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Bentum J. K.*[1], Acquah-Baidoo, D[1] Boafo F[1] , Agor-Woananu, S[2] and Cole[3], P.K.

[1]*Department of Chemistry, University of Cape Coast Ghana*
[2]*Department of Laboratory Technology, University of Cape Coast Ghana*
[3]*Department of Mathematic and Statistics, University of Cape Coast Ghana*
*(*Corresponding Author jbentum@ucc.edu.gh)*

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Abstract

Fruit juices are a highly valued, tasty food and usually have exceptional nutritional qualities. However, they can be a probable source of toxic elements. The presence of potentially toxic heavy metals in fruits is of intense public interest and requires rapid assessment of the levels of these contaminants and their potential to pose hazard. The aim of this study was to assess the levels and potential health of three essential heavy metals Cu, Zn and Fe in sugarcane juice sold by vendors in the Cape Coast Metropolis. Samples of sugarcane were obtained from ten vendors over a period of five weeks in November 2013. Three samples were collected weekly. The juice were quizzed from the fruits. The weekly samples from each vendor were composited and then filtered.10 mL of the homogenized juice was digested with Nitric acid and the metals analyzed using 210VGP Flame Atomic Absorption Spectrophotometer. The risk was assessed by evaluating the hazard quotient, HQ and hazard index, HI. The overall mean concentration of metals in the fifty samples sugarcane juice were Cu, 0.31 mg/L; Zn, 1.37 mg/L and Fe, 9.79 mg/L. The mean concentration of metals in the sugarcane collected from the ten vendors over the period ranged between 0.20 and 0.46 mg/L for Cu; 0.30- 2.80 mg/L and for Zn 7.13 -13.04 for Fe. The HQs and HI were all less than 1, suggesting that adverse effects are unlikely.

Keywords: *Sugarcane juice, Hazard, Risk, Heavy metals*

Introduction

Sugarcane is an important industrial crop cultivated in tropical and subtropical regions of the world. Sugarcane juice is the opaque and viscous liquid, brownish to deep-green in colour, obtained by pressing sugarcane stalks (Heuzé, 2012). It has been used as a sweetener for many years.) (Phanikumar, 2011), and possesses therapeutic value (Banerji et al., 1997). Sugarcane juice, a nutritious juice, is used as a drink in both urban and rural areas. It contains natural sugars (fructose), carotene, minerals like chromium, cobalt, copper, iron, magnesium, potassium, calcium, zinc, phosphorous and some organic acids such as malic, succinic, acotinic, amino acids, protein, starch, gums, waxes, non-sugar phosphatides (Parvathy, 1983; Swaminathan, 1995).

Sugarcane juice has broad biological effects in raising innate immunity to infections (Lo et al., 2005). Conditions, loss of milk production, cough, anaemia, constipation as well as general debility. Some texts advise its use for jaundice and low blood pressure (Kadam et al., 2008). It has been used in the Ayurveda and Unani systems of medicine in India, since time immemorial. Sugarcane extract has displayed a wide range of biological effects including immunostimulation (El-Abasy et al., 2002), anti-thrombosis activity, anti-inflammatory activity, and vaccine adjuvant modulation of acetylcholine release and anti-stress effects (Barocci et al., 1999). It has observed that sugarcane juice is more effective rehydration drink than plain water (PW), and sports drink (SpD) in post exercise as it enhances muscle glycogen resynthesis (Kalpana et al., 2013). It is beneficial long-term health effects, such as decreasing the risk of cancer and heart disease (AMES, 1998: Hollman et al., 1996). Juice from artisanal production and surplus juice from sugar factories (when sugar prices are low) often goes to animal feeding (OECD, 2011; Myer et al., 2001). The apparent energy digestibility of sugarcane juice is close to 100%, due to its high content of water soluble sugars, which are entirely digestible (Xandé et al., 2010).

