

The Heavy Metals Content in Leaves of the Forest Fruits (*Hippophae rhamnoides* and *Rubus fruticosus*) from the Tailings Dumps Mining

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In almost all areas of the world where there is a mining practice, this activity has led in time to a formation of tailings/sterile dumps, as a result of copper ore preparation by flotation. Prolonged exposure of plants to high concentrations of heavy metals within the sterile determine their accumulation in quantities that can affect plant metabolism and by trophic transfer, it will be a potential danger to the consumers' health. The purpose of this research was to determine the influence of tailings on bioaccumulation of potentially toxic metals in leaves of products of an eco and health importance (sea-buckthorn and blackberry), and econometric modeling data. For this purpose it was useful to analyze the medium level, the variant, and the homogenization degree of the data. The need for these studies resulted as a fact that sea-buckthorn and blackberry leaves are harvested by locals and used in popular medicine for rheumatism, stomach and skin diseases. . The heavy metals (Fe, Mn, Zn, Cu, Ni, Cd, Pb) of the sea buckthorn and of the blackberry leaves were determined by atomic absorption spectrophotometry method. The statistical data taken were performed by using econometric software EViews 7.0. Our research reveals that mineralization of heavy metals in leaves is much higher than in fruit and a slow accumulation and during a period of time of these substances will negatively influence their quality and the consumers' metabolism. In the case of fruit shrubs (sea-buckthorn and blackberry) cropped/cultivated on copper dumps mining, potentially toxic metals bioaccumulate in much larger quantities in the leaves of blackberry, in comparison to those of the sea-buckthorn's, so the recommendation is that these leaves were not used for medical purposes.

Keywords: heavy metals , dumps mining, *Rubus fruticosus*, *Hippophae rhamnoides*

On the international level, a number of researchers have been concerned with the assessment of heavy metals from mining wastes and their influence on vegetation.

In Romania, flotation tailings were expanded and began to form focuses/sources of damage to the environment and ecological imbalances. At Moldova Noua are the largest deposits of tailings in Romania, forming like some ponds/lakes of "field" and being the most under privileged in terms of frequency and wind speed, from all areas with deposits of sterile [1, 2].

The physico-chemical analyzes of sands that make up our dumps provided information and data concerning the presence of unfavorable features of vegetation installation, generally sterile samples collected from the lake Tău^oani from Moldova Noua, Caras-Severin that showed high concentrations of zinc and nickel [2].

Prolonged exposure of plants to high concentrations of heavy metals in tailings causes their accumulation in quantities that can affect plant metabolism and the trophic transfer will be a potential health hazard to consumers [3, 4].

Slow and in time accumulation of heavy metals and possible toxic in shrubs' leaves will negatively influence their quality and consumer's metabolism. Metals such as

zinc, iron, copper, cobalt and chromium are essential nutrients to plants exhibiting toxic only in high concentrations [1, 4].

The need for these studies resulted as the fact that sea-buckthorn and blackberry leaves are harvested by locals and used in folk medicine for rheumatism, stomach and skin diseases. For example, sea buckthorn leaves are rich in protein, and they have a remarkable effect on the central nervous system, which stimulates it very effective.

Experimental part

Materials and methods

Plant material: The leaves of *Hippophae rhamnoides* and *Rubus fruticosus* was harvested in September 2012, from the waste dumps from Moldova New, from the area where it was planted from about 23 years ago [7].

Chemical analysis methods: Chemical analysis were performed in the Department of Agricultural Chemistry at the University of Agricultural Sciences and Veterinary Medicine of Banat, Timisoara, Romania.

The heavy metals: Fe, Mn, Zn, Cu, Ni, Cd, Pb of the sea buckthorn and of the blackberry leaves were determined by atomic absorption spectrophotometry method. Sample preparation procedure was like that described above,

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except that after cooling the sample were added 3 drops of HCl 50%. Then evaporated on a sand bath and then added 10 ml 0.5N HCl, stirring with a glass rod. The heavy metals content was read on an atomic absorption spectrophotometer [8].

Statistical analysis

For data processing was used econometric software EViews 7.0.

When calculating indicators and methodologies were used statistical methods: analysis series gives statistics, regression analysis and correlation of factors included in the research. For each model the correlation coefficient R is greater than the value of 0.5 which indicates the relationship between the analyzed variables, recording a moderate and direct connection [9, 10].

