Toxicology Reports 8 (2021) 1220–1225

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Ethnic food beverages with heavy metal contents: Parameters for associated risk to human health, North-East India

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ARTICLE INFO

Handling Editor Dr. Aristidis Tsatsakis

Keywords:
Food beverage
Jou
Estimated daily intakes
Target hazard quotient
Metal content

ABSTRACT

Food beverage consumption is a worldwide age-old practice. It is also a common run-through habit among the tribes of North Eastern parts of India. The food beverage group *Jou* is a traditionally fermented rice-based beverage by *Bodo* community of Assam, the largest plain tribe. It is not only consumed by *Bodos* on regular basis but also widely used in their socio-cultural activities. *Joubidwi* (JB, fermented product), *Joufinai* (JF, preserved product) and *Jougwran* (JG, distilled) are the three varieties of *Jou*. Herewith, it had been reported; the concentrations of metals such as Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, Pb and Zn in the three varieties of *Jou* to make people aware about the possible health benefits as well as the risks associated with the consumption of *Jou*. The metal contents were estimated using ICP-OES after digesting the samples with 10:1 mixture of concentrated nitric and concentrated sulfuric acid. The estimated daily intakes (EDI) of metals from consumption of JB, JF and JG were also within the recommended daily allowances (RDA). From the estimated target hazard quotient (THQ) values, it was observed no health risk associated with the consumption of *Jou*; whereas total target hazard quotient (TTHQ) of *Joufinai* were associated with more health risks. Metal concentrations of all metals in *Bodo* beverages was lower in comparison with barely based and wheat based beverages.

1. Introduction

Metals in food beverages play a significant role on different aspects concerning the quality and acceptability of the beverages by consumers.[1] The metal content in beverages affects consumption and preservation. [1] Both positive and negative effects are possible. Dietary intake of minerals and removal of bad odour include positive effects but beer hazing, sensorial health consequences, toxicity from excessive intake of metals, etc. are some of the negative effects. [2,3] Brewing procedure, raw materials used, storage or aging, equipment/ utensils used are the main sources of metals which find their way into food beverages [4]. Essential metals like Ca, K, Cu, Zn, Co, Fe, Mn etc. have pivotal role in human biological system [5]. Copper and iron ions are required for metalloproteins synthesis [6,7]. However, excessive intake of Fe may lead to Parkinson's disease due to deposition of iron oxide [8]. Again, excessive intake of Cu may cause oxidative stress, diminished activity of antioxidant enzymes and subsequent tissue damage [9,10]. Even small amount of Cd and Pb are toxic. Cadmium accumulation in biological systems can cause hypertension and tumors [9,12]. Because of long biological half- life, longer accumulation of Cd may lead to kidney damage [13]. Higher level of Pb is responsible for kidney and liver damage, mental retardation, impaired hearing and shortened gestation period [7,14,18]. Elevated intake of essential metals also produce toxic effects, for instance, excessive Zn is considered responsible for increased prevalence of obesity [5]. Heavy metals can enter the human body trough ingestion, inhalation and dermal absorption [15]. For example, 15 % inorganic Pb is absorbed to human body as compared to 80 % of organic Pb by ingestion [15].

Food beverage production and consumption is a popular practice among tribal communities of North-East India. [14] *Bodo* community of North-East India also uses their traditional food beverage *Jou* for their livelihood. Consumption is highly acceptable in various socio-cultural activities. Despite of wide range of consumption among the community people, metal profile of *Jou* is not reported yet. The traditional manufacturing protocol is shown in Fig. 1. Herein, we are reporting the metal concentrations of Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, Pb and Zn in all the three varieties of *Jou*. Provisional tolerable intakes are also estimated to assess the risk factors to human health from metal intake arising from the consumption of *Jou*. Target Hazard Quotients (THQ) provided by Environmental Protection Agency (EPA, Washington DC)

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https://doi.org/10.1016/j.toxrep.2021.06.013
Received 13 December 2020; Received in revised form 5 June 2021; Accepted 14 June 2021
Available online 16 June 2021

[14], which is the ratio between the exposure dose and the reference dose (separately estimated values for various metals), is also estimated for each *Jou* sample to express the risk of non-carcinogenic effects of daily intake of metals. The objective of the study is to make people aware of the possible health benefits as well as the risks associated with the consumption of *Jou* and to explore the ethnic beverage comprehensively.

