Heavy Metal Content of Selected Some Turkish Beers

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Abstract
In this study, concentration of Cr, Fe, Ni, Cu, Zn, Cd, Sn and Pb heavy metals present in 23 different beers produced by the largest beer maker company of Turkey is determined using the electrothermal atomic absorption spectrometry (ETAAS) technique. Results were compared by considering can-bottle, low alcohol containing- high alcohol containing and dark-light beers. Comparison has been made on results with the help of the t test. It is concluded that only Cr concentration of can beers is higher than that in bottle packaging. All the results are below the permissible values. Average values for 23 beers are 50.9 for Cr, 82.4 for Fe, 57.5 for Ni, 82.5 for Cu, 425 for Zn, 3.70 for Cd and 12.5 μg/L for Pb. Standard addition technique has been used with arbitrary beer samples for accuracy of the method and recovery values are found between 92% and 104%.

Keywords: Beer, alcoholic beverage, heavy metal determination, ETAAS, metal contamination

1. Introduction

In literature, studies devoted to heavy metal analyses of alcoholic beverages are fewer than other food samples. First studies in this field appeared in literature at early 70's [1,2]. Numerous studies followed in literature after 1995. Concentrations of heavy metals in wine have been reported in most of these studies [3-21]. In these studies, mostly atomic spectroscopic methods have been used. These methods include flame atomic absorption spectroscopy (FAAS) [3-8], electrothermal atomic absorption spectroscopy (ETAAS) [9-12], inductively coupled plasma–optical emission spectroscopy (ICP-OES) [13-17] and hydride generation atomic absorption spectrometry (HGAAS) [18-19]. Since limit of quantitation (LOQ) is relatively high on FAAS method, enrichment processes have been executed at first in these studies which were mostly applied on wines. In addition, the number of analyzed elements is limited in HGAAS method where applications for as and Pb elements are frequent in literature. Due to their limits of quantification, ETAAS and ICP-OES are more appropriate methods.
Recently, electrochemical and X-ray fluorescence techniques have also been reported in literature. Especially, electrochemical stripping techniques and differential pulse polarography have been used for this purpose [20, 21]. In addition, energy dispersive X-ray fluorescence method (EDXRF) is recommended because it is fast and practical [22, 23].
Heavy metal studies for wine exist but similar studies devoted to heavy metal analysis for beers remain very limited and local. The limited studies about heavy metal determinations for beers have been published in literature [24-28]. Only few studies have been made on the Turkish wines in literature [19, 29, and 30]. Any study about heavy metal ingredient of beers produced in Turkey has not been reported yet. At 2008, Ibanez et al. have submitted a good review about the issue of alcoholic beverages including heavy metal ingredient [31] where, many references about heavy metal studies have been cited from literature. Heavy metals of alcoholic beverages might be derived from raw materials, additives, process and bottling. Especially old boilers are seen as the most important reason of heavy metal ingredient [32]. Data from literature have been evaluated in Ibanez's article and it concluded that Cu concentration in many alcoholic beverages spread in a wide range for Beer and Cognac [31]. Zn, Cu, Fe and Pb values of beers are slightly over than other alcoholic beverages [31].

This study has been carried out made to obtain data regarding the status of heavy metals in Turkish beers. For this purpose, Cr, Fe, Ni, Cu, Zn, Cd, Sn and Pb values of 23 different beers, which are produced by the largest beer maker company of Turkey, obtained from markets were determined using ETAAS technique and results were compared by considering can-bottle, low alcohol containing-high alcohol containing and dark-light beers. Alcohol strength comparison was considered for the effect of fermentation duration on heavy metal concentration and dark-light distinction was considered for the effect of blast process on metal concentration.

Beer Maker Company makes production in many parts of the world, especially several plants in Russia. For beer samples that have been randomly selected from the market, 8 of them have can and 15 of them have bottle packaging. Alcoholic strength for 5 of these beers is bigger than %6.1 and alcoholic strength for other 18 beers is %5 or less. Also, four of these beers are dark. 23 beers, which their heavy metal analyses have been done, are given in Table 1.

