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TRACE METAL CONCENTRATION AND HUMAN HEALTH RISK ASSESSMENT IN DISTILLED ALCOHOLIC BEVERAGES IN ROMANIA

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ABSTRACT. The concentration of 12 metals (Mg, Ca, K, M, Fe, Co, Ni, Cr, Cu, Pb, Cd, and Zn) in 14 classes of alcoholic beverages were determined by ICP-MS after HNO_3/H_2O_2 digestion. The mean concentration of metals (µg/mL) in these alcoholic beverages varied in the ranges 0.26-15.43, 0.94-234.43, 0.56-278.02, 0.02-2.69, 0.18-2.64, 0.03-0.13, 0.03-0.13, 0.02-0.29, 0.04-2.51, 0.03-0.30, 0.02-0.04, and 0.13-0.88 for Mg, Ca, K, Mn, Fe, Co, Ni, Cr, Cu, Pb, Cd, and Zn respectively. The concentration of metals found in these particular alcoholic beverages was below the International Statutory Limits for metals in alcoholic beverages. The estimated daily intake of the metals based on a per capita consumption of 14.4 L per annum pure alcohol was lower than the tolerable daily intake of each metal. The individual and combined target hazard quotients of the metals were <1, indicating no long-term health concerns from the consumption of these alcoholic beverages based on their metal content alone.

Keywords: *alcoholic beverages, daily intake, target hazard quotients, Romania*.

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INTRODUCTION

The concentration of metals in many alcoholic beverages can be a significant parameter affecting their consumption and conservation. This derives from the negative and positive effects caused directly or indirectly by the presence of metals. Negative effects include beverage spoilage and hazing, as well as sensorial and health consequences [1]. Positive effects include the removal of bad odors and tasters [2], participation in fermentative processes [3], provision of pathways for dietary intake of some essential mineral [4], and usefulness for authentication purpose [5].

Metals find their way into alcoholic beverages at different stages and through various sources including raw materials, brewing, process type and equipment, bottling, aging/storage, and adulteration.

Several metal ions can be taken up from the surrounding soil by plants from which an alcoholic beverage is prepared. For instance, type of soil (i.e., it's geogenic), it's agrochemical treatments (e.g., the use of pesticides and fungicides), and the surrounding environmental pollution implies the mineral content of many beverages [6]. In this way, wines from vineyards in coastal areas are richer in Na [7]. Fungicides, pesticides, and fertilizers containing Cd, Cu, Mn, Zn and Pb, compounds can derive in increased contents of these metals in the alcoholic beverage [7]. Most of the Mg found in beer comes mainly from raw materials [1]. Cu in beer comes mainly from raw materials [4]; on the contrary, only a small percentage of the final Cu content in whiskey comes from the barely from which the spirit is distilled [8].

Hops, acids, silica gel, bases, flavoring agents, dilution water, additives, and stabilizers are potential sources of metal ions in the brewing process [4]. For example, the main source of Cu in wine is the $CuSO_2$ added to remove sulfidic odors [2]. The acidity of the liquor to be distilled may be important in this regard (e.g., in whiskeys), since mare acidic beverage tend to contain more Cu [8]. The addition of fining and clarifying substances (e.g., flocculants) to reduce turbidity can bring about an increase in Al, Ca, Na in wine [7].

Major differences in metal content (e.g., Ni) have been found among alcoholic beverages depending on their processing. In this way, certain fermented beverages (e.g., beer and wine) contain several times more Ni than distilled beverages (e.g., brandy, vodka, and whiskey) [9].

Process equipment is frequently a key source of metal ions in the final products. Several examples follow: (1) the concentration of Cu comes from process equipment in vodka is twice as much that coming from raw materials [10]; in whiskey the main source of Cu is the copper still used for distillation process [8]; corrosion of tequila distillation equipment (made also from Cu), provokes the presence of this in the final product [11]; storage of vodka in metal containers (e.g., Cu alloys or low-quality steel) results in their corrosion with the concomitant introduction of metals into the liquors [1], this is also the case with acidic wine vinegar [12]; the Cu, Fe, and Zn contained in home-produced alcoholic drinks can be essentially unrelated to the material fermented as it primarily depends on the several materials [13]; degree of still and the temperature in the distillate affect the Cu content in whiskey [1]; extremely high concentration of Fe, Zn, and Cu in home-produced beers and spirits can be largely traced to use of galvanized metal fermentation drums, when these replace old clay and wooden vessels [14]; the Fe content from musts and pulps increases due to the Fe concrete tanks used for the storage of raw materials [1]; contact of wine with process equipment, barrels, casks, and pipes is the usual source of Al, Cd, Cu, Cr, Fe, and Zn [7]; Pb plumbing can add Pb to beverages [2].

Metal addition from process equipment (e.g., stills) can be prevented by the use of high-quality steel or glass, although some organoleptic properties may be altered by the absence of certain metal ions added during distillation (e.g., the lack of minute amounts of Cu in tequila affects negatively its flavor).

Bottling equipment may also introduce metals in beverages. The content of Ca, Mg and Na brandies depends on the quality of water used for dilution after distillation [1]. The modification of certain imported alcoholic beverages „to bottle and sale by the addition of distilled or otherwise purified water to adjust the beverage to a required strength" is sometimes allowed. It is noteworthy that when metallic capsules seal alcoholic beverage bottles, some Pb may be carried over [15].

Possible effects caused by metals during these stages are multiple. Fe (III) and Mn (II) affect the stability of old wines and modify their sensorial quality after bottling since they are believed to activate molecular oxygen by forming reactive oxygen species (e.g., hydroxyl radicals) this is possible due to their electronic configurations involving unpaired electrons that may interact quantum mechanically with the dioxygen triplet [7]. Likewise, Fe catalyzes the oxidation of polyphenolic substances and Mn facilitates acetaldehyde formation, the products of these reactions yield undesirable precipitates. Cu and Zn can be introduced into beer by welded cans [4].

In the case of adulteration process, Pb and other metallic impurities can enter beverages during adulteration practices, e.g., adulterated vodka has been found to contain an excess of Ca and Mn ions [1]. The objective of the present study was to determine the concentration of 12 metals (Mg, Ca, K, Fe, Co, Ni, Cr, Cu, Pb, Cd, and Zn) in some alcoholic beverages, with a view to providing information on the metal profiles and risks associated with the consumption of these products.

