was depicted in Figure 1.

3.2 Bioaccessible heavy metals

Health risks caused by heavy metal contaminated food materials are well known, however, only a limited extent was used to assess the health risks (Versantvoort et al., 2005). The boxplot of the 20 sampling points with bioaccessible metals were displayed in Figure 2. The 75% of sampling points recorded the bioaccessible Pb, Ni, Cd, and Cr less than 0.088, 0.033, 0.073, and 0.08 mg kg^{1} respectively. Bioaccessible Pb in rice ranged between 0.01 to 0.22 mg kg^{-1} with the median of 0.05 mg kg^{-1}. Samples showed significant variations in the bioaccessible lead content, which was reflected in the accessible percent (1.43 to 59.46%). In the present study, there was a reduction in lead content in bioaccessible fraction, when compared to lead content in polished rice (from 0.01 to 0.22 to 0.1 to 0.82 mg kg^{-1}). The same observation was

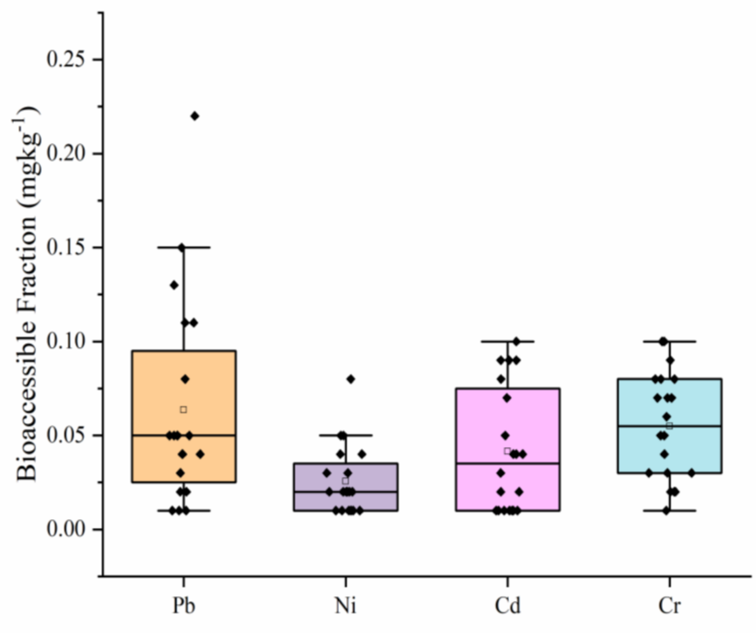

Figure 2 The boxplots of samples representing bioaccessible Pb, Ni, Cd and Cr.

made by Praveena & Omar (2017), who reported total concentration of Cr, Cd, and Pb in rice was 2.7 mg kg^{-1}, 0.16 mg $kg^{-1,}$ and 0.11 mg kg^{-1} respectively, on the other hand, the mean of bioaccessible heavy metal concentrations were decreased to 0.11 mg kg^{-1}, 0.027 mg kg^{-1} and 0.022 mg kg^{-1}, respectively. The long upper whisker in bioaccessible Pb denoted that bioaccessible fractions are varied higher among the greater values and very similar for lower values.

The bioaccessible Ni content was between 0.01 to 0.08 mg kg^{-1} with the median of 0.02 mg kg^{-1} and the bioccessible percentage ranged from 1.12 to 38.46. So far no studies on the bioaccessibility of Ni were reported in rice. Bioaccessible Cd ranged from 0.01 to 0.09 mg kg^{-1} with the median of 0.035 mg kg^{-1} and the percent of bioaccessibility was between 2.33 to 42.86. Omar et al. (2015) reported bioavailable Cd concentration was the lowest as compared to other metals in all cooked rice samples. Cadmium can be easily released from plants during the gastric phase of *in vitro* digestion process due to the presence of the pepsin enzyme and low pH (Zhuang et al., 2016). Also, pancreatic and bile extracts might precipitate most of the soluble Cd may be the result in decreased bioaccessible Cd. Bioaccessible Cr ranged between 0.01 to 0.10 mg kg^{-1} with the median of 0.055 mg kg^{-1} and the percent of bioaccessibility was 1.02 to 45.45. The box plot of bioaccessible Cr indicating that data sets are symmetric or normal distribution while bioaccessible Pb, Ni, and Cd are skewed towards the right indicating that the distribution is positively skewed. There are outliers only in bioaccessible Pb and Ni (Figure 2).

