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Metal Contents in Traditional Alcoholic Rice Beers Prepared by Rabha and Sonowal Kachari Tribes of Assam, India

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Consumption of food that contains heavy metals poses a risk to the health of humans. The Rabhas and Sonowal Kacharis, indigenous tribal communities residing in the Assam state of India, produce their traditional alcoholic beverages for personal consumption in their daily lives. However, there is a potential risk of heavy metal contamination in these beverages due to the various substances utilized throughout the production process. Using atomic absorption spectroscopy, an investigation to determine whether heavy metals are present in the alcoholic beverages of these two communities reveals the concentration of eleven metals *viz.* zinc, potassium, sodium, chromium, cobalt, copper, iron, lead, manganese, nickel and zinc. The metal contents (mg/L) in the samples were in the range of 0.002-0.008 for cadmium, 0-0.012 for chromium, 0-0.008 for cobalt, 0.092-0.121 for copper, 1.09-1.56 for iron, 0.09-0.19 for lead, 1.67-2.53 for manganese, 0.024-0.094 for nickel, 175.8-290.8 for potassium, 9.7-25.8 for sodium, 0.639-1.678 for zinc. This study revealed that all the samples can be marked as safe as the metal concentrations are within the permissible limits of WHO. Sticky rice beer of Pati Rabhas has highest total hazard quotient, but still it can be marked as safe by comparing with WHO permissible limit and almost free from contamination of toxic metals like cobalt and chromium.

Keywords: Rabhas, Sonowal Kacharis, Traditional alcoholic beverage, Target hazard quotient, Metal content.

INTRODUCTION

Rabhas and Sonowal Kacharis of Assam state, India are the two major indigenous tribal communities in the north-eastern region of India and they prepare their traditional alcoholic beverages (rice beers) for their own consumption in day-to-day life [1]. Rice beers can contain heavy metals due to the wide variety of ingredients used in their production. These metals can enter the food product through several food processing steps. The alcoholic beverages of these two communities are therefore investigated to find the presence of metals. Public Health Department often express their concern with the unscientific way of preparation and consumption of traditional alcoholic beverages by the indigenous communities.

Metals like cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), manganese (Mn), sodium (Na), nickel (Ni) and zinc (Zn) have a lot of pivotal roles in human health. The trace element cobalt is an essential component of vitamin B_{12} [2,3]. But the excessive intake of cobalt causes goiter disorder by reducing thyroid activity [4]. Nausea, vomiting, abdominal pain are also caused by excess ingestion of cobalt [4]. Chromium helps maintain normal blood sugar levels and helps cells to draw energy from blood sugar [5]. Chromium exists in different oxidation states in different compounds [6]. Chromium with oxidation state +3 is an essential nutrient for human body whereas +6 oxidation state of chromium is believed to cause several forms of cancer [6]. For metalloprotein synthesis, copper and iron ions are required [5,7-9]. However, consumption of excessive amount of copper leads to oxidative stress and tissue damage [9]. Similarly, higher level of iron consumption causes Parkinson disease [5,8,9]. Manganese helps bone formation and metabolizing amino acid, cholesterol and carbohydrates [5], but its toxicity can lead to a chronic neurological condition called manganism, characterized by symptoms such as tremors, impaired mobility, and facial muscle spasms [10]. Deficiency of zinc during developmental stages of children causes growth failure [11,12], but the higher level of zinc in the body may cause nausea, epigastric pain, vomiting, lethargy, *etc*. The higher level of nickel may cause genotoxicity, carcinogenicity, immunotoxicity, *etc*. [13]. Among the heavy metal ions, cadmium is a toxic metal; even small amount of cadmium may lead to hypertension and tumors [5,7]. Similarly, lead metal is responsible for kidney damage, liver damage, impaired hearing, mental retardation, *etc*. [5,7,8].