2. Materials and methods

2.1. Sample collection

Samples of three *Jou* varieties i.e. *Joubidwi* (JB), *Joufinai* (JF) and *Jougwran* (JG) were collected from the Kokrajhar district of Assam, North-East India. Two samples of each variety were examined, and these are labelled as JB1, JB2, JF1, JF2, JG1 and JG2 for *Joubidwi*, *Joufinai* and *Jougwran* respectively. The manufacturing protocol adapted for the samples were identical.

2.2. Sample preparation for analysis

Each rice-beer sample was digested with a mixture of HNO_3 and H_2SO_4. 10 mL of conc. HNO_3 and 1 mL conc. H_2SO_4 were added to 25 mL of each beer sample in a beaker and digested on a hot plate until the appearance of straw yellow colour. During heating, the beaker was covered with a watch glass. The straw yellow coloured sample obtained after heating was allowed to cool to room temperature, filtered and diluted to 25 mL with distilled water [11,12,14].

2.3. Chemical analysis

All digested samples were analysed in triplicate using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy, Perkin Elmer).

2.4. Method for determination of estimated daily intake (EDI)

EDI of metals for a 60 kg adult was estimated based on average consumption of 250 mL per day. EDI in *Jou* samples are calculated using the following formula [12,14]:

EDI (μg/kg bw/day) = [C (μg/mL) x V (mL)]/W (kg)

where C is the concentration of metals found in samples, V is volume consumed (average 250 mL considered from field survey), W is the body weight for 60 kg adult. The mean concentrations of metals are considered for EDI value determination for beer samples. Daily intake of Cd for *Bodo* rice beers ranges from 0.016 to 0.041 μg/kg bw/day.

2.5. Methods for determination of risk associated with beverage

THQ is established by EPA [17] and calculated by using the equation

$$THQ = \frac{Efr \times Ed \times Sfi \times C}{RfD \times Bw \times ATn} \times 10^{-3}$$

where, Efr is exposure frequency (365 days/year); Ed is the exposure duration (year); Sfi is food ingestion rate (g/day); C is the concentration of inorganic species in the dietary components (considered mean values only); RfD, oral reference dose (mg/kg bw/day); Bw, average adult body weight (assuming 60 kg); ATn, averaging time for non-carcinogens (day), and 10^{-3} is unit conversion factor. Reference dose (mg/kg bw/day) calculations are carried out using assumptions from integrated United States EPA risk analysis.[17] Oral reference doses for metals used for health risk calculation of THQ are listed in Table 4. For estimating THQ of heavy metals in *Jou* samples, 250 mL/day ingestion rate is assumed. Daily intake calculation was based on per capita consumption of 3.6 L of pure alcohol per annum [5,7]. The length of exposure is set to 12,191 days based on average life expectancy of 48.4 years consumption from 15 years of age as estimated by WHO, 2004 [5,7]. Target Hazard Quotients (THQ) signify the ratio between exposure doses of heavy metals to the reference doses. It is used to explain the risk of non-carcinogenic effects. THQ values less than 1 and greater than or equal to 1 are likely to have influence on health risk factor. When it is