RESULTS AND DISCUSSION

Wine Mineral Content

The mean concentration of Mg in the studied samples ranged from 0.26 to 15.43 µg/mL with an average of 3.84 µg/mL. The highest concentration of Mg was observed in cream liquors (15.43±1.57 µg/mL). The cream liquors, aperitif, local brandy, and local cider samples showed significantly higher mean concentrations (*p≤0.005*). The lowest mean level of Mg in the drinks samples was observed in rum (0.26±0.04 µg/mL). Iwegbue et al. [16] and Cameán et al. [17] reported Mg concentrations in the range of 0.26-25.45 µg/mL [16] and 0.24-11.20 µg/mL [17]. The levels of Mg recorded in the present study were lower than the levels reported in the wine literature 98.20 mg/L [18], 95-73 mg/L [19], 75.20 mg/L [20] however, the concentration of Mg in these samples was comparable to the levels found in spirits, liquor and whiskey [21].

The mean concentration of Ca in the alcoholic beverages varied from 0.94 to 234.43 µg/mL with an average of 23.77 µg/mL. Again, the highest concentration of Ca was observed in cream liquors (234.43±10.58 µg/mL). Cameán et al. [17] reported Ca levels ranging from 'not detected' to 14.80 µg/mL, while Iwegbue et al. [16] reported Ca levels from 1.43 µg/mL to 162.86 µg/mL. The high Ca concentration in the cream liquors could be due to enrichment of this type of drink with milk, which is known to contain substantial amounts of minerals [16]. The lowest mean level of Ca in the drinks samples was observed in dry gin (0.94±0.05 µg/mL). Lower level of Ca has been reported in the scientific literature in distilled products, 'not detected' to 14.80 mg/L (brandy) and from 6.00 to 11.00 mg/L (cognac) [22], 1.00 mg/L (gin) [23], 4.00 mg/L (rum) [23], 3.00 mg/L (rum) [22]. The concentration of Ca recorded in the present study was lower than the levels reported in the wine literature 83.50 mg/L [18], average values of 37.00 mg/L [19] and 65.90 mg/L [20]. In the coconut/orange liquor, showed significantly higher levels (*p≤0.005*) of Ca than the other brands analysed in this group. Apart from a few brands of coconut/orange liquor, the concentration of Ca in other classes of drinks was comparable to the concentration of reported in Brazilian cachaça and spirits [24].

The mean concentration of K in the alcoholic drinks ranged between 0.02 µg/mL to 278.02 µg/mL. The highest mean concentration of K was observed in cream liquors. The highest mean concentration of K varied significantly (*p≤0.005*) within the same class, as well as in other classes. The lowest mean level of Mg in the drinks samples was observed in aromatic schnapps. Cameán et al. [17] reported a K concentration ranging from 0.11 µg/mL to 70.06 µg/ml and Iwegbue et al. [16] reported K levels from ‘not detected‘ to 322.58 µg/mL. Except for cream liquors, the K concentration in these alcoholic drinks was similar to the levels of K reported in Brazilian sugar cane spirit [24]. The concentration of K recorded was lower than the levels reported in the wine literature from 491.12 mg/L to 633.74 mg/L for red wines and from 148.66 mg/L to 327.64 mg/L for white wines [25] and 819.61 mg/L average value [26].

It was observed that the cream liquors contained a higher concentration of Mg, Ca and K compared with the other classes of alcoholic beverages. This suggests that persons who drink cream liquors in preference to sprits and other types of alcoholic drinks studied in this research would be likely to be exposed to more metals.

The highest mean level of Mn was observed in cognac (0.39 µg/mL) and the lowest in aromatic schnapps (0.02 µg/mL). The maximum permissible limit of Mn in drinking water is 0.40 µg/ml Iwegbue et al. [16]. The concentrations of Mn in the alcoholic beverages were lower than the permissible level in drinking water. Iwegbue et al. [16] reported Mn levels from ‘not detected‘ to 0.33 µg/mL. Lower levels of Mn were observed in this study in comparison with the levels reported in wines 0.83 mg/L [25], 1.89 mg/L [18] and 2.04 mg/L [26], but were comparable to the levels reported from Brazilian cachaça and other international spirits [16].

The Fe concentration in the alcoholic beverages varied from 0.18 µg/mL to 2.64 µg/mL with an average of 1.03 µg/mL. The highest concentration of Mn was observed in spirit, while the lowest mean level was observed in aromatic schnapps. The guide provides a concentration of Fe in drinking water of 0.30 µg/mL [16]. The concentrations of Fe were higher than the permissible level in drinking water. Cameán et al. [17] reported Fe levels varying from ‘not detected‘ to 2.03 µg/mL in Spanish brandy and Iwegbue et al. [16] reported Fe levels varying from 0.28 µg/mL to 1.48 µg/mL. The concentration of Fe found in the alcoholic drinks was comparable to the levels in Fe reported in the literature for other alcoholic beverages in beer and wine 0-25 mg/L [26; 15], in brandy ‘not detected‘ to 2.30 mg/L [28; 15; 22], cognac 0.1 mg/L [28], gin ‘not detected‘ [28] rum 1.00 mg/L [28], vodka ‘not detected‘ [28], whiskey ‘not detected‘ [28], 1.48 mg/L spirits [16], 0.29 mg/L brandy [16], 0.29 mg/L aromatic schnapps [16].

Co concentration in the beverages varied from 0.03 µg/mL to 0.16 µg/mL, with whiskey and aromatic schnapps having the maximum and minimum mean levels, respectively. The highest mean concentration of Co varied significantly (*$p \le 0.005$*) within the same class, as well as in other classes. Xuebo et al. [29] reported a Co concentration ranging from 0.37 mg/L to 0.89 mg/L in baijiu (Chinese liquors) and Iwegbue et al. [16] reported Co levels varying from ‘not detected‘ to 0.12 µg/mL. Lower levels of Co were observed in this study in comparison with the levels reported in wines from 2.60 mg/L to 7.63 mg/L [25].

The mean concentration of Ni in the drinks varied between 0.02 µg/mL and 0.13 µg/mL. The highest mean concentration of Ni was observed in brandy and the lowest mean concentration was observed in aromatic schnapps. The maximum permissible limit of Ni in drinking water is 0.02 µg/mL Iwegbue et al. [16]. The mean concentration of Ni in most classes of these alcoholic beverages exceeded the maximum prescribed limit for Ni in drinking water. Iwegbue et al. [16], Xuebo et al. [29] and Ibanez et al. [1], also reported Ni mean concentration in alcoholic beverages which exceeded the maximum prescribed limit for Ni in drinking water. Ni concentration reported in this research were higher than the 0.0812-0.115 µg/mL reported in Brazilian cachaça and were comparable to levels reported for other alcoholic beverages 0.13 µg/mL in aromatic schnapps and 0.05 cognac 16]. Lower levels of Ni were observed in alcoholic beverages (this study) in comparison with the levels reported in wines from 0.073 mg/L to 19.40 mg/L [1].