The majority of the native tribal people in North-East India produce rice-based beers using a yeast culture as a starter, which requires a significant amount of medicinal plants for its preparation [14,15]. These homemade alcoholic rice beers have attracted attention of researchers because of their biochemical and antioxidant properties and most importantly the metal profiles. As per WHO guidelines the presence of different metals in different concentrations is desirable in diet for a good health although excessive consumption of metals may lead to metal toxicity. Besides this, metals like Na, K, Fe, Mn, Cu and Zn are also necessary for human bodies as essential nutrients for the formation of bones and for balanced carbohydrate and cholesterol metabolism.

Natural inquisitiveness led us to speculate on the possible origins of metals in rice beers. The field study revealed that indigenous peoples do not add metal or metallic salt directly to their alcoholic drinks. Thus, the possibilites through which metals can enter alcoholic beverages are *via* the components and raw materials as well as the brewing equipments and storage utensils used throughout the preparation process. Therefore, it is crucial to thoroughly examine both the raw materials and the brewing and storage equipments that the metal content exceed the critical limit.

EXPERIMENTAL

Sample collection: Rice beer samples were collected from different parts of Assam state, India. Rice beer 'Janga' prepared by Rangdani Rabha was collected from Goalpara district, Assam, whereas sticky rice beer of Pati Rabhas was collected from Baksa and Bongaigaon districts. Sonowal Kacharis tribal people in Dibrugarh district prepare two types of fermented alcoholic beverages *viz*. Sepa and Rohi; however, Rohi is the most common and popular among them, which was collected.

Sample preparation and analysis: Metal contents in rice beer samples were analyzed by the reported standard protocols. Samples were degassed by ultrasonic bath for 30 min and then 20 mL of aliquot of degassed sample was mixed with 2 mL of conc. HNO_3 and 2 mL H_2O_2 in a digestion tube. The metal contents in digested samples were analyzed by atomic absorption spectroscopy [9].

Statistical analysis: The statistical interpretation of the data was done using Duncan test, SPSS version 24 (SPSS Inc., Chicago, USA) software. Data were analyzed by one way analysis of variance (one way ANOVA) and the levels of significance were set as $p \leq 0.05$.

RESULTS AND DISCUSSION

Metal contents of analyzed different rice beer samples are compared in Fig. 1 and found within WHO maximum permissible limit of metals in drinking water. Two toxic metals cobalt and chromium are absent in rice beer prepared by Pati Rabhas

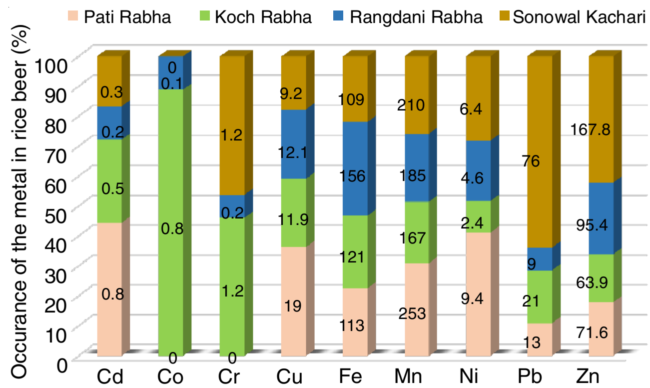

Fig. 1. Bar diagram of % of metals in traditional rice beers

and toxic metal cobalt is absent in rice beer of Sonowal Kacharis. All metals except iron and manganese are within the permissible limit of WHO in all alcoholic beverages. During the field survey, high level of iron content was observed in underground water. Although conventional low-cost filtration techniques are used to remove iron from underground water before consumption, still iron makes its way to alcoholic beverages through contamination from vessels used for storing or fermentation processes. Iron contamination is highest in Rangdani Rabha beer, which is above the WHO permissible limit. Cadmium is present in higher concentration in rice beer prepared by Pati Rabhas tribal community (0.008 mg/L) as compared to other alcoholic beverages, but still it is within WHO permissible limit; so it can be marked as safe for consumption. Cobalt content is in very minimal in rice beer prepared by Rangdani Rabhas (0.001 mg/L), but slightly high in the rice beer prepared by Koch Rabhas (0.008 mg/L). Chromium content in Rangdani Rabha beer is found in a trace amount (0.002 mg/L) and for Koch Rabha Beer or Sonowal Kachari beer, its content is almost same (0.012 mg/L). In all the samples, chromium content is far below WHO maximum permissible limit and thus suitable for comsume.