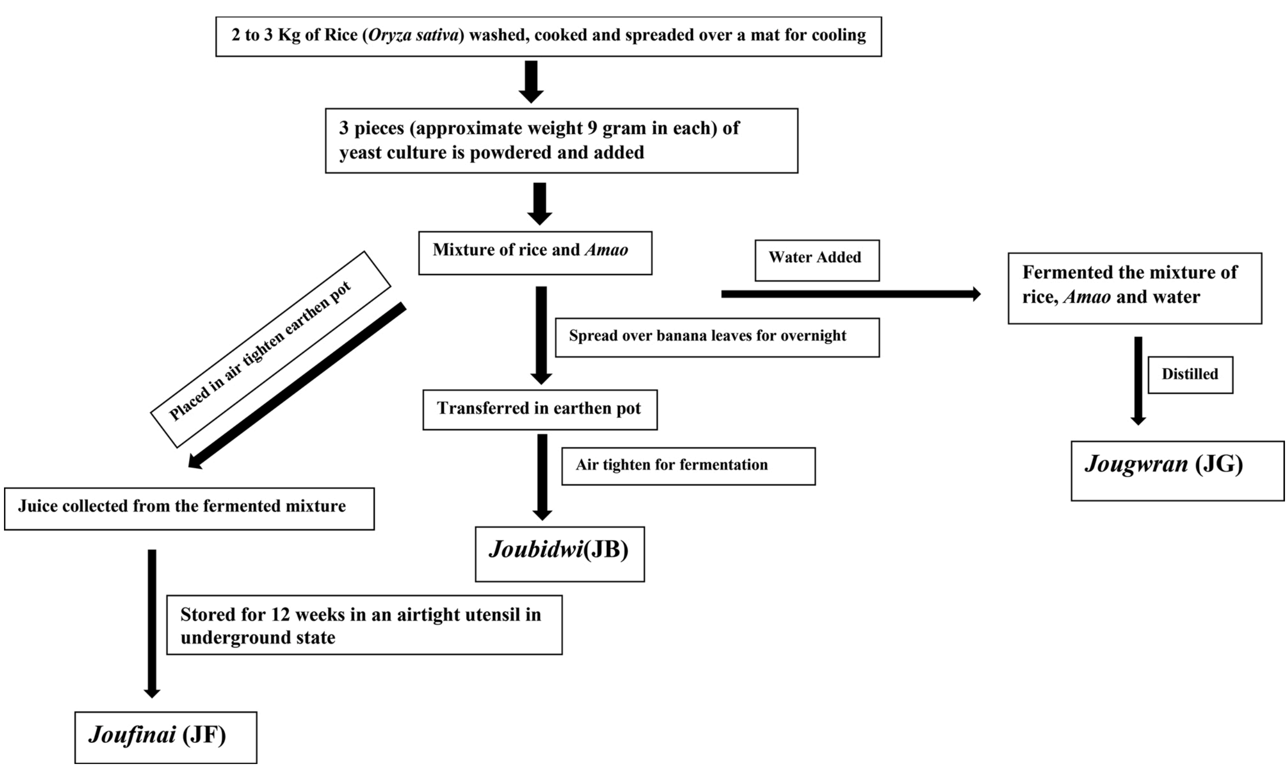

Fig. 1. Flow chart for schematic representation for *Jou* preparation procedure.

Table 4
Oral reference doses (RfD) for metals [5,7,34].

Metals	RfD (mg/kg bw/day) for heavy metals
Cd	1×10^{-3}
Co	3×10^{-4}
Cr	1.5
Cu	4×10^{-2}
Fe	7×10^{-1}
Mn	1.4×10^{-1}
Ni	2.0×10^{-3}
Pb	4×10^{-3}
Zn	3×10^{-1}

less than 1, there is non-obvious risk; however greater than 1 or equal to 1 signifies a potential health risk [15].

2.6. Reproducibility of analysis

Reproducibility of results was checked by analysis of each samples in triplicate. Permissible limits of metals were expressed as mean ± standard deviation.

