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THE INFLUENCE OF THE DISTILLATION PROCESS ON THE CONTENT OF METALS IN HOME- AND INDUSTRIALLY-BREWED ALCOHOLIC BEVERAGES – RISK ASSESSMENT TO HUMAN HEALTH

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ABSTRACT. The aim of this study was to assess the influence of the distillation processes on the content of Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn in 18 home-brewed fruit spirits, originating from different locations of Romania, and 3 industrially-brewed spirits and 19 cognacs. Metals quantification was achieved by inductively coupled plasma optical emission spectrometry (ICP-OES) after sample digestion. The study revealed higher metals concentration in the home-brewed spirits, compared to the industrially-brewed ones, with concentrations of Cu, Fe, Al and Zn in most of the samples above the Alcohol Measures for Public Health Research Alliance (AMPHORA) project set threshold in recorded alcoholic beverages. However, no risk to human health was found by a moderate consumption of the analyzed alcoholic beverages (100 mL/day) as respects to long-term non-carcinogenic health risk. The Principal Components Analysis (PCA) indicated a wide dispersion of the analyzed alcoholic beverages according to their elemental composition. The two-dimensional PCA representation after Varimax rotation indicated a group of elements of natural origin (Ca, Mg, Al, Cd, Mn, Pb), and another of trace elements (Co, Cr, Ni, Zn, Fe) originating from the distillation equipment. Copper however, was associated both with the raw material and the distillation equipment.

Keywords: *alcoholic beverage, human health risk assessment, Principal Component Analysis*

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INTRODUCTION

According to the World Health Organization (WHO) the yearly average pure alcohol consumption in Romania is 12.6 L, from which 56%, 28% and 16% as beer, wine and spirits, respectively. In the same study it was revealed that of the 12.6 L alcohol consumed 10.4 L originates from recorded sources (store-bought) and 2.2 L from unrecorded ones (non-commercial alcohols), obtained in small scale distilleries or at home [1]. Moreover, until recently there were no legislation related to maximum admitted concentrations of metals in distilled beverages, except for wines, set by the International Organization of Vine and Wine (OIV) [2]. The maximum limits of metals in recorded alcohol were established for the first time in 2011 by the Alcohol Measures for Public Health Research Alliance (AMPHORA) project, funded by the European Community's Seventh Framework Programme [3].

Unrecorded alcoholic beverages may pose a great health risk concern as they could contain many toxic compounds, such as methanol, acetaldehyde, ethyl carbamate and even toxic metals [4]. The metals in alcoholic beverages may originate from the raw materials, substances added during brewing, the brewing equipment, bottling, aging and storage [4–6]. The metal concentration in raw materials is influenced by the soil [7], pollution of the environment and agrochemical treatments, like fungicides, pesticides and fertilizers [6]. These treatments contribute to the increase of Cd, Cu, Mn, Pb and Zn content in the final product [8]. In several studies it was pointed out that in industrially produced brandies Mn and Cu may also originate in higher quantities from the oak chips used for aging [9], or from the oak wood casks that they are stored in for aging [10].

Although Fe, Mn and Zn are essential for human health, in elevated concentrations they may be harmful, and thus, the United States Environmental Protection Agency (U.S. EPA) advises an oral reference dose (RfDo) of 0.7, 0.14 and 0.3 mg/kg body weight/day, respectively [11–13]. On the other hand, Cd and Pb are considered as priority hazardous metals with RfDo of 0.001 and 0.00015 mg/kg body weight/day [14,15], which could cause kidney damage, anaemia, cancer and neurological disorders [16]. Cadmium is considered by the International Agency for Research and Cancer (IARC) group 1 carcinogen, while Pb group 2B carcinogen [17]. Elemental composition of alcoholic beverages, besides their verification from the point of view of human health risk, it can also be used to construct elemental fingerprints of different kinds of beverages, such as whiskies [18,19], Spanish brandies [20] or *orujo* distillates [21], that could be used in their authentication and differentiation.

The study of metal content and health risk assessment by the consumption of unrecorded alcohol is of interest not only to the Romanian population, but also to the international ones, as they may be shipped across borders. Pantani *et al.* [22] reported that a large proportion of unrecorded alcohol, produced in Finland, Sweden and other northern Countries, is shipped across borders. In some countries home-made alcohol is even slowly transitioning to mass production. Furthermore, the recent trend of online shopping, which circumvents alcohol availability regulations, increases their availability and affordability, as they are sold at a much cheaper price [3,23]. Thus, the quality assessment of home or small scale brewed alcoholic beverages could be considered an issue at global level.

The aim of this study was to evaluate the influence of the distillation processes on the content of several elements, including priority hazardous metals (Cd, Pb), in home- and industrially-brewed spirits, and cognacs. The determinations were carried out by inductively coupled plasma optical emission spectrometry (ICP-OES) after bringing the samples to dryness and redissolving in nitric acid. The risk assessment to human health, especially of the priority hazardous metals, ingested with the alcoholic beverages under study, was evaluated according to the target hazard quotient (THQ) and total THQ (TTHQ) approach. Also, possible correlations between different metals in the analysed alcoholic beverages were investigated using Principal Component Analysis.

RESULTS AND DISCUSSION

Figures of merit and method validation

Table 1 presents the limits of detection (LODs) in alcoholic beverages obtained by the ICP-OES method for 12 metals. The LODs of elements were in the range 0.0003(Mg)–0.0055(Ni) mg L^{-1}. The LODs were 5 and 7 times lower than the maximum recommended concentration of Cd and Co in recorded alcohol, and more than 10 times lower for the other metals, set by the AMPHORA project [3]. Therefore, the ICP-OES method is adequate for the quantification of the 12 metals in distilled alcoholic beverages.

Table 1. Figures of merit of the ICP-OES method for the determination of 12 metals in alcoholic beverages

Element	Wavelength (nm)	Parameters of the calibration curve		LOD (mg L^{-1})[a]	LOQ (mg L^{-1})[b]
		Slope (L mg^{-1})	Correlation coefficient		
Al	396.152	0.0000129	0.9986	0.0014	0.0042
Ca	422.673	0.0000024	0.9996	0.0004	0.0012
Cd	214.438	0.0000375	0.9999	0.0014	0.0042
Co	237.862	0.0000752	0.9997	0.0021	0.0063
Cr	267.716	0.0000330	0.9999	0.0015	0.0045
Cu	324.754	0.0000054	0.9997	0.0030	0.0090
Fe	259.940	0.0000252	0.9999	0.0006	0.0018
Mg	285.213	0.0000244	0.9995	0.0003	0.0009
Mn	260.569	0.0000091	0.9998	0.0004	0.0012
Ni	341.476	0.0000281	0.9991	0.0055	0.0165
Pb	220.351	0.0000040	0.9994	0.0032	0.0096
Zn	213.856	0.0000179	1.0000	0.0042	0.0126

[a] LOD was calculated using the 3σ criterion and sample preparation protocol [24];
[b] LOQ was considered as 3*LOD.