Presence of potassium and sodium is highest in Rangdani Rabha beer, but they are non-toxic metals. Potassium balances fluid in the body, helps to regulate blood pressure [5]. On the average an adult needs 500 mg of sodium per day in the body to conduct nerve impulses, contract and relax muscles. Nickel and lead contents are highest respectively in Pati Rabha beer and Koch Rabha beer, still values are within the WHO permissible limit. As per WHO maximum permissible limit, presence of zinc as nutrient in food is 5.0 mg/mL, but all rice beer samples contain much less amount of zinc than WHO limit. Metal count and total load of metals in different alcoholic beverages are shown in Table-1. Population mean of metals is highest for Rangdani Rabha rice beer, *i.e.* it signifies that mean or average of all values in Rangdani Rabha population is highest as compared to other beer samples.

Statistical analysis: In Table-1, a comparable statistical data for all types of beers are listed. The average mean value for Pati Rabha beer is 28.33, Koch Rabha beer is 23.30, Rangdani Rabha beer is 29.20 and Sonowal Kachari beer is 19.13. In Pati Rabha beer, cobalt and chromium is below estimated limit. In Sonowal Kachari beer, cobalt is also in below

TABLE-1
STATISTICAL ANALYSIS FOR SIGNIFICANCE OF DIFFERENCES AMONG TRADITIONAL RICE BEERS

Types of alcoholic beverages	Metal count	Metal content total (µg/mL)	Population mean	Standard deviation (S)	Variance (S^2)	Sum of squares (SS)
Pati Rabha rice beer	09	254.998	28.33	71.23	5074.86	45673.81
Koch Rabha rice beer	11	256.297	23.30	67.00	4489.73	49387.07
Rangdani Rabha rice beer	11	321.226	29.20	83.04	6896.05	75856.51
Sonowal Kachari rice beer	10	191.299	19.13	52.30	2734.92	27349.20

estimated limit. The sum of square value is highest for Rangdani Rabha beer, which indicates that there is higher variability of results from the mean value. This is attributed because of high potassium content in Rangdani Rabha's beer.

Estimated daily intake (EDI) of metals through alcoholic beverages: The estimated daily intake (EDI) of various metals present in alcoholic beverages are listed in Table-2. The EDI of toxic metals like cadmium and chromium in all the beer samples ranges from 0.0083-0.05 µg/kg bw/day, which is below the tolerable EDI value for cadmium (1 µg/kg bw/day) and chromium (2.2 µg/kg bw/day) [2,3]. The concentration of cobalt in Pati Rabha rice beer and Sonowal Kachari rice beer was also below the permissible limit whereas the EDI of cobalt for Koch Rabha Beer and Rangdani Rabha beer ranges from 0.004-0.033 µg/kg bw/day, which is below the tolerable intake value (20-30 mg per day) of cobalt. EDI for chromium ranges from 0.0083-0.05 2.2 µg/kg bw/day [2,3]. The estimated daily intake is calculated by the following pre-reported formula [8,17,18]:

$$\text{EDI } (\mu\text{g/kg bw/day}) = \frac{\text{C } (\mu\text{g/mL}) \times \text{V (mL)}}{\text{W (kg)}}$$

where C is the concentration of metals found in the samples; V is the volume consumed (average 250 mL considered from field survey); W is the body weight for a 60 kg adult.