Invariably all the chosen metals showed a reduction in the bioaccessible fraction of heavy metal compared to total metals in the polished rice. Praveena & Omar (2017) also reported the same and this may be due to the process adopted while cooking, digestion process, pH of the simulated condition, enzymes, etc.

3.3 Correlation

Correlation analysis was done to understand the relationship between metals in the polished rice and bioaccessible fraction (Figure 3). The present study reveals a significantly negative correlation which was observed among total heavy metals concentration and bioaccessibility in rice. Among the tested metals, Nickel and Chromium showed moderate negative correlation with bioaccessibility percentage (Figure 3b,d; r = - 0.669*** and r = - 0.668***). A significant weak negative correlation between lead and its bioaccessibility was noticed (Figure 3a; r = - 0.479*) whereas cadmium showed no significant weak negative correlation (Figure 3c). The present study revealed that the bioaccessibility of the analysed metals were independent of their total content. The observed variation in metal concentrations for analyzed foodstuffs might be due to variable capabilities of absorption and accumulation of metals by the crops (Kumari & Kalpana 2017).

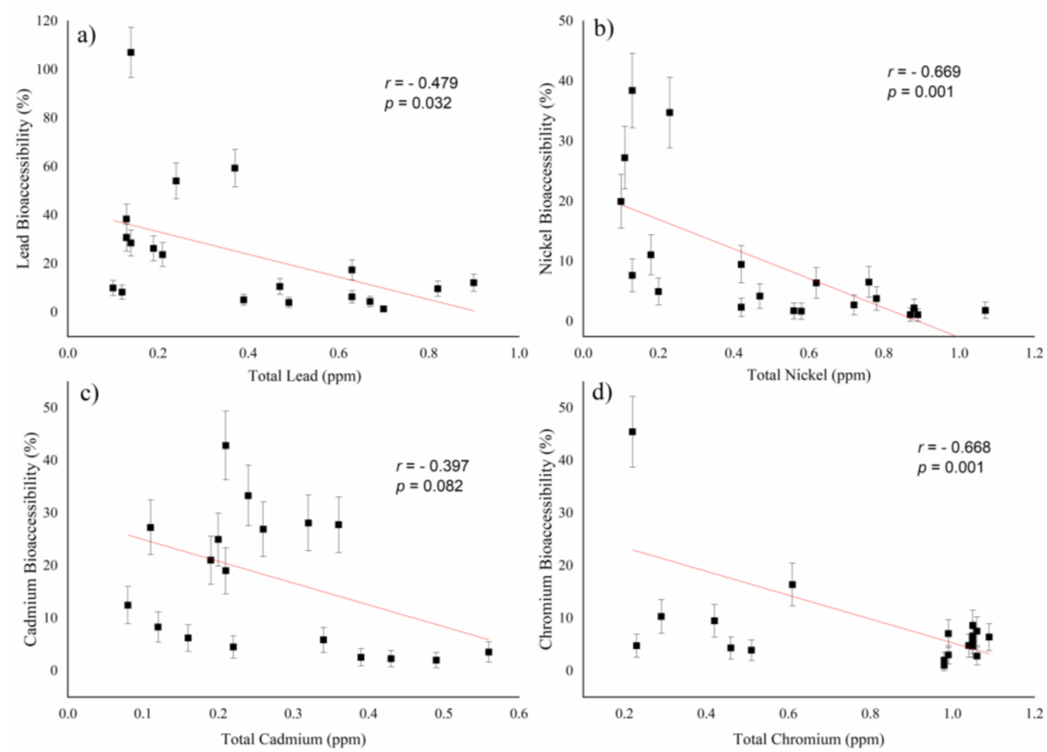

Figure 3 The correlation between total heavy metals concentration and bioaccessibility percentage in rice

3.4 Target hazard quotient (THQ)

Hazard quotient is an index of risk associated with long term exposure to a pollutant. It is the ratio of the potential exposure to a pollutant and the level at which no adverse effects are expected (USEPA, 2011). In the present study, few samples exceeded the total metal concentration above the permissible limit, however, THQ values were less than 1 indicating non-carcinogenic health risks (Figure 4). The target hazard quotient ranged from 1.18 to 8.83 x 10^{-4} for lead, 1.1 to 92.2 x 10^{-4} for nickel, 0.10 to 9.42 x 10^{-4} for cadmium, and 1.73 to 8.56 x 10^{-4} for chromium.