Table 1. Characteristics of beer samples used in the study.

Selected Beer Number	Manufacturer	Manufacturing location, geographical area and factory name	Can-bottle Trade name	Dark-light	Alcohol content %(V/V)	The expiry date
1	Anadolu Biracılık Efes Pilsen	Kazan Factory Ankara	Bottle (Efes Pilsen)	Light	5	09.11.2013
2		Kazan Factory Ankara	Bottle (Efes Pilsen)	Light	5	09.12.2013
3		Kazan Factory Ankara	Bottle (Efes Dark Brown)	Dark	6.1	23.07.2013
4		Kazan Factory Ankara	Bottle (Efes Extra)	Dark	7.5	19.05.2013
5		Kazan Factory Ankara	Bottle (Efes Light)	Light	3	15.07.2013
6		Kazan Factory Ankara	Bottle (Efes Malt)	Light	5	06.09.2013
7		Kazan Factory Ankara	Can (Efes Pilsen Fıçı)	Light	5	02.03.2013
8		Kazan Factory Ankara	Can (Efes Pilsen)	Light	5	11.12.2013
9		Kazan Factory Ankara	Can (Efes High alcohol containing)	Light	6.1	12.04.2013
10		Kazan Factory Ankara	Can (Efes Extra)	Dark	7.5	01.12.2013
11		Kazan Factory Ankara	Bottle (Efes Draft)	Light	5	01.12.2013
12		Kazan Factory Ankara	Bottle (Efes Dark)	Dark	6.1	16.09.2013
13		Haznedar Factory Istanbul	Bottle (Beck's)	Light	5	20.06.2013
14		Haznedar Factory Istanbul	Bottle (Beck's)	Light	5	18.02.2013
15		Haznedar Factory Istanbul	Can (Beck's)	Light	5	22.03.2013
16		Kazan Factory Ankara	Bottle (Miller)	Light	4.7	11.09.2013
17		Haznedar Factory Istanbul	Bottle (Bomonti)	Light	4.8	11.09.2013
18		Haznedar Factory Istanbul	Can (Bomonti)	Light	4.8	14.02.2013
19		Kazan Factory Ankara	Can (Miller)	Light	4.7	07.09.2013
20		Evrensekiz Factory Kırklareli	Bottle (Marmara Gold)	Light	4.1	03.02.2013
21		Haznedar Factory Istanbul	Bottle (Bomonti)	Light	4.8	03.02.2013
22		Kazan Factory Ankara	Bottle (Efes Pilsen Unfiltered)	Light	5	09.11.2013
23		Kazan Factory Ankara	Bottle (Gusta)	Light	5	02.02.2013

2. Experimental Part

2.1. Apparatus:

GBC Avanta PM model AAS (Atomic Absorption Spectrometer) with GF 3000 power supply and PAL 3000 auto sampler was used and atomization was achieved by graphite furnace electrothermally (GBC Scientific Equipment Pty. Ltd., Braeside, Victoria, Australia).

The elements were determined by ETAAS only. The matrix modifier has been used in only Sn determination [33,34]. For the Sn determination, 1% (v/w) $Mg(NO3)2$ was added to the standard and analyte solutions as matrix modifier.

All solutions were prepared with de-ionized water with 0.55 µS/cm conductivity. Calibration curves were obtained for 1 to 200 µg/L standard solutions prepared from 1000 mg/L commercial stock solutions (Merck, Darmstadt, Germany). The graphite oven temperature programs are shown in Table 2.