The accuracy of the ICP-OES method, was evaluated by fortifying the samples with the 12 metals. The recovery degrees were in the range 84–117% with a trueness of up to ± 20%. Thus, the ICP-OES method ensures accurate results in terms of metals determination in digested spirits and cognacs. The relative standard deviation was in the range 1.1–10.9%.

Content of major and trace metals in spirits and cognacs

Tables 2–5 presents the concentration of major and trace metals in home- and industrially-brewed spirits, and cognacs, respectively. It can be seen that the concentrations of metals were generally higher in home-brewed spirits compared to the industrially-brewed ones. The *t test* [24] revealed no significant differences in terms of major metals concentration (Ca, Mg, Cu, Fe, Al and Zn) in the home-brewed spirits from plums (samples 1–6), apples (samples 7–11) and grapes (samples 12–16), for 95% confidence level (t_{calc} = 0.020–1.232 < t_{tab} = 2.32). Also, it was revealed that there were no differences between beverages from different sources produced between 2016 and 2020. The concentrations of Ca and Mg in the home-brewed spirits were much higher (0.73–25.8 and 0.076–15.1 mg L^{-1}), compared to the industrially-brewed ones (0.13–0.65 and <0.0003(LOD)–0.11 mg L^{-1}). The difference could be attributed to the water used for the dilution of distillates.

The concentration of Ca and Mg in the home-brewed spirits under study were similar to those reported by Iwegbue *et al.* [25] and Bora *et al.* [26] (0.62–21.74 mg L^{-1} Ca and 0.09–11.26 mg L^{-1} Mg) in distillates originating from Nigeria.

Table 2. Concentrations ± C.I.[a] (mg L^{-1}) of major metals in home- and industrially-brewed spirits

	Sample nr.	Ca	Mg	Cu	Fe	Al	Zn
Home-brewed	1	10.3 ± 0.4	2.90 ± 0.30	4.74 ± 0.58	7.55 ± 0.44	0.41 ± 0.04	0.22 ± 0.01
	2	2.16 ± 0.18	0.66 ± 0.04	7.88 ± 0.46	1.57 ± 0.14	0.46 ± 0.05	0.073 ± 0.004
	3	5.48 ± 0.54	2.27 ± 0.17	9.58 ± 1.09	1.80 ± 0.08	0.14 ± 0.01	2.69 ± 0.30
	4	7.59 ± 0.61	0.93 ± 0.03	1.56 ± 0.18	22.06 ± 2.01	1.50 ± 0.05	0.64 ± 0.06
	5	5.97 ± 0.39	1.96 ± 0.12	5.54 ± 0.86	2.87 ± 0.24	0.46 ± 0.05	2.30 ± 0.24
	6	2.48 ± 0.14	0.35 ± 0.03	5.47 ± 0.41	1.68 ± 0.17	0.076 ± 0.008	0.048 ± 0.004
	7	12.0 ± 0.7	3.84 ± 0.24	0.53 ± 0.10	21.03 ± 1.41	1.23 ± 0.15	0.46 ± 0.03
	8	1.13 ± 0.09	0.15 ± 0.01	3.14 ± 0.31	2.42 ± 0.13	0.17 ± 0.02	0.17 ± 0.02
	9	0.73 ± 0.06	0.076 ± 0.005	1.86 ± 0.16	0.066 ± 0.004	0.020 ± 0.001	0.061 ± 0.004
	10	6.16 ± 0.57	1.13 ± 0.04	7.61 ± 0.35	16.7 ± 0.63	1.09 ± 0.04	0.96 ± 0.05
	11	1.42 ± 0.10	0.19 ± 0.02	8.73 ± 0.73	0.18 ± 0.01	0.06 ± 0.01	0.18 ± 0.02
	12	12.4 ± 0.9	1.89 ± 0.18	3.09 ± 0.15	3.97 ± 0.24	0.26 ± 0.04	0.20 ± 0.01
	13	8.17 ± 0.40	0.53 ± 0.04	7.55 ± 0.53	21.7 ± 0.8	1.15 ± 0.10	2.57 ± 0.19
	14	16.1 ± 1.8	2.19 ± 0.16	6.97 ± 0.47	5.65 ± 0.46	0.59 ± 0.03	0.38 ± 0.04
	15	3.31 ± 0.12	0.61 ± 0.05	6.69 ± 0.46	7.19 ± 0.41	0.34 ± 0.03	0.28 ± 0.01
	16	1.60 ± 0.16	0.27 ± 0.03	6.38 ± 0.63	0.030 ± 0.002	0.086 ± 0.005	0.20 ± 0.02
	17	25.8 ± 1.8	15.1 ± 0.64	0.50 ± 0.07	4.64 ± 0.46	2.24 ± 0.17	0.76 ± 0.04
	18	8.89 ± 0.37	2.38 ± 0.16	5.97 ± 0.24	22.1 ± 1.2	1.35 ± 0.07	0.78 ± 0.07
	Conc. range	*0.73–25.8*	*0.076–15.1*	*0.50–9.58*	*0.030–22.1*	*0.020–2.34*	*0.048–2.69*
	Average	*7.32*	*2.08*	*5.21*	*7.96*	*0.65*	*0.72*
	SD	*6.41*	*3.43*	*2.82*	*8.50*	*0.63*	*0.87*
Industr.-brewed	19	0.13 ± 0.01	<0.0003	0.016 ± 0.002	<0.0006	0.055 ± 0.004	0.017 ± 0.003
	20	0.30 ± 0.03	0.059 ± 0.005	0.022 ± 0.002	0.020 ± 0.003	0.088 ± 0.004	0.037 ± 0.005
	21	0.65 ± 0.04	0.11 ± 0.01	0.061 ± 0.007	0.019 ± 0.003	0.116 ± 0.009	0.032 ± 0.004
	Conc. range	*0.13–0.65*	<0.0003–*0.11*	*0.016–0.061*	<0.0006–*0.020*	*0.055–0.116*	*0.017–0.037*
	Average	*0.36*	*0.086*	*0.033*	*0.020*	*0.086*	*0.028*
	SD	*0.26*	*0.038*	*0.024*	*0.001*	*0.031*	*0.010*

