J. Elem. s. 67–78 DOI: 10.5601/jelem.2012.17.1.06

EFFECT OF TRAFFIC POLLUTION ON CHEMICAL COMPOSITION OF RAW ELDERBERRY (*SAMBUCUS NIGRA* L.)

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Abstract

The aim of the study was to determine the effect of road traffic on the chemical composition of elderberry flowers and fruits. The raw material for the study was collected from sixteen different wild stands in south-east Poland. Each stand was located at a different distance from roads, carrying different loads of traffic. The raw material was collected from June (flower) to August (fruit) 2009 and dried at 30°C. Determination of the content of flavonoids (flavonols converted into quercetin) was performed by spectrophotometry according to the Polish Pharmacopoeia VIII (2008) procedure. Some minerals, including heavy metals (Cr, Zn, Pb, Cu, Cd, Ni, Fe and Mn), were determined with the ICP-AES method after dry digestion of the plant material.

A closer distance to transportation routes and heavier traffic had a significant impact on the chemical composition of raw elderberry, which had a lower total content of flavonoids than the material collected from sites further away from roads. Significant correlation was found between the content of Cr, Fe, Cd and Cu in elderberry fruit or the content of Fe, Zn, Cu, Cr, Cd, Pb and Mg in elderberry flowers and the influence of traffic, where closer proximity and heavier road traffic contributed significantly to an increase in the concentration of these elements in the analyzed raw material. The chemical composition of elderberry flowers and fruits was significantly affected by the traffic and depended on a harvest site.

Key words: elderberry, *Sambucus nigra*, traffic pollution, content of flavonoids, macro- and micronutrients.

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WPŁYW ZANIECZYSZCZEŃ KOMUNIKACYJNYCH NA SKŁAD CHEMICZNY SUROWCA BZU CZARNEGO (*SAMBUCUS NIGRA* L.)

Abstrakt

Celem badań było określenie wpływu zanieczyszczeń komunikacyjnych na skład chemiczny kwiatów i owoców bzu czarnego. Surowce pozyskano z 16. stanowisk naturalnych w południowo-wschodniej części Polski. Stanowiska znajdowały się w różnym oddaleniu od ciągów komunikacyjnych o zróżnicowanym natężeniu ruchu drogowego. Surowiec zbierano od czerwca (kwiat) do sierpnia (owoc) 2009 r. i natychmiast po zbiorze suszono w temp. 30°C. Zawartość flawonoidów (w przeliczeniu na kwercetynę) oznaczono spektrofotometrycznie wg Farmakopei Polskiej VIII (2008), a wybranych składników mineralnych – włącznie z metalami ciężkimi (Cr, Zn, Pb, Cu, Cd, Ni, Fe i Mn) – po mineralizacji materiału roślinnego na sucho metodą IPC.

Bliska odległość od ciągów komunikacyjnych oraz większa intensywność ruchu drogowego wpłynęły w istotny sposób na skład chemiczny surowca bzu czarnego, który zawierał mniej flawonoidów w stosunku do surowców pozyskiwanych z terenów bardziej odległych. W doświadczeniu stwierdzono istotną zależność między zawartością Cr, Fe, Cd i Cu w owocu bzu czarnego oraz Fe, Zn, Cu, Cr, Cd, Pb i Mg w kwiecie bzu czarnego a miejscem ich pozyskiwania. Bliskość ciągów komunikacyjnych i większa intensywność ruchu drogowego znacznie przyczyniły się do zwiększenia zawartości tych pierwiastków w analizowanym surowcu. Na skład chemiczny obydwu przebadanych rodzajów surowca bzu czarnego istotny wpływ ma miejsce ich pozyskiwania ze stanu naturalnego.

Słowa kluczowe: bez czarny, *Sambucus nigra*, zanieczyszczenia komunikacyjne, zawartość flawonoidów, zawartość makro- i mikroelementów.

