*Bulgarian Chemical Communications, Volume 50, Special Issue B, (pp. 32 – 37) 2018*

# Correlation between Cambisols soil characteristics and lead content in wild edible mushrooms (*Cantharellus cibarius, Tricholoma equestre, Craterellus cornucopioides*)

L. Dospatliev[1*], M. Ivanova[2], K. Gavazov[3,4]

[1]*Department of Pharmacology, Animal Physiology and Physiological Chemistry, Trakia University, Stara Zagora, Bulgaria;*
[2]*Department of Informatics and Mathematics, Trakia University, Stara Zagora, Bulgaria;*
[3]*Faculty of Chemistry, University of Plovdiv Paisii Hilendarski, Plovdiv, Bulgaria;*
[4]*Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria*

Received, July 10, 2017; Revised, October 25, 2017

The study was conducted on Cambisols soils and wild edible mushrooms from the Batak Mountain, Bulgaria. The total lead content in the soils was determined after their decomposition with HF, $HClO_4$, and $HNO_3$ acids. The mushroom samples were prepared by dry ashing and subsequent dissolution in 3 M HCl. All lead analyses were performed by inductively coupled plasma atomic emission spectroscopy (ICP-OES) at a wavelength of 220.353 nm. Certified reference materials (three soils and tobacco leaves) were also analysed for verification of the accuracy of Pb determination. A correlation/regression analysis was carried out to reveal possible associations between pH, humus content, and total lead content of the soils and the concentration of this element in the mushroom samples.

**Keywords:** Pb, Correlation, Cambisols soil, Mushroom

## INTRODUCTION

Environmental pollution is one of the most serious problems in industrialized countries. Emissions of heavy metals from anthropogenic sources have been constantly increasing in recent decades. Heavy metals are very persistent in the environment and, due to the ability of accumulation, threat to living organisms [1-3]. Mushrooms are known to accumulate high concentrations of toxic metallic elements, metalloids, and radionuclides [4,5].

Lead is a naturally occurring heavy metal with an average content in the Earth's crust of 14 ppm. Its widespread use in various industrial processes has resulted in extensive environmental contamination, human exposure and significant public health problems in many parts of the world [6]. Important sources of environmental contamination include mining, smelting, recycling activities, metal plating, effluents from storage batteries, and, in some countries, the continued use of leaded paint, leaded gasoline and aviation fuel [6,7].

When Pb enters the body, it distributes throughout the organs such as brain, kidneys, liver, and bones. It is particularly harmful to young organisms and can cause irreversible health effects [6,8,9].

Although a number of investigations have been carried out on heavy metal accumulation by mushrooms, to the best of our knowledge there is no clearness concerning the correlation between soil characteristics and lead content in mushrooms.

The purpose of this study is to provide information on the relationship between pH, humus, and total content of lead in Cambisols soils from one hand, and the concentration of this element in wild-grown edible mushroom species (*Cantharellus cibarius*, *Tricholoma equestre, Craterellus cornucopioides)* from the other hand.

## EXPERIMENTAL

Fifteen Cambisols soil samples (from Batak mountain, Bulgaria) were taken from a depth of 0 – 20 cm. The following soil characteristics were determined: pH in water, humus according to Turin [9], total content of lead through decomposition by HF, $HClO_4$, and $HNO_3$ acids, following ISO (International Organization for Standardization) 14869-1 standard [11].

Forty five mushroom samples (found in the regions of the soil sampling sites) were collected in 2014 and 2015 by the authors themselves. All samples were washed so as to remove any adhering soil particles and rinsed with distilled water, after which they were dried at 105°C for 24 h and ground. The further processing of the samples included dry ashing and dissolution in 3 M HCl.

An inductively coupled plasma atomic emission (ICP-OES) system HORIBA Jobin Yvon ULTIMA 2 (Jobin Yvon, Longjumeau, France) was

* To whom all correspondence should be sent.
E-mail: lkd@abv.bg

used for determination of Pb content in the Cambisols soil and mushroom samples. The operating wavelength was 220.353 nm.