[a]C.I. – is the confidence interval for n = 3 replicate measurements for 95% confidence level

Table 3. Concentrations ± C.I.[a] (mg L^{-1}) of trace metals in home- and industrially-brewed spirits

	Sample nr.	Cr	Cd	Co	Mn	Ni	Pb
Home-brewed	1	<0.0015	0.012 ± 0.001	<0.0021	0.13 ± 0.01	<0.0055	<0.0032
	2	0.070 ± 0.10	<0.0014	<0.0021	0.026 ± 0.001	<0.0055	<0.0032
	3	<0.0015	<0.0014	<0.0021	0.032 ± 0.002	<0.0055	<0.0032
	4	0.008 ± 0.002	0.005 ± 0.001	0.015 ± 0.003	0.44 ± 0.04	0.008 ± 0.002	0.038 ± 0.004
	5	<0.0015	0.004 ± 0.001	<0.0021	0.058 ± 0.003	<0.0055	<0.0032
	6	0.025 ± 0.002	<0.0014	0.020 ± 0.002	0.015 ± 0.001	0.051 ± 0.004	0.065 ± 0.008
	7	0.067 ± 0.006	0.008 ± 0.001	0.008 ± 0.002	0.18 ± 0.01	<0.0055	<0.0032
	8	<0.0015	<0.0014	<0.0021	0.029 ± 0.002	<0.0055	<0.0032
	9	<0.0015	<0.0014	<0.0021	0.014 ± 0.001	0.007 ± 0.001	<0.0032
	10	0.016 ± 0.002	<0.0014	0.015 ± 0.001	0.29 ± 0.02	0.017 ± 0.004	0.064 ± 0.005
	11	<0.0015	<0.0014	<0.0021	0.032 ± 0.003	<0.0055	<0.0032
	12	<0.0015	<0.0014	<0.0021	0.030 ± 0.001	<0.0055	<0.0032
	13	0.019 ± 0.003	0.005 ± 0.001	0.017 ± 0.004	0.17 ± 0.01	0.018 ± 0.002	0.43 ± 0.03
	14	0.022 ± 0.004	0.005 ± 0.001	<0.0021	0.16 ± 0.01	<0.0055	0.042 ± 0.006
	15	<0.0015	<0.0014	<0.0021	0.059 ± 0.005	<0.0055	<0.0032
	16	0.008 ± 0.005	<0.0014	0.010 ± 0.001	0.016 ± 0.002	0.024 ± 0.002	<0.0032
	17	<0.0015	0.042 ± 0.003	<0.0021	0.65 ± 0.06	<0.0055	0.67 ± 0.07
	18	<0.0015	0.009 ± 0.002	0.010 ± 0.002	0.26 ± 0.02	<0.0055	0.038 ± 0.007
	Conc. range	*<0.0015–0.070*	*<0.0014–0.042*	*<0.0021–0.020*	*0.014–0.65*	*<0.0055–0.051*	*<0.0032–0.67*
	Average	*0.029*	*0.011*	*0.014*	*0.14*	*0.020*	*0.19*
	SD	*0.025*	*0.013*	*0.005*	*0.17*	*0.016*	*0.25*
Industr.-brewed	19	<0.0015	<0.0014	0.012 ± 0.002	<0.0004	0.027 ± 0.003	<0.0032
	20	<0.0015	<0.0014	<0.0021	<0.0004	0.025 ± 0.003	<0.0032
	21	0.108	<0.0014	<0.0021	<0.0004	0.033 ± 0.002	<0.0032
	Conc. range	*<0.0015–0.108*	*<0.0014*	*<0.0021–0.012*	*<0.0004*	*0.025–0.033*	<0.0032
	Average	-	*<0.0014*	-	-	*0.028*	<0.0032
	SD	-	-	-	-	*0.004*	-

[a]C.I. – is the confidence interval for n = 3 replicate measurements for 95% confidence level

Table 4. Concentrations ± C.I.[a] (mg L^{-1}) of major metals in industrially produced cognacs

Sample nr.	Ca	Mg	Cu	Fe	Al	Zn
22	0.67 ± 0.07	0.11 ± 0.01	7.65 ± 0.40	1.21 ± 0.11	0.046 ± 0.004	0.078 ± 0.009
23	1.19 ± 0.10	2.59 ± 0.33	11.1 ± 1.1	1.52 ± 0.14	0.079 ± 0.006	0.11 ± 0.01
24	0.59 ± 0.07	0.21 ± 0.03	8.66 ± 0.61	6.29 ± 0.49	0.038 ± 0.002	0.18 ± 0.02
25	0.59 ± 0.05	0.066 ± 0.010	7.88 ± 0.42	0.96 ± 0.10	0.032 ± 0.003	0.10 ± 0.01
26	0.36 ± 0.02	0.072 ± 0.006	11.9 ± 1.3	3.76 ± 0.41	0.030 ± 0.003	0.15 ± 0.01
27	0.20 ± 0.01	0.020 ± 0.003	11.6 ± 0.8	7.08 ± 0.50	0.032 ± 0.004	0.14 ± 0.01
28	0.57 ± 0.05	0.086 ± 0.013	12.5 ± 1.0	11.4 ± 0.65	0.19 ± 0.02	0.25 ± 0.02
29	1.09 ± 0.09	0.17 ± 0.02	10.0 ± 0.6	9.58 ± 1.13	0.078 ± 0.005	0.24 ± 0.02
30	0.25 ± 0.01	0.081 ± 0.009	7.29 ± 0.60	0.81 ± 0.11	0.029 ± 0.003	0.26 ± 0.01
31	0.89 ± 0.09	0.071 ± 0.008	11.4 ± 0.6	1.20 ± 0.10	0.028 ± 0.004	0.15 ± 0.02
32	0.57 ± 0.06	0.11 ± 0.01	11.1 ± 1.0	3.10 ± 0.30	0.060 ± 0.008	0.14 ± 0.01
33	0.73 ± 0.09	0.12 ± 0.01	50.6 ± 3.2	0.31 ± 0.03	0.041 ± 0.003	0.36 ± 0.04
34	0.46 ± 0.04	0.16 ± 0.01	51.3 ± 2.7	1.94 ± 0.18	0.054 ± 0.007	0.38 ± 0.03
35	4.65 ± 0.30	2.57 ± 0.19	21.5 ± 2.0	2.75 ± 0.31	0.072 ± 0.003	0.24 ± 0.02
36	4.88 ± 0.43	2.48 ± 0.27	20.3 ± 1.6	1.01 ± 0.09	0.11 ± 0.01	0.20 ± 0.02
37	4.25 ± 0.27	2.38 ± 0.23	14.0 ± 1.4	0.20 ± 0.02	0.081 ± 0.011	0.11 ± 0.01
38	5.03 ± 0.20	2.64 ± 0.21	22.1 ± 2.0	1.31 ± 0.11	0.11 ± 0.01	0.25 ± 0.02
39	4.91 ± 0.39	2.35 ± 0.24	35.2 ± 2.5	0.38 ± 0.03	0.080 ± 0.013	0.29 ± 0.04
40	5.07 ± 0.34	2.52 ± 0.28	23.3 ± 3.3	0.32 ± 0.03	0.13 ± 0.01	0.32 ± 0.04
Conc. range	*0.20–5.07*	*0.020–2.64*	*7.29–51.3*	*0.20–11.4*	*0.028–0.19*	*0.078–0.38*
Average	*1.94*	*0.99*	*18.4*	*2.90*	*0.070*	*0.21*
SD	*2.01*	*1.19*	*13.5*	*3.30*	*0.043*	*0.09*