INTRODUCTION

Common elderberry (*Sambucus nigra* L.) has many medicinal properties. Elder flowers contain mainly flavonoids (min. 0.8% acc. to Polish Pharmacopoeia VIII (2008)), organic acids and essential oils. Elderberry fruits have anthocyanins, flavonoids and phenolic acids. Elderberry extract and extract-containing preparations demonstrate antiviral properties, reducing the duration of flu symptoms to 3-4 days. They stimulate the immune system by increasing production of inflammatory cytokines, and are probably immunoprotective when administered to cancer and AIDS patients. Elderberry extract acts as an antioxidant, neutralizing free radicals and inhibiting the cooxidation reactions of linolenic acid and b-carotene(Barak et al. 2001, Dawidowicz et al. 2006, Simonovik et al. 2007). Elderberry fruits have been used for making preserves, wines, winter cordials, and for adding flavor and color to other products (ice cream, beverages, yoghurt). Dietary supplements containing elderberry extracts, juices or syrups have become popular as remedies for treating cold and flu symptoms (*The ABC*... 2003). Elderberry plants are also grown as ornamentals. Unfortunately, wild elderberry shrubs often grow in areas polluted by vehicles. Elderberry grows mostly in ruderal habitats (landfill, rubble), in thickets, on roadsides and along busy streets (Atkinson, Atkinson 2002). Harvesting flowers and fruits from such sites raises concern about the quality of elderberry extracts and efficacy of treatment. Increasing pollution leads to a steady rise in levels of toxic heavy metals in plants, which precludes their optimal use as medicinal raw produce. Using contaminated herbs, apart from the desired pharmaceutical effect, causes intoxication of a patient with metals (MIROSŁAWSKI et al. 1995, OGNIK et al. 2006). Thus, it seems necessary to examine medicinal plants for their content of heavy metals. This is dictated by the fact that our current knowledge does not reflect the negative effects of toxic heavy metals and their possible interactions (MIROSŁAWSKI et al. 1995).

The purpose of this study is to determine the effect of road traffic on the chemical composition of elderberry raw material. The scope of work includes the determination of heavy metals as well as active substances (flavonoids) and their relation to the distance from the nearest roads.

MATERIAL AND METHODS

The raw material for the study consisted of flowers and fruits of common elderberry harvested in 2009 from sixteen different wild stands in the south-eastern part of Poland. The stands were located at a different distance from roads (Table 1). The raw material was collected from June (flower) to August (fruit) 2009 and then dried at 30°C. Voucher specimens have been deposited at the Department of Industrial and Medicinal Plants, University of Life Sciences, Lublin.

Determination of flavonoid content (flavonoles converted into quercetine) was performed by spectrophotometry according to the Polish Pharmacopoeia VIII (2008) procedure. In addition, the content of selected minerals, including heavy metals (Cr, Zn, Pb, Cu, Cd, Ni, Fe and Mn), after digestion of plant material to dryness with the ICP-AES method was determined at the Department of Agricultural and Environmental Chemistry, the University of Agriculture in Krakow. For chemical analysis, elderberry samples (5 g dry weight) of plant material from each replication were collected. The plant samples, after drying at 70°C in an oven with forced air flow, were dry-mineralized in a muffle furnace at 450°C. The ash was dissolved in HNO_3 (1:2) and evaporated to dryness in sand bath. After evaporation, the ash was re-mineralized (in order of total ash) in a muffle furnace. The burned ash was dissolved first in 20% HCl, followed by evaporation to dryness, dissolution in HNO_3 (1:2) and boiling. Thus prepared, the plant material samples were transferred to volumetric flasks. During the study, the content of heavy metals (Cr, Zn, Pb, Cu, Cd, Ni and Mn) and macroelements (K, Ca, Mg) was determined with the inductively coupled plasma atomic emission spectroscopy (ICP-AES) method. Chemical analyses were performed in three replications.

Table 1

Location of the stands of obtaining elderberry raw material along with an indication of their distance from the route and traffic intensity