3. Results and discussion

Metal concentrations in alcoholic beverages depend on certain sources like (a) brewing procedure, (b) raw materials used, (c) storing or aging, (d) equipment/ utensils used etc. [14] The mean concentrations (±standard deviations) for Cd, Co, Cr, Cu, Fe, K, Mn, Na, Ni, Pb and Zn in *Jou* samples are summarized in Table 1. Maximum permissible limits (MPL) of some of the metals as specified by International Organisation for Grapes and Wines are also shown in Table 1 [17]. Levels of Cd, Cr, Cu, Na, Pb and Zn were observed within MPL in all the samples. Concentrations of Fe and Ni are found to exceed MPL in all the samples and those of Mn exceed MPL in *Joubidwi* (JB) and *Joufinai* (JF) samples. Concentrations of metals in distilled beverage *Jougwran* (JG) are observed much lower as compared to other two varieties JB and JF. Distillation have a positive discrimination towards transfer of alcohol, low molecular weight compounds, aromatics etc. leaving behind the heavy metals with the liquor in the distillation flask. But in the undistilled beverages, metals are transferred directly from the raw materials and responsible for higher metal concentrations.

3.1. Resemblances and variations of metal concentrations of Jou to some alcoholic beverages of North-East India

Mean values of metal concentrations for Cd, Co, Cr, Cu, Fe, K, Mn, Na, K, Pb and Zn in rice beer samples of *Bodo* community were compared to those of rice beers by other communities, namely *Deoris*, *Ahoms* and *Misings* of North-East India [14] as shown in Table 2. Metal levels for Cu, K, Mn, Na and Pb in *Jou* were slightly differing from others. Higher level of Cu (0.120−0.255 mg/L) was observed in *Jou* as compared to rice based alcoholic beverages of other three communities. But values were within the permissible limit of Cu (1 mg/L) as specified by International Organisation for Grapes and Wines [21].

The metal concentrations for Cd, Co, Ni in *Deori*, *Ahom* and *Missing* alcoholic beverages were quite higher than *Bodo* alcoholic beverage *Jou* (Table 2). Again, Fe content of *Jou* (0.859−1.992 mg/mL) and *Missing* community (1.992−1.233 mg/mL) based beverage were comparable, whereas lesser Fe metal content was observed for *Deori* alcoholic beverages.

Lead content in *Jou* (0.064−0.256 mg/mL) was only in the permissible limit, whereas metal concentration of Pb in other alcoholic beverages were in higher range than permissible ranges (Table 2) for heavy metals [22,23].

Na (5.59–5.920 mg/L) and K (160−231.9 mg/L) concentrations in

Table 1
Metal concentrations in *Jou* samples with maximum permissible limits.

SCN	Metal concentrations (mg/L) in *Jou* samples										
	Cd	Co	Cr	Cu	Fe	K	Mn	Na	Ni	Pb	Zn
JB1	0.004 ± 0.02	0.008 ± 1.01	0.019 ± 0.40	0.120 ± 0.67	0.859 ± 0.65	167.6 ± 0.09	1.135 ± 2.10	5.579 ± 0.08	0.062 ± 0.04	0.064 ± 0.02	0.855 ± 0.11
JB2	0.005 ± 0.15	0.009 ± 0.11	0.110 ± 0.05	0.189 ± 0.01	1.621 ± 0.07	160.0 ± 0.03	2.195 ± 0.11	5.991 ± 0.35	0.078 ± 1.20	0.075 ± 1.55	2.124 ± 0.30
JF1	0.007 ± 0.04	0.007 ± 0.02	0.008 ± 0.44	0.278 ± 2.11	1.992 ± 0.22	231.9 ± 0.15	3.238 ± 1.11	5.652 ± 1.32	0.094 ± 0.55	0.107 ± 0.65	3.034 ± 0.21
JF2	0.010 ± 0.02	0.014 ± 0.01	0.143 ± 0.05	0.255 ± 0.03	1.926 ± 0.23	271.3 ± 0.01	3.262 ± 0.03	5.920 ± 0.02	0.102 ± 0.25	0.250 ± 1.15	2.848 ± 0.01
JG1	0.001 ± 1.10	0.010 ± 0.35	0.011 ± 0.01	0.119 ± 0.11	0.612 ± 0.01	4.026 ± 0.01	0.048 ± 0.01	4.292 ± 0.25	0.058 ± 0.11	0.067 ± 0.12	0.174 ± 0.55
JG2	0.001 ± 0.08	0.008 ± 1.21	0.014 ± 0.08	0.112 ± 0.70	0.579 ± 0.12	3.821 ± 0.02	0.036 ± 0.01	4.199 ± 0.05	0.046 ± 0.55	0.052 ± 0.11	0.159 ± 0.01
MPL	0.01[a]	—	0.05[b]	1[a]	0.30[b]	—	0.40[b]	60[a]	0.02[b]	0.2[a]	5[a]