Many scientists have reported varying levels of THQ for different metals (Singh et al., 2014; Praveena & Omar 2017). Similarly, Chandorkar & Deota (2013) reported the THQ for Cd was 15 and Pd 78.6. Also, 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}) were showed no evidence of health risk.

3.5 Principle component analysis (PCA)

The Principle component analysis plot explained 49.10% of the variation pattern. The total metal concentration and total hazard quotient of Cd and Cr (Cd_T, Cr_T Cd_THQ, and Cr_THQ) and Ni bioaccessibility percentage (Ni-BAP) were major contributors to PC1. Whereas, Pb bioaccessible fraction (Ni-BF) was a major contributor to PC2. The two factors were well separated the Cr and Cd contaminated sampling locations in one group (green ellipse) and another group (pink ellipse) with Ni and Pd contaminated sampling locations. Based on studied variables, the sampling locations lies in positive quadrates of PC1 and PC2 denoted the high health risk while samples positioned in negative quadrates were associated with a low level of health risk. The rice sampling locations scattered in low health risk quadrants were mostly originated from Gobichettipalayam and in high health risk quadrants were obtained from Thanjavur, Villupuram, and Thiruvannamalai (Figure 5).

3.6 Source of contamination

This study has opened a wide scope for assessing the actual health risk in rice by using *in vitro* digestion model. Even though the origin of the samples were identified by the label, it may not be possible to conclude the source of heavy metals contamination. Further, there are few reasons by which the metals would have reached the rice grains. There may be few reasons by which metal would have reached the rice grains via cultivated soil, irrigation water, processing, handling, and cooking. There are evidences that the crops cultivated in heavy metal contaminated soils showed the highest accumulation than those cultivated in uncontaminated soils (Arao et al., 2010; Boyd, 2010; Zhang et al.. 2013).

To figure out the reasons associated with the presence of metal residues, a systematic investigation was carried out in the contaminated site. The site chosen for the study was a tannery contaminated site at Vellore, Tamil Nadu. The delineated 'hot-spots' of chromium contamination in the Vellore district identified by our previous workers (Sunitha et al., 2015) where six taluks (Walajahpet, Arcot, Vellore, Tirupathur, Vaniyambadi, and Gudiyatham) were selected for present study. From each spot, four rice growing farmers field were identified for sample collection (soil, water and rice grain).