No	Stand	Location	Distance from the oad (m)	Traffic intensity near the stand	Possible impact of road traffic on raw material on a conventional scale (1-6)
1	Lublin Konopnicka estate	51°14'22.34"N 22°31'09.29"N	150	+/-	2
2	Lublin Nałęczowska street	51°15'01.29"N 22°30'41.65"E	0.5	+++	6
3	Lublin Skromna street	51°14'57.40"N 22°30'33.01"E	1.5	+	4
4	Lublin Głęboka street	51°14'50.92"N 22°31'14.70"E	5	+++	5
5	Lublin Słowacki estate	51°14'44.23"N 22°31'11.02"N	21	+	3
6	Goraj Lubelski	50°43'14"N 22°40'9"E	60	-	1
7	Kocudza	50°40'9"N 22°36'52"E	4	-	1
8	Lublin Morwowa street	51°14'57.11"N 22°30'32.68"E	15	++	5
9	Lublin Kolorowa street	51°15'02.08"N 22°30'59.04"E	10.5	+	3
10	Lublin Kraśnicka street	51°14'47.35"N 22°30'56.44"E	1.5	+++	6
11	Świdnik	51°13'42"N 22°39'56" E	10	+	3
12	Żyrzyn	51°30'24" N 22°04'28" E	2	+/-	2
13	Syczyn	51°16'36" N 23°14'34" E	3	+/-	2
14	Bekiesza	51°17'44" N 23°12'05" E	50	-	1
15	Pacanów	50°24'19" N 21°03'06" E	5	+	3
16	Ławy	50°12'18" N 20°38'49" E	150	++	2

Key:

+++ very large, ++ large, + medium, +/- small, - very small

6 – maximum, 5 – very big, 4 – big, 3 – average, 2 – small, 1 – minimum

Statistical analysis

The data were analyzed with the SAS general linear model procedure (version 8.2 SAS Institute, Cary, N.C.). Testing for significance of mean effects and interactions on all variables was calculated using ANOVA analysis of variance. Statistical significance was set at *P*=0.05. The strength of the linear relationship between two variables was expressed using Pearson's linear correlation coefficient (Rxy), and taking values from –1.0 to 1.0 (where -1.0 is a perfect negative correlation, 0.0 is no correlation, and 1.0 is a perfect positive correlation). The test of significance is based on the assumption that the distribution of residual values (i.e. deviations from the regression line) for dependent variable y follows normal distribution, and that the variability of residual values is the same for all values of independent variable x. In the interpretation of Pearson's correlation coefficient, the following scale was used: 0 <Rxy <0.25 low degree of correlation; 0.25 <Rxy <0.75 moderate correlation; 0.75 = <Rxy <1 high degree of correlation (Stanisz 2000).

RESULTS

Mineral composition analysis of elderberry flowers and fruits showed significant differences in the concentration of individual elements (including heavy metals) in examined samples of plant material (Tables 2, 3, 4).

The content of macroelements in elderberry flowers ranged from 32.4 (stand number 16) to 83.1g K kg^{-1} d.m. (stand number 12); from 4.15 (stand number 7) to 8.83 g Mg kg^{-1} d.m. (stand number 3), and from 1.99 (stand Nº 6) to 5.15 g C kg^{-1} d.m. (stand number 15) – Table 2. It is worth noticing that the content of each analyzed element was significantly related to the site of raw material collection (the least significant difference *P*=0.05), which implies that it was most likely influenced by the composition of soil on which elderberry plants grew.

A similar relationship between the mineral content and the from the place from which the raw material originated was observed for heavy metals (Table 2). The analyzed flower samples contained the highest levels of iron, followed by zinc and manganese. Elderberry flowers accumulated also copper: from 5.28 (stand 16) to 16.10 mg kg^{-1} d.m. (stand 10), chromium: from 0.63 (stand 3) to 2.31 mg kg^{-1} d.m. (stand 2), nickel: from 0.252 (stand 7) to 2,264 mg kg^{-1}d.m. (stand 12) and cadmium: from 0 (stands 6, 12, 13, 14 and 15) to 0.04 mg kg^{-1} d.m. (stand 11). The lead content was the highest in the raw material gathered closer to roads. Generally, the raw material gathered near the busiest routes (stands 2 and 10) had the highest total content of most heavy metals (Tables 3, 4). The lowest total content of heavy metals was observed in elderberry flowers from rural areas far from roads. In general, elderberry flowers contained almost twice as much of the analyzed

Table 2

The content of selected macronutrients in elderberry flowers and fruits (g kg^{-1} d.m.)