For the past few years, research on sugar cane are focused on the effect of application of sewage sludge and fertilizers (Thiago Nogueira et al., 2013; Marques et al., 2007; Nogueira et al., 2007; Camilotti et al., 2009), phytoavailability of high natural soil borne heavy metal (Blanche and Doelsch, 2010); adsorption of heavy metal by sugarcane bagasse or chemically modified sugarcane bagasse and chemically modification of sugarcane bagasse (Homagai et al., 2010; Gurgel and Laurent Fre´de´ric Gil, 2009; Khosravi-Darani and Zoghi, 2008; Osvaldo et al, 2007)

The composition of sugarcane juice varies within limits according to the variety, age and health of the sugarcane, environment, agricultural planning (maturation, harvest period, handling, transportation and storage), pests and diseases (OECD, 2011). It is reported that, sugar cane bioaccumulates and biomagnifies toxic metals. Some metals have been detected in the sugarcane juice (Collin & Doelsch, 2010), some at levels above recommended limits (Abdus-Salam et al., 2008). The accumulation of heavy metals is potentially most harmful in cereals and vegetables rather than to sugarcane which is not used for immediate human consumption, reducing the risk to human health. Consequently, sewage sludge his been applied on sugarcane field (Marques et al., 2007; Nogueira et al., 2007; Camilotti et al., 2009). However, sugarcane juice is consumed worldwide, and due to its known health benefits, it is a traditional food with a profound presence in the local cultures where it is grown Brazil and India are the top two producers, though it is grown in over 100 countries. Sucking or chewing on an exposed end of the raw sugarcane is one way to consume the juice, although the juice can also be extracted in larger quantities for drinking (McCaffrey, 2011),

Though sugarcane juice has numerous health benefits, consumption of the raw juice has the potential to cause hazards if the juice is contaminated with high levels of toxic heavy metals as diet is the main source of toxic metals exposure to humans (Al-Jedah and Robinson, 2002). Several cases of human disease, disorders, and malformation of organs due to metal toxicity have been reported (Jarup et al., 1998). Metals such as lead, and copper are cumulative poisons, and cause environmental hazards. Chronic low-level intakes of heavy metals have been found damaging effects on human beings (Ellen et al., 1990). In Ghana the analysis of heavy metals in fruit juice, especially sugarcane, their toxicity and contribution to the recommended intake have not been studied extensively. The determination of heavy metal ions in sugarcane juice is not only of interest for environmental monitoring but also for purposes of and health impacts as high concentrations of essential elements like Cu and Zn can be very toxic. It is important therefore to monitor the dietary intake of metals in sugar cane. The objectives of this study were to determining the levels of three essential heavy metals Cu, Zn and Fe in sugarcane juice sold by vendors in the Cape Coast Metropolis, and to assess the hazards pose to consumers.

Materials and Methods

Sampling and Treatment

Samples of sugarcane were obtained from ten vendors over a period of five weeks in November 2013. Three samples were collected weekly. The juice were quizzed from the fruits. The sugarcane juice obtained from the weekly samples from each vendor were composited and then filtered. 10 mL of homogenously mixed juice samples were taken for analysis. 5 mL of sample was measured into platinum crucibles. The samples were evaporated on hot plate 105°C till dryness, and subsequently ashed in the Muffle furnace at a temperature 500ºC for 6 hours, and then cooled in desiccator. 5 mL (65 %, HNO_3) was added to the ashed sample and the solution heated on hot plate until the ash dissolved. Deionized water was added and filtered into 50 ml volumetric flask. The resultant solution was top up to the mark. Blank solution was treated the same way as the sample. Buck Scientific 210VGP Flame Atomic Absorption Spectrophotometer was used to analyzed Cu Fe and Zn and standard solutions and the samples at appropriate wavelength.

Quality Control

The recovery test of the total analytical procedures was carried out for the metals analyzed in the selected samples by spiking replicate samples with aliquots of metal standards and then reanalyzed the samples. The recoveries obtained for Cu, Fe and Zn were 95±1%, 96±1%, and 97±1% respectively. Relative Percent Different (RPD%) was used to evaluate the precision from analysis of spike duplicate. The RPD% were less than 10% for the metals. Samples were handled carefully to avoid contamination and to ensure reliability of results. Three standards with a minimal values of determination coefficient of 0.99, 0.98 and 0.99 were obtained for Cu, Fe, and Zn standard curves respectively. The metal content of the samples were derived from calibration curves.

