Carpathian Journal of Earth and Environmental Sciences, August 2016, Vol. 11, No 2, p. 519 - 528

HEAVY METAL CONTENTS IN THE SOIL OF THE BOTANICAL GARDEN IN IAŞI, ROMANIA

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Abstract. Although detailed research on heavy metal contents in urban area soil has been carried out, green areas and especially botanical gardens from Romania have been granted little attention so far. This is why we have carried out a study focused on the distribution of heavy metals in the soil of the Botanical Garden in Iaşi and on the assessment of potential anthropic output sources. Based on the 126 samples collected from the soil of the Botanical Garden in Iaşi, we have determined its contents of Fe, Mn, Cr, Co, Ni, Cu, Zn, Pb, Cd and As via X-ray Fluorescence Spectrometry. By comparison with the average Romanian soil, the tests run on soils from the Botanical Garden indicate higher contents of Pb, Cr, As, Ni and Cu, while Zn and Fe show only mildly richer concentrations. While the studied soils have a deficit of Cd and Co content, Mn is present in a percentage that is almost equal to the natural geochemical make-up of the Romanian soil. Starting from correlation coefficients, a principal component analysis and a hierarchical cluster analysis, we have established the existence of two potential sources of anthropic outputs whose impact on the soil of the Botanical Garden was determined with the help of heavy metal enrichment factors. The natural geochemical Cr, As and Cu background has been altered due to the use of agricultural chemicals whereas Pb and partially Ni contents reflect anthropic input generated by fossil fuel combustion (Pb and Ni), combined with the use of agricultural chemicals (Ni). Iron, Mn, Co, Cd and Zn contents indicate their belonging to the natural geochemical background, and in the case of Zn one can notice a slightly higher level generated by the use of agricultural chemicals.

Keywords: Botanical Garden, Iaşi, Romania, soil, heavy metals, pollution

1. INTRODUCTION

The growth rate of the world population, which is estimated to reach approximately 9 billion in the year 2050, as well as the ample phenomenon of urbanization that has been exerting high pressure on the environment (EEA, 2014), have imposed the concept of green growth in cities (OECD, 2013). In this context, the role of the green infrastructure in great urban agglomerations becomes essential (EC, 2012), offering multiple benefits to city dwellers by improving physical health (Maas et al., 2009; EEA, 2010; Coutts & Hahn, 2015**),** mental health (Abkar et al., 2010; Curtis, 2010) and by facilitating social cohesion (de Oliveira & Ward Thompson, 2015). To this we could add the preservation of biodiversity, the decrease in pollution levels through the absorption of carbon dioxide, the moderation of the urban microclimate, as well as the increase in the price of land adjacent to green spaces (Bark et al., 2011; Wolch et al., 2014). As part of the green infrastructure from urban agglomerations, the more than 3,000 botanical gardens existing in the world and defined by BGCI (2012) as institutions holding documented collections of living plants, constitute unique ecosystems that confer multiple valences to the advantages of the presence of green areas in urban agglomerations, that are didactic, scientific, recreative-cultural, hygienic-sanitary, contributing to the preservation of the indigenous genetic plant fund (BGCI, 2012; Tănase & Oprea, 2013) and that bring economic benefits (Ward et al., 2010). Although, theoretically, they are protected ecosystems, botanical gardens are subject to anthropic stress factors that are characteristic of urban agglomerations, to which specific factors are added. This aspect is reflected in the abnormal levels of high metal content in the soils of botanical gardens (Meng et al., 2011; Scanferla et al., 2012) and in some plant species (Abou El Saadat et al., 2011; Słomka et al., 2011). Nevertheless, a hierarchy of the degree of high metal pollution of urban soils

(Meng et al., 2011) indicate the fact that botanical garden < suburbs farmland < traffic area < industrial area.