Table 2. Temperature programming of graphite cuvette using ETAAS method

Determined Element	Drying			Ashing			Reading			Cleaning		Inert Gas
	Ramp Time(s)	*°C*	*Hold. Time(s)*	*Ramp Time(s)*	*°C*	*Hold Time(s)*	*Ramp Time(s)*	*°C*	*Time (s)*	*°C*	*Time (s)*	
Sn	5	80	5	5	350	0	1.5	2400	1.1	2500	1	Ar
	5	120	10									
Cr	5	80	5	5	750	0	1.5	2500	1.5	2500	1	Ar
	5	120	10									
Fe	5	80	5	5	750	0	1.5	2400	1.5	2500	1	Ar
	5	120	10									
Ni	5	80	5	5	700	0	1.5	2000	1.5	2200	1	Ar
	5	120	10									
Cu	5	80	5	5	600	0	1.5	2000	1.5	2400	1	Ar
	5	120	10									
Zn	5	80	5	5	550	0	1.5	2000	1.5	2400	1	Ar
	5	120	10									
Cd	5	80	5	5	450	0	1.5	2000	1.4	2400	1	Ar
	5	120	10									
Pb	5	80	5	5	350	0	1.5	2200	1.5	2300	1	Ar

LOQ values were assessed with respect to standard methods designated in literature [35,36]. The value corresponding to 10 times the standard deviation of the blank solution has been designated as LOQ.

The obtained LOQ values are as shown in Table 3:

Table 3. The obtained LOQ values

Element	LOQ (µg/L)	Element	LOQ (µg/L)
Cr	1.95	Sn	3.60
Cu	1.34	Cd	0.36
Fe	2.42	Ni	2.20
Zn	0.68	Pb	2.70

In studies with the standards, the found regression values (R2) for calibration curves are 0.9950 for Cr, 0.9995 for Fe, 0.9524 for Ni, 0.9958 for Cu, 0.9935 for Zn, 0.9961 for Cd, 0.9166 for Sn and 0.9942 for Pb. In addition, due to the lack of a reference standard material, accuracy of the analysis and the effect of the matrices in the media were controlled with the standard addition method. All studied elements were tested with standard addition method for 8 randomly selected samples.

2.2. Preparation of the Beer Samples for Analysis

The beer samples were treated with hot HNO3 – H2O2 for decomposition of organic matrix [33, 34]. For each sample; 25.00 mL of beer was placed in a Kjeldahl flask. Then, 5.00 mL of the certificated HNO3 (63%, d=1.43 g/mL) and 5.00 mL of H2O2 were put in the same flask and the mixture was boiled for about half an hour until colorless. Later, this solution was placed in a 50.00 mL volumetric flask and diluted to 50 mL from where the samples were used for ETAAS analysis.

In this study, two different samples were taken from each beer. After separate digestions, two different solutions were obtained for each sample and two replicates were measured three times with ETAAS.

3. Results and Discussion:

Results are given in **Table 4, 5** and **6** according to can-bottle, dark-light and high alcohol containing (higher than 6.1%) low alcohol containing (5% or less) criteria. Results are given uncertainties as standard deviations. t test has been used to specify any significant difference between the results of every metal in groups above. Results of T test are under the tables. All results are in µg/L NO, ppb is equal to these values used only when density is 1.0 g/mL Since standard deviations in Fe determinations are high, results of Fe are not decimal but integer.