Hazard analysis

Risk to human health by the intake of metal-contaminated fruit juice was characterized using a hazard quotient (HQ) (US EPA, 1989). It is the ratio between exposure and the reference oral dose (R_fD). If the ratio is lower than 1, there will be no obvious risk. An estimate of the potential hazard to human health through consumption metal-contaminated sugarcane juice was calculated using the equation below (Wang *et al.,* 2005; U.S. EPA 20):

$$HQ = EDI/\ R_fD = ADD/\ R_fD$$

Where *(EDI)* is the Estimated Daily Intake of fruit juice (mg/kg- day), ADD is Average Daily Dose (mg/kg-day) R_fD is the oral reference dose for the metal (mg/kg of body weight per day), R_fD is an estimate of a daily oral exposure for the human population, which does not cause deleterious effects during a lifetime, generally used in EPA's noncancerous health assessments.

The estimated daily intake (EDI) of each heavy metal in this exposure pathway was determined by the equation

$$EDI = \frac{(C_m \times E_D \times E_F \times F_{IR})}{W_{AB} \times T_A}$$

Where E_F is the exposure frequency (365 days/year); E_D is the exposure duration, equivalent to average lifetime (70 years); F_{IR} is the ingestion rate (g/person/day); C_m is the heavy metal concentration in foodstuffs (mg/kg Dw.); W_{AB} is the average body weight (bw) (average adult body weight was considered to be 75 kg; and TA is the average exposure time for non-carcinogens (equal to $E_F \times E_D$) (Saha and Zeman, 2012). To evaluate the potential risk to human health through more than one heavy metal, the hazard index (HI) has been developed (US EPA, 1989). The hazard index is the sum of the hazard quotients, as described below:

$$HI = \sum HQ \quad = HQ_{Cu} + HQ_{Zn} + HQ_{Fe}$$

It assumes that the magnitude of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ.

Data Analysis

The data obtained were evaluated statistically using Microsoft Excel 2007 and SPSS computer software package version 16. Descriptive statistics, Analysis of Variance (ANOVA) and Pearson's product moment correlation were used to ascertain the mean levels and effects. The analyses were done at 0.01 and 0.05 levels. All other calculation were done using Microsoft Excel 2007.

Results and Discussion

The descriptive statistics of the data obtained from the analysis of Fe, Zn and Cu metals in sugarcane juice from ten different vendors from the Cape Coast Metropolis (Table 1) showed that the concentrations (mean ± sd) of Fe, Z n and Cu were 9.78±2.88 mg/L, 1.37±1.18 mg/L, and 0.31±0.11 mg/L respectively. Zn showed the greatest variation in the levels of the metals in the 50 samples of juice with a coefficient of variation of 85.87% followed by Cu 36.585 and Fe the least, 29.47%. Table1 is the descriptive statistics for metals in sugar cane juice.

Table 1: Descriptive statics for metals in sugar cane juice

	Cu	*Fe*	*Zn*
Mean (mg/L)	0.31	9.78	1.37
Median (mg/L)	0.30	10.00	1.05
Mode (mg/L)	0.30	11.00	0.15
SD (mg/L)	0.11	2.88	1.18
Sample Variance	0.01	8.31	1.38
CV (%)	36.58	29.47	85.87
Range (mg/L)	0.75	14.65	5.50
Minimum (mg/L)	0.10	4.75	0.05
Maximum (mg/L)	0.85	19.40	5.55
n	50	50	50