Moderate connection means the trend towards marginal value, but there are other factors that influence coverage, but not included in the survey [9].

The same thing is confirmed by the values of the coefficient of determination, which also have values that exceed the value of 0.5 which means there is a direct and moderate connection.

The Durbin-Watson test (DW) (framing interval between the value 0 and 4) is used to verify the null hypothesis (Ho), which is nonexistent autocorrelation of the residual values.

$$d = \frac{\sum_{i=2}^n (\varepsilon_i - \varepsilon_{i-1})^2}{\sum_{i=1}^n \varepsilon_i^2} \quad (1)$$

where ε_i - residual values.

F (test F) = variance intergroup / intragroup variance (F calculated).

$$F_c = \frac{S_{y/x}^2}{S_e^2} \quad (2)$$

where: S12 and S22 - variance estimators, calculated based on data.

F-test is greater as:

- distance between groups is greater or dispersion media groups around the general average is greater;
- groups are more homogeneous or error represented by scattering within the groups is less;

Thus, relatively high F's are strong arguments against Ho (null hypothesis).

P value is the probability of obtaining a value of F which is at least as great as that observed by us if Ho were true. Therefore, the smaller the P the chance that Ho to be fair are lower. For $P < 0.05$ to reject Ho.

Check the formula: $F_{calculated} > F_{tabular}$
 . (F calculated > F tabular)

The general indicator of the correlation between quantitative variables is the covariance. It is used as an intermediate indicator measuring the intensity of the linear relationship between two variables x and y and simultaneously quantify their variation according to the relationship:

$$cov(x, y) = \frac{1}{n} \sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y}) \quad (3)$$

Sample	Fe [ppm]	Mn [ppm]	Zn [ppm]	Cu [ppm]	Ni [ppm]	Cd [ppm]	Pb [ppm]
White sea buckthorn	8.6	2.15	15.5	0.25	0.96	-	1.22
blackberry	20.2	19	22	12	-	0.03	19

Table 1
HEAVY METAL CONTENT IN THE LEAVES OF WHITE SEA-BUCKTHORN AND BLACKBERRY

The covariance matrix indicates the tendency of each pair of paths (vector positions) to vary together, or to covary. Covariance has some important property:

- If x_i and x_k grow together, then $cov_{ik} > 0$;
- If x_i tends to decrease when x_k increases, the $cov_{ik} < 0$;
- If x_i and x_k are uncorrelated, then $cov_{ik} = 0$.

Results and discussions

Heavy metal content in the leaves of the white sea buckthorn and blackberry

In table 1 are mean values of the most significant heavy metals determined in leaves of spontaneous white sea buckthorn and blackberry on dumps.

- After the contamination of the leaves, playing the medium concentrations of iron, very clear from table 1, blackberry leaves accumulate too much iron compared with those of sea-buckthorn. Mean value of total iron content of the leaves of the blackberry bush in the dumps area at Moldova Noua is influenced by the position of blackberry leaf on the tailings surface.

- The degree of contamination with manganese of blackberry leaves is about 9 times higher than sea buckthorn leaves, and this can be explained by the fact that blackberry bush is a species that is very close to sterile leaves, unlike the sea-buckthorn leaves that are located in the upper level of vegetation.

- Regarding the ability of zinc accumulation in leaves it was observed the superiority of blackberry bush species analyzed in (table 1). The average concentrations of zinc in blackberry leaves and sea-buckthorn are similar, 15.5 ppm zinc in sea-buckthorn leaves and 22 ppm zinc in the blackberry leaves.

- Also, blackberry leaves have a higher average content of copper than the sea buckthorn leaves (table 1). The fact that sea buckthorn leaves copper retention was low (0.25%) compared with blackberry leaves that have accumulated at the rate of 12%, advantages this sanogenic resource.

- Nickel was accumulated only in leaves of sea buckthorn, the average concentration of 0.96 ppm.

- Cadmium was not found in the leaves of the sea-buckthorn, and the blackberry in extremely low concentration (0.03 ppm).

- Taking into consideration the accumulation of lead in the sea-buckthorn and blackberry leaves, shown in Table 1. the average concentrations is observed that this element is found in appreciable quantities in the leaves of blackberry (19 ppm) compared with those of sea-buckthorn that were detected only 1.22 ppm.