SCN = Sample code name, JB, JF and JG stand for *Joubidwi*, *Joufinai* and *Jougwran*. 1 and 2 stands for first and second samples respectively. [a]MPL, maximum permissible limits of metals specified by International Organisation for Grapes and Wine (OIV 2008). [b]MPL specified by World Health Organisation (WHO,1993) in drinking water.

Table 2
Resemblances and variations of metal concentrations of *Jou* with other alcoholic beverages of North_ East India.

Metal concentrations (mg/L) in rice based alcoholic beverages of tribal communities of North-East India					Permissible limits of |heavy metals (mg/L)
Metals	*Deori* community [3]	*Ahom* Community [3]	*Mising* community [3]	*Bodo* community	
Cd	0.044–0.003	0.051–0.022	0.02–0.049	0.004–0.010	0.01
Co	0.196–0.207	0.304–0.01	0.153–0.180	0.007–0.014	———
Cr	ND	ND	ND	0.008–0.143	0.05
Cu	0.074–0.068	0.053–0.002	0.008–0.017	0.120–0.255	1
Fe	0.709–3.313	1.311–4.15	1.922–1.233	0.859–1.992	0.30
K	300–450	440–460	330–400	160–231.9	———
Mn	0.044–0.325	0.011–0.112	0.028–0.311	1.135–3.262	0.40
Na	20–60	40–50	40–50	5.579–5.929	60
Ni	0.305–0.419	0.554–0.451	0.280–0.324	0.062–0.102	0.02
Pb	0.68–0.71	0.48–1.13	0.48–1.10	0.064–0.256	0.2
Zn	3.968–3.643	3.231–0.740	0.674–1.598	0.855–0.034	5

ND : Not Detected.

Jou were lower than rice beers of *Deori*, *Ahom* and *Mising* communities (Table 2). The concentrations of Mn in *Jou* were observed in the range 1.135–3.262 mg/L as compared to 0.044–0.325, 0.011–0.112 and 0.028–0.311 mg/L in rice beers of other communities. The concentration of Mn in *Bodo* rice beer was higher than permissible limits for Mn (0.2 mg/L) in drinking water specified by SON [24], but almost similar to the values reported by Li and Hardy [25], Sauvage et al. [26], Kmet et al., [27] Alvarez et al. [28], Pohl [20], Sass-Kiss et al. [29], Woldemariam and Chandranvanshi [30]. Chromium concentrations were observed in the range of 0.008–0.143 mg/L for *Bodo* rice beer but not detected in other rice beers of NE India origin. The maximum permissible limit for Cr in drinking water is set at 0.05 mg/L by SON. [24]

3.2. Comparison of Bodo traditional rice based alcoholic beverage with wheat and barley beverages

Metal contents of *Bodo* rice based beverage (freshly prepared) was compared with wheat and barley based beverages (Fig. 2). [14] We observed that the concentrations of almost all metals were lower in *Bodo* traditional beverage in comparison to wheat and barley beverages; within the MPL; provide a positive discrimination of its intake. Mn concentration of both barley based beverage and rice based beverage was same. Whereas, the Fe concentration of wheat based beverage was comparable with *Bodo* traditional one. With reference to other metals Na, K, Cr, Co, Ni, Zn, Cd and Pb; differences were observed in the concentration of metals.