The highest mean concentration of Cr was observed in cream liquor (0.29 µg/mL) and the lowest mean concentration was observed in spirit (0.02 µg/mL). The highest mean concentration of Cr varied significantly ($p \leq 0.001$) within the same class, as well as in other classes. Similar level of Cr has been reported in the literature [16] for alcoholic beverages, namely 0.28 µg/mL in cream liquor, 0.03 µg/mL in cognac and0.05 µg/mL in punch, while level of Cr concentration in wine was higher (872.42 µg/L) than in the studied alcoholic beverages.

The mean concentration of Cu in the samples ranged from 0.04 µg/mL to 2.51 µg/mL, with an average of 0.43 µg/mL. The highest mean concentration of Cu was observed in cream liquor, while the lowest mean level was observed in the punch. The permissible limit of Cu in alcoholic beverages is set at 5.0 µg/mL [16]. The mean concentration of Cu in these alcoholic beverages was below the permissible limit. Cu concentration in the range of 1.64 to 4.40 µg/mL [16] has been reported for Brazilian cachaça and other international spirits [1].

Similar, in Spain the Cu concentration was in the range of 0.10 µg/mL to 8.01 µg/mL for brandy, gin, rum, liquor, and whiskey, in Denmark, Cu leaves was in the rage of ‘not detected‘ to 0.12 µg/mL, were reported in for gin, rum, brandy and liquor [16]. The concentration of Cu found in the alcoholic drinks was comparable to the levels in Cu reported in the literature for other alcoholic beverages in spirits 0.40 mg/L [15], sherry brandy 0.22 to 5.31 mg/L [17], and in wine, fruit wine, cocktails from ‘not detected‘ to 7.62 mg/L [1].

Table 1. Metal concentration (µg/mL) in distilled alcoholic beverages and liquors purchases in Romania (the values in parentheses represent the range)