*Accuracy and precision*

Soil and plant materials used for accuracy and precision tests included three certified soil samples corresponding to two main soil types in Bulgaria and one certified reference material of tobacco leaves as follows:

i) Light Alluvial–deluvial Meadow Soil PS-1,SOOMET No. 0001-1999 BG, SOD No. 310[a]-98.

ii) Light Meadow Cinnamonic Soil PS-2,SOOMET No. 0002-1999 BG, SOD No. 311[a]-98.

iii) Light Alluvial–deluvial Meadow Soil PS-3,SOOMET No. 0003-1999 BG, SOD No. 312[a]-98.

iv) Polish reference material CTA-VTL-2 (Virginia tobacco leaves).

For evaluation of the correctness of the results (Table 1), three generally accepted criteria were used as follows:

1. $D = X - X_{CRM}$, where $X$ is the measured value and $X_{CRM}$ is the certified value. When $D$ is within the borders of $\pm 2\sigma$ , where $\sigma$ is the standard deviation from the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$ - satisfactory, and beyond these limits the result is unsatisfactory.

2. $D\% = D / X_{CRM} \cdot 100$ - percentage difference. When the values of $D$% are in the limits $\pm 200\sigma / X_{CRM}$, the result is considered to be good; when the value is in the limits $\pm 200\sigma / X_{CRM}$ and $\pm 300\sigma / X_{CRM}$ - satisfactory; and when it is out of the limits $\pm 300\sigma / X_{CRM}$, the result is unsatisfactory.

3. $Z = X - X_{CRM} / \sigma$ . When $Z \leq 2$, the result is considered to be good; when $2 \leq Z \leq 3$ – satisfactory; when $Z > 3$ – unsatisfactory.

For evaluation of the accuracy of the digestion and measuring procedures, we have used R criterion showing the extent of extraction of the element in percent from the certified value. When the measured value $X$ is within the borders of $X_{CRM} \pm U_{CRM}$, where $U_{CRM}$ is the indefiniteness of the certified value, we accept an extent of extraction to be 100%. In all the remaining cases, the extent of extraction is equal to $X / X_{CRM} \cdot 100$.

*Statistical processing*

SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing.

## RESULTS AND DISCUSSION

*Soils*

Table 2 presents the pH values for the tested Cambisols soils. As evident from the table, the Cambisols soil reaction is medium acid.

**Table 1.** Analytical results of the certified materials for Pb

| Element | Sample | Certified value | $X \pm \sigma_x$ mg kg$^{-1}$ | *D* | *D*, % | *Z* | *R* |
|---|---|---|---|---|---|---|---|
| Pb | PS-1 | 120.48 ± 5.20 | 117.39 ± 3.04 | - 3.09[b] | - 2.57[b] | 0.59[b] | 100 |
| | PS-2 | 51.21 ± 5.09 | 52.40 ± 3.71 | - 1.87[b] | 2.32[b] | 0.23[b] | 100 |
| | PS-3 | 87.50 ± 11.68 | 88.65 ± 5.46 | 1.15[b] | 1.32[b] | 0.1[b] | 100 |
| | CTA-VTL-2 | 22.1 ± 1.2 | 21.7 ± 7.6 | - 0.4[b] | - 1.81[b] | 0.33[b] | 100 |