[a]C.I. – is the confidence interval for n = 3 replicate measurements for 95% confidence level

The content of Cu in home-brewed spirits was in the range 0.50–9.50 mg L^{-1}, higher than in store-bought alcoholic beverages (0.016–0.061 mg L^{-1}). According to OIV and Italian legislation the maximum permissible concentration of Cu in wine is set to 1 mg L^{-1} [2] and 10 mg L^{-1}[27], respectively. The content of Cu in home-brewed spirits did not exceed the permissible level set by the Italian legislation, but was over the OIV level. The limit set by the AMPHORA project of 2 mg L^{-1} [3] was also exceeded by the majority of home-brewed spirits.

Table 5. Concentrations ± C.I.[a] (mg L^{-1}) of trace metals in industrially produced cognacs

Sample nr.	Cr	Cd	Co	Mn	Ni	Pb
22	<0.0015	<0.0014	0.016 ± 0.003	0.022 ± 0.002	0.035 ± 0.004	<0.0032
23	0.012 ± 0.003	<0.0014	0.014 ± 0.003	0.128 ± 0.014	0.035 ± 0.001	<0.0032
24	0.018 ± 0.002	<0.0014	0.027 ± 0.004	0.039 ± 0.004	0.043 ± 0.005	<0.0032
25	<0.0015	<0.0014	<0.0021	0.028 ± 0.003	0.017 ± 0.003	<0.0032
26	<0.0015	<0.0014	0.011 ± 0.003	0.026 ± 0.003	0.029 ± 0.006	<0.0032
27	0.050 ± 0.005	<0.0014	0.018 ± 0.004	0.079 ± 0.008	0.044 ± 0.002	<0.0032
28	0.048 ± 0.006	<0.0014	0.010 ± 0.002	0.081 ± 0.011	0.025 ± 0.004	<0.0032
29	0.036 ± 0.003	<0.0014	0.011 ± 0.003	0.083 ± 0.006	0.021 ± 0.004	<0.0032
30	<0.0015	<0.0014	<0.0021	0.021 ± 0.002	0.023 ± 0.005	<0.0032
31	<0.0015	<0.0014	<0.0021	0.030 ± 0.002	0.019 ± 0.003	<0.0032
32	0.011 ± 0.002	<0.0014	0.020 ± 0.003	0.029 ± 0.003	0.043 ± 0.004	<0.0032
33	0.027 ± 0.001	<0.0014	<0.0021	0.007 ± 0.001	0.035 ± 0.005	0.013 ± 0.002
34	0.024 ± 0.005	<0.0014	<0.0021	0.016 ± 0.001	0.021 ± 0.003	<0.0032
35	0.011 ± 0.002	0.008 ± 0.001	<0.0021	0.040 ± 0.004	<0.0055	<0.0032
36	0.018 ± 0.003	<0.0014	<0.0021	0.071 ± 0.005	<0.0055	0.030 ± 0.006
37	<0.0015	0.008 ± 0. 001	<0.0021	0.075 ± 0.006	<0.0055	<0.0032
38	0.019 ± 0.002	0.006 ± 0. 001	<0.0021	0.093 ± 0.009	<0.0055	0.013 ± 0.002
39	0.030 ± 0.003	0.006 ± 0. 001	0.052 ± 0.009	0.048 ± 0.004	0.019 ± 0.004	0.039 ± 0.008
40	0.015 ± 0.002	0.005 ± 0. 001	<0.0021	0.081 ± 0.004	<0.0055	<0.0032
Conc. range	*<0.0015–0.050*	*<0.0014–0.008*	*<0.0021–0.052*	*0.007–0.128*	*<0.0055–0.044*	*<0.0032–0.039*
Average	*0.024*	*0.007*	*0.020*	*0.052*	*0.029*	*0.023*
SD	*0.013*	*0.001*	*0.013*	*0.033*	*0.010*	*0.013*

[a]C.I. – is the confidence interval for n = 3 replicate measurements for 95% confidence level

The high Cu concentration could be explained by the fact that home brewers mainly use copper stills, while industrial distilleries use stainless steel. Adam *et al.* [19] found that the largest amount of Cu (97%) in whiskies originate from the copper stills, while a proportion of only 3% from the barley that is distilled from. Iwegbue *et al.* [25] and Bora *et al.* [26] found concentrations of Cu in store bought spirits in the range 0.71–1.33 and 0.56–1.89 mg L^{-1}, respectively. van Wyk *et al.* [28] found concentrations of Cu in pot stilled spirits up to 8.6 mg L^{-1}, attributed mainly to the distillation process. The Cu concentration in cognacs in our beverages was extremely high, between 7.29 and 51.3 mg L^{-1}.

Ibanez *et al.* [5] pointed out that besides the distillation equipment, Cu may originate from the metallic containers (low-quality steel or Cu alloys) in which the alcoholic beverages are stored, the bottling process, or from added Cu that improves their organoleptic properties.