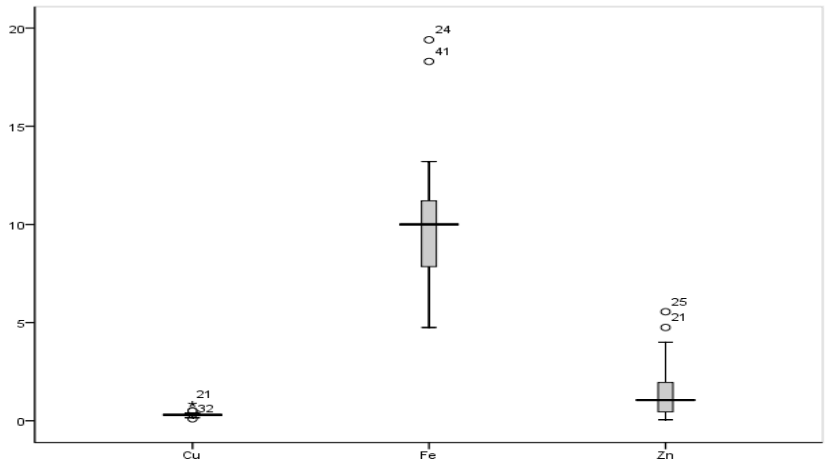

Fig 1: Box plot for Cu, Zn and Fe in sugarcane Juice

The box plot(Fig. 1) indicates that the distribution of Cu was almost normal and symmetric with the mean, median and mode being 0.31 mg/L, 0.30 mg/L and 0, 30 mg/L respectively. The distribution of Zn was slightly positively skewed with a mean of 1.37 mg/L > median 1.05 mg/L > mode 0.15 mg/L. The distribution of Fe was slightly negatively skewed with a mean of 9.79 mg/L, median 10.0 mg/L and mode 11.0 mg/L. There was a significant differences in the concentrations of Cu and Fe, and Fe and Zn in the sugarcane juice at the $p<.001$ level for the three conditions [$F (42, 7) = 0.385$, $p = 0.975$] and [$F (42, 7) = 1,712$, $p = 0.235$], respectively. However there was no significant difference between the levels of Cu and Zn [$F (33, 16) = 4, 1,26$, $p = 0.002$].

Table 2 Concentration of Fe in sugar cane juice from Cape Coast

		Fe (mg/L)			
Sample	week 1	week 2	week 3	week 4	week 5
AM	11.7	11.05	11.8	10.9	18.3
AB	11	7.1	11.25	5.1	7.8
AK	7.95	8.3	8.65	12.25	12.4
PD	11.8	10.6	19.4	12.3	11.1
AP	7.8	5.8	8.4	9.5	10.75
SD	6.6	11.2	10.65	9.25	10
KT	8	10	9.05	8.25	11
KY	8	8.5	6.15	7.65	6.9
EW	5.25	4.75	6.8	7.85	11
ADS	11.1	10.05	11.75	13.2	13
Mean	8.92	8.74	10.39	9.63	11.23
SD	2.31	2.25	3.73	2.54	3.11
CV	25.85	25.78	35.94	26.38	27.68

The analysis of Fe, Zn and Cu metals in sugarcane juice from ten different vendors from the Cape Coast Metropolis for five consecutive weeks are shown in tables 2-4.

Table 3 Concentration of Zn in sugar cane juice from Cape Coast

	Zn (mg/L).				
	week 1	week 2	week 3	week 4	week 5
AM	1.1	0.5	4.75	2.85	2.8
AB	0.55	0.05	0.3	0.25	0.3
AK	0.95	1	0.15	1.05	2.55
PD	1.8	1.3	1	0.5	2
AP	0.8	1.8	5.55	2.25	2
SD	0.45	0.4	2.3	0.35	0.4
KT	0.15	1.45	0.5	4	1.05
KY	0.6	1.95	1.7	2.4	0.4
EW	0.15	1.9	1	2.05	1.75
ADS	1.45	1.45	0.3	0.55	1.6
Mean	0.80	1.18	1.76	1.63	1.49
SD	0.54	0.67	1.92	1.27	0.91
CV	67.25	56.75	109.43	78.33	61.17

Table 4: Concentration of Cu in sugar cane juice from Cape Coast **.**

	Cu (mg/L).				
	week 1	week 2	week 3	week 4	week 5
AM	0.35	0.25	0.85	0.5	0.35
AB	0.25	0.35	0.35	0.1	0.25
AK	0.3	0.25	0.3	0.3	0.25
PD	0.3	0.4	0.35	0.35	0.4
AP	0.2	0.2	0.5	0.4	0.3
SD	0.35	0.35	0.35	0.35	0.3
KT	0.35	0.3	0.3	0.35	0.4
KY	0.2	0.2	0.15	0.3	0.15
EW	0.2	0.2	0.25	0.25	0.2
ADS	0.3	0.3	0.3	0.3	0.4
Mean	0.28	0.28	0.37	0.32	0.30
SD	0.06	0.07	0.19	0.10	0.09
CV	22.59	25.53	51.44	32.27	29.40