beverage type	Mg	Ca	K	Mn	Fe	Co	Ni	Cr	Cu	Pb	Cd	Zn
Whiskey	2.48±0.12^{e}	6.99±0.55^{def}	6.18±0.68^{ef}	0.03±0.01^{cde}	1.38±0.01^{c}	0.16±0.06^{a}	0.06±0.02^{cd}	0.05±0.02^{c}	0.19±0.06^{ef}	0.28±0.06^{a}	0.03±0.01^{ab}	0.21±0.04^{cd}
	(1.19-2.99)	(3.65-8.96)	(4.08-8.54)	(<0.0001-0.07)	(0.57-2.87)	(0.05-0.45)	(0.03-0.21)	(0.02-0.11)	(0.11-0.34)	(0.14-0.52)	(0.01-0.10)	(0.13-0.38)
Brandy	3.07±0.47^{e}	8.19±2.25^{cde}	27.99±0.56^{d}	0.07±0.02^{c}	0.87±0.12^{de}	0.05±0.02^{bc}	0.03±0.01^{d}	0.03±0.01^{c}	0.24±0.09^{e}	0.14±0.02^{b}	0.03±0.01^{ab}	0.45±0.11^{b}
	(0.79-11.21)	(4.67-20.09)	(0.89-68.89)	(0.02-0.31)	(0.35-2.01)	(0.01-0.06)	(0.01-0.06)	(0.01-0.09)	(0.08-0.78)	(0.10-0.17)	(0.007-0.05)	(0.09-0.76)
Local Brandy	6.48±0.78^{c}	7.99±0.86^{cde}	32.59±1.75^{c}	0.07±0.02^{c}	1.22±0.13^{cd}	0.08±0.04^{b}	0.05±0.01^{cd}	0.13±0.02^{b}	0.67±0.11^{c}	0.15±0.06^{b}	0.02±0.01^{b}	0.28±0.05^{c}
	(4.53-18.32)	(3.87-12.05)	(18.23-54.98)	(0.01-0.09)	(0.76-3.24)	(0.03-0.10)	(0.02-0.08)	(0.09-0.21)	(0.61-0.84)	(0.11-0.18)	(0.01-0.03)	(0.16-0.38)
Cream liquor	15.43±1.57^{a}	234.43±10.58^{a}	278.02±9.97^{a}	0.16±0.03^{b}	2.10±0.21^{b}	0.05±0.02^{bc}	0.07±0.02^{bcd}	0.29±0.10^{a}	0.15±0.04^{ef}	0.18±0.05^{b}	0.03±0.02^{ab}	0.88±0.10^{a}
	(6.55-23.42)	(135.67-354.09)	(79.56-632.11)	(0.09-0.32)	(0.89-5.61)	(0.03-0.07)	(0.04-0.10)	(0.09-0.39)	(0.01-0.25)	(0.13-0.24)	(0.001-0.04)	(0.59-1.13)
Cognac	0.59±0.14^{f}	3.07±0.66^{efg}	5.41±0.90^{efg}	0.39±0.06^{a}	1.48±0.25^{c}	0.03±0.02^{bc}	0.11±0.04^{ab}	0.06±0.02^{c}	2.51±0.31^{a}	0.30±0.13^{a}	0.03±0.02^{ab}	0.19±0.02^{cd}
	(0.21-0.72)	(2.18-4.08)	(3.44-7.89)	(0.12-0.43)	(0.56-1.89)	(<0.0001-0.06)	(0.02-0.26)	(0.04-0.18)	(1.17-3.21)	(0.04-0.76)	(0.02-0.08)	(0.15-0.34)
Dry Gin	0.50±0.05^{f}	0.94±0.05^{g}	4.33±0.09^{efg}	0.03±0.01^{cde}	0.39±0.06^{fg}	0.04±0.01^{bc}	0.12±0.06^{a}	0.02±0.02^{c}	0.06±0.02^{ef}	0.14±0.03^{b}	0.03±0.01^{ab}	0.16±0.02^{d}
	(0.28-1.76)	(0.57-4.21)	(3.97-6.28)	(0.067-0.06)	(0.06-0.98)	(0.01-0.08)	(0.03-0.19)	(0.01-0.08)	(0.01-0.24)	(0.08-0.22)	(0.02-0.11)	(0.08-0.65)
Rum	0.26±0.04^{f}	2.90±0.07^{efg}	1.51±0.24^{fg}	0.02±0.02^{de}	0.85±0.06^{de}	0.03±0.02^{bc}	0.06±0.02^{cd}	0.26±0.06^{a}	0.20±0.01^{ef}	0.11±0.01^{bc}	0.02±0.01^{ab}	0.18±0.01^{cd}
	(0.08-0.36)	(0.78-4.57)	(0.19-2.44)	(0.001-0.06)	(0.32-1.45)	(0.02-0.12)	(0.05-0.23)	(0.08-0.30)	(0.12-0.34)	(0.06-0.17)	(0.01-0.18)	(0.10-0.21)
Vodka	0.55±0.10^{f}	1.96±0.06^{fg}	2.29±0.27^{efg}	0.03±0.02^{cde}	0.59±0.12^{efg}	0.14±0.05^{a}	0.09±0.02^{abc}	0.03±0.02^{c}	0.50±0.05^{d}	0.18±0.06^{b}	0.03±0.01^{ab}	0.20±0.03^{cd}
	(0.23-1.89)	(0.23-7.32)	(1.09-5.76)	(0.01-0.04)	(0.19-1.98)	(0.05-0.16)	(0.04-0.13)	(0.01-0.08)	(0.43-1.01)	(0.09-0.28)	(0.01-0.07)	(0.13-0.28)
Aromatic schnapps	0.28±0.05^{f}	2.50±0.30^{efg}	0.56±0.09^{g}	0.02±0.01^{cde}	0.18±0.06^{g}	0.03±0.01^{c}	0.13±0.01^{a}	0.03±0.02^{c}	0.11±0.02^{ef}	0.16±0.06^{b}	0.02±0.01^{ab}	0.13±0.01^{d}
	(0.13-0.49)	(0.68-3.07)	(0.36-3.08)	(0.001-0.06)	(0.12-2.76)	(0.02-0.04)	(0.07-0.35)	(0.02-0.05)	(0.06-0.25)	(0.05-0.27)	(0.01-0.04)	(0.09-0.21)
Punch	0.69±0.05^{f}	9.32±0.57^{cd}	7.48±0.87^{e}	0.05±0.01^{cde}	0.36±0.06^{fg}	0.05±0.02^{bc}	0.07±0.02^{bcd}	0.06±0.01^{c}	0.04±0.01^{f}	0.03±0.02^{c}	0.01±0.01^{ab}	0.52±0.06^{b}
	(0.47-4.80)	(7.04-26.97)	(4.76-13.54)	(0.04-0.09)	(0.09-1.09)	(0.03-0.08)	(0.04-0.11)	(0.05-0.14)	(0.01-0.09)	(0.01-0.03)	(0.01-0.03)	(0.24-0.79)
Local Cider	4.82±1.18^{d}	12.77±1.56^{c}	7.19±0.63^{e}	0.04±0.02^{cde}	0.74±0.06^{ef}	0.14±0.02^{a}	0.05±0.02^{cd}	0.06±0.01^{c}	0.23±0.11^{ef}	0.09±0.05^{bc}	0.02±0.02	0.29±0.05^{c}
	(3.68-9.13)	(10.00-29.95)	(2.12-16.89)	(0.01-0.05)	(0.53-2.01)	(0.11-0.26)	(0.03-0.15)	(0.03-0.09)	(0.20-0.57)	(0.06-0.16)	(0.01-0.03)	(0.14-0.35)
Spirit	2.56±0.19^{e}	5.74±0.28^{defg}	25.09±1.49^{d}	0.03±0.02^{cde}	2.64±0.71^{a}	0.03±0.01^{c}	0.03±0.01^{d}	0.02±0.01^{c}	0.95±0.05^{b}	0.13±0.02^{b}	0.02±0.02	0.16±0.05^{d}
	(0.11-6.23)	(0.78-18.66)	(5.87-86.67)	(0.01-0.12)	(0.45-7.34)	(0.001-0.08)	(0.01-0.09)	(0.01-0.05)	(0.56-1.89)	(0.09-0.24)	(0.01-0.07)	(0.09-0.22)
Coconut/Orange liquor	3.25±0.24^{e}	26.34±2.95^{b}	23.74±2.12^{c}	0.03±0.02^{cde}	0.28±0.05^{g}	0.12±0.01^{a}	0.08±0.04^{bcd}	0.03±0.01^{c}	0.05±0.03^{f}	0.03±0.02^{c}	0.04±0.01^{a}	0.28±0.05^{c}
	(0.87-11.09)	(5.35-102.45)	(10.12-135.54)	(0.01-0.07)	(0.12-3.89)	(0.05-0.23)	(0.07-0.12)	(0.02-0.10)	(0.04-0.13)	(0.02-0.07)	(0.03-0.05)	(0.21-0.29)
Aperitif	12.74±1.63^{b}	9.33±0.50^{cd}	49.18±2.26^{b}	0.06±0.01^{cd}	1.40±0.12^{c}	0.05±0.01^{bc}	0.05±0.01^{cd}	0.02±0.01^{c}	0.13±0.01^{ef}	0.16±0.03^{b}	0.02±0.01^{b}	0.45±0.11^{b}
	(4.34-47.76)	(8.09-32.98)	(32.87-66.52)	(0.008-0.009)	(0.87-5.32)	(0.03-0.12)	(0.08-0.13)	(0.01-0.05)	(0.08-0.19)	(0.13-0.32)	(0.01-0.04)	(0.16-0.57)

Average value ± standard deviation (n = 3). Romans letters represent the significance of the variety difference ($p \le 0.05$). The difference between any two values, followed by at least one common letter, is insignificant

The mean levels of Pb in alcoholic beverages ranged from 0.03 µg/mL to 0.30 µg/mL. The highest mean concentration was observed in cognac, while the lowest mean value is in a punch, a home-made drink made from alcohol and fruits or fruit juice. These values are in accordance with Iwegbue et al. [16]. The acceptable limits for Pb in alcoholic beverages in some European countries are in the range of 0.20 µg/mL to 0.50 µg/mL. The mean concentration of Pb in these alcoholic beverages are below the permissible limit. The concentration of Pb found in the alcoholic drinks was comparable to the levels of Pb reported in the literature for other alcoholic beverages, which are from 'not detected' to 0.46 mg/L in beer [14], 'not detected' to 0.22 mg/L in spirits [15], 0.008 to 0.420 mg/L in cognac [22], 'not detected' to 0.035 mg/L in gin [15], and 'not detected' to 1.125 mg/L in wine, fruit wine, cocktails [1].