2.MATERIALS AND METHODOLOGIES

2.1.Research site

Founded in 1856 by Anastasie Fătu, a physician and a naturalist, and situated on its current site since 1963, the Botanical Garden from Iaşi (BGI) owns approximately 83.18 ha (Tănase & Oprea, 2013) at present, following some land restitutions (Mititiuc & Oprea, 2004), which makes it the largest botanical garden in Romania. On cambic chernozem soils (Secu, 2008) - the Botanical Garden's soil (BGS), one can now find more than 6,000 vegetal taxa originating from various biogeographical areas (Tănase & Oprea, 2013), that attract, on average, more than 145,000 visitors yearly (Mititiuc & Oprea, 2004).

2.2. Sampling and analysis

The samples were collected between April 1st and June 15th 2014 so as to avoid the impact of the urban microclimate (extreme temperatures, heavy rain or snowfall) upon the representativeness of the heavy metal contents of the soils. A total of 126 soil samples were collected from the nodes of a virtual square network (Fig. 1). The size of the basic sampling cell was 100 x 100 m the sampling interval was between 0-30 cm, while the mass of the samples ranged between 1.5 and 2.0 kg. The samples were collected manually, using a stainless steel spade shovel, and they were then put in plastic bags.

After extracting gravel and coarse organic matter or plant root residues, soil samples were oven dried at a temperature of 50^0C for 72 hours and ground to pass through a 0.01-mm sieve.

Sample reduction was performed through the coning and quartering method (Pitard, 1993), finally retaining 25 sample grams.

In order to determine the Fe, Mn, Cr, Co, Ni, Cu, Zn, Pb, Cd and As contents, an EDXRF spectrometer (Epsilon 5 PANalytical) was used. The 25 sample grams were mixed with a binder at a 5:1 ratio and homogenized for 20 minutes in an agate mortar using a mechanical mill. Approximately 15 g of the mixture were pressed into 40 mm-diameter aluminum pellets, using a hydraulic press operating at a pressure of 20 tons. Standardization was performed by using 23 Certified Reference Materials (SO1-4, RT, RTH, GSD and LKSD). The exposure time was 50 seconds, with the exception of As, for which the exposure interval was 90 seconds. Thus, the total concentration represents the mean value of both counts. Result accuracy was tested via Certified Reference Materials.

2.3. Descriptive analysis and correlation coefficient

A descriptive data analysis was carried out, including minimum and maximum values, mean, geometric mean, median, mode, standard deviation (SD), range of concentrations, skewness, kurtosis, coefficient of variation (CV). The coefficient of variation (CV) was used to reflect the degree of discrete distribution of different metal element concentrations, and to indicate indirectly the activeness of the selected element in the examined environment. Skewness and kurtosis were also utilized to reflect different distributions of the metals. The Spearman correlation coefficients (SCC), very robust about outliers (Vittinghoff et al., 2012), were calculated to determine relations among different metals.

2.4. Multivariate analysis

Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are multivariate statistical methods used in environmental studies especially in order to separate potential heavy metal sources: lithogenic, anthropogenic or mixed (Yuan et al., 2013). Before running PCA and HCA, the distribution of heavy metals was tested, and log transformation of data was performed if the concentration of one element was not normally distributed. PCA is used to reduce the large number of variables into a smaller number of principal components (PC) that will account for most of the variance in the observed variables (Verma, 2013). We used the VARIMAX normalized rotation which maximizes the sum of the variances of the squared loadings. To simplify the interpretation, the VARIMAX normalized rotation was used, which maximizes the sum of the variances of the squared loadings. When PCA with VARIMAX normalized rotation is performed, each PC score contains information on all of the metal elements combined into a single number, while the loadings indicate the relative contribution that each element makes to the score. The PC loadings are plotted and the plot is inspected for similarities observed as clusters in the PC loading plot.