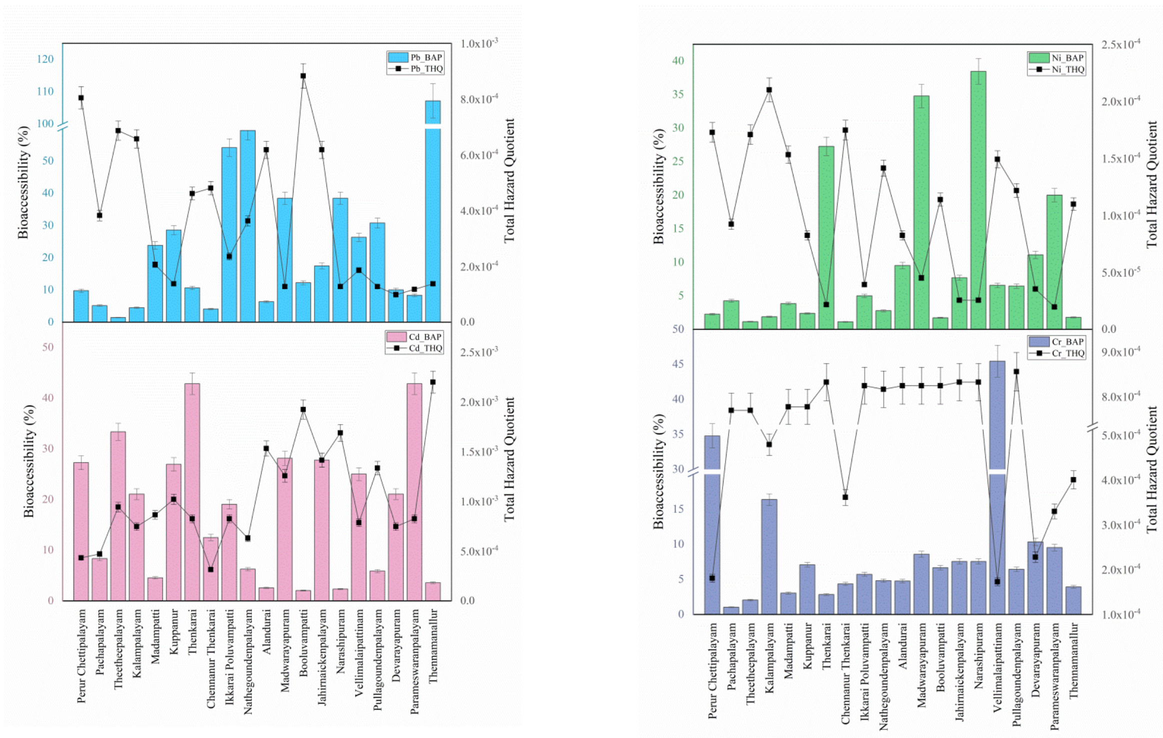

Figure 4 The bioaccessibility percentage and target hazard quotient of in rice samples collected from various locations

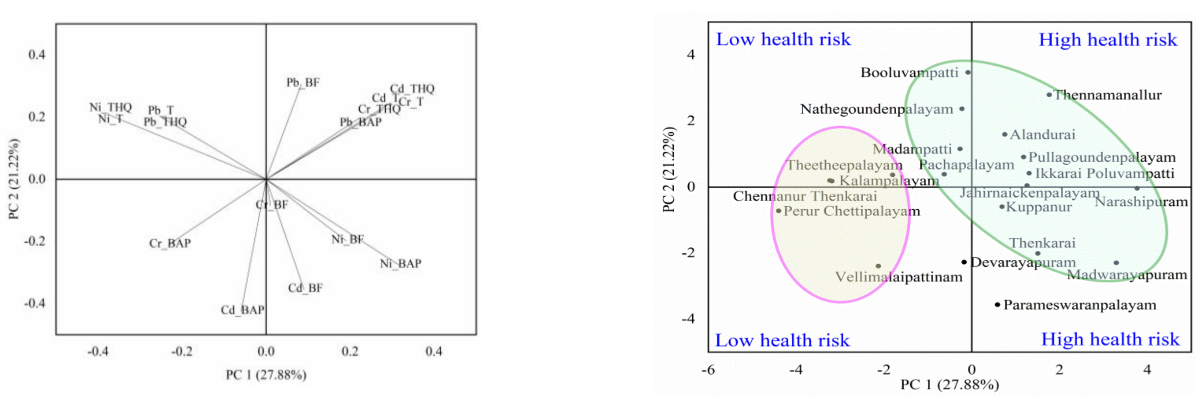

Figure 5 Biplot of PC1 and PC2 for heavy metal contaminated rice collected from various location (Where T is total metal concentration, BF is bioaccessible fraction, BAP is bioaccessibility percentage, THQ is total hazard quotient)