Stand number	Flower			Fruit		
	K	Mg	Ca	K	Mg	Ca
1*	53.4	6.87	3.50	30.6	4.05	2.28
2	66.8	6.41	3.43	41.4	5.46	2.55
3	48.5	8.83	3.60	26.7	5.75	2.83
4	59.2	6.05	4.04	29.4	4.08	2.44
5	62.1	6.11	2.23	37.4	4.50	3.02
6	53.3	4.30	1.99	34.6	3.26	1.53
7	60.2	4.15	2.42	35.3	4.57	1.97
8	59.5	5.97	3.08	40.4	3.29	1.37
9	56.2	7.07	2.91	32.7	4.53	2.29
10	66.2	5.84	3.55	38.0	2.98	1.59
11	61.5	6.92	4.76	35.1	4.58	2.72
12	83.1	5.62	2.60	49.6	2.95	1.66
13	56.6	4.50	3.14	37.0	5.04	4.22
14	45.4	6.31	3.75	27.2	3.67	1.93
15	71.3	6.85	5.15	49.0	5.87	3.86
16	32.4	6.33	3.54	33.0	3.82	2.53
$LSD^{**}_{0.05}$	7.99	0.777	0.781	7.75	1.091	1.573

*key under Table 1, $^{**}LSD_{0.05}$ – least significant difference at P=0.05

elements as fruits harvested from the same plants growing in the wild (Tables 2, 3, 4).

Differences in the mineral composition of elderberry fruit as well as a significant effect of the location from which the raw material had been harvested were evidenced during the experiment (Tables 2, 4). The content of macroelements in elderberry fruits ranged from 26.7 (stand 3) to 49.6 g K kg^{-1} d.m. (stand 12), from 2.95 (stand 12) to 5.87 g Mg kg^{-1} d.m. (stand 15) and from 1.37 (stand 8) to 4.22 g Ca kg^{-1} d.m. (stand 13). It is important that the content of each macronutrient in elderberry fruits, like in the flowers, significantly depended on the place where the raw material had been gathered.

A relationship was also found between the content of heavy metals in elderberry fruits and the site where the raw materials had been collected (Table 4). As in the flowers, elderberry fruit samples contained the highest amounts of iron, zinc and manganese of all the heavy metals analyzed. Elderberry fruits also accumulated copper: from 3.93 (stand 6) to 8.54 mg

Table 3

The content of heavy metals in elderberry flowers (mg kg^{-1} d.m.)

Stand number	Cu	Zn	Mn	Fe	Cr	Ni	Cd	Pb
1*	11.26	35.63	30.82	60.23	0.68	0.410	0.007	0
2	9.94	38.35	25.21	214.58	2.31	1.227	0.018	0.232
3	9.23	24.34	26.46	65.61	0.63	1.396	0.034	0
4	9.10	37.22	27.38	75.70	0.72	0.421	0.025	0.369
5	7.71	23.92	26.57	55.16	1.08	1.531	0.014	0
6	5.47	26.97	13.85	48.67	0.83	0.396	0	0
7	7.11	25.22	14.93	35.77	0.77	0.252	0.002	0.009
8	8.93	38.58	22.38	76.36	0.86	0.582	0.017	0.013
9	13.21	36.13	23.61	50.84	0.84	1.474	0.015	0
10	16.10	39.29	27.80	192.47	1.48	0.634	0.008	0.202
11	9.11	37.01	31.714	111.21	0.78	0.652	0.040	0.310
12	11.45	36.36	38.266	53.84	1.19	2.264	0	0.063
13	6.81	30.15	20.562	52.80	0.68	0.843	0	0.004
14	6.88	31.78	33.39	54.75	0.70	0.909	0	0.212
15	6.58	26.33	30.08	78.93	0.91	0.855	0	0.202
16	5.28	32.04	30.17	68.18	0.67	0.838	0.002	0.328
LSD**$_{0.05}$	1.009	8.333	4.5089	15.5334	0.229	0.7749	0.0042	0.1321

*key under Table 1, **LSD$_{0.05}$ – least significant difference at P=0.05

kg^{-1} d.m. (stand 4), chromium: from 0.388 (stand 5) to 0.714 mg kg^{-1} d.m. (stand 2), nickel: from 0.100 (stand 7) to 0.803 mg kg^{-1} d.m. (stand 3) and cadmium: from 0 (stands 3, 9, 11, 15) to 0.076 mg kg^{-1} d.m. (stand 10) The raw material from stands 15, 2 and 3, gathered in close proximity to busy roads, possessed the highest total content of all the heavy metals (121.03, 99.59 and 97.91 mg kg^{-1} d.m., respectively). Generally, elderberry fruits contained half the concentrations of the analyzed elements determined in flowers but accumulated them in a similar way. Cadmium was exceptional in that it did not tend to rise in the raw material (Table 2).