a"Satisfactory" result
b"Good" result

**Table 2.** Soil properties and content of Pb in Cambisols soil and mushrooms (n = 15)

| Statistical index | pH | Humus | Content of Pb in soil, mg kg$^{-1}$ Total | Content of Pb in *Cantharellus cibarius*, mg kg$^{-1}$ | Content of Pb in *Tricholoma equestre*, mg kg$^{-1}$ | Content of Pb in *Craterellus cornucopioides*,mg kg$^{-1}$ |
|---|---|---|---|---|---|---|
| Mean | 5.76 | 2.60 | 17.10 | 0.27 | 1.16 | 0.41 |
| Standard Error | 0.17 | 0.14 | 0.46 | 0.03 | 0.04 | 0.01 |
| Median | 5.85 | 2.68 | 16.45 | 0.27 | 1.12 | 0.42 |
| Mode | 5.45 | 3.15 | #N/A | #N/A | #N/A | #N/A |
| Standard Deviation | 0.65 | 0.56 | 1.80 | 0.11 | 0.16 | 0.06 |
| Sample Variance | 0.42 | 0.31 | 3.23 | 0.01 | 0.03 | 0.00 |
| Kurtosis | -1.52 | -1.38 | -0.66 | -1.38 | -1.47 | -1.28 |
| Skewness | -0.15 | -0.16 | 0.78 | 0.23 | 0.36 | 0.19 |
| Range | 1.84 | 1.70 | 5.17 | 0.32 | 0.43 | 0.15 |
| Minimum | 4.81 | 1.75 | 15.15 | 0.13 | 0.96 | 0.34 |
| Maximum | 6.65 | 3.45 | 20.33 | 0.44 | 1.40 | 0.50 |
| Sum | 86.41 | 39.06 | 256.55 | 4.09 | 17.36 | 6.19 |
| Count | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 | 15.00 |
| Con. Level (95.0%) | 0.36 | 0.31 | 1.00 | 0.06 | 0.09 | 0.03 |
| CV. % | 11.28 | 21.54 | 10.53 | 40.74 | 13.79 | 14.63 |

The average arithmetic value is 5.76, as pH of most of the Cambisols soils is close to this value, i.e., they are very suitable for mushrooms growing. The humus content is within the limits from 1.75 to 3.45 (low to medium), as most of the soils have low humus content and they are suitable for the mushroom variety group [12].

The total lead content in Cambisols soil ranges from 15.15 to 20.33 mg $kg^{-1}$. According to the requirements of the Bulgarian standards for allowable lead content in the Cambisols soil depending on the active soil reaction (pH) is below the limit concentration (90 mg $kg^{-1}$). The arithmetic mean is $\overline{X}$ = 17.10 mg $kg^{-1}$, as its value is greater than the median (*Me* = 16.45), therefore there is a right-tail distribution. This is proved by the positive coefficient of skewness (*Sk* = 0.78). The coefficient of kurtosis is negative (*Kr* = – 0.66), i.e. observed low peak height of the distribution. Not greater dispersion of the values of Pb, the mean value leads to no higher values of standard deviation (σ = 1.80) and coefficient of variation, which reaches 10.53% (Table 2).

*Mushrooms*

Heavy metal contents in mushrooms are largely dependent on their trophic pattern, hysiology of mushroom species, area of sample collection, mushroom accumulation of other metals, and the distance from the pollution sources. Moreover, the age of mycelium and lag between fructification seem to be further factors affecting metals content [13].

Toxicity of lead is enhanced by its ability to bio-concentrate and bio-transform in the tissues [14, 15]. Lead ions produce physiological poisoning by becoming attached or absorbed on the cellular enzymes, causing inhibition of enzymatic control of respiration, photosynthesis and poor respiration [14, 15]. Lead is primarily absorbed via respiration and ingestion, circulated through the bloodstream and thereafter enters all tissues of the body [13]. Lead can displace calcium from the bones causing them to be brittle [14-16]. Bioaccumulation in the liver or kidney can occur leading to liver or kidney malfunction, disruption of the central nervous system (encephalopathy) resulting in uncoordinated muscular control and poor eyesight has also been reported [15-19]. However, these symptoms do not become noticeable until the level in a particular organism exceeds its tolerance limit for the metal. Every organism has a different limit for each toxic substance for example lead can be tolerated in plants at a higher concentration than mercury [15].

The total lead content in the samples of the three investigated mushrooms species (*Cantharellus cibarius, Tricholoma equestre, Craterellus cornucopioides)* varied in the range from 0.13 to 1.40 mg $kg^{-1}$. These values are several times lower than those specified in The Annex to Regulation (EC) No 1881/2006 [20] (0.3 mg $kg^{-1}$ wet weight), having in mind that the average water content in our samples was 90%. These results comply with the data published by other authors [21-33].