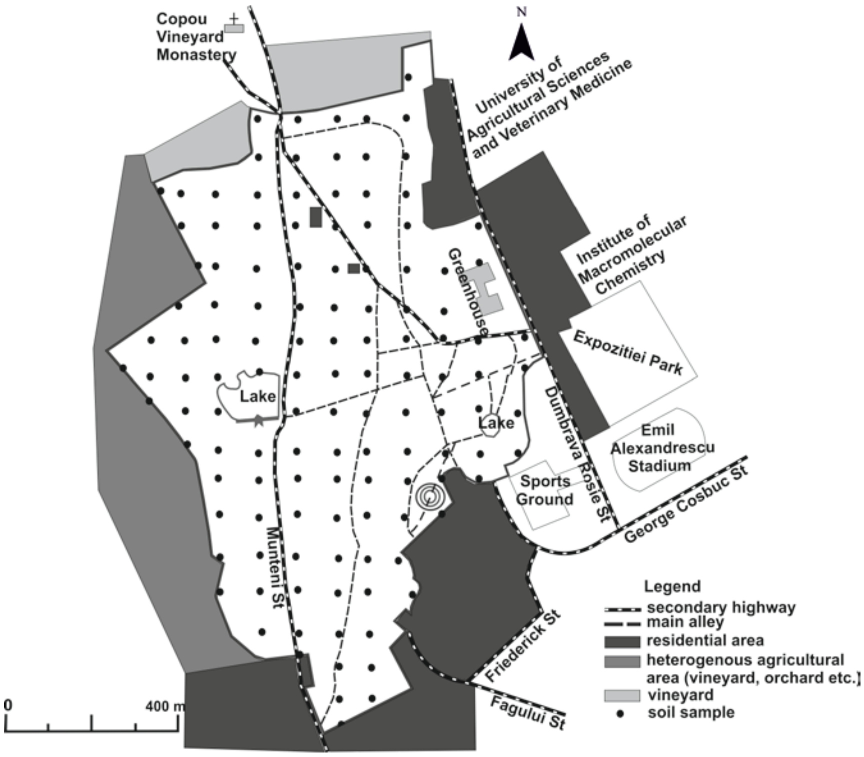

Figure 1. Sample locations of Botanical Garden soil

HCA was performed for the partitioning of the contents of the heavy metals set into subsets (clusters), so that the data in each subset share some common traits – often proximity according to some defined distance measure. HCA was undertaken according to the Ward-algorithmic method. Results are shown in a dendrogram, where steps in the hierarchical clustering solution and values of the distances between clusters (squared Euclidean distance) are represented.

In our study, we have used SPSS for Windows (version SPSS 19) for the descriptive analysis, SCC and for multivariate statistical analysis.

2.5. Enrichment factor

The enrichment factor (EF) can be used to differentiate between metals originating from anthropogenic activities and those from natural procedures, and to assess the degree of anthropogenic influence (Olubunmi & Olorunsola, 2010). In order to establish EF values, a frequently used method consists in the normalization of a tested element against a reference one. One such element, which is also sometimes used as a conservative lithogenic reference element, is Mn (Reimann et al., 2008; Bu et al., 2016). EF value was calculated by the modified formula suggested by Buat-Menard & Chesselet (1979):

$$EF = [C_n \text{ (sample)} / C_{ref} \text{ (sample)}]/[B_n \text{ (baseline)}/B_{ref} \text{ (baseline)}] \quad (1)$$

where C_n(sample) is the concentration of the examined element in the studied soil, C_{ref} (sample) is the concentration of the reference element in the studied soil, B_n(baseline) is the content of the examined element in Romanian soils and B_{ref} (baseline) is the content of the reference element in Romanian soils. Based on the EF value, Sutherland (2000) distinguish the following five intervals: (0÷2) - depletion to minimal enrichment; (2÷5) - moderate enrichment; (5÷20) - significant enrichment; (20÷40): very high enrichment; (40÷+∞): extremely high enrichment.