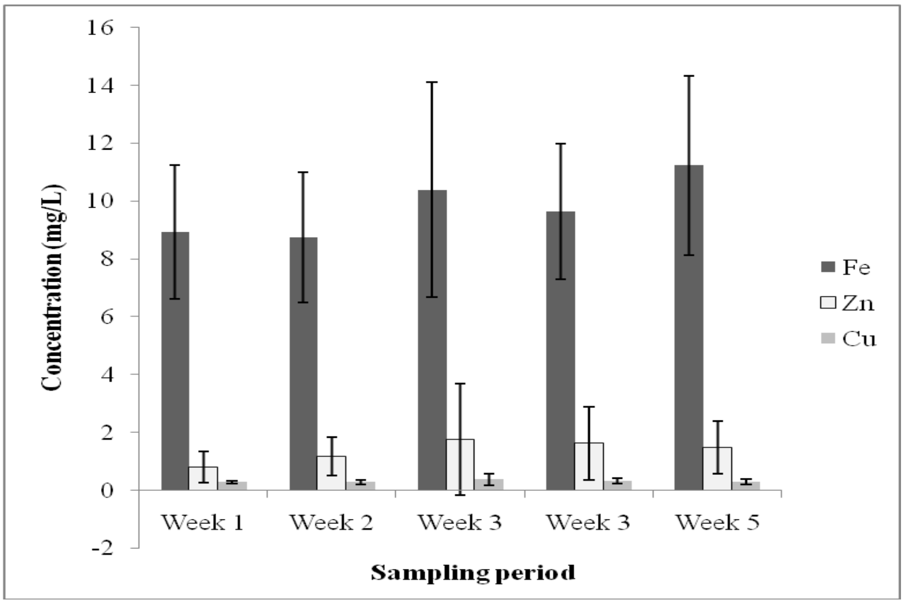

Fig 1: Mean weekly distribution of Fe, Zn and Cu metals in sugarcane juice sampled from vendors in the Cape Coast Metropolis.

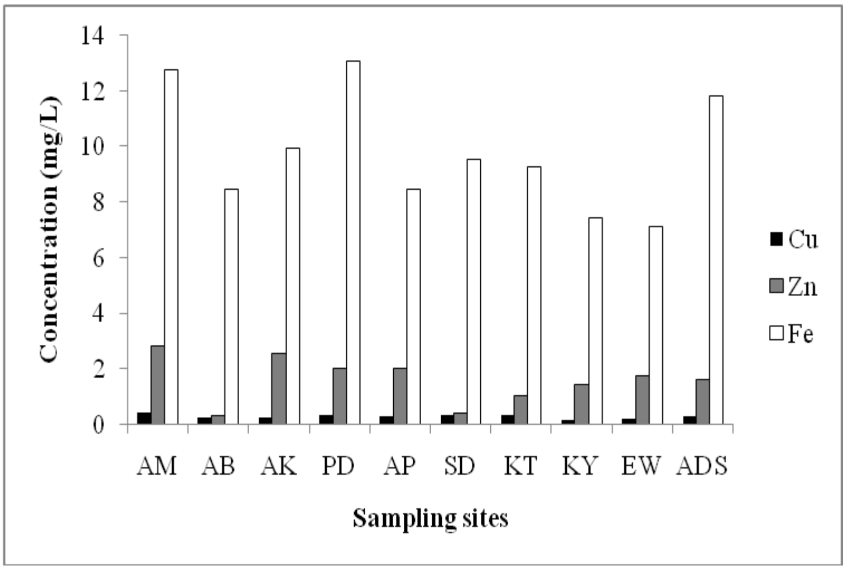

Figure 2: Mean levels of Fe, Zn and Cu metals in sugarcane juice sampled from vendors in the Cape Coast Metropolis.

The concentrations (mean ± CV) of the metals in the sugarcane juice collected from the individual vendors for five consecutive weeks (Fig. 2) ranges from 0.20±12.30 -0.46±51.21 mg/L Cu; 0.30±59.40 - 2.80±59.7 mg/L for Zn and 7.13±34.93 -13.04±27.72 for Fe. The median for the metals Cu, Zn and Fe were 0.32, 1.68 and 9.40 mg/L respectively. Generally samples from AM recorded very high mean concentration of the metals 0.46± mg/L, 2.80± mg/L and 12.75± for Cu , Zn and Fe respectively, whilst samples from AB recorded relatively lower mean concentrations (Fig. 2). The variation in the mean levels of the metals for the five week period were greatest for Zn, 217. 66% at site SD, Cu 51.21 at site AM and Fe 24.53%at site EW. The lowest variations were Cu, 9.78%; Zn, 34.14% and Fe 11.15% recorded at site ADS, AK and AK respectively. There were significant correlation among some of the metals at 0.01 level (Table 5); between Cu and Fe (n= 50, r = 0.396) and between Cu and Zn (n= 50, r = 0.528).