*Correlation dependencies among pH, humus, and total quantities of Pb in cambisols soil and in the mushrooms.*

Correlation coefficients among soil parameters and concentration of lead in mushroom are summarized in Table 3 and Figures 1 – 3.

The results of the conducted correlation/regression analysis show that there are statistically significant dependencies determined between the humus content, total forms and Cambisols soil pH and lead concentration in the mushrooms.

Lead concentration in mushrooms increases linearly with the increase of the total element content in the Cambisols soil. The observed level of significance has a lower value than the critical level of significance of 0.01; the correlation coefficients are high and statistically significant. Determination coefficients show that nearly 85 – 97% of the lead concentration in mushrooms depend on the total lead content in the Cambisols soils.

**Table 3.** Correlation at the 0.05 level between the soil parameters and concentration of lead in the mushrooms (*n* = 15)

| Element | Soil parameters | *Cantharellus cibarius* | *Tricholoma equestre* | *Craterellus cornucopioides* |
|---|---|---|---|---|
| Pb | pH | $0.95^{**}$ | $0.96^{**}$ | $0.81^{**}$ |
| | Humus | $0.86^{**}$ | $0.82^{**}$ | $0.67^{**}$ |
| | Total | $0.97^{**}$ | $0.90^{**}$ | $0.85^{**}$ |

*ns* no significant correlation; $^{**}$ Correlation is significant at the 0.05 level; $^{*}$Correlation is significant at the 0.01 level