Results show that ranges for metals are 4.92-205.88 for Cr, 252-15 for Fe, 5.49-109.52 for Ni, 12.49-231.01 for Cu, 344.02-487.80 for Zn, 1.39-14.57 for Cd and 3.77-34.84 µg/L for Pb. Average values for 23 beers are 50.90 for Cr, 82.4 for Fe, 57.49 for Ni, 82.52 for Cu, 425.36 for Zn, 3.70 for Cd and 12.50 µg/L for Pb. These values are compatible with the results given in literature and correspond to ranges given in literature [24-28]. Heavy metal concentration of beers is higher for Cu, Zn, Cd and Pb; lower for Ni and Cr in literature [27]. There is an opportunity to compare Cu, Zn, Cd and Pb. Studies in literature show that Cu concentration is between 40-300 µg/L, Zn concentration is between 100-680 µg/L, Cd concentration is between 0.2-15 µg/L and Pb concentration is between 4-50 µg/L [24, 25, 27, 28, and 30]. No anomaly exists in our results. A definite result is that the heavy metal ion with higher concentration in Turkish beers is Zn. Results and literature clearly proves this. Nowadays, stainless steel boilers are used for production in all brewery plants. Stainless steel includes Cr, Fe and Ni where Zn is trace element. The facts that galvanized boilers were used and they caused high Zn concentration in old times were reported in literature. The cause of the high level of Zn concentration in our days cannot be metal components. Sources of several metals in alcoholic beverages have been examined before [32]. Previous studies have suggested that production and can packaging cause to Zn and Cu elements [28], Ni concentration does not differ between can-bottle [27]. Mayer has found and reported that Cu concentration is between 28-50 µg/L through electroanalytic methods, Pohl has found and reported that Cu concentration differs between 71-114 µg/L through FAAS method and Dugo has found and reported that Ni concentration in beers differs between 55.5-105.0 µg/L through electroanalytic methods. Their results are comparable to Cu and Ni results of this study but the observation for Ni is the opposite to this study's result. In our study, there is no difference between can and bottle for Zn and Cu but there is a difference for Ni. Zn and Cu are derived from potentially used material in beers because Zn results of all beers are approximately equal. The reason of this could be the process but also it could be the grain used because Zn and Cu are essential elements. It is stated that Zn concentration is particularly less in phytonutrient but especially in grain and barley, it is stated in literature that Zn is in form of Zn-phytate with phytic acid [37]. According to this and the results, we can say that the source of Zn and Cu are raw materials. Since Cd and Pb are not essential elements, the interpretation of Cd and Pb is the pollution of heavy metal in raw material. These metals have low melting temperatures and their environmental pollution would be on plants and soil through the vaporization - condensation. For this reason, it is possible that Pb and Cd concentrations are derived from environmental pollution.

All the results are below the permissible values. Acceptable limit values provided by Organization of International Vine and Wine (OIV) are 1000 µg/L for Cu, 5000 µg/L for Zn, 10 µg/L for Cd, 150 µg/L for Pb [38]. Only one of the results exceeds these limit values. Cd concentration of beer number 16 was found as 14.57 µg/L. This is different than others. It could be an experimental mistake but its standard deviation is low (0.43) and that is the reason of its addition to the list. OIV's limit values are high according to drinking water standards. Limit values are lower for drinking water standards. Permissible values in WHO Guidelines for Drinking-water Quality data are 50 µg/L for Cr, 20 µg/L for Ni, 100 µg/L for Cu, 5 µg/L for Cd and 10 µg/L for Pb [39]. In our average values, concentrations are high for Ni and Pb. But this is normal since there is no heavy metal removal action during the process; on the other hand a treatment process exists in city waters.

t test values are calculated to see any difference between the results of groups in Tables 4, 5 and 6 [40]. There is a significant difference between Cr values only for can and bottle beers. If $t_{calculated}$ values and $t_{critical}$ values given in Table 4 would be examined, it would be seen that $t_{calculated}$ value is higher than $t_{critical}$ for Cr. Cr concentration for cans is higher than bottles. Apart from that, concentrations of Fe, Ni, Cd, Zn and Pb have not affected can packaging. There is no difference in all other results. Alcoholic strength and beer being dark have not affected the heavy metal concentration. This can be seen in values of Table 5 and 6. For can packaging, the high level of this Cr concentration may be resulted from two sources. First, it could be the metallic boiler and pipes in production and second, it could be aluminum can itself. But if the production had caused that, Cr would be high also in bottle packaging. That is why the reason of this high level is probably Cr ions which have passed to beer from can surface. Due to this, three cans have been selected and their surfaces have been analyzed by both ETAAS and XRF methods. A part of approximately 1 cm^2 has been cut from selected cans and it has been weighed, after that it has been dissolved by boiling in HCl/HNO_3 (v/v: 3/1) mixture. Later, it has been diluted and concentrations of some metals were calculated with ETAAS. Inner surface of other 1 cm^2 can samples were analyzed with XRF method and results are given in Table 7.