3.3. Estimated daily intake (EDI) of metals in Bodo traditional alcoholic beverage Jou

The estimated daily intakes (EDI) of metals in JB, JF and JG samples were reported in μg/kg bw/day (Table 3). EDI of Cd in present study was within the tolerable ranges as assigned by WHO 1993(1 μg/kg bw/day).722 Higher intake of Cd comes from the consumption of JF (0.029–0.041 μg/kg bw/day) as compared to JB (0.016–0.020 μg/kg bw/day) and JG (4×10^{-3} μg/kg bw/day).

The recommended daily allowance (RDA) for Co is 100 μg/day [14,30,22]. Daily intakes of Co from rice beer samples varied from 0.033 to 0.037, 0.029 to 0.058 and 0.153 to 0.033 μg/kg bw/day for JB, JF and JG respectively. Cobalt intakes from the three categories of *Jou* were below the RDA values. As specified by WHO (1993), daily intake of Cu ranges from 15–500 μg/kg bw/day [7,22]. In the present study, it was observed that EDI for Cu were within the RDA prescribed by WHO, 1993.

The recommended daily allowances for Fe and Mn are 10–18 and 2–5 mg/day/person, respectively [7,14]. EDI of Fe for consumption of

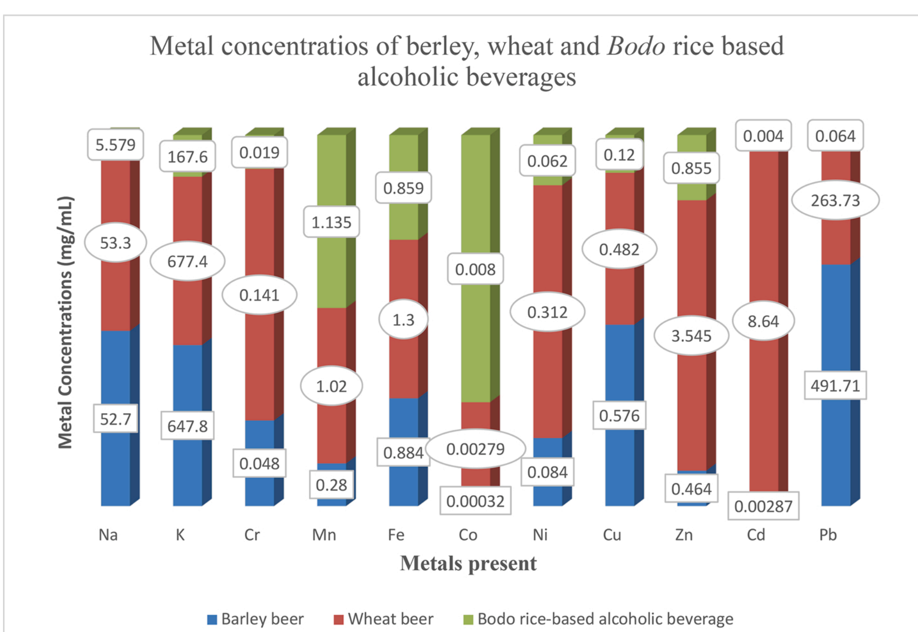

Fig. 2. Comparison metal contents in rice based *Jou* with other cereal based *beverages.*

Table 3
EDI in μg/kg bw/day based on 250 mL per person per day consumption and permissible RAD for metals.