Health risk assessment analysis: Health risk assessment analysis is the anticipated risk measure of the adverse health effects on human due to exposure to chemicals like metals. Average daily dose (ADD), target hazard quotient (THQ), hazard index (HI) are the few parameters to assess the health risk. The average daily dose of heavy metals was calculated by the following formula [16,17] and the values are tabulated in Table-3.

$$\text{ADD} = \frac{C_i \times \text{MDI} \times \text{EF} \times \text{ED}}{\text{ABW} \times \text{AT}}$$

TABLE-2
ESTIMATED DAILY INTAKE (EDI) OF METALS IN DIFFERENT RICE BEERS

Name of the metal	EDI (µg/kg bw/day) in different rice beers by various communities: Pati Rabha	Koch Rabha	Rangdani Rabha	Sonowal Kachari
Cadmium	0.033	0.021	0.0083	0.0125
Cobalt	–	0.033	0.004	–
Chromium	–	0.05	0.0083	0.05
Copper	0.79	0.49	0.504	0.38
Iron	4.71	5.04	6.5	4.54
Potassium	954	977	1211	732.5
Manganese	10.54	6.96	7.71	8.75
Sodium	88.33	74.10	107.5	40.41
Nickel	0.39	0.1	0.192	0.27
Lead	0.54	0.79	0.375	0.67
Zinc	2.98	2.66	3.975	6.99

where C_i is the metal concentration in vegetable, MDI is the ingestion rate, EF is the exposure frequency, ABW is the body weight of the consumer, AT is the average time.

$$\text{Hazard quotient (HQ)} = \frac{\text{ADD}}{\text{RfD}} \quad [16]$$

where ADD is the average daily dose and RfD is the oral reference dose. Hazard index was calculated as the total hazard quotients. HI = ΣTHQ, [16] *i.e.*

$$\text{HI} = \text{THQ}_1 + \text{THQ}_2 + \text{THQ}_3 + \text{----------} + \text{THQ}_n$$

THQ is the estimation of non-carcinogenic risk level of human health with metals [18] as established by Environmental Protection Agency (EPA). Rice beer consumption 250 mL per day having THQ less than 1 indicates safe level, while THQ greater than or equal to 1 indicates level of concerns for human health [18]. THQ can be expressed by the following equation [17,18]:

TABLE-3
AVERAGE DAILY DOSE OF HEAVY METALS IN RICE BEER SAMPLES

Name of the metal	ADD of heavy metals in rice beer samples — EDI (µg/kg bw/day) in different rice beers by various communities: Pati Rabha from sticky rice	Koch Rabha from sticky rice	Rangdani Rabha Jonga	Sonowal Kachari Rohi
Cadmium	0.033	0.020	0.008	0.012
Cobalt	–	0.033	0.004	–
Chromium	–	0.050	0.008	0.050
Copper	0.791	0.495	0.504	0.383
Iron	4.708	5.041	6.500	4.541
Manganese	10.541	6.958	7.708	8.750
Nickel	0.039	0.100	0.192	0.267
Lead	0.541	0.792	0.375	0.667
Zinc	2.983	2.663	3.975	6.992
Total ADD value (ΣADD)	19.636	16.152	18.694	21.662

$$THQ = \frac{EF \times ED \times MDI \times C_i}{RfD \times BW \times AT} \times 10^{-3}$$

where, EF stands for exposure frequency (365 days/year), ED is the exposure duration (year) which is 53.88 years, MDI is the mass of selected dietary ingestion (250 mL/day), C is the concentration of inorganic species in the dietary components, RfD is the oral reference dose (mg/kg/day), BW is the average adult body weight (assuming 60 kg), AT is the average time for non-carcinogens (day) (365 days/year multiplied by number of exposure years, assuming 68.88 years [19], consumption starts at 15 years of age in average from the information of field survey) and 10^{-3} is the unit conversion factor. Length of exposure is set to 19666.20 days (intake years = Life expectancy - life time before consumption starts). Reference dose (mg/kg/day) calculations were carried out using assumptions from Integrated United States EPA risk analysis [9].