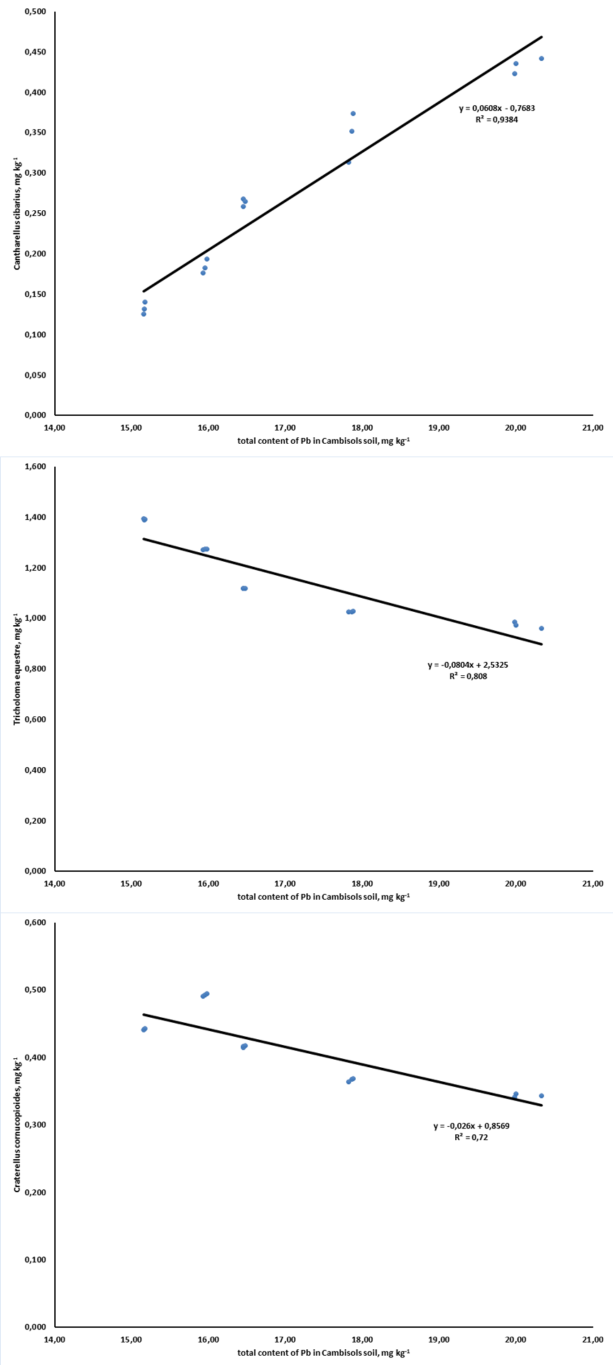


**Fig. 1.** Correlation between the total Pb content in Cambisols soil and Pb content in mushrooms

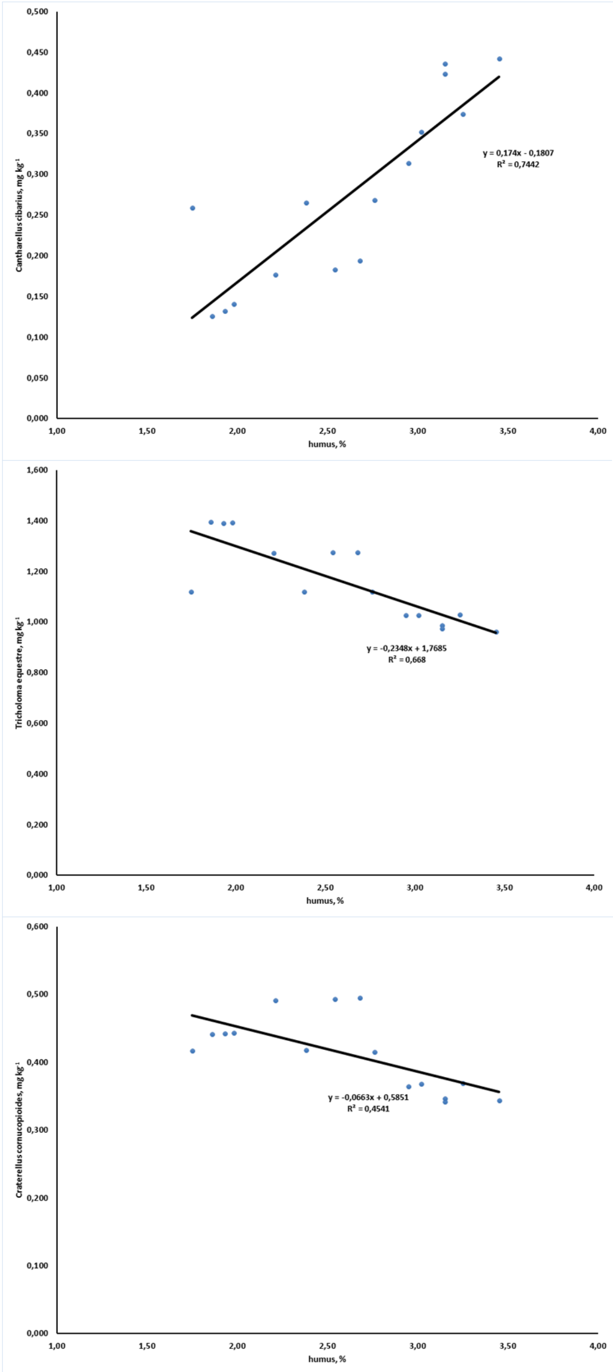


**Fig. 2.** Correlation between the Cambisols soil humus and Pb content in mushrooms

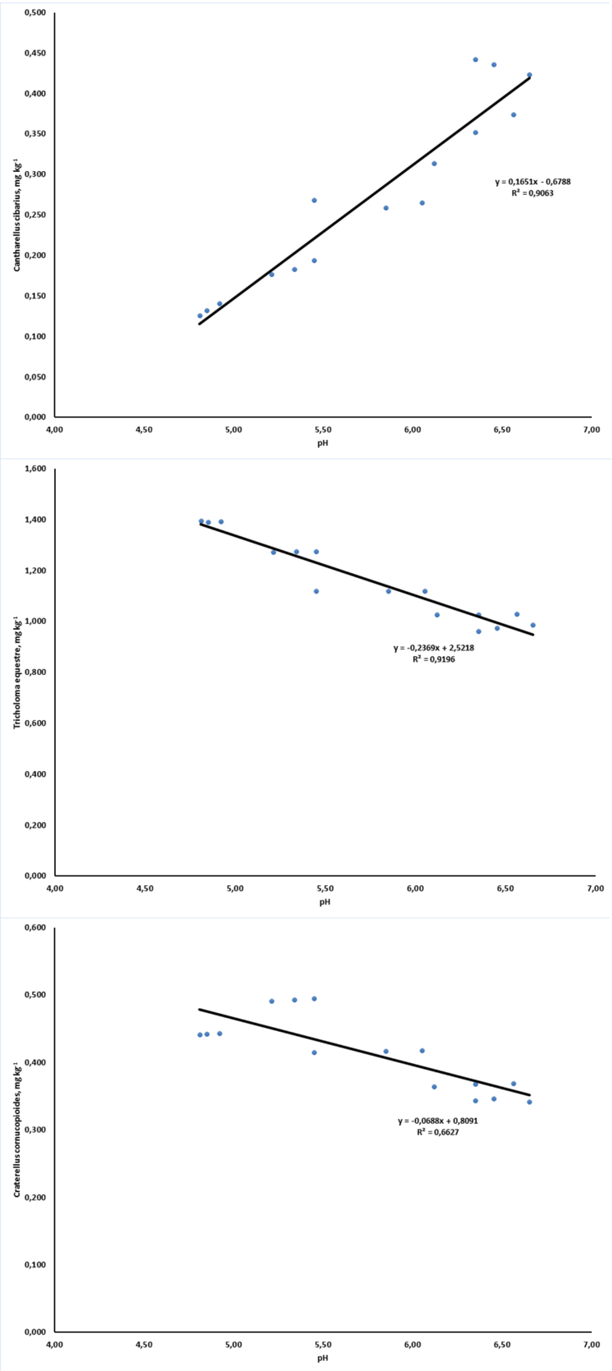


**Fig. 3.** Correlation between the Cambisols soil pH and Pb content in mushrooms

## CONCLUSION

A correlation/regression analysis was conducted between pH, humus, total content of lead in the Cambisols soil, and the concentration of these elements in the mushrooms. The results obtained show that:

- There are statistically significant dependencies determined between the Cambisols soil pH and lead concentration in the mushrooms. Pb concentration in the Cantharellus cibarius mushrooms increase linearly with the increase of the pH in the Cambisols soils. Pb concentration in the Tricholoma equestre and Craterellus cornucopioides mushrooms decrease linearly with the increase of the pH in the Cambisols soils.

- Pb concentration in the Cantharellus cibarius mushrooms increase linearly with the increase of the total element content in the Cambisols soils. Pb concentration in the Tricholoma equestre and Craterellus cornucopioides mushrooms decrease linearly with the increase of the total element content in the Cambisols soils.

- There are statistically significant dependencies determined between the Cambisols soil humus and lead concentration in the mushrooms. Pb concentration in the Cantharellus cibarius mushrooms increase linearly with the increase of the humus in the Cambisols soils. Pb concentration in the Tricholoma equestre and Craterellus cornucopioides mushrooms decrease linearly with the increase of the humus in the Cambisols soils.

From the obtained concentrations of heavy metals one can say that the locality Batak mauntain is ecologically clean area and very suitable for collecting wild edible mushrooms that we can use in our daily menu.