As is seen, Ni and Pb concentrations are low if any in can composition. Composition of aluminum alloys like EN AW3004, EN AW3104, EN AW5086 given as can packaging material are similar in literature [41]. In many sources, it is stated that some sort of surface process has been made with chromate and phosphate ions for the purpose of surface hardening [41]. According to above results, only metal which can be affected by can packaging is Cr. Same interpretation is valid for Cu and Zn but our findings show that no significant difference exists among can and bottle. A similar situation is valid for Fe. Fe, Cu and Zn are the most important essential element for all living creatures. These elements are the richest essential elements of all livings [42]. That is why these elements are probably derived from raw material and process, not from packaging. It is not definite that Cr has been affected from can packaging but it can be said that the effect of can packaging to Cr does exist. This result shows that chromate is derived from both process and packaging because some Cr exists in bottle packaging, although all results are below the permissible concentrations.

As is seen from Table 1, beer being high alcohol containing or low alcohol containing or dark does not affect heavy metal concentration. Alcohol strength affecting heavy metal concentration is already an unexpected situation. The objective was to understand the effect of fermentation process but according to our results it is out of question. As it is stated at introduction, malt is more roasted in dark beers and this can affect the heavy metal concentration. Dark-light beer distinction is considered with this remark. Iron surplus stands out in dark beers but due to big standard deviation t test results show that Fe concentration is not different in dark beers, Table 6.

In this study, Sn determination has been performed in the beer. If the Sn determination is considered, LOQ value is high and that is why it is below the Sn detection limit for many beers. Sn has been found only in a few samples and standard deviation of these results is high. Results are not fit to be statistically evaluated. For instance, Sn value of beer number 19 was 4.27±0.64 µg/L on device but standard deviation is quite big and this value is at the limit of LOQ value with standard deviation.

Standard addition method has been used for accuracy of results. We did not have an appropriate standard reference material (SRM) for beer that is why standard addition has been done. Therefore, 8 beer samples were randomly selected at the end of each element analysis. Standard metal ion (10, 20 and 40 µg/L) has been added on top them and the absorbance has been checked. Concentrations found were estimated with concentrations calculated and recoveries % were achieved. Average recovery % values for each metal are given below. It is seen that recovery% values are in sufficient level.

Analyzed Metal	**Cr**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Sn**	**Pb**
Recovery values %	104.6	95.9	95.6	98.5	96.7	102.2	91.3	93.8

Table 4. The results according to can-bottle criteria (µg/L)