Oral reference doses for metals used for health risk calculation of THQ are listed in Table-4. THQ for all metals in different types of rice beers is listed in Table-5, THQ less than 1 signifies the safe level of metal exposure in different types of alcoholic beverages. Sticky rice beer of Pati Rabhas has highest total hazard quotient (Fig. 2), still it can be marked as safe by comparing with WHO permissible limit and it is almost free from contamination of toxic metals like cobalt and chromium.

TABLE-4
ORAL REFERENCE DOSES (RfD) FOR DIFFERENT METALS [8]

Element	Oral reference dose for heavy metals (mg/kg/day)
Copper	4×10^{-2}
Cadmium	1×10^{-3}
Nickel	2×10^{-3}
Iron	7×10^{-1}
Zinc	3×10^{-1}
Lead	1.5
Manganese	1.4×10^{-1}
Chromium	1.5
Cobalt	3×10^{-4}

Conclusion

Metals in food can provide both nutritional values as well as toxicity, both of which depend on the concentration of the metals. The tribal peoples in north-eastern states of India have maintained a long tradition of rice beer culture which has deep socio-cultural meanings. In this work, the permissible limits of heavy metals in different rice beer samples prepared by Rabhas and Sonowal Kacharis, indigenous tribal peoples of Assam state, India were analyzed. All the parameters related to metal load like concentration, estimated daily intake (EDI), target hazard quotient (THQ), average daily dose (ADD) studied for the traditional alcoholic beverages were analyzed and it was observed that in all the alcoholic rice beer beverages, hazardous metals are in lower concentrations than maximum permissible limits as prescribed by WHO.

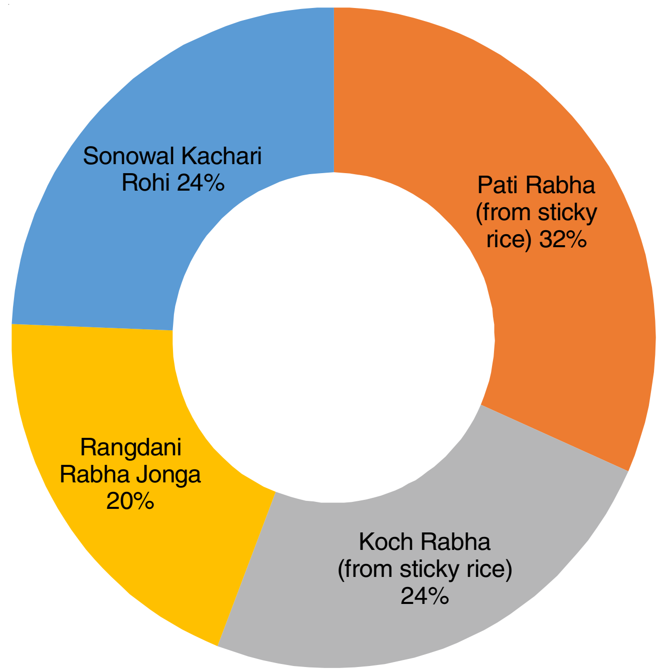

Fig. 2. Van diagram showing %THQ of heavy metals in beer of different communities

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TABLE-5
TARGET HAZARD QUOTIENT (THQ) OF HEAVY METALS IN RICE BEER SAMPLES

Name of the metal	THQ of heavy metals in rice beer samples			
	Pati Rabha from sticky rice	Koch Rabha from sticky rice	Rangdani Rabha Jonga	Sonowal Kachari Rohi
Cadmium	0.033	0.021	0.008	0.012
Cobalt	–	0.110	0.013	–
Chromium	–	0.000	0.000	0.000
Copper	0.020	0.012	0.012	0.009
Iron	0.007	0.007	0.009	0.007
Manganese	0.075	0.050	0.055	0.062
Nickel	0.196	0.050	0.096	0.133
Lead	0.001	0.001	0.000	0.001
Zinc	0.019	0.018	0.027	0.046
Total hazard quotient (ΣTHQ)	0.351	0.269	0.220	0.270

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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