Metals	EDI (μg/kg bw/day) in *Jou* samples						Recommended Daily Allowance (RDA) values
	Joubidwi (JB)		*Joufinai* (JF)		*Jougwran* (JG)		
	JB1	*JB2*	*JF1*	*JF2*	*JG1*	*JG2*	
Cd	0.016	0.020	0.029	0.041	4×10^{-3}	4×10^{-3}	1 μg/kg bw/day [7,22]
Co	0.033	0.037	0.029	0.058	0.153	0.033	100 μg/kg bw/day [14,30,31,32]
Cr	0.079	0.458	0.033	0.595	0.045	0.058	2.2 μg/kg bw/day [7]
Cu	0.500	0.787	1.158	1.062	0.495	0.466	15 - 500 μg/kg bw/day [7,22]
Fe	3.579	6.754	8.300	8.025	2.550	2.415	10 −18 mg/day/person [7,14]
K	696	667	966	1129	16.775	15.920	3510 mg/day/ adult person [33]
Mn	4.729	9.145	13.49	13.59	0.200	0.150	2−5 mg/day/person [7,14]
Na	23.24	24.96	23.55	23.66	17.883	17.495	2000 mg/day/ adult person [16]
Ni	0.258	0.325	0.310	0.425	0.241	0.191	5 μg/kg bw/day [7,14,22]
Pb	0.266	0.312	0.445	1.041	0.279	0.216	7.14 μg/kg bw/day [7,14,22]
Zn	3.563	8.850	12.641	11.866	0.725	0.662	1000 μg/kg bw/day [14,34,35]

all samples of *Jou* ranged from 2.550 to 8.330 μg/kg bw/day and Mn varied from 0.150 to 13.59 μg/kg bw/day. The daily intake values of Fe and Mn in the present study were within the range of 0.85–2.77 % and 0.24–16.308 % of upper limit of RDA for Fe and Mn respectively. The highest level of EDI was observed for JF samples.

EDI values for K for *Jou* consumption ranged from 696 to 667, 966–1129 and 16.775 to 15.920 μg/kg bw/day for JB, JF and JG respectively. Highest daily intake of K was observed with JF samples as compared to JB and JG. Na consumption daily intake ranged from 17.495 to 24.96 kg bw/day for all *Jou* samples. Highest intake of Na was observed with JF.

Tolerable daily intake of Ni and Pb are 5 μg/kg bw/day and 7.14 μg/kg bw/day for a 60 kg adult, respectively [7,13,22]. EDI of Ni ranged from 0.258 to 0.325 μg/kg bw/day, 0.310 to 0.425 μg/kg bw/day and 0.241 to 0.191 μg/kg bw/day for JB, JF and JG respectively. All values were within the tolerable range of Ni as specified by WHO, 1993 [22]. Higher intake of Pb is observed with JF (0.445–1.041 μg/kg bw/day) as compared to JB (0.266−0.312 μg/kg bw/day) and JG (0.279−0.216 μg/kg bw/day). The EDI values from the consumption of alcoholic beverages in this study ranged from 0.216 to 1.041 μg/kg bw/day [19]. The highest intake of Pb was associated with *Joufinai* (JF). The values were within RDA of Pb (Table 3). The consumption of average 250 mL of *Jou* per day an adult will consume 3–14 % of daily tolerable intake. The RDA for Zn is 1000 μg/kg bw/day as per guidelines of WHO, 1982 (Table 3). EDI values for consumption of *Jou* range from 3.563 to 8.850 μg/kg bw/day for JB,12.641 to 11.866 μg/kg bw/day for JF and 0.725 to 0.662 μg/kg bw/day for JG, and all values were within the tolerable intake values of Zn as specified by FAO/WHO and EVM.

3.4. Health risk assessments

Health risk assessments with heavy metals associated with *Jou* consumption was estimated by target hazard quotient (THQ) and total target hazard quotient (TTHQ). The results of target hazard quotients (THQ) for *Jous* were presented in Table 5. Estimated THQ values from consumption of 250 mL per day of JB, JF and JG were less than 1 for heavy metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. THQ values interpretations are binary; THQ is <1 or >1, when THQ > 1; indicates a health concern but THQ is not a measure of risk [5]. We observed for each individual metals Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn; for *Jous*; THQ < 1 indicated the safe level for the consumption with an indication of no matter of concern or health risk as per guidelines of USEPA, 2000 and WHO, 2004 [35,36].