The influence of heavy metals on the chemical composition of leaves of sea buckthorn and blackberry

If shrubs (sea-buckthorn and blackberry) grown on cuprifer tailings, potentially toxic metals bioaccumulate in much larger quantities in the leaves of blackberry, compared with those of sea-buckthorn, so the recommendation is that the leaves are not used for medical purposes.

Whereas in sea buckthorn leaves, as no fruit was not determined in large amounts of these heavy metals, and especially the fact that elements such as nickel, cadmium, copper and lead were detected in very small quantities in chemical composition of leaves and fruits of this shrub

	Fe	Mn	Zn	Cu	Ni	Cd	Pb
Mean	19467.13	270.6889	230.8189	252.7678	8.682222	0.288889	45.70222
Median	17618.00	142.0000	252.8000	214.2100	9.350000	0.000000	40.69000
Maximum	27887.00	1409.000	415.0000	337.2000	11.50000	1.000000	78.62000
Minimum	12897.00	48.70000	24.25000	202.3000	5.300000	0.000000	17.88000
Std. Dev.	6636.798	431.7859	169.1525	63.28403	2.301949	0.425572	26.38196
Skewness	0.417556	2.366779	-0.202316	0.695864	-0.316387	1.030906	0.292699
Kurtosis	1.500000	6.824184	1.500129	1.500720	1.632380	2.342787	1.500308
Homogeneous coefficient(%)	34,09%	159,51%	73,28%	25,04%	26,51%	147,31%	57,73%
Jarque-Bera	1.105280	13.88661	0.905002	1.569279	0.851546	1.756125	0.971912
Probability	0.575429	0.000965	0.636035	0.456284	0.653265	0.415587	0.615109

Table 2
STATISTICAL ANALYSIS OF ELEMENT Fe

Dependent Variable: Fe				
Method: Least Squares				
Fe=C(1)+C(2)*Mn+C(3)*Cu+C(4)*Pb+C(5)*Zn+C(6)*Ni+C(7)*Cd				
	Coefficient	Std. Error	t-Statistic	Prob.
C(1)	20416.43	1713.810	11.91289	0.0070
C(2)	-0.036885	0.082200	-0.448726	0.6976
C(3)	1.708609	14.58856	0.117120	0.9175
C(4)	194.8768	20.60143	9.459383	0.0110
C(5)	-45.90587	2.526267	-18.17142	0.0030
C(6)	37.41182	102.0555	0.366583	0.7491
C(7)	-22.06506	107.4227	-0.205404	0.8563
R-squared	0.999978	Mean dependent var	19467.13	
Adjusted R-squared	0.999913	S.D. dependent var	6636.798	
S.E. of regression	61.74847	Akaike info criterion	11.13549	
Sum squared resid	7625.747	Schwarz criterion	11.28889	
Log likelihood	-43.10972	Hannan-Quinn criter.	10.80446	
F-statistic	15402.60	Durbin-Watson stat	1.892077	
Prob (F-statistic)	0.000065			

Table 3
ECONOMETRIC MODELING FOR Fe

entitles us to say that from this plant can be harvested both leaves and fruits for medical purposes. Especially since leaves of sea buckthorn have a remarkable effect on the nervous system, which stimulates it very effectively.

Statistical analysis

Statistical interpretation Fe

An important role in statistical analysis occupies the preliminary analysis of the database. In our case we considered the followed purpose it is useful to analyze the environment and the degree of homogeneity of variance data.

We conclude the first phase that the data are asymmetric right (table 2). The homogeneous data can be considered - Cu and Ni.

Econometric modeling:

For econometric modeling of the variables included in this study we considered factor Fe as a resultative factor and independent factors are Mn, Cu, Pb, Zn, Cd, Ni (table 3). We believe that the relationship between these factors show.

Connecting equation in this case is:

$$Fe = C(1) + C(2) * Mn + C(3) * Cu + C(4) * Pb + C(5) * Zn + C(6) * Cd + C(7) * Ni \quad (4)$$

From the estimates made (it was applied the method of small squares) the resulted model is:

$$Fe = 20416.432 - 0.037 * Mn + 1.709 * Cu + 194.877 * Pb - 45.906 * Zn - 22.0651 * Cd + 37.412 * Ni \quad (5)$$

The model shows a very strong relationship between factors, a correlation of 0.999978. Fisher F-test statistic calculated is worth a probability of 0.000065 15402.6. Tabular value given by test is 4.26 for a probability of 0.05 (5%), which means that the resulting equation was: $F_{calculated} > F_{tabular}$ (F calculated > F tabular).