The mean concentration of Cd in these alcoholic beverages were similar except for local brandy, which had a mean level that was twice the level found in the other classes. The mean concentration of Cd ranged from 0.02 µg/mL to 0.04 µg/mL. The guideline value for Cd in drinking water is 0.05 µg/mL according to Iwegbue et al. [16]. The mean concentration of Cd in these alcoholic beverages was below the upper boundary these limits. The results of these studies indicate that persons who consume local brandy, in preference to other alcoholic beverages, are likely to be exposed to more Cd. Iwegbue et al. [16] came to the same conclusion in terms of coconut liquor consumption. The levels of Cd observed in these drinks were comparable to the levels of Cd reported for other alcoholic beverages in the scientific literature <0.005 mg/L in berry liquors [1], sherry brandy from 5.31-0.30 mg/L [17] and from 'not detected' to 0.052 mg/L in wine, fruit wines, cocktails. Iwegbue et al. [16] reported Cd levels from 'not detected' to 0.04 µg/mL in alcoholic beverages.

The mean concentration of Zn ranged from 0.13 µg/mL to 0.88 µg/mL with 0.31 µg/mL average value. The highest mean level of Zn was observed in cream liquor, while the lowest mean level was observed in aromatic schnapps. The permissible limit for Zn in alcoholic beverages is 5 µg/mL according to Iwegbue et al. [16]. The mean concentration of Zn in these alcoholic beverages was below the upper boundary these limits. Zn level concentration of 44 µg/mL to 69 µg/mL in rum and from 0124 µg/mL to 0.151 µg/mL in cachaça and international spirits from Brazil have been reported by Iwegbue et al. [16]. The concentration of Zn found in the alcoholic drinks was comparable to the levels in Zn reported in the literature for other alcoholic beverages, like beer 0.1 mg/L - 68 mg/L [14; 15], brandy 3.0 mg/L [15; 22;

28], cognac 0.016 mg/L to 3.00 mg/L [28], gin 0.5 mg/L [28], rum 3.00 mg/L [28], vodka 'not detected' [28], whiskey 0.50 mg/L [28], spirits 3.0 mg/L [16], brandy 3.00 mg/L [16] and 0.12 mg/L aromatic schnapps [16].

Dietary intake of metals and target hazard quotients (THQs)

The estimated daily intake of metals based on a per capita consumption of 14.4 L per annum of pure alcohol is displayed in Table 2. The estimated THQs of the metals are displayed in Table 3. The intake values of Mg in this study were in the range of 0.14-8.60 µg/kg b.w. per day. Higher intakes of Mg are likely for consumers of whiskey, brandy, local brandy, cream liquor, local cider, spirit, coconut/orange liquors, and aperitif. The recommended dietary allowance (RDA) values for male and female healthy adults are 400-420 mg/day and 310-320 mg/day, respectively [16]. Based on the results obtained, the dietary intake of Ca from the consumption of these drinks is in the range of 0.27-130.61 µg/kg b.w. per day. Higher intakes of Ca are likely for consumers of whiskey, brandy, local brandy, cream liquor, cognac, rum, vodka, aromatic schnapps, punch, local cider, spirit, coconut/orange liquors, and aperitif. The RDA value of Ca is set at 1000 mg per day [16]. The dietary intake of K from the consumption of these alcoholic beverages ranged from 0.31-154.90 µg/kg b.w. per day. Persons who drink cream liquor in preference to other types of the drink have higher K intake. Iwegbue et al. [16] reported dietary intake of Mg, Ca and K from consumption of distilled alcoholic beverages and liquors for international origin as 0.07-4.24, 0.23-27.14 and 0.08-53.76 µg/kg b.w. per day based on 3.6 L per annum per capita consumption. The estimated intake of Mn and Fe were 0.01-0.09 and 0.10-117.00 µg/kg b.w. per day, respectively. Higher intakes of Mn and Fe are likely for consumers of cream liquors and spirits. The recommended dietary allowances value for Fe and Mn are 2-5 and 10-18 mg per day, respectively, according to Iwegbue et al. [16].

The RDA value for Co is 100 µg per day according to Iwegbue et al. [16]. The estimated dietary intake of Co from these types of alcoholic beverages was lower than the RDA for Co. The recommended dietary allowance value for Ni is in the range of 35-700 µg/kg b.w. per day. The estimated intake values for Ni varied from of 0.02-0.07 µg/kg b.w. per day. The recommended dietary allowances value for Ni is 5 µg/kg b.w. per day.

The estimated dietary intake of Cu and Cr was 0.02-1.40 and 0.01-0.16 μg/kg b.w. per day. The highest intake values of Cr and Cu was observed in cream liquor and cognac. The RDA values for Cu and Cr per person are 900-30 mg per day (15-500 μg/kg b.w. per day) and 130 μg/kg b.w. per day, respectively according to Iwegbue et al. [16].

The estimated daily intake of Pb from consumption of any type of these alcoholic beverages ranged between 0.02-0.16 μg/kg b.w. per day. The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) established a limit for Pb (3.6 μg/kg b.w. per day). The dietary intake of Cd from the consumption of these classes of alcoholic beverages is in the range of 0.01-0.02 μg/kg b.w. per day. The tolerable dietary intake of Cd is set at 1 μg/kg b.w. per day according to Iwegbue et al. [16]. The intakes of Zn from the consumption of these alcoholic drinks were 0.07-0.49 μg/kg b.w. per day. Higher intakes of Zn are likely for consumers of cream liquor, punch, and aperitif. The JECFA provisional maximal tolerable daily intake of Zn is 1000 μg/kg b.w. per day [16].

Table 2 presents the results of the estimated THQ from the consumption of these alcoholic drinks. The interpretation of the THQ values is binary: THQ is either ≥1 or <1, where THQ >1 indicates a health concern [30; 31; 32; 33; 16]. It must be noted that THQ is not a measured risk [30; 16; 34], but rather indicates a level of concern, and while THQ values are additive, they are not multiplicative, for example, the level of concern at THQ=20 is larger than, but not 10-hold, that at THQ=2 according to Iwegbue et al. [16]. The estimated THQ values for the individual and combined metals from consumption of these drinks were <1 (Table 3). The THQ values reveal no significant concern to health for people with a 14.4 L per annum per capita consumption rate.

Risk assessment for a specific contaminant intake comprehensive consideration of all intake mechanisms, and alcoholic beverages consumption was just one such path, the amount of wine consumption was, therefore, more important for health risk assessment of wine in the daily diet of drinkers.