The concentration of Fe, Al and Zn in the home-brewed spirits were between 0.030–22.1, 0.020–2.34 and 0.048–2.68 mg L^{-1}, respectively, while in those of commercially sold in stores were around 10 times lower. None of the three metals in the store-bought spirits surpassed the threshold of 2 mg L^{-1}, set by the AMPHORA project [3]. The values of Fe, Al, Zn in the majority of home-brewed spirits surpassed this threshold value. In the cognac samples, the concentration of Fe, Al and Zn was found to be up to 11.4 ± 0.65, 0.19 ± 0.02 and 0.38 ± 0.03 mg L^{-1}, respectively, similar to those found by Iwegbue *et al.* [25] and Bora *et al.* [26]. The content of Fe was above the AMPHORA set limit in seven cognac distillates, while the concentrations of Al and Zn were below the threshold values in all distillates of this type.

The concentration of Cd and Pb in the home-brewed spirit samples were in the range <0.0014–0.042 (mean 0.011) and <0.0032–0.67 (mean 0.19) mg L^{-1}. Cd concentration in the analyzed home- and industrially-brewed spirits were below the 0.010 mg L^{-1} AMPHORA limit [3], with the exception of the home-brewed spirit from plums (sample 1, 0.012 ± 0.001 mg L^{-1}) and cherry (sample 17, 0.042 ± 0.003 mg L^{-1}). Pb concentrations were also below the 0.2 mg L^{-1} AMPHORA limit [3], with the exception of the home-brewed spirit from grapes (sample 13, 0.43 ± 0.03 mg L^{-1}) and cherry (sample 17, 0.67 ± 0.07 mg L^{-1}). The elevated Pb concentrations in the two samples may originate from the soldering material used in the copper stills. Iwegbue *et al.* [25] found concentrations of Cd and Pb within the permissible levels in their spirit and cognac samples, 0.001–0.030 mg L^{-1} Cd and 0.08–0.20 mg L^{-1} Pb, respectively. On the other hand, Bora *et al.* [26] found, in average, Cd and Pb concentrations in their spirits of 0.03 ± 0.02 and 0.30 ± 0.13 mg L^{-1}, respectively, surpassing both the OIV [13] and AMPHORA [3] set limits.

The concentrations of Cr, Co, Mn and Ni were in the <0.0015–0.108, <0.0021–0.020, <0.0004–0.65 and <0.0055–0.051 mg L^{-1} range, respectively, below the AMPHORA limits, with the exception of one home-brewed spirit (sample 17) in case of Mn (0.65 ± 0.06 mg L^{-1}). Our results were similar to those found by Iwegbue *et al.* [25] and Bora *et al.* [26].

Human health risk assessment

Table 6 presents a summary overview of the oral reference dose (RfDo), the recommended daily intake (RDA) and the maximum admitted concentrations (MACs) of the studied metals in wine, according to German, Polish, Italian and Australian legislations, in recorded alcohol, according to the AMPHORA project, and in drinking water, set by the U.S. EPA, European Commission and European Food Safety Authority (EFSA).

Table 6. The oral reference dose (RfDo), the recommended daily intake (RDA) and the maximum admitted concentrations (MAC) of the studied elements in wine, recorded alcohol and drinking water

Metal	RfDo (mg/kg b.w./ day)	RDA (mg/day)	MAC in wine (mg L^{-1})					MAC in recorded alcohol (mg L^{-1})	MAC in drinking water (mg L^{-1})
			OIV	DE	PL	IT	AU		
Ca	-	1000 [29]	-	-	-	-	-	-	50 [30]
Mg	-	220 in females [29] 260 in males [29]	-	-	-	-	-	-	12 [30]
Cu	0.04 [31]	1.3 in females [32] 1.6 in males [32]	1.00 [2]	5.00 [27]	-	10.00 [27]	5.00 [27]	2.00 [3]	2.0 [33] 0.02 [30]
Fe	0.7 [12]	19.6–58.8 in females [29] 9.1–27.4 in males [29]	-	-	-	-	-	2.00 [3]	0.3 [30,34]
Al	1.0 [35]	-	-	8.0 [27]	-	-	-	2.0 [3]	0.05–0.2 [34]
Zn	0.3 [11]	3.0–9.8 in females [29] 4.2–14.0 in males [29]	5.0 [2]	5.0 [27]	-	-	-	5.0 [3]	5.0 [34] 0.1 [30]
Cr	0.3 [36]	-	-	-	-	-	-	0.5 [3]	0.050 [33] 0.030 [30]
Cd	0.001 [14]	-	0.01 [2]	0.01 [27]	0.03 [27]		0.05 [27]	0.01 [3]	0.005 [33] 0.0005 [30]
Co	0.0016 [37]	0.12 [37]	-	-	-	-	-	-	0.01 [28]
Mn	0.14 [13]	3 [38]	-	-	-	-	-	0.50 [3]	0.05 [33,37]
Ni	0.013 [40]	-	-	-	-	-	-	0.20 [3]	0.020 [33] 0.010 [30]
Pb	0.00015 [15]	-	0.15 [2]	0.30 [27]	0.30 [27]	0.30 [27]	0.20 [27]	0.20 [3]	0.010 [33] 0.005 [33]

OIV - International Organization of Vine and Wine; DE – Germany; PL – Poland; IT – Italy; AU – Australia.

Table 7 presents the target hazard quotients (THQ) of each individual metal, and the total THQ (TTHQ) for the home- and industrially-brewed spirits. In terms of industrially-brewed alcohol, there were found no risk of exposure to metals by the consumption of these types of beverages, not even at a high consumption rate (300 mL/day), the highest TTHQ value being 0.0616. On the other hand, in case of the home-brewed spirits, at moderate consumption rate (100 mL/day) three home-brewed spirits were found to pose some health risk (TTHQ>1), samples 10, 13 and 17, due to the high content of Pb, which resulted in THQ values of 0.7144, 4.7611 and 7.4611, representing 65, 92 and 98% of TTHQ, respectively. Thus, with the exception of these three samples, there is no health concern by a moderate consumption of the home-brewed spirits for 365 days/year and an exposure period of 57 years.