So the null hypothesis (H0) is rejected and the variances values included in the study differ significantly between them. A direct influence, increasing the factor resultative Fe, it has the factors Cu, Pb, Ni.

Classification ranges given by model coefficients are presented in table 4. Calculations are performed with a probability of 90% and respectively 95%. Pb and Zn variables ranges are relevant in both cases of the evaluation.

In figure 1 you can see the differences in the calculation of theoretical versus actual values, which indicate the ability to perform some calculations for different values of the variable Fe (quantity) of the factors included in the presented model.

Statistical interpretation Cu

For econometric modeling of the variables included in this study we considered the factor Cu factor resultative and independent factors are Mn, Fe, Pb, Zn, Cd, Ni (table 5). Connecting equation in this case is:

Variable	Coefficient	90%		95%	
		Low	High	Low	High
C(1)	20416.43	NA	NA	13042.50	27790.36
C(2)	-0.036885	-0.276909	0.203138	-0.390565	0.316794
C(3)	1.708609	-40.88978	44.30700	-61.06090	64.47812
C(4)	194.8768	134.7209	255.0327	106.2360	283.5176
C(5)	-45.90587	-53.28253	-38.52921	-56.77552	-35.03622
C(6)	-22.06506	-335.7378	291.6077	-484.2676	440.1375
C(7)	37.41182	-260.5888	335.4124	-401.6976	476.5212

Table 4
COEFFICIENT CONFIDENCE INTERVALS

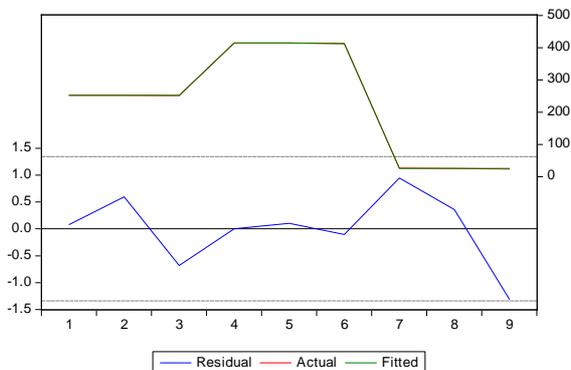


Fig. 1. Actual, theoretical/fitted and residual values of the variable Fe

$$Cu = C(1) + C(2) * Mn + C(3) * Fe + C(4) * Pb + C(5) * Zn + C(6) * Cd + C(7) * Ni \quad (6)$$

$$\text{Substitution coefficients of this model are: } Cu = 31.682 - 0.0023 * Mn + 0.004 * Fe + 0.5362 * Pb + 0.3529 * Zn - 0.0613 * Cd + 4.4462 * Ni \quad (7)$$

The model shows a very strong relationship between factors, a correlation of 0.999445. Fisher F-test statistic calculated is worth a probability of 0.001665 599.8693.

So the null hypothesis (H0) is rejected and the variance values included in the study differ significantly between them.

A direct influence, increasing the factor result, it has the factors Fe, Pb, Zn, Ni.

Table 5
ECONOMETRIC MODELING FOR Cu

Dependent Variable: CU				
Method: Least Squares				
CU=C(1)+C(2)*MN+C(3)*FE+C(4)*PB+C(5)*ZN+C(6)*CD+C(7)*NI				
	Coefficient	Std. Error	t-Statistic	Prob.
C(1)	31.68170	701.8930	0.045138	0.9681
C(2)	-0.002903	0.003625	-0.800718	0.5073
C(3)	0.003987	0.034040	0.117120	0.9175
C(4)	0.536200	6.719599	0.079796	0.9437
C(5)	0.351665	1.552942	0.226451	0.8419
C(6)	-0.061309	5.243276	-0.011693	0.9917
C(7)	4.446151	4.006395	1.109763	0.3827
R-squared	0.999445	Mean dependent var	252.7678	
Adjusted R-squared	0.997779	S.D. dependent var	63.28403	
S.E. of regression	2.982734	Akaike info criterion	5.075036	
Sum squared resid	17.79340	Schwarz criterion	5.228433	
Log likelihood	-15.83766	Hannan-Quinn criter.	4.744006	
F-statistic	599.8693	Durbin-Watson stat	2.093788	
Prob(F-statistic)	0.001665			