3. RESULTS AND DISCUSSIONS

3.1. Heavy metal concentrations

Descriptive statistics of heavy metal concentrations of BGS, as well as background values of Romanian soils (Law 756/1997) which are considered to be the reference values (NVS), are presented in table 1. The values of mean concentrations in BGS decrease in the order of Fe > Mn > Zn > Cu > Cr > Ni > Pb > Co > As > Cd, approximately similar to data presented by Tomašević et al., (2004) and Kuzmanoski et al., (2014). Asymmetry (skewness) (SK) and excess (kurtosis) (KU) values, standard errors (SE) of SK and KU, as well as SK/SE and KU/SE ratios (Doane & Seward, 2011) and the Shapiro-Wilk test ($p>0.05$) (Shapiro & Wilk, 1965; Razali & Wah, 2011) indicate the fact that in the case of Fe, Mn, Co, Ni, Cu, Cd and Cr, one can estimate the presence of normal distributions while the right skewed and/or leptokurtic distributions of Zn, Pb and As suggest a high probability for extreme values (lognormal distributions). The relations between mean, median and mode confirm the presence of negative asymmetries (mode > median > mean) in the cases of Fe, Mn and Co, or positive ones (mean > median > mode) in the cases of Cu, Cd, Ni, Zn, Pb and As (Sharma, 2006). Based on CV, the studied elements can be classified in 2 groups: Fe, Mn, Co, Cr, Ni, and Cd, whose CVs belong to the interval (0.0-0.40), which suggests that those elements are dominated by a natural source (Yongming et al., 2006); Pb, Cu, Zn and As, whose CVs belong to the interval (0.4-1.0], which suggests the presence of a weak anthropic input, up to average values. Although the value of CV calculated for Cr contents indicate its belonging to the natural geochemical background, bimodal distribution shows the presence of two subpopulations (Sinclair & Blackwell, 2002): one subpopulation that belongs to the natural geochemical background, on which anthropic inputs were overlaid.

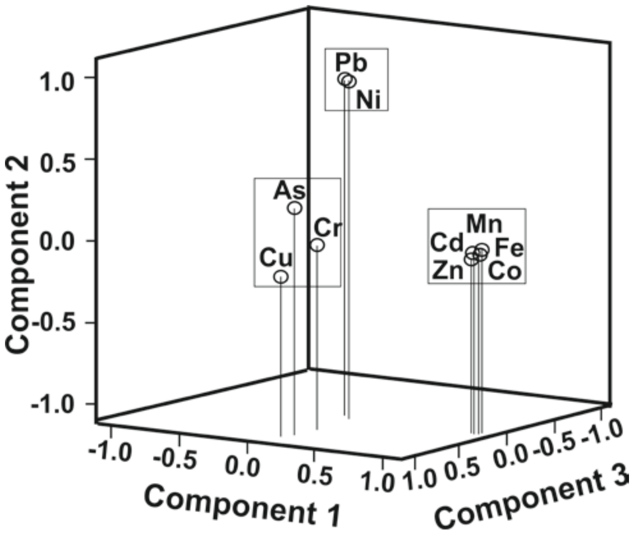

Figure 2. PCA loading 3-D plot for the heavy metals

3.2. Spearman correlation coefficient analysis

SCC values (Table 2) indicate the presence of 3 groups of chemical elements that, via strong positive associations, suggest common sources of origin for the elements belonging to the same group: Fe-Mn-Zn-Co-Cd, Ni-Pb and Cu-Cr-As. The Cr-Fe-Co positive correlation, though weak, suggests a dual source of origin for Cr.

3.3. Principal component analysis

PCA was applied to identify the association between the heavy metals studied, as well as to highlight the presence of potential anthropic inputs that have affected the geochemical make-up of BGS. Table 3 features the factor loadings and the eigenvalues. 3-D plot of PCA loadings (Fig. 2) facilitates the identification of relations between the heavy metals studied. Via PCA, three factors were obtained, accounting for more than 80% of the total variance. Factor 1 is dominated by Fe, Mn, Zn, Co and Cd, accounting for 46.836% of the total variance. Factor 2, dominated by Ni and Pb, accounts for 19.172% of the total variance. Factor 3 is dominated by Cu, As and Cr, accounting for 14.006% of the total variance. In this case, the Cr loading (0.551) is not as high as the loadings of the other elements of the group, which suggests a quasi-independent behavior within the group.

3.4. Hierarchical Cluster Analysis

HCA was performed to validate PCA results for the heavy metals studied. According to the dendrogram in figure 3, the 10 heavy metals were classified and merged into three distinct clusters. The first cluster includes Co, Cd, Fe, Mn and Zn, the second cluster includes Ni and Pb, and the third cluster includes Cu, As and Cr. On the whole, the results provided by HCA concur with information provided by PCA (section 3.3).