Table 7. Estimated THQ and TTHQ values from metals exposure by the consumption of the home- and industrially-brewed spirits

	Sample nr.	THQ*										TTHQ*
		Cu	Fe	Al	Zn	Cr	Cd	Co	Mn	Ni	Pb	
Home-brewed	1	0.1975	0.0180	0.0007	0.0012	0.0000	0.0198	0.0000	0.0015	0.0000	0.0000	0.2387
	2	0.3281	0.0037	0.0008	0.0004	0.0004	0.0000	0.0000	0.0003	0.0000	0.0000	0.3337
	3	0.3990	0.0043	0.0002	0.0149	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.4188
	4	0.0651	0.0525	0.0025	0.0036	0.0000	0.0088	0.0158	0.0053	0.0010	0.4183	0.5730
	5	0.2306	0.0068	0.0008	0.0128	0.0000	0.0065	0.0000	0.0007	0.0000	0.0000	0.2582
	6	0.2277	0.0040	0.0001	0.0003	0.0001	0.0000	0.0213	0.0002	0.0065	0.7228	0.9830
	7	0.0221	0.0501	0.0021	0.0026	0.0004	0.0127	0.0088	0.0022	0.0000	0.0000	0.1008
	8	0.1306	0.0058	0.0003	0.0009	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000	0.1379
	9	0.0776	0.0002	0.0000	0.0003	0.0000	0.0000	0.0000	0.0002	0.0009	0.0000	0.0791
	10	0.3171	0.0398	0.0018	0.0053	0.0001	0.0000	0.0152	0.0035	0.0022	0.7144	**1.0994**
	11	0.3638	0.0004	0.0001	0.0010	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.3656
	12	0.1288	0.0094	0.0004	0.0011	0.0000	0.0000	0.0000	0.0004	0.0000	0.0000	0.1401
	13	0.3146	0.0517	0.0019	0.0143	0.0001	0.0083	0.0181	0.0020	0.0022	**4.7611**	**5.1744**
	14	0.2902	0.0135	0.0010	0.0021	0.0001	0.0076	0.0000	0.0019	0.0000	0.4672	0.7836
	15	0.2788	0.0171	0.0006	0.0016	0.0000	0.0000	0.0000	0.0007	0.0000	0.0000	0.2987
	16	0.2658	0.0001	0.0001	0.0011	0.0000	0.0000	0.0101	0.0002	0.0030	0.0000	0.2805
	17	0.0208	0.0110	0.0037	0.0042	0.0000	0.0705	0.0000	0.0077	0.0000	**7.4611**	**7.5791**
	18	0.2485	0.0527	0.0023	0.0043	0.0000	0.0150	0.0102	0.0030	0.0000	0.4183	0.7544
	Min.	*0.0208*	*0.0149*	*0.0008*	*0.0055*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0791*
	Max.	*0.3990*	*0.0527*	*0.0037*	*0.0149*	*0.0004*	*0.0705*	*0.0213*	*0.0077*	*0.0065*	***7.4611***	***7.5791***
	Average	*0.2170*	*0.0189*	*0.0011*	*0.0040*	*0.0001*	*0.0083*	*0.0055*	*0.0017*	*0.0009*	*0.8313*	*1.0888*
Industrially-brewed	19	0.0007	0.0000	0.0001	0.0001	0.0000	0.0000	0.0127	0.0000	0.0034	0.0000	0.0170
	20	0.0009	0.0000	0.0001	0.0002	0.0000	0.0000	0.0000	0.0000	0.0032	0.0000	0.0045
	21	0.0025	0.0000	0.0002	0.0002	0.0006	0.0000	0.0000	0.0000	0.0043	0.0000	0.0078
	Min.	*0.0007*	*0.0000*	*0.0001*	*0.0001*	*0.0000*	*0.0000*	*0.0000*	*0.0000*	*0.0032*	*0.0000*	*0.0041*
	Max.	*0.0025*	*0.0000*	*0.0002*	*0.0002*	*0.0006*	*0.0000*	*0.0127*	*0.0000*	*0.0043*	*0.0000*	*0.0205*
	Average	*0.0014*	*0.0000*	*0.0001*	*0.0002*	*0.0002*	*0.0000*	*0.0042*	*0.0000*	*0.0036*	*0.0000*	*0.0098*

*THQ and TTHQ values >1 are marked in bold face.

Figure 1 presents the exposure frequency to metals of the analyzed home-brewed spirits at moderate (100 mL/day), high (200 mL/day) and very high consumption (300 mL/day) rate. Exposure frequency was calculated as the number of samples in which THQ for Cu, Cd and Pb, and TTHQ value exceeded the limit value of 1 from the total number of samples. According to our analysis, at moderate consumption, 17% (3 samples) of the home brewed spirits present risk to human health, mainly due to the high content of Pb found in the samples. On the other hand, at high and very high consumption rate, the exposure frequency was 33 and 50%, respectively, due to the cumulative effects of the metals, where the main contribution was found to be Pb and Cu. At a high consumption rate of the home brewed spirits, TTHQ was found being up to 15.1048, while at very high consumption rate, up to 22.6571.

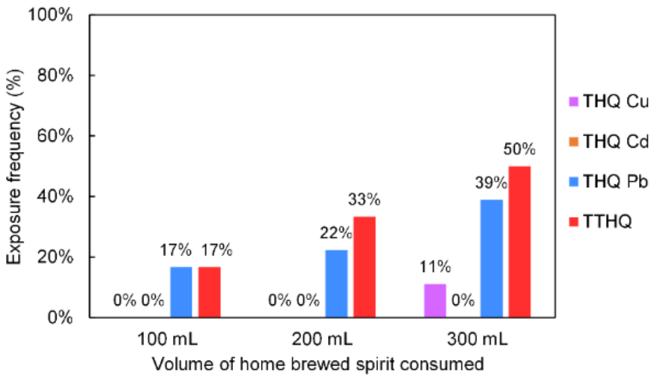

Figure 1. Exposure frequency to metals by consumption of home-brewed spirits in which the THQ and TTHQ values exceeded the value of 1

Elemental profiling of alcoholic beverages by PCA

Table 8 presents the results of the PCA analysis performed on the home- and industrially-brewed spirits and cognacs. The alcoholic beverages are characterized by four PCs, which explains ~79% of the elemental composition variability. The PC1 (45.5%), containing Ca, Mg, Al, Cd, Mn and Pb (0.75–0.95), was attributed to natural factors, such as water used in the distillation process. It can be observed the presence of the two priority hazardous elements (Cd, Pb), which leads to the idea that they could have a natural origin, but could also be associated with the fermentation and distillation conditions, considering that they were found in higher concentrations in the home-brewed spirits. The next 3 factors (PC2–PC4), describing 33.7% of the variability, was associated with the distillation equipment material, because it contains Co and Ni (PC2, 14.1%), Fe, Cu and Al (PC3, 11.2%), and Zn and Cr (PC4, 8.4%). These three factors characterize primarily the industrially-brewed beverages, in which higher concentrations have been found, and which suggests that the distillation equipment was made of stainless steel. It is interesting to note that although Cu was found in high concentrations in the home-brewed spirits and in the industrially-brewed cognacs from wine, it has only an average influence on the characteristics of the alcoholic beverages analyzed (loading factor –0.61 in PC3, 11.2%). This demonstrates that the source of Cu is not well defined, and could be attributed to the raw material (wine) and the distillation equipment material. This is also supported by the fact that two of the elements (Fe and Al) in PC3 could be associated with their natural presence in wine.