Table 2. Estimated dietary intake of metals (µg/kg b.w. per day) based on a 14.4 L per annum per capita consumption

beverage type	Mg	Ca	K	Mn	Fe	Co	Ni	Cr	Cu	Pb	Cd	Zn
Whiskey	**1.38**	**3.89**	**3.44**	0.02	0.77	0.09	0.03	0.03	0.11	0.16	0.02	0.12
Brandy	**1.71**	**4.56**	**15.59**	0.04	0.48	0.03	0.02	0.02	0.13	0.08	0.02	0.25
Local Brandy	**3.61**	**4.45**	**18.16**	0.04	0.68	0.04	0.03	0.07	0.37	0.08	0.01	0.16
Cream liquor	**8.60**	**130.61**	**154.90**	0.09	**117.00**	0.03	0.04	0.16	0.08	0.10	0.02	0.49
Cognac	0.33	**1.71**	**3.01**	0.03	0.82	0.02	0.06	0.03	**1.40**	0.17	0.02	0.11
Dry Gin	0.28	0.27	**2.41**	0.02	0.22	0.02	0.07	0.01	0.03	0.08	0.02	0.09
Rum	0.14	**1.62**	0.84	0.01	0.47	0.02	0.03	0.14	0.11	0.06	0.01	0.10
Vodka	0.31	**1.09**	**1.28**	0.02	0.33	0.08	0.05	0.02	0.28	0.10	0.02	0.11
Aromatic schnapps	0.16	**1.39**	0.31	0.01	0.10	0.02	0.07	0.02	0.06	0.09	0.01	0.07
Punch	0.38	**5.19**	**4.17**	0.03	0.20	0.03	0.04	0.03	0.02	0.02	0.01	0.29
Local Cider	**2.69**	**7.11**	**4.01**	0.02	0.41	0.08	0.03	0.03	0.13	0.05	0.01	0.16
Spirit	**1.43**	**3.20**	**13.98**	0.02	**1.47**	0.02	0.02	0.01	0.53	0.07	0.01	0.09
Coconut/Orange liquor	**1.81**	**14.68**	**13.23**	0.02	0.16	0.07	0.04	0.02	0.03	0.02	0.02	0.16
Aperitif	**7.10**	**5.20**	**27.40**	0.22	0.78	0.03	0.03	0.01	0.07	0.09	0.01	0.25

Table 3. Estimated target hazard quotients (THQ) from the consumption of these alcoholic drinks

beverage type	Mg	Ca	K	Mn	Fe	Co	Ni	Cr	Cu	Pb	Cd	Zn
Whisky	N/A	N/A	N/A	0.01	0.01	0.001	0.30	0.002	0.00002	0.003	0.0001	0.02
Brandy	N/A	N/A	N/A	0.01	0.01	0.001	0.09	0.001	0.00001	0.002	0.0001	0.02
Local Brandy	N/A	N/A	N/A	0.03	0.03	0.001	0.15	0.002	0.00005	0.009	0.0001	0.01
Cream liquor	N/A	N/A	N/A	0.06	0.06	0.002	0.09	0.001	0.00010	0.002	0.0001	0.02
Cognac	N/A	N/A	N/A	0.002	0.002	0.001	0.06	0.0003	0.00002	0.030	0.0001	0.02
Dry Gin	N/A	N/A	N/A	0.02	0.02	0.000	0.07	0.0004	0.00001	0.001	0.0001	0.02
Rum	N/A	N/A	N/A	0.001	0.001	0.001	0.06	0.0003	0.00010	0.003	0.00004	0.01
Vodka	N/A	N/A	N/A	0.002	0.002	0.001	0.26	0.002	0.00001	0.007	0.0001	0.02
Aromatic schnapps	N/A	N/A	N/A	0.001	0.001	0.0001	0.06	0.0003	0.00001	0.002	0.0001	0.01
Punch	N/A	N/A	N/A	0.003	0.003	0.0003	0.09	0.001	0.00002	0.001	0.00001	0.01
Local Cider	N/A	N/A	N/A	0.02	0.02	0.001	0.26	0.002	0.00002	0.003	0.00003	0.01
Spirit	N/A	N/A	N/A	0.01	0.01	0.002	0.06	0.0003	0.00001	0.010	0.0001	0.01
Coconut/Orange liquor	N/A	N/A	N/A	0.01	0.01	0.0002	0.22	0.001	0.00000	0.001	0.00001	0.02
Aperitif	N/A	N/A	N/A	0.05	0.05	0.001	0.09	0.001	0.00004	0.002	0.00006	0.01

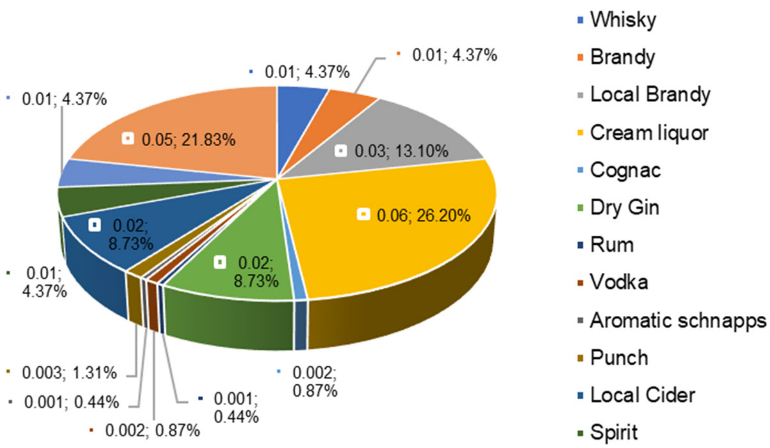

Figure 1. The average proportion of the total THQ for all the alcoholic beverages

In this research the THQ also represented the contribution of alcoholic beverages to contaminants in the acceptable range for daily diet, the average THQ of cream liquor was 26.20% (Figure 1) and Co 89.15% (Figure 2) which meant that the contribution of cream liquors consumption to the tolerable daily intake of Co was 89.15%.