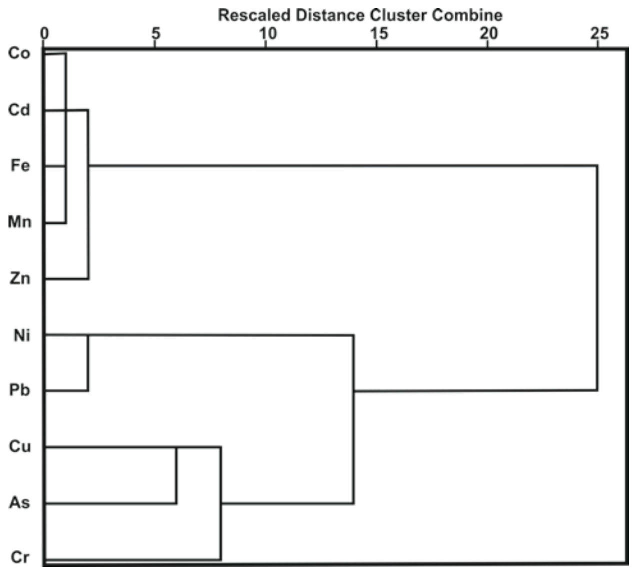

Figure 3. Hierarchical dendrogram for the heavy metals

Table 1. Heavy metal concentrations of BGS (Fe in %; Mn, Co, Ni, Cu, Zn, Pb, Cd, Cr and As in mg/kg)

	Fe	Mn	Co	Ni	Cu	Zn	Pb	Cd	Cr	As
Mean	3.54	896.51	12.63	54.78	73.47	113.06	33.81	0.36	55.53	12.36
Geom. Mean	3.53	893.62	12.56	54.54	70.23	109.66	31.99	0.33	55.37	12.21
Median	3.50	896.90	12.80	54.63	65.00	107.00	31.90	0.33	55.00	12.30
Mode	3.60	981.00	13.20	54.60	59.00	105.00	31.01	0.29	51.00 59.00	12.27
Minimum	2.70	671.00	8.70	45.00	39.00	73.00	22.00	0.08	45.00	9.70
Maximum	4.20	1051.00	14.90	75.00	137.00	236.00	234.00	0.82	69.00	18.50
Std.Dev.	0.29	70.87	1.26	5.13	33.06	47.48	19.51	0.14	4.19	5.07
CV	0.08	0.08	0.10	0.09	0.45	0.42	0.58	0.39	0.08	0.41
Skewness	-0.41	-0.56	-0.76	0.03	0.76	2.21	8.87	0.61	0.01	0.97
Kurtosis	0.62	0.49	0.80	-0.49	-0.53	5.90	90.16	0.26	0.75	6.05
NVS[a]	3.089[b]	900	15	20	20	100	20	1	30	5
Mean/NVS	1.14	0.99	0.84	2.74	3.67	1.13	1.69	0.36	1.85	2.45
Distribution	N	N	N	N	N	LN	LN	N	N	LN

a: Law 756/1997

b: average concentration in the upper crust (Reimann et al., 2008)

Table 2. Spearman's correlation matrix for the metal concentrations

	Fe	Mn	Zn	Co	Cd	Ni	Cu	Pb	Cr	As
Fe	1									
Mn	0.990**	1								
Zn	0.669**	0.702**	1							
Co	0.650**	0.609**	0.797**	1						
Cd	0.501**	0.807**	0.405**	0.671**	1					
Ni	-0.133	-0.125	-0.121	-0.299**	-0.182*	1				
Cu	-0.143	-0.130	0.132	-0.124	0.134	-0.077	1			
Pb	-0.154	-0.151	-0.147	-0.216*	-0.204*	0.793**	-0.301**	1		
Cr	0.188*	0.124	0.123	0.196*	0.137	0.112	0.152	0.143	1	
As	0.154	0.151	0.150	0.142	0.169	0.090	0.411**	0.110	0.239**	1