Table 8. Principal components with eigenvalues > 1 and factor loadings of parameters after auto-scaling and Varimax rotation[a] that describe the elements variability in the home- and industrially brewed spirits and cognacs

Element/Parameter	PC1	PC2	PC3	PC4
Ca	**0.84**	-0.29	0.25	0.14
Mg	**0.95**	-0.18	-0.06	0.01
Cu	-0.06	0.31	*-0.61*	0.18
Fe	0.20	0.21	**0.85**	0.29
Al	**0.75**	-0.02	*0.59*	0.17
Zn	0.15	0.03	0.31	**0.80**
Cr	-0.07	0.27	0.30	*-0.61*
Cd	**0.97**	-0.13	-0.02	0.00
Co	0.01	**0.87**	0.07	0.02
Mn	**0.84**	0.01	0.41	0.11
Ni	-0.27	**0.75**	-0.16	-0.30
Pb	**0.85**	0.13	0.09	0.19
Eigenvalue	5.45	1.69	1.35	1.01
Total variance (%)	45.5	14.1	11.2	8.4
Cumulative (%)	45.5	59.6	70.8	79.3

[a] Strong relationship loading values > 0.70 are in bold face; moderate values between 0.50–0.70 are marked in italics; the values < 0.50 corresponding to a weak relationship are written with regular font [40]

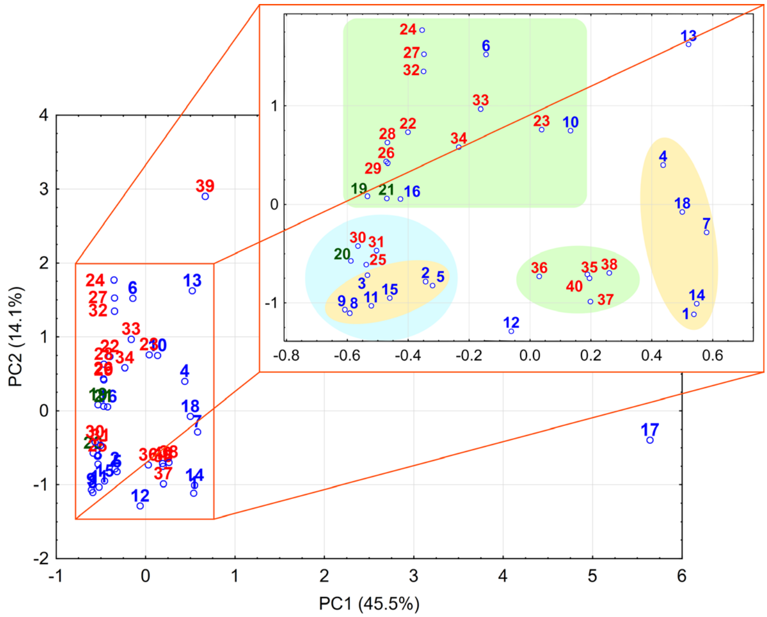

Figure 2. Two-dimensional PCA after auto-scaling and Varimax rotation of the analyzed home-brewed (marked in red) and industrially-brewed spirits (marked in green), and industrially-brewed cognacs (marked in blue)

Figure 2 shows the beverages clustering based on the two-dimensional Varimax rotated PCA after auto-scaling of the original data. According to Figure 1, there is a wide dispersion of alcoholic beverages, but can be observed a classification on clusters and sub-clusters according to their origin. Thus, a well-defined cluster of home-brewed spirits, and two of the industrially-brewed alcoholic beverages can be observed, one that groups cognacs and spirits, and another that groups only cognacs. A third group contains both industrially- and home-brewed beverages.

Figure 3 presents the two-dimensional PCA grouping of the parameters after auto-scaling and Varimax rotation, which characterize the analyzed alcoholic beverages. Two distinct groups could be observed, that of the elements of natural origin (Ca, Mg, Al, Mn, Cd, Pb), which have a great influence on the variability of alcoholic beverages based on the first factor (45.5%). The second group includes elements that show the influence of the distillation equipment material on the elemental content of the alcoholic beverages (Cu, Fe, Cr, Zn, Ni, Co). Of these elements, the greatest influence is observed for Ni and Co, while Cu present only a small influence in comparison. Its presence in alcoholic beverages, especially in the cognacs, is more influenced by the wine content, and less by the distillation equipment.

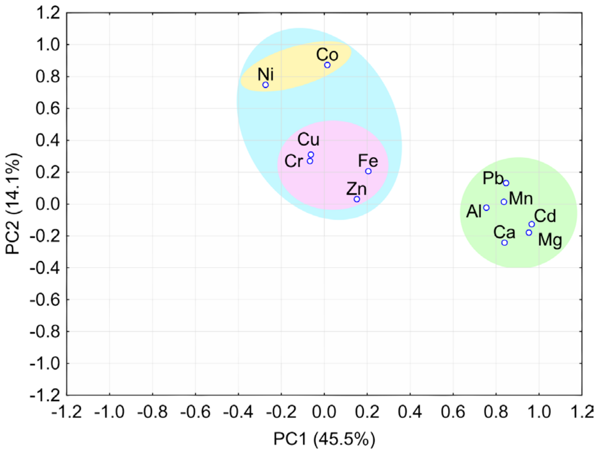

Figure 3. Two-dimensional PCA after auto-scaling and Varimax rotation of the analyzed alcoholic beverages

CONCLUSIONS

The results of the present study revealed that the concentrations of the 12 metals in the home-brewed spirits were significantly higher than in the industrially-brewed ones. In most of the analyzed samples the AMPHORA set threshold values for spirits were surpassed in case of Cu, which was attributed to the copper stills used by the home brewers. Nonetheless, health risk assessment by the THQ and TTHQ approach did not indicate non-cancerogenic long term health risk in case of moderate spirits consumption, with the exception of three home-brewed ones, that had very high Pb concentration. The results of this study also indicated no significant differences in terms of metals concentration in spirits home distilled from plums, apples and grapes. The statistical PCA analysis highlighted the fact that alcoholic beverages are characterized by a great variability of their elemental composition, the first four factors describing ~79% of the variability. However, a few groups of alcoholic beverages have been observed according to their origin (home- or industrially-brewed). Also, two clusters of the elements could be highlighted, one that includes elements of natural origin (Ca, Mg, Al, Mn, Cd, Pb), and the second, the trace elements, whose origin was associated with the material of the distillation equipment, or in the case of Cu, its presence in the cognacs was associated more with its content in wine, than the one resulting from the distillation equipment. However, it was observed that it has only an average influence, in proportion of around 11% on the variability of alcoholic beverages.