BEER		*Determined Metals*							
		Cr	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Sn**	**Pb**
CAN	**7**	51.60±0.21	ND	68.48±6.12	74.72±0.81	418.39±21.28	2.46±0.32	5.55±0.82	17.71±9.16
	8	77.16±1.92	122±2	109.52±3.08	144.92±2.01	466.07±12.58	2.14±0.16	ND	12.06±0.72
	9	78.60±1.28	21±7	83.43±2.34	66.62±10.40	420.82±17.58	4.69±0.45	ND	34.84±2.37
	10	76.56±0.28	28±5	78.87±14.56	46.89±5.76	417.34±26.16	2.06±0.23	5.40±0.37	12.80±1.09
	15	41.88±1.06	15±3	70.64±1.55	12.45±1.32	448.65±11.66	3.45±0.13	4.35±0.51	8.85±0.36
	18	205.88±2.08	119±10	73.69±10.60	71.36±0.90	487.80±22.44	1.39±0.27	ND	8.44±1.12
	19	182.92±6.24	49±9	94.82±0.89	60.90±0.93	388.87±16.89	1.89±0.03	4.27±0.64	5.75±0.30
	20	162.36±2.12	90±7	89.72±2.72	72.49±1.17	377.42±13.35	1.85±0.12	ND	8.70±1.06
	Average	**109.91±2.78**	**55.50±6.29**	**83.64±7.43**	**68.79±4.64**	**428.17±18.40**	**2.49±0.25**		**13.64±3.42**
BOTTLE	**1**	26.44±3.48	149±79	94.44±8.10	128.66±1.08	403.88±4.68	4.48±0.73	ND	22.14±3.59
	2	26.08±1.00	210±37	57.42±10.92	160.04±3.80	442.28±20.16	3.47±0.32	ND	13.11±0.68
	3	26.24±1.68	252±44	106.93±3.32	94.92±2.56	487.72±24.85	8.48±0.77	ND	5.64±1.02
	4	49.64±0.78	144±27	52.28±8.05	117.66±0.31	431.92±9.52	6.08±0.11	ND	16.89±2.34
	5	27.76±0.96	44±12	81.28±3.47	64.98±7.56	344.02±0.66	8.97±2.34	ND	11.73±1.08
	6	18.60±1.68	42±9	51.55±6.80	189.72±22.52	395.76±2.18	2.61±0.52	ND	17.07±0.86
	11	4.92±0.20	ND	38.30±3.97	42.10±4.36	420.80±18.16	2.40±0.12	4.51±0.17	13.66±1.07
	12	12.04±1.32	95±6	38.81±4.04	63.35±3.68	436.91±8.96	2.04±0.22	ND	12.10±0.22
	13	9.64±0.48	ND	64.85±2.72	22.20±0.95	374.88±3.71	2.99±0.34	ND	9.36±0.59
	14	7.44±0.16	ND	73.13±2.43	72.98±0.90	382.17±26.50	2.05±0.06	4.78±0.70	8.80±1.76
	16	24.92±1.40	ND	80.19±1.71	231.01±10.06	455.67±12.17	14.57±0.43	ND	10.38±1.43
	17	11.80±1.12	ND	32.86±4.56	61.81±2.88	487.96±22.44	1.41±0.23	ND	ND
	21	20.68±0.52	58±7	56.16±1.54	32.92±4.15	471.33±7.59	2.42±0.15	ND	3.77±0.07
	22	25.28±0.28	21±8	65.49±1.55	40.14±3.21	372.12±24.98	2.14±0.24	5.11±0.31	8.86±1.06
	23	9.60±0.40	37±11	66.74±3.72	25.35±12.68	452.19±8.72	1.19±0.15	ND	ND
	Average	**19.43±1.36**	**101.2±30.7**	**64.02±5.39**	**89.85±8.14**	**423.87±15.66**	**4.35±0.71**		**11.80±1.51**
	S_{pooled}	**33.83**	**61.18**	**17.78**	**53.49**	**40.08**	**2.14**		**6.45**
	$t_{calculated}$	**6.11**	**1.61**	**2.52**	**0,90**	**0.24**	**1.21**		**0.64**
	$t_{critical}$	**2.95-2.84**	**3.17-2.95**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**		**2.95-2.84**

ND: Not Detected

Table 5. The results according to high alcohol containing- (higher than 6.1%) - low alcohol containing (5% or less) criteria (µg/L)