******: correlation is significant at the 0.01 level (2-tailed).

*****: correlation is significant at the 0.05 level (2-tailed).

Table 3. Rotated component matrix for data of BGS

Factors		F1		F2		F3
Elements		Fe,Mn,Zn,Co,Cd		Ni,Pb		Cu,Cr,As
Fe		**0.987**		0.006		0.063
Mn		**0.976**		-0.015		0.066
Zn		**0.906**		-0.017		0.032
Co		**0.956**		-0.005		0.113
Cd		**0.957**		-0.042		0.125
Ni		0.013		**0.942**		0.077
Cu		0.028		-0.150		**0.809**
Pb		-0.063		**0.936**		0.001
Cr		0.075		0.014		**0.551**
As		0.092		0.267		**0.745**
Initial Eigenvalues						
Variance %		46.836		19.172		14.006
Cumulative %		46.836		66.008		80.014

Extraction method: principal component analysis

3.5. Enrichment factor analysis

Considering Mn as a conservative lithogenic reference element, a premise that is also supported by the ratio $\overline{\mathrm{Mn}}/\mathrm{NVS}_{\mathrm{Mn}} \approx 1$, we have calculated the enrichment factor (EF) values for the studied chemical elements (Fig. 4). Figure 4 shows that Fe, Co and Cd feature subunitary or close to unity EF values, which suggests that they belong to the natural geochemical background. In the case of Zn, Cr, Ni and As, the moderated anthropic contribution increases in the order Zn > Cr > Ni ≥ As, respectively 3.17% > 21.43% > 100% ≥ 100%. In the case of Pb, there is a predominance of contents that belong to the natural geochemical background (89.68% of EF values), with occasional records of moderate and significant anthropic inputs (9.52%, respectively 0.79% of EF values). Cu mainly comes from anthropogenic sources, which is also highlighted by EF values, 87.30%, respectively 11.90% of the sample, indicating a moderate, and respectively a significant anthropic contribution.

3.6. Sources of anthropic outputs

3.6.1. Analysis of potential sources of anthropic outputs

In our opinion, a hierarchy of possible sources that can generate anthropic stress on BGS would be the following: a) main sources: a_1) specific treatments to maintain plant species that include the use of various agricultural chemicals (fertilizers, pesticides, fungicides, insecticides) (Forman, 2008), and which favor the accumulation of heavy metals in the soil (Kelepertzis, 2014): this aspect, corroborated with GBI's length of operation (class A: history < 100 years – by extending the criterion used to classify parks (Chen et al., 2005) to botanical gardens), as well as with the previous use of the field where BGI is currently located, includes a multitude of factors that can potentially disturb the natural geochemical background, whose evolution is difficult to quantify in time; a_2) car traffic on adjoining streets and on the streets inside BGI, combined with BGI equipment; b) secondary sources: b_1) the urbanization process that has affected BGS by the construction of residential buildings inside the garden; b_2) the use of BGI adjoining fields.

3.6.2. The estimation of the impact of anthropic output sources on BGS

Fe, Mn, Co and Cd come mainly from soil sources; this argument is confirmed by descriptive statistical analysis, by mean content ratios against subunitary or close to unity NVS, by SCC, PCA, HCA and EF. Though SCC and multivariate analysis indicate the strong association of Zn with Fe, Mn, Co and Cd, the $\overline{Zn} \cdot NVS_{Zn}^{-1}$ ratio, CV and EF suggest the presence of another anthropic contribution that, in our opinion, is slightly generated by the use of fertilizers and pesticides (Oertli, 2008).The non-correlation between the contents of Zn with those of Cu and Pb (Meuser, 2010) suggests that car traffic has a totally subordinate role in modifying the natural geochemical background of Zn (Fig. 5a).

The Cr-As-Cu association is indicated by SCC, PCA and HCA. The ratios of mean contents against supraunitary NVS, as well as EF values, suggest moderate anthropic inputs (As>Cu>Cr) up to significant ones (Cu), that are generated, in our opinion, by the use of certain pesticides, fertilizers