Variable	Coefficient	90%	
		Low	High
C(1)	31.68170	-1122.840	1186.204
C(2)	-0.002903	-0.013488	0.007682
C(3)	0.003987	-0.095409	0.103383
C(4)	0.536200	-19.08493	20.15733
C(5)	0.351665	-4.182905	4.886234
C(6)	-0.061309	-15.37160	15.24898
C(7)	4.446151	-7.252466	16.14477

obs	Actual	Fitted	Residual	Residual Plot
1	202.3	204.7641908109028	-2.464190810902807	. * . .
2	210.1	209.5113553841955	0.5886446158044691	. * .
3	208.9	207.0259769729554	1.874023027044643	. * .
4	337	336.9999989932978	1.006702177619445e-06	. * .
5	336.8	336.1877084876579	0.6122915123421535	. * .
6	337.2	337.8102634584327	-0.6102634584327689	. * .
7	213.98	215.624905934031	-1.644905934031044	. * .
8	214.42	212.3613455177541	2.058654482245885	. * .
9	214.21	214.6242544407729	-0.4142544407728792	. * .

Classification ranges given by model coefficients are presented in Table 6. Calculations are performed with a probability of 90% and respectively 95%. Pb and Zn variables ranges are relevant in both cases of the evaluation.

In figure 2 and table 7 you can see differences in the calculation of theoretical versus actual values, which indicate the possibility to perform some calculations with different values of the variable (quantity) of the factors included in the presented model.

Statistical interpretation: Zn

For econometric modeling of the variables included in this study we considered as a resultative factor- Zn and as independent factors- Mn, Fe, Pb, Cu, Cd, Ni (table 8). We believe that the relationship between these factors indicates.

Zn variable estimation equation is:

$$Zn = C(1) + C(2) * Mn + C(3) * Fe + C(4) * Pb + C(5) * Cu + C(6) * Cd + C(7) * Ni \quad (8)$$

The substitution coefficients calculated in the resulted model are:

$$Zn = 438.372 - 0.0007 * Mn - 0.0216 * Fe + 4.1777 * Pb + 0.0711 * Cu - 0.4362 * Cd + 0.619 * Ni \quad (9)$$

The model shows a very strong relationship between factors, a correlation of 0.999984. Fisher F-test statistic calculated is worth a probability of 0.000047 21212.67. Tabular value by given test is 4.26 for a probability of 0.05 (5%).

So the null hypothesis (H0) is rejected and the variance values included in the study differ significantly between them.