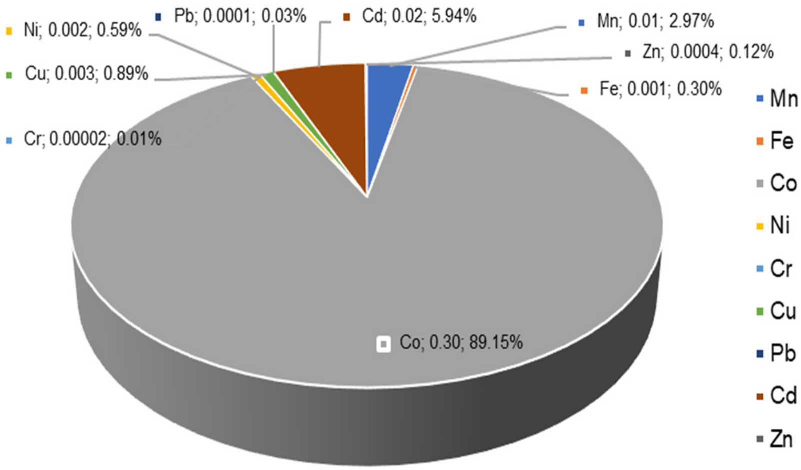

Figure 2. The average proportion of the total THQ for all the metals

CONCLUSIONS

Relatively low levels of both essential and potential hazardous metal ions were found in these types of alcoholic beverages. Based on a 14.4 L per capita consumption of pure alcohol, the local and international drinks instigated gave low dietary intakes of the essential and potentially toxic metals. It was observed that the cream liquors and cognac contained a higher concentration of Mg, Ca, K, Fe, and Cu compared with the other classes of alcoholic beverages. This suggests that persons who drink cream liquors in preference to spirits and other types of alcoholic drinks studied in this research would be likely to be exposed to more metals.

The THQ values may not present any detrimental health concerns for a lifetime based on the metal content alone. However, the hazardous and harmful use of alcohol is a major global contributing factor, such as liver cirrhosis, cancers, alcohol dependences, injuries, and others through the dangerous actions of intoxicated people.

EXPERIMENTAL SECTION

Sampling

Samples of different brands of alcoholic beverages with different batch numbers and manufacturing dates were collected from retail operations in Bucharest, Cluj, Maramureș, Constanța, Galați, Braila, Satu-Mare, Salaj, Vâlcea, Alba, Covasna, Hargita and Mureș. The choice of brands was carefully made to reflect popular brands consumed by different income classes and influenced by availability at the time of purchase. The categorization and other information on the bottles of the alcoholic drinks are displayed in Table 4. The samples were stored at 3-5 °C until the analysis was made.

Table 4. Information on the samples studied

Alcoholic beverage	Percentage (v/v) alcohol	Class	Country of origin
Johnnie Walker	40	Whisky	UK
Small Batch	46.85	Whisky	America
Ardreg Ulgeadail	46	Whisky	Scotland
Arran Lochranza	43	Whisky	Scotland
Ballantine's	40	Whisky	Italy
Balvenie	40	Whisky	Scotland
Benriach	40	Whisky	Scotland

Alcoholic beverage	Percentage (v/v) alcohol	Class	Country of origin
Big Peat	46	Whisky	Scotland
Blanton's	51.5%	Whisky	America
Braeval	48.4	Whisky	Scotland
Baffalo Trace	40%	Whisky	America
Bulleit Bourbon	45.6	Whisky	America
Dalmore	40	Whisky	Scotland
Dramsylvania	40	Whisky	Scotland
Afinată	40	Brandy	Romania
Caisată	40	Brandy	Romania
Căpșunată	40	Brandy	Romania
Vișinată	40	Brandy	Romania
Plums brandy	40	Brandy	Romania
Pears brandy	40	Brandy	Romania
Quince brandy	40	Brandy	Romania
Apricots brandy	40	Brandy	Romania
Nuts liqueur	35	Cream liquor	Romania
Black blueberry liqueur	31	Cream liquor	Romania
Jägermeister	35	Cream liquor	Germany
Amaretto Disaronno	28	Cream liquor	Italy
Aperol	11	Cream liquor	Italy
Unicum	40	Cream liquor	Hungary
De kuyper	40	Cream liquor	Netherlands
Courvoisier Cognac	40	Cognac	France
Hennessy	40	Cognac	France
Martel	40	Cognac	France
Remy Martin	40	Cognac	France
Wembley London	40	Dry Gin	England
Beefeater London	40	Dry Gin	England
Gordons	37.5	Dry Gin	UK
Finsbury	40	Dry Gin	UK
London Hill	43	Dry Gin	UK
Havana Club	40	Rum	Cuba
Matusalem	40	Rum	Dominican Republic
Stroh 80	80	Rum	Austria
Bacardi	40	Rum	Cuba
Captain Morgan	35	Rum	Jamaica
Absolut Vodka	40	Vodka	Sweden
Finlandia Vodka	40	Vodka	Finland
Rasputin Vodka	40	Vodka	Germany

Alcoholic beverage	Percentage (v/v) alcohol	Class	Country of origin
Stolichnaya	40	Vodka	Russia
Zubrowka	40	Vodka	Poland
Wyborowa	40	Vodka	Poland
Eagles	42	Aromatic schnapps	Nigeria
Seamans	40	Aromatic schnapps	Nigeria
Kp	42	Aromatic schnapps	Nigeria
Crown	40	Aromatic schnapps	Nigeria
Garvey	30	Punch	Spain
Freihof Jagertee	40	Punch	Austria
Stroh Jagertee	40	Punch	Austria
Local cider	4.5	Cider	Romania
Tequila Blanco	38	Spirit	Mexico
Absolute Citron	40	Sprit	Sweden
Gordons Spark	5.5	Sprit	Nigeria
Malibu	21	Coconut liquor	UK
Calypso	28	Coconut liquor	Nigeria
Blue Curaçao	33.8	Coconut liquor	USA
Sweet „n” sour mix	32	Coconut liquor	USA
Cointreau	40	Orange liquor	France
Campari	20	Aperitif	Italy
Bacardi	40	Aperitif	Germany
Vino din tavola	10.5	Aperitif	Italy
Ricard	45	Aperitif	France

Reagents and solutions

Twelve elements (Mg, Ca, K, Mn, Fe, Co, Ni, Cr, Cu, Pb, Cd, and Zn) were determined in 14 classes of alcoholic beverages. The analysis was made using multielement analysis and ICP-MS technique, after appropriate dilution, using the external standard calibration method (Table 5). The calibration was performed using XXICertiPUR multielement standard and from an individual standard solution of Cr and Hg. The working standards and the control sample were prepared daily from the intermediate standards that were prepared from the stock solution. The intermediate solutions stored in polyethylene bottles and glassware was cleaned by soaking in 10% v/v HNO_3 for 24 hours and rinsing at least ten times with ultrapure water (18.2 MΩ cm^{-1} ultrapure water-Types 1). The accuracy of the methods was evaluated by replicate analyses of fortified samples (10 µL-10 mL concentrations) and the obtained values ranged between 0.8-13.1 percent, depending on the element. The global recovery for each element was estimated and the obtained values were between 84.6-100.9% [35].