BEER		Determined Metals							
		Cr	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Sn**	**Pb**
High alcohol containing (higher than 6.1%)	**9**	78.60±1.28	21±7	83.43±2.34	66.62±10.40	420.82±17.58	4.69±0.45	ND	34.84±2.37
	10	76.56±0.28	28±5	78.87±14.56	46.89±5.76	417.34±26.16	2.06±0.23	5.40±0.37	12.80±1.09
	3	26.24±1.68	252±44	106.93±3.32	94.92±2.56	487.72±24.85	8.48±0.77	ND	5.64±1.02
	4	49.64±0.78	144±27	52.28±8.05	117.66±0.31	431.92±9.52	6.08±0.11	ND	16.89±2.34
	12	12.04±1.32	95±6	38.81±4.04	63.35±3.68	436.91±8.96	2.04±0.22	ND	12.10±0.22
	average	**48.62±1.17**	**108.0±23.6**	**72.06±7.87**	**77.89±5.68**	**438.94±18.88**	**4.67±0.43**		**16.45±1.64**
Low alcohol containing (5% or less)	**1**	26.44±3.48	149±79	94.44±8.10	128.66±1.08	403.88±4.68	4.48±0.73	ND	22.14±3.59
	2	26.08±1.00	210±37	57.42±10.92	160.04±3.80	442.28±20.16	3.47±0.32	ND	13.11±0.68
	7	51.60±0.21	-	68.48±6.12	74.72±0.81	418.39±21.28	2.46±0.32	5.55±0.82	17.71±9.16
	8	77.16±1.92	122±2	109.52±3.08	144.92±2.01	466.07±12.58	2.14±0.16	ND	12.06±0.72
	5	27.76±0.96	44±12	81.28±3.47	64.98±7.56	344.02±0.66	8.97±2.34	ND	11.73±1.08
	6	18.60±1.68	42±9	51.55±6.80	189.72±22.52	395.76±2.18	2.61±0.52	ND	17.07±0.86
	11	4.92±0.20	ND	38.30±3.97	42.10±4.36	420.80±18.16	2.40±0.12	4.51±0.17	13.66±1.07
	15	41.88±1.06	15±3	70.64±1.55	12.45±1.32	448.65±11.66	3.45±0.13	4.35±0.51	8.85±0.36
	14	7.44±0.16	ND	73.13±2.43	72.98±0.90	382.17±26.50	2.05±0.06	4.78±0.70	8.80±1.76
	16	24.92±1.40	ND	80.19±1.71	23.01±10.06	455.67±12.17	14.57±0.43	ND	10.38±1.43
	17	11.80±1.12	ND	32.86±4.56	61.81±2.88	487.96±22.44	1.41±0.23	ND	ND
	21	20.68±0.52	58±7	56.16±1.54	32.92±4.15	471.33±7.59	2.42±0.15	ND	3.77±0.07
	22	25.28±0.28	21±8	65.49±1.55	40.14±3.21	372.12±24.98	2.14±0.24	5.11±0.31	8.86±1.06
	23	9.60±0.40	37±11	66.74±3.72	25.35±12.68	452.19±8.72	1.19±0.15	ND	ND
	18	205.88±2.08	119±10	73.69±10.60	71.36±0.90	487.80±22.44	1.39±0.27	ND	8.44±1.12
	19	182.92±6.24	49±9	94.82±0.89	60.90±0.93	388.87±16.89	1.89±0.03	4.27±0.64	5.75±0.30
	20	162.36±2.12	90±7	89.72±2.72	72.49±1.17	377.42±13.35	1.85±0.12	ND	8.70±1.06
	13	9.64±0.48	ND	64.85±2.72	22.20±0.95	374.88±3.71	2.99±0.34	ND	9.36±0.59
	average	**51.94±2.02**	**79.72±7.65**	**70.51± 5.18**	**83.82±7.10**	**421.68±15.99**	**3.44±0.63**		**11.27±2.62**
	S_{pooled}	**55.66**	**66.41**	**19.97**	**82.39**	**38.78**	**9.46**		**5.95**
	$t_{calculated}$	**0.14**	**0.80**	**0.16**	**0.14**	**0.88**	**0.25**		**1.72**
	$t_{critical}$	**2.95-2.84**	**3.17-2.95**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**		**2.95-2.84**

ND: Not Detected

Table 6. The results according to dark-light criteria (μg/L)