The content of flavonoids was higher in elderberry flowers than in fruit (Figure 1). The percentage of these active substances in elderberry flowers of ranged from 0.55% to 1.56% of air dry matter, while in fruits it varied from 0.028% to 0.116%. The highest content of flavonoids in flowers appeared in samples nos 6 and 15 (1.45 and 1.56% respectively). These samples originated from stands in a rural area, far from any roads. The lowest content of flavonoids was observed in the raw material from the most con-

Table 4

The content of heavy metals in elderberry fruit (mg kg^{-1} d.m.)

Stand number	Cu	Zn	Mn	Fe	Cr	Ni	Cd
1*	7.28	15.63	13.60	32.08	0.440	0.242	0.013
2	5.73	17.65	15.78	58.96	0.714	0.248	0.014
3	8.51	19.65	14.01	54.05	0.542	0.803	0
4	8.54	25.79	11.30	50.42	0.504	0.186	0.029
5	7.99	16.39	30.66	38.02	0.388	0.160	0.009
6	3.93	12.71	7.71	37.37	0.443	0.176	0.018
7	5.53	11.86	11.50	29.52	0.431	0.100	0.034
8	5.68	13.14	9.99	31.43	0.465	0.168	0.015
9	6.16	13.99	11.79	27.63	0.482	0.231	0
10	5.99	8.76	9.81	54.47	0.629	0.261	0.076
11	4.70	18.59	9.96	58.46	0.517	0.407	0
12	7.31	14.29	27.72	34.03	0.407	0.523	0.001
13	4.94	17.28	19.63	51.89	0.642	0.462	0.011
14	5.55	13.37	30.03	39.98	0.476	0.458	0.017
15	6.27	16.08	18.41	79.12	0.630	0.523	0
16	4.68	15.35	18.86	45.37	0.608	0.500	0.011
$LSD^{**}_{0.05}$	1.110	4.774	1.709	8.112	0.1087	0.0768	0.0049

*key under Table 1, **$LSD_{0.05}$ – least significant difference at P=0.05

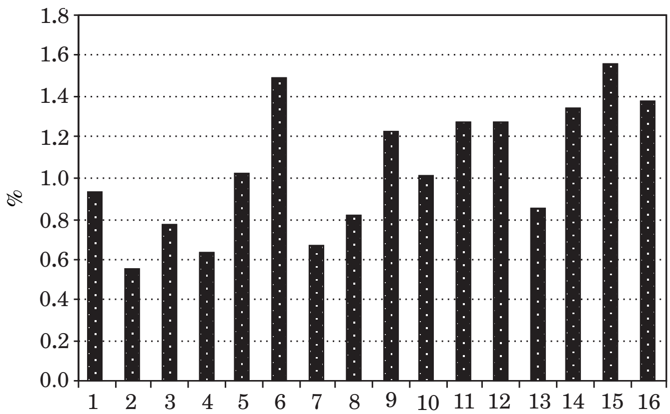

Fig. 1. Total content of flavonoids in elderberry flower (in % of dry weight) depending on a stand from which raw material has been obtained (N° 1-16)

taminated stands (nos 2, 4), where it was lower than required by Polish Pharmacopoeia VIII (2008)). The largest content of flavonoids in elderberry fruits was determined in the raw material harvested from stands 5, 9, 14 and 15 (0.101 and 0.115%, respectively). The lowest content of flavonoids was found in the raw material from stands 10 (0.028%) and 2 (approx. 0.029%) (sites most severely contaminated with heavy metals, near to roads) (Figure 2).