## REFERENCES

1. P. Kalač, *Food Chem*., **75**, 29 (2001).
2. P. Zaprjanova, L. Dospatliev, V. Angelova, K. Ivanov, *Environ Monit Assess*., **163**, 253 (2010).
3. L. Dospatliev, P. Zaprjanova, K. Ivanov, V. Angelova, *Bulg. J. Agric. Sci*., **20**, 1380 (2014).
4. J. Vetter, *Eur. Food Res. Technol*., **219**, 71 (2004).
5. L. Svoboda, B. Havlickova, P. Kalač, *Food Chem*., **96**, 580 (2006).
6. World Health Organization (WHO). Lead poisoning and health, 2014. http://www.who.int/mediacentre/factsheets/fs379/en/
7. P. Sharma, R.S. Dubey, *Braz. J. Plant. Physiol*., **17**, 35 (2005).
8. L. Dospatliev, M. Ivanova, *CR Acad Bulg Sci*, **70**, 795 (2017).
9. G. Flora, D. Gupta, A. Tiwari, *Interdiscip. Toxicol*., **5**, 47 (2012).
10. T. Totev, P. Gribachev, H. Nechev, N. Artinova, Soil science, Zemizdat, Sofia, 1987.
11. ISO 14869-1. Quality of the soils. Mineralization for determining the total content of elements. Part 1: Mineralization with fluoride hydrogen and perchloric acid. (2002).
12. E. Tanov, K. Lukanov, I. Miljanchev, P. Penchev, A. Andonov, A. Konarev, District-division, concentration and specialisation of tobacco-cultivation and tobacco-processing in Bulgaria, Hristo G. Danov, Plovdiv, 1978.
13. O. Isildak, I. Tukekul, M. Elmastas, H.Y. Aboulenein, *Anal. Lett*., **40**, 1099 (2007).

14. O. Adriana, B. Hudson, *J. of Environ. Monit*., **6**, 36 (2004).
15. N. Evert, G.G. Fisher, Y. Thomassen, *J. of Environ. Monit*., **1**, 1 (1999).
16. B.A. Brown, A.A. Brown, G.C. Lalor, *Environ. Geochem. Health*, **17**, 51 (1995).
17. A.M. Moir, I. Thornton, *Environ. Geochem. Health*, **11**, 113 (1989).
18. J.E. Ferguson, The Heavy Elements, Chemistry, Environmental Impact and Health Effects, London: Pergamon Press, 1990, p. 614.
19. L. Dospatliev, M. Ivanova, *Bulg. Chem. Commun*., **49**, G, 5 (2017).
20. Commission Regulation (EC) 1881/2006. Setting maximum levels for certain contaminants in foodstuffs.
21. A. Brzostowski, J. Falandysz, G. Jarzynska, Z. Dan, *J. Environ. Sci. Health*. A, **46**, 378 (2012).
22. A. Chojnacka, M. Drewnowska, G. Jarzynska, I.C. Nnorom, J. Falandysz, *J. Environ. Sci. Health*. A, **47**, 2094 (2012).
23. J. Falandysz, A. Mazur, A.K. Kojta, G. Jarzynska, M. Drewnowska, A. Dryzalowska, I.C. Nnorom, *J. Sci. Food Agric*., **93**, 853 (2012).
24. M. Aloupi, G. Koutrotsios, M. Koulousaris, N. Kalogeropoulos, *Ecotoxicol. Environ. Saf*., **78**, 184 (2012).
25. M. Gucia, G. Jarzynska, A. Kojta, J. Falandysz, *J. Environ. Sci. Health*. B, **47**, 81, (2012).
26. M.A. Garcia, J. Alonso, M.J. Melgar, *Food Chem. Toxicol*., **58**, 249 (2013).
27. S.S. Petkovšek, B. Pokorny, *Sci. Total Environ*., **443**, 944 (2013).
28. I. Širić, I. Kos, D. Bedeković, A. Kaić, A. Kasap, *Period. Boil*., **116**, 319 (2014).
29. J. Falandysz, M. Drewnowska, *J. Environ. Sci. Health*. B, **50**, 374 (2015).
30. G. Toncheva, K. Gavazov, Z. Georgieva, L. Dospatliev, A. Peltekov, B. Boyanov, *Bull. Chem. Soc. Ethiop*., **30**, 325 (2016).
31. M. Saba, J. Falandysz, I.C. Nnorom, *Environ. Sci. Pollut. Res*., **23**, 2749 (2016).
32. V. Stefanović, J. Trifković, J. Mutić, Ž. Tešić, *Environ. Sci. Pollut. Res*., **23**, 13178 (2016).
33. L. Dospatliev, M. Ivanova, *Oxid. Commun*., **40**, 993 (2017).