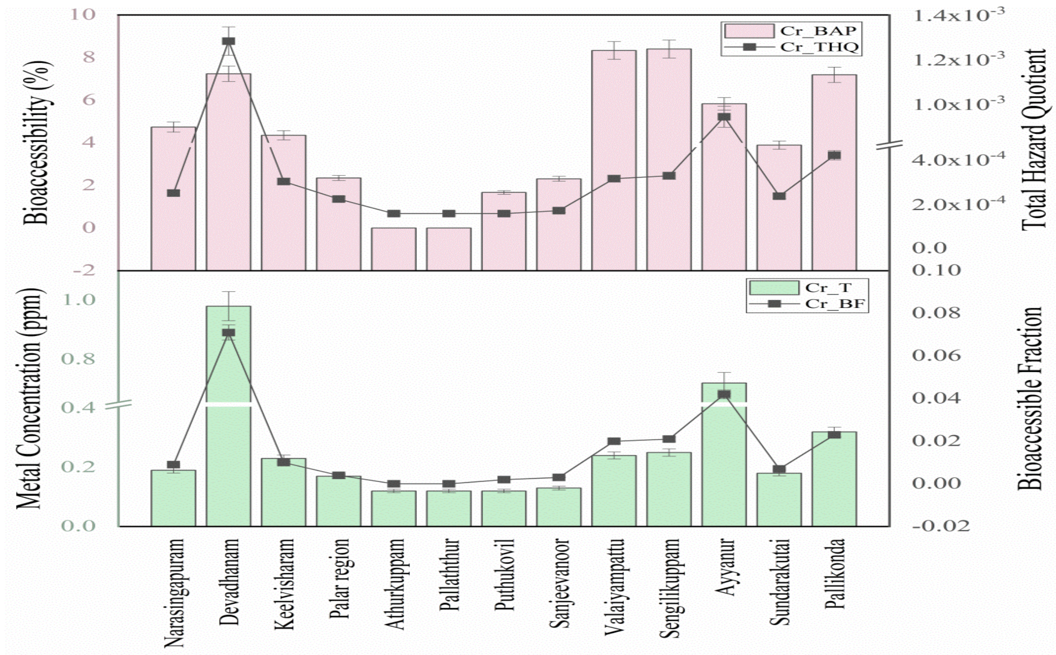

Figure 6 Chromium contamination in rice collected from various sites of Vellore district

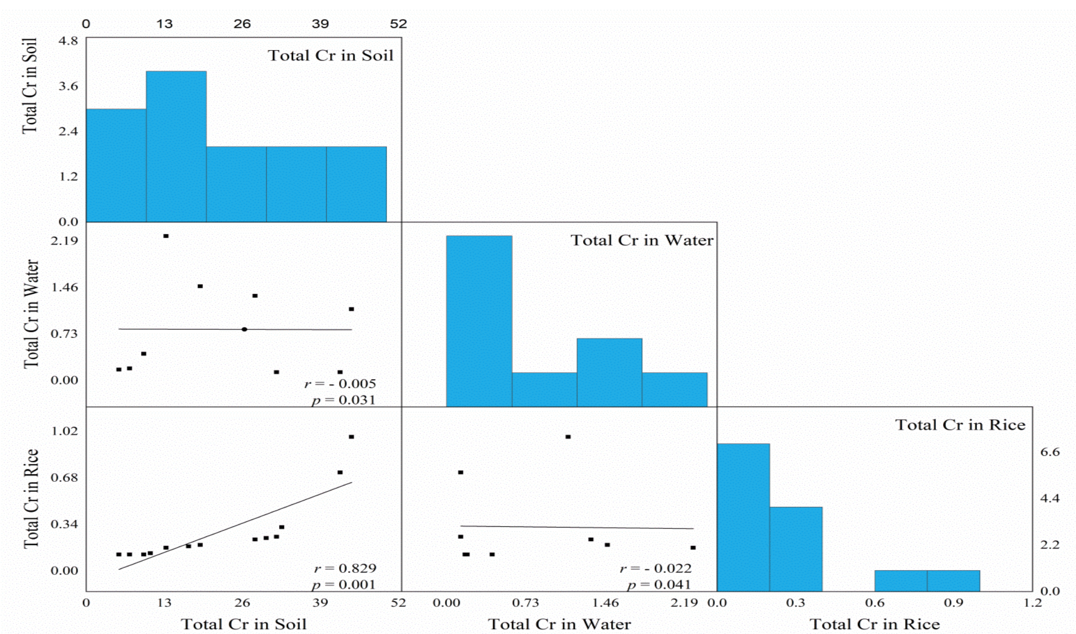

Figure 7 Scatter matrix of total chromium content in soil, water and rice grain.

The maximum chromium content in soil was registered in Devadhanam (44.16 mg kg^{-1}) followed by Ayyanur (42.29 mg kg^{-1}) and Pallikonda (32.57 mg kg^{-1}) while the minimum was recorded in Athurkuppam (5.45 mg kg^{-1}). The chromium content in the water sample recorded maximum in Palar region (2.27 mg L^{-1}) followed by Narasingapuram (1.48 mg L^{-1}) and Keelvisharam (1.33 mg L^{-1}) whereas samplings from Sanjeevanoor, Valaiyampattu, Sundarakutai, and Pallikonda were registered below the detectable limit.