But, summation of THQs or total target hazard quotients (TTHQ) for individual metals against JF (*Joufinai*) beverages were found >1. Therefore, both JFs are associated with possible health risk for consumption in terms of TTHQ. For JBs and JGs the TTHQ values were <1; or very close to 1; presented less possible health risk for consumption. The level of TTHQ for *Bodo* alcoholic beverages could be summarized as TTHQ of JF > TTHQ of JB > TTHQ of JG. Less associated hazardous health risk was observed in distilled variety as compared to freshly prepared one alcoholic beverages (Fig. 3).

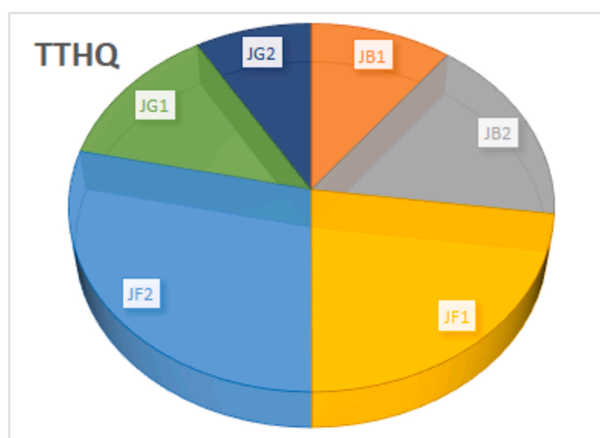

Fig. 3. TTHQ for heavy metals from consumption of *Jous*.

Table 5
THQ for heavy metals based on 250 mL per day consumption for an adult of 60 kg.

AB's	THQ of heavy metals in *Jou* samples										
	Cd	Co	Cr	Cu	Fe	K	Mn	Na	Ni	Pb	Zn
JB1	0.024	0.160	0.000	0.003	0.007	0.004	0.187	0.010	0.017	0.412	0.003
JB2	0.030	0.184	0.000	0.028	0.014	0.009	0.235	0.110	0.042	0.652	0.028
JF1	0.042	0.143	0.000	0.042	0.017	0.139	0.283	0.162	0.061	0.889	0.042
JF2	0.060	0.287	0.001	0.038	0.016	0.140	0.307	0.377	0.057	0.906	0.038
JG1	0.000	0.205	0.000	0.018	0.005	0.002	0.175	0.101	0.003	0.509	0.018
JG2	0.000	0.164	0.000	0.016	0.005	0.001	0.138	0.078	0.003	0.266	0.016

AB's stands for alcoholic beverages. JB, JF and JG stand for *Joubidwi*, *Joufinai* and *Jougwran*, respectively. 1 and 2 stand for first and second samples respectively. THQ = Target Hazard Quotients, TTHQ = Total Target Hazard Quotients.

4. Conclusion

The present study indicates that the metals are present in rice beer *Jou* (*Joubidwi*, *Jougwran* and *Joufinai*) at concentrations below the maximum permissible limits as specified by WHO for drinking water. In comparison to other alcoholic beverages of NE-India (*Deori*, *Ahom* and *Mising* rice beers), *Bodo* rice beer contains Cr in the range 0.008−0.143 mg/L while for other varieties Cr content is not detected [3]. The estimated daily intakes of metals from consumption of *Joubidwi*, *Jougwran* and *Joufinai* are within the recommended daily intakes. From the estimated target hazard quotient values, we observe no health risk associated with the consumption of *Bodo* rice beer.

Declaration of Competing Interest

Authors have no conflict of interest.

Acknowledgement

The authors would like to thank the Department of Environmental Science, Tezpur University, Assam, India for instrumental support.

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