Table 5. Instrumental conditions for the determination of each element (ICP-MS technique)

Element	Correlation coefficient	LoD* (µg/L)	LoQ*** (µg/L)	BEC** (µg/L)
Mg	0.9999	2.7320	9.0990	9.0990
Ca	0.9999	5.6640	18.8640	20.8200
K	0.9999	2.1860	7.2790	31.7281
Mn	0.9999	0.0100	0.0340	0.0850
Fe	0.9999	5.2100	17.3501	71.3990
Co	0.9999	0.0365	0.1215	0.152
Ni	0.9999	0.0591	0.1968	0.091
Cr	0.9999	1.6630	5.5378	0.636
Cu	0.9999	0.0402	0.1339	0.237
Pb	0.9999	0.0003	0.0010	0.002
Cd	0.9999	0.0202	0.0673	0.027
Zn	0.9999	0.3780	1.2580	5.401

*Detection limit; **Background equivalent concentration; ***Quantification limit.

For quality control purposes, blanks and triplicates samples (n = 3) were analysed during the procedure. The variation coefficient was under 5% and detection limits (ppb) were determined by the calibration curve method. Limit of detection (LoD) and Limit of quantification (LoQ) limits was calculated according to the next mathematical formulas: LoD = 3SD/s and LoQ = 10 SD/s (SD = estimation of the standard deviation of the regression line; s = slope of the calibration curve).

Sample preparation for determination of metals using ICP-MS

For the determination of elements from wine samples 0.5 mL wine were mixed with 7 mL of HNO_3 65% and 1 mL of H_2O_2 and were mineralized in a clean Teflon digestion vessel using a microwave system Milestone START D Microwave Digestion System. Mineralization was done in three steps: step I (time 10 min., temperature 200ºC), step II (time 15 min., temperature 200ºC) and step III (time 40 min., ventilation - temperature 32ºC). After mineralization, samples were filtered through a 0.45 mm filter paper and the volume was adjusted to a volume of 50 mL.

To confirm the best-chosen conditions for wine digestion standard additions for checking the accuracy of the microwave digestion and recoveries were calculated (Table 6). The digestion seemed visually completed in all of the combinations, but the spiked recoveries showed significant differences for total elements content (p = 0.005).

Table 6. Accuracy of the ICP-MS determination of metals in reference materials (NIST SRM 1572) (n=7)

Element	Certified Concentration (mg/L)	Measured Concentration (mg/L)
Mg	5.80±0.30	5.74±0.01
Ca	31.10±1.10	31.05±2.11
K	1.82±0.06	1.79±0.05
Mn	23.00±2.00	23.05±0.09
Fe	90.00±10.00	89.08±6.78
Co	20.00±0.01	21.45±2.33
Ni	600.00±300.00	612.34±27.98
Cr	0.80±0.20	0.79±3.22
Cu	16.50±1.00	16.49±1.22
Pb	13.30±2.40	13.30±2.56
Cd	36.00±0.10	36.67±1.05
Zn	29.00±2.00	29.34±0.99

Instrumentation

The elements were determined by using ICP-MS (iCAP Q Thermo scientific mode). The sample solution was pumped by a peristaltic pump from tubes arranged on autosampler (CETAC ASX-520), which was combined with a quartz cyclonic spray chamber (water-cooled 2°C). The instrumental setting and operative conditions are reported in Table 7.

Table 7. ICP-MS instrumental parameters

Parameter	Setting
RF-Power	1550
Reflected power	<5
Carrier gas flow (mL/min.)	1.0
Plasma gas flow (L/min.)	15
Auxiliary gas flow (mL/min.)	1.0
Spray chamber	Water cooled double pass
Spray-Chamber temperature (°C)	2
Lens voltage (V)	6.25
Mass range (AMU)	3-209
Mass resolution	0.7
Integration time points/ms.	3
Points per peak	3
Replicates	3

The instrument was daily optimized to give maximum sensitivity for M^+ ions and the double ionization and oxides monitored by the means of the ratios between Ba^{2+}/Ba^+ and Ce^{2+}/CeO^+, respectively, these always being less than 2%.

Estimation of dietary intake (EDI) and THQ

The adult per capita consumption rate of pure alcohol in Romania is 14.4 L per annum. This value is from the calculation based on the Food and Agriculture Organization of the United Nations data, which includes fermented beverages and estimates of beer produced locally from sorghum, millet, and other agricultural products [36]. In this study, an adult per capita consumption rate of 14.4 L per annum of spirits, which is equivalent to 39 mL per day, and an average weight of 70 Kg per adult was adopted. EDI is measured in µg/kg b.w. [37].

$$EDI = (FIR \times C) / Bwa$$

where EDI is estimated daily intake (µg analysed element/kg body weight/day), FIR is average daily consumption of alcohol (mL/kg), C is average concentration of the heavy metals in the samples (µg/mL) and Bwa is average body weight (Kg) [37, 16].

To assess the human risk from consumption of alcoholic beverages with metals, the target Hazard Quotient (THQ) was calculated as per the US EPA Region III Risk-Based Concentration Table [USEPA] (United States Environmental Protection Agency) 2011] [37]. The THQ is an estimate of the non-carcinogenic risk level due to pollutant exposure and calculated by the following equation:

$$THQ = 10^{-3} \times (Efr \times EDtot \times Fir \times C) / (RfDo \times Bwa \times ATn)$$

where, THQ is target hazard quotient, Efr – exposure frequency (365 days/year), EDtot – exposure duration (70 years), FIR – average daily consumption of alcohol (mL/kg), C – average concentration of the metals in samples (µg/mL), RfDo – oral reference dose (mg/kg/day), Bwa – average body weight (kg) and ATn – average exposure for non-carcinogens in year (365 days/year × 70 years). THQ value below 1 indicant no adverse effect on human health.

Statistical analysis

The statistical interpretation of the results was performed using the Duncan test, SPSS Version 24 (SPSS Inc., Chicago, IL., USA). The statistical processing of the results was primarily performed to calculate the following statistical parameters: average and standard deviation. This data was interpreted with the analysis of variance (ANOVA) and the average separation was performed with the DUNCAN test at $p \leq 0.05$.

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