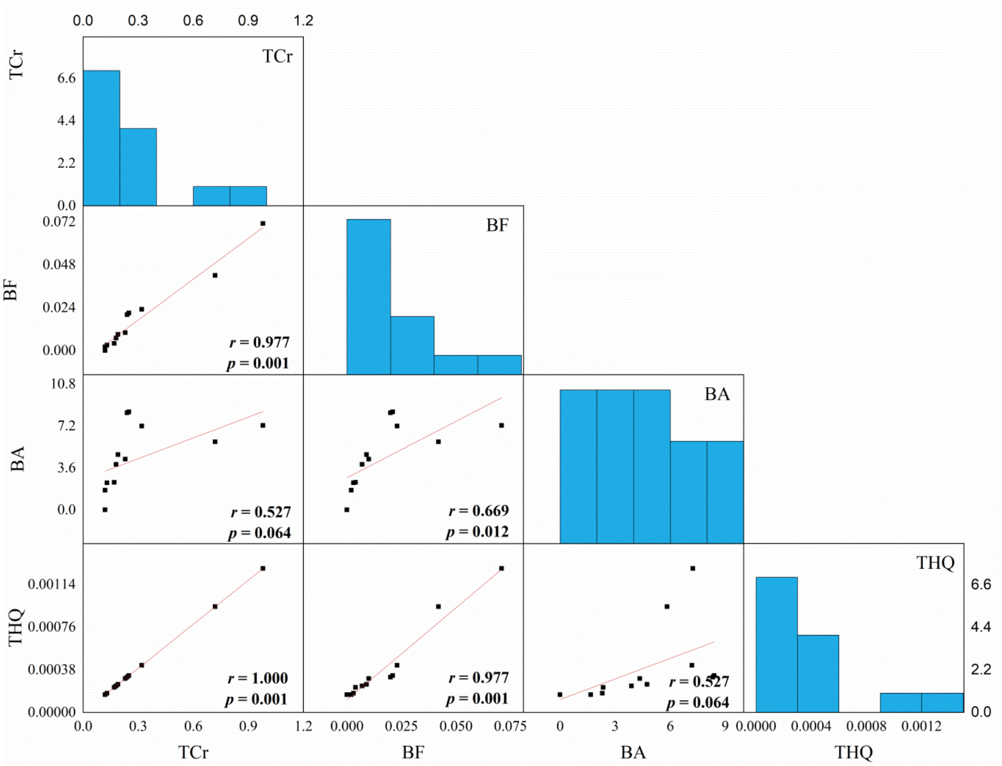

Figure 8 The correlation between metal bioaccessibility and target hazard quotient in rice

Total chromium content ranged from 0.12 to 0.98 mg kg^{-1} (Median 0.19 mg kg^{-1}) for polished rice and 0 to 0.071 mg kg^{-1} (Median 0.009 mg kg^{-1}) for bio accessible fraction. The bioaccessible percentage varied from 0 to 8.40 (Median 4.35). Target hazard quotient ranged from 1.57 to 1.28 × 10^{-3} (Median 2.49 × 10^{-4}). However, in present study chromium concentrations were well within the permissible limit to food grains (1 mg kg^{-1}) (Figure 6).

A significant positive correlation between total chromium content in rice samples and soil total chromium was evident for biomagnifications of chromium from soil to rice grains. Furthermore, a significant positive correlation was observed between total chromium content and target hazard quotient and also with bioaccessible fractions in rice samples (Figure 7 & 8). High chromium in the soil is directly correlated with the total chromium in rice even though it is below the tolerable level.

Conclusion

The study showed that the total and bioaccessible heavy metals were higher than the maximum permissible limits in few polished rice samples. Consumption of foodstuff with elevated levels of heavy metals for the long term may lead to health disorders. Therefore regular monitoring of heavy metals is essential to reduce the intensive accumulation of heavy metals in the human food chain. Because of the paucity of data on bioaccessible heavy metals in rice, no comparison with heavy metals by other researchers can be drawn. However, the present study confirms that bioaccessible metals were independent of total content and soil-water contaminated environment may be the reasons for the entry of heavy metals in rice grains.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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