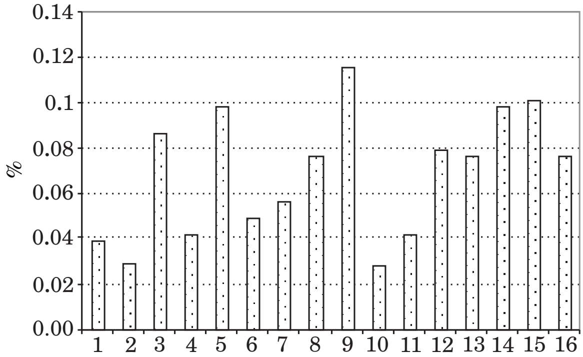

Fig. 2. Total content of flavonoids in elderberry fruit (in % of dry weight) depending on a stand from which raw material has been obtained (N° 1-16)

Table 5

Pearson's correlation coefficient r_{xy} between the effect of traffic routes and content of minerals in elderberry raw material

Component	Effect of traffic routes on a conventional (1-6) scale (see Table 1)	
	flowers	fruits
K	0.03	0.14*
Mg	0.37*	0.11
Ca	0.28*	-0.05
Cu	0.53**	0.33**
Zn	0.64**	0.23*
Mn	0.14	-0.33**
Fe	0.75**	0.38*
Cr	0.50*	0.48**
Ni	- 0.05	- 0.10
Cd	0.48**	0.35**
Pb	0.41**	-
Flavonoids in total	- 0.47**	- 0.36*

**significant at $P \leq 0.01$, * significant at $P \leq 0.05$

A high positive correlation was verified in the experiment between the impact of road traffic (the distance from the nearest road and the load of traffic) and the content of Fe, Zn, Cu and Cr in elderberry flowers (Pearson's correlation coefficient reached 0.75, 0.64, 0.53 and 0.50; Table 5). A weaker correlation was obtained between the impact of transportation routes and the content of Cd, Pb and Mg in elderberry flowers (Pearson's correlation coefficient was 0.48, 0.41, 0.37, respectively) or Cr, Fe, Cd and Cu in elderberry fruit (r=0.48, 0.38, 0.35, 0.33; Table 5). Positive correlation was also noted between the negative impact of transportation routes and the total content of flavonoids in elderberry flowers and fruits or the manganese content in elderberry fruits.

DISCUSSION

Increasing environmental pollution causes continuous rise in the content of toxic heavy metals in plants, which may precludes their optimal use as medicinal raw material (SROGI 2005). There are many factors influencing the content of heavy metals in the plant. The most important one is the degree of contamination and the distance from its source. Possible sources of environmental contamination with heavy metals are industry, transportation, public facilities, landfill waste and fertilizers (GAMBUŚ, GORLACH 2001). The study has shown that the distance from transport routes and traffic load intensity affects the mineral composition of the analyzed material. Elderberry fruit and flowers collected far from roads in rural areas were characterized by the smallest total content of heavy metals, a finding which coincides with the results reported by OGNIK et al. (2006). However, TRĘTOWSKA et al. (1998) suggested that the place where elderberry plants grow has no effect on the content of Cd, Zn, Fe, Mg and Ca in its fruit. According to these authors, an increasing traffic flow correlated positively with the content of Pb and Cu, but negatively with Mn. Our experiment showed a clear relationship between the content of Fe, Zn, Cu and Cr in elderberry raw material and road traffic. Analogously to the study by KUŹNIEWSKI et al. (1993), we found out that the amount of accumulated heavy metals depends on the anatomical parts of plants (flowers of *S. nigra* contained almost twice as much of the analyzed elements as fruits).

Sambuci flos is characterized by a relatively high content of flavonoids (up to 1.8%). In our experiment, the content of flavonoids ranged from 0.55 to 1.56% in flowers and from 0.03 to 0.12% in elderberry fruits. Similar amount of flavonoids was found in elderberry flowers by DAWIDOWICZ et al. (2005) and WACH et al. (2007) and in fruits by RIEGER et al. (2008). Moreover, the lowest content of flavonoids was observed in the raw material from the most contaminated stands (2, 3, 4), where it was below the amount required by Polish Pharmacopoeia VIII (2008).

CONCLUSIONS

1. The chemical composition of both types of elderberry raw material was significantly affected by the place of harvest.

2. Close proximity to transportation routes and more intensive traffic had a significant impact on the chemical composition of raw elderberry, which was characterized by a lower total content of flavonoids in comparison to the material collected from uncontaminated areas.

3. A significant correlation was found between the content of Cr, Fe, Cd and Cu in elderberry fruit, and Fe, Zn, Cu, Cr, Cd, Pb and Mg in elderberry flowers versus the influence of traffic routes, where proximity and higher intensity of road traffic contributed significantly to an increase in the content of these elements in the analyzed raw material.

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