Table 5: Correlation matrix for metals in fifty samples of sugarcane juice

	Cu	*Fe*	*Zn*
Cu	1		
Fe	0.396**	1	
Zn	0.528**	0.135	1

**Correlation is significant at the 0.01 level (2-tailed).

Table 6: Correlation matrix for mean concentrations of metals in sugarcane juice from ten sampling sites

	Cu	Zn	Fe
Cu	1		
Zn	0.500*	1	
Fe	0.809**	0.532*	1

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Very high significant correlation was observed between Fe and Cu (n=10, r =0.809, p<0.01) for the mean concentrations of metals at the sites (Table 6). Accumulation of high levels Fe and Cu could serve as a dietary risk factors for Alzheimer's disease, which has become a global health concern (Loef & Walach, 2012). Moderate correlation were observed between Fe and Zn n=10, r =0.532, p<0.05), and Cu and Zn (n=10, r =0.50, p<0.05).

Health Hazards

The exposure pathway of heavy metals to human through ingestion of contaminated food has been studied by many researchers (Copat *et al.*, 2012; Xue *et al.*, 2012; Chary *et al.*, 2008). Although the HQ-based risk assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of the risk level due to exposure to pollutants (Chary et al., 2008). Many researchers consider the risk estimation method reliable (Chary et al., 2008; Khan et al., 2008; Wang et al., 2005) and it has been proven to be valid and useful.

The HI for adult was calculated by using the reference oral doses (RfD) for, Cu 4.0 x 10^{-2}, Zn, 3.0 x 10^{-1}, Fe, 7.0 x 10^{-1} mg/kg/day respectively (USEPA, 2009). The ingestion rate (F_{IR}) the used was based on daily intake of 4 oz (114 g/person/day) for 100% fruit juice recommended by panel of expert (Popkin et al., 2006). The HQ for the metals ranges from 0.07 to 0.62. HQs for means and weekly sampled sugarcane juice in this study were all below 1.0 for all HMs. The HRI were all below 1.0 The (Table 7).

Table 7: Hazard Quotient and Hazard Index for Fe, Zn and Cu in Sugarcane juice .

	Fe (mg/L)	HQ	Cu (mg/L)	HQ	Zn (mg/L)	HQ	∑HQ
Week 1	8.92	0.49	0.28	0.16	0.8	0.04	0.69
Week 2	8.74	0.48	0.28	0.16	1.18	0.07	0.71
Week 3	10.39	0.58	0.37	0.21	1.76	0.10	0.88
Week 3	9.63	0.53	0.32	0.18	1.63	0.09	0.80
Week 5	11.23	0.62	0.3	0.17	1.49	0.08	0.87
mean	9.78	0.54	0.31	0.17	1.37	0.08	0.79

The sequence of mean HQ for adults followed the decreasing order Fe (0.54) > Cu (0.31) > Zn (0.08). Since the HQ and HI were all less than 1, it means the exposed population is unlikely to experience obvious adverse effects; there is no apparent chance of non-carcinogenic effects. The chance for effect increases with an increasing probability as the value increases.

Our study has limitations. Firstly, vendors could not indicate exactly where the sugar cane were cultivated. Secondary, since the juice obtained from sugar cane collected from each vendor on the day of sampling were composited, even though the sources of the sugar cane were unknown, there might be mixing of sugar cane from different sources. Hence discussion on the sources and levels of metals extruded into the sugar cane juice from different farms is restricted.

Conclusions

The overall mean concentration of metals in the fifty samples sugarcane juice were Cu, 0.31 mg/L; Zn, 1.37 mg/L and Fe, 9.79 mg/L. The weekly means concentration of the metals Cu, Zn, and Fe ranges from 0.28-0.37, 0.80-1.76 and 8.74-11.23 respectively. The mean concentration of metals in the sugarcane collected from the ten vendors over the period ranged between 0.20±12.30 -0.46±51.21 mg/L for Cu; 0.30±59.40 - 2.80±59.7 mg/L for Zn and 7.13±34.93 -13.04±27.72 for Fe. Significant correlation were observed at 0.01 level between Cu and Fe (n= 50, r = 0.396) and between Cu and Zn (n= 50, r = 0.528). The HQs for Cu, Fe and Zn were 0.17, 0.54 and 0.08 respectively. The hazard index was less than 1, suggesting that adverse effects are unlikely.

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