BEER		**Determined Metals**							
		Cr	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Sn**	**Pb**
Dark	**3**	26.24±1.68	252±44	106.93±3.32	94.92±2.56	487.72±24.85	8.48±0.77	ND	5.64±1.02
	4	49.64±0.78	144±27	52.28±8.05	117.66±0.31	431.92±9.52	6.08±0.11	ND	16.89±2.34
	12	12.04±1.32	95±6	38.81±4.04	63.35±3.68	436.91±8.96	2.04±0.22	ND	12.10±0.22
	10	76.56±0.28	28±5	78.87±14.56	46.89±5.76	417.34±26.16	2.06±0.23	5.40±0.37	12.80±1.09
	Average	41.12±1.14	129.7±26.1	69.22±8.72	80.71±3.65	443.47±19.18	4.66±0.42		11.86±1.39
Light	**1**	26.44±3.48	149±79	94.44±8.10	128.66±1.08	403.88±4.68	4.48±0.73	ND	22.14±3.59
	2	26.08±1.00	210±37	57.42±10.92	160.04±3.80	442.28±20.16	3.47±0.32	ND	13.11±0.68
	15	41.88±1.06	15±3	70.64±1.55	12.45±1.32	448.65±11.66	3.45±0.13	4.35±0.51	8.85±0.36
	18	205.88±2.08	119±10	73.69±10.60	71.36±0.90	487.80±22.44	1.39±0.27	ND	8.44±1.12
	5	27.76±0.96	44±12	81.28±3.47	64.98±7.56	344.02±0.66	8.97±2.34	ND	11.73±1.08
	6	18.60±1.68	42±9	51.55±6.80	189.72±22.52	395.76±2.18	2.61±0.52	ND	17.07±0.86
	11	4.92±0.20	ND	38.30±3.97	42.10±4.36	420.80±18.16	2.40±0.12	4.51±0.17	13.66±1.07
	7	51.60±0.21	-	68.48±6.12	74.72±0.81	418.39±21.28	2.46±0.32	5.55±0.82	17.71±9.16
	8	77.16±1.92	122±2	109.52±3.08	144.92±2.01	466.07±12.58	2.14±0.16	ND	12.06±0.72
	9	78.60±1.28	21±7	83.43±2.34	66.62±10.40	420.82±17.58	4.69±0.45	ND	34.84±2.37
	19	182.92±6.24	49±9	94.82±0.89	60.90±0.93	388.87±16.89	1.89±0.03	4.27±0.64	5.75±0.30
	20	162.36±2.12	90±7	89.72±2.72	72.49±1.17	377.42±13.35	1.85±0.12	ND	8.70±1.06
	14	7.44±0.16	ND	73.13±2.43	72.98±0.90	382.17±26.50	2.05±0.06	4.78±0.70	8.80±1.76
	16	24.92±1.40	ND	80.19±1.71	231.01±10.06	455.67±12.17	14.57±0.43	ND	10.38±1.43
	17	11.80±1.12	ND	32.86±4.56	61.81±2.88	487.96±22.44	1.41±0.23	ND	ND
	21	20.68±0.52	58±7	56.16±1.54	32.92±4.15	471.33±7.59	2.42±0.15	ND	3.77±0.07
	22	25.28±0.28	21±8	65.49±1.55	40.14±3.21	372.12±24.98	2.14±0.24	5.11±0.31	8.86±1.06
	23	9.60±0.40	37±11	66.74±3.72	25.35±12.68	452.19±8.72	1.19±0.15	ND	ND
	13	9.64±0.48	ND	64.85±2.72	22.20±0.95	374.88±3.71	2.99±0.34	ND	9.36±0.59
	Average	**53.34±1.98**	**69.8±24.6**	**71.17±5.07**	**82.91±6.77**	**421.63±17.02**	**3.50±0.62**		**12.66±2.63**
	S_{pooled}	**55.82**	**63.82**	**20.29**	**54.04**	**39.25**	**3.06**		**6.59**
	$t_{calculated}$	**0.40**	**1.65**	**0.17**	**0.07**	**1.01**	**0.69**		**0.22**
	$t_{critical}$	**2.95-2.84**	**3.15-2.95**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**	**2.95-2.84**		**2.95-2.84**

ND: Not Detected

Table 7. The heavy metal contents of three randomly selected beer cans.

Analyzed metal	Found with ETAAS %	Found on the surface with XRF %
Cr	0.05±0.01	0.033
Fe	0.48±0.06	0.309
Ni	UDL*	<0.01
Cu	0.14±0.1	0.139
Zn	0.72±0.02	0.343
Pb	UDL	<0.01
Cd	UDL	<0.01
Sn	UDL	<0.01

*UDL: Under Detection Limit
*Selected beer numbers are 7, 12, and 15

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