EXPERIMENTAL SECTION

Reagents and solutions

The ICP-OES instrument calibration was achieved using standard solutions obtained by dilution with 5% (v/v) HNO_3 of an ICP multi-elemental standard solution IV 1000 mg L^{-1} produced by Merck (Darmstadt, Germany). Samples acidulation and preparation of the 5% (v/v) HNO_3 solution was prepared from 69% (w/w) HNO_3 for analysis (Merck, Germany). For the dilution of samples doubly distilled water was employed, obtained with the Fistreem Cyclon Double (Bi-) Distiller (Cambridge, United Kingdom).

Samples and sample preparation

Samples consisted of 18 home- and 3 industrially-brewed spirits, and 19 industrially obtained cognacs. The home brewed spirits were distilled from plums (samples 1–6), apples (samples 7–11), grapes (samples 12–16), cherry (sample 17) and peach (sample 18) between 2016 and 2020 in small scale distilleries or in private homes. They originated from different locations across Romania, namely Tăutelec (Bihor County), Satu Mare (Satu Mare County), Carastelec (Sălaj County), Vama (Suceava County), Vaslui (Vaslui County), Sfântu Gheorghe (Covasna County), Viile Satu Mare (Satu Mare County), Viișoara (Cluj County) and Panciu (Vrancea County). The industrially-brewed spirits (samples 19–21) originated from local stores of Cluj-Napoca, while the cognacs (samples 22–40) were obtained industrially from an unadulterated producer, that were not intended for human consumption.

Samples preparation consisted in evaporation of aliquot volumes of 50 mL alcoholic beverage on a sand bath to dryness, retaking it in 10 mL HNO_3 69% (w/w), boiling for 1 h in order to digest the organic compounds and dilution to 25 mL with doubly distilled water. Along with the samples, a blank solution was also prepared.

Instrumentation

The concentrations of metals in the alcoholic beverages were measured using the Spectro CIROS^{CCD} ICP-OES spectrometer (Spectro, Kleve, Germany) using the following conditions: 27.12 MHz radiofrequency, 1400 W plasma power, 12/0.6/1 L min^{-1} outer/auxiliary/nebulaztion Ar flow rate, axial plasma viewing (X = -3.9 mm, Y = 3.6 mm, Z = +2.6 mm). The samples were pumped by a peristaltic pump, at a flow rate of 5 mL min^{-1}, into the cross-flow nebulizer (flushing time 40 s, delay time 20 s). The emission signals of the elements were separated by the double grating Paschen Runge polychromator with Ar filled chamber and were detected simultaneously by the 22 charge coupled devices (CCD). Signals were processed as peak height with background correction in two-points. Quantitative determinations were realized after external calibration in the 0–10 mg L^{-1} range (n = 8 points) for all elements.

Method validation

Method validation consisted of figures of merit (LOD, LOQ), accuracy and precision evaluation. LODs of the elements were calculated as the ratio of 3 times the standard deviation of 11 measurements of a blank sample and the slope of the calibration curve (3σ criterion), while LOQ was considered as 3*LOD [24]. Methods accuracy was verified by spike – recovery testing, using a concentration of 0.1 mg L^{-1} ICP IV standard solution for elements in the <LOD–1 mg L^{-1} concentration range, and 5 mg L^{-1} ICP IV solution for 1–50 mg L^{-1} concentration range. Methods precision was verified by relative standard deviation (RSD, %) calculation from samples replicate measurements.

Human health risk assessment

According to WHO, in Romania, the adult per capita consumption of pure alcohol is 12.6 L per year [1], which results in a consumption of 100 mL alcoholic beverage (40%, v/v) for a consumption frequency of 365 days/year.

The THQ values, for the assessment of non-carcinogenic health risk posed by the metals present in the alcoholic beverages, with the exception of Ca and Mg, was calculated using equation (1), while TTHQ was calculated as the sum of individual THQ values [14], taking into account 100 mL alcohol/day for moderate consumption, 200 mL for high consumption and 300 mL for very high alcohol consumption. If THQ and TTHQ are <1, then there is no health risk to non-carcinogenic diseases, while at THQ value >1, some detrimental health effects may appear due to exposure to metals in the analyzed alcoholic beverages. It can be considered that TTHQ is much more suitable for assessing the long-term risk exposure, because it reflects the cumulative effect of potentially toxic elements present in drinks or foods.

$$THQ= \frac{Efr \times EDtot \times Fir \times c}{RfDo \times Bwa \times ATn} \times 10^{-3} \quad (1)$$

where, Efr – is the exposure frequency (365 days/year),

EDtot – is the exposure duration (57 years, based on an average life expectancy in Romania of 75 years and consumption starting at age 18),

Fir – is the daily alcohol ingestion (100; 200; 300 mL/day),

c – is the concentration of the element in the alcoholic beverage (mg/L),

RfDo – is the oral reference dose (mg/kg/day),

Bwa – is the average body weight of an adult (60 kg),
ATn – is the exposure time for non-carcinogens (20,805 days)
10^{-3} – is the unit conversion factor.

Elemental profiling of alcoholic beverages by PCA

The elemental profile of the studied alcoholic beverages was obtained by the unsupervised multivariate Principal Component Analysis (PCA) method after Varimax rotation of the auto-scaled data. In the PCA approach, a data set is transformed by combining the original parameters into a multidimensional space of new variables called principal components (PCs) or factors. Each PC contains a linear combination of the original variables. Only dominant PCs that have an eigenvalue greater than 1 according to the Kaiser criterion, that have the largest variance and describe the system's variability were considered in the Varimax rotation. Maximization of the variance of the retained PCs was achieved by Varimax rotation. The absolute loading values provided the influence of each parameter in a PC. A strong influence of a parameter was considered for loading values (> 0.70), while values in the range 0.50–0.70 or 0.30–0.50 indicate a moderate or weak influence [40]. The PCA analysis was performed considering all elements and all beverages. In the beverages in which the metals concentration was lower than the method LODs, the considered value was one half of LOD.

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