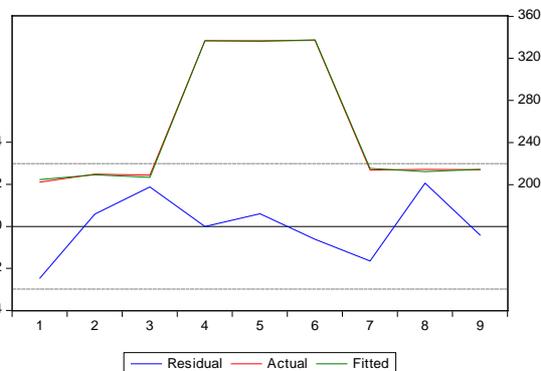


Fig. 2. Actual, residual and theoretical/fitted values of the variable Cu

Table 6
COEFFICIENT CONFIDENCE INTERVALS

Table 7
ACTUAL, RESIDUAL AND THEORETICAL/FITTED VALUES OF THE VARIABLE Cu

Table 8
ECONOMETRIC MODELING FOR Zn

Dependent Variable: Zn				
Method: Least Squares				
Zn=C(1)+C(2)*Mn+C(3)*Fe+C(4)*Pb+C(5)*Cu+C(6)*Cd+C(7)*Ni				
	Coefficient	Std. Error	t-Statistic	Prob.
C(1)	438.3722	60.03471	7.301980	0.0182
C(2)	-0.000687	0.001809	-0.379979	0.7405
C(3)	-0.021653	0.001192	-18.17142	0.0030
C(4)	4.177676	0.655786	6.370484	0.0238
C(5)	0.071087	0.313921	0.226451	0.8419
C(6)	-0.436227	2.337222	-0.186643	0.8692
C(7)	0.619003	2.247472	0.275422	0.8088
R-squared	0.999984	Mean dependent var		230.8189
Adjusted R-squared	0.999937	S.D. dependent var		169.1525
S.E. of regression	1.341055	Akaike info criterion		3.476269
Sum squared resid	3.596859	Schwarz criterion		3.629666
Log likelihood	-8.643210	Hannan-Quinn criter.		3.145239
F-statistic	21212.67	Durbin-Watson stat		1.853360
Prob(F-statistic)	0.000047			

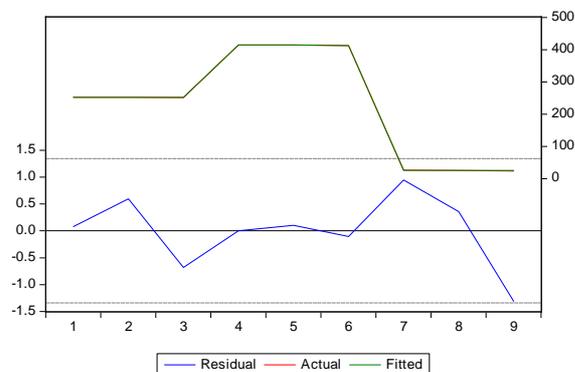


Fig. 3. Actual, theoretical/fitted and residual values of the variable Zn

Variable	Coefficient	90%		95%		99%	
		Low	High	Low	High	Low	High
C(1)	438.3722	339.6230	537.1214	180.0637	696.6807	-157.4628	1034.207
C(2)	-0.000687	-0.005969	0.004594	-0.008470	0.007095	-0.018639	0.017265
C(3)	-0.021653	-0.025132	-0.018173	-0.026779	-0.016526	-0.033479	-0.009826
C(4)	4.177676	2.262790	6.092562	1.356055	6.999296	-2.330899	10.68625
C(5)	0.071087	-0.845556	0.987731	-1.279604	1.421779	-3.044525	3.186700
C(6)	-0.436227	-7.260883	6.388429	-10.49248	9.620030	-23.63279	22.76034
C(7)	0.619003	-5.943584	7.181590	-9.051090	10.28910	-21.68681	22.92481

Table 9
COEFFICIENT
CONFIDENCE INTERVALS

obs	Actual	Fitted	Residual	Residual Plot
1	252.8	252.718028101985	0.08197189801504124	* .
2	253	252.4020242610453	0.597975738954716	* .
3	252	252.6798189673557	-0.6798189673556862	* .
4	415	414.9993345838711	0.0006654161289248	* .
5	415	414.8967148425971	0.1032851574029223	* .
6	413	413.1038919182396	-0.103891918239583	* .
7	26.45	25.50242114101472	0.947578858985274	* .
8	25.87	25.50665169013561	0.3633483098643921	* .
9	24.25	25.56111449375587	-1.311114493755866	* .

Table 10
ACTUAL, RESIDUAL AND
THEORETICAL/FITTED VALUES
OF THE VARIABLE Zn

A direct influence, increasing the resultative factor Zn, it has Pb, Ni.

Classification ranges given by model coefficients are presented in table X8. Calculations are performed with a probability of 90, 95 and 99%. At variables Fe and Pb ranges are relevant for assessing cases of 90 and 95%.

In figure 3 and table 10, you can see differences in the calculation of theoretical versus actual values, which indicate the ability to perform some calculations for different values of the variable Zn (quantity) of the factors included in the presented model.

Conclusions

Our research reveals that mineralization of heavy metals in leaves is much higher than in fruit and slow accumulation over time of these substances will negatively influence their quality and consumer metabolism, while the leaves are used in teas for blackberry in different conditions.

Following chemical analysis performed shows that blackberry leaves harvested from the tailings of Moldova Noua have an average content of iron, manganese, copper and lead superior to sea-buckthorn leaves from the same area.

In conclusion, species *Rubus caesius* (blackberry) leaves proved to be intoxicated with heavy metals and therefore should be avoided harvesting and the use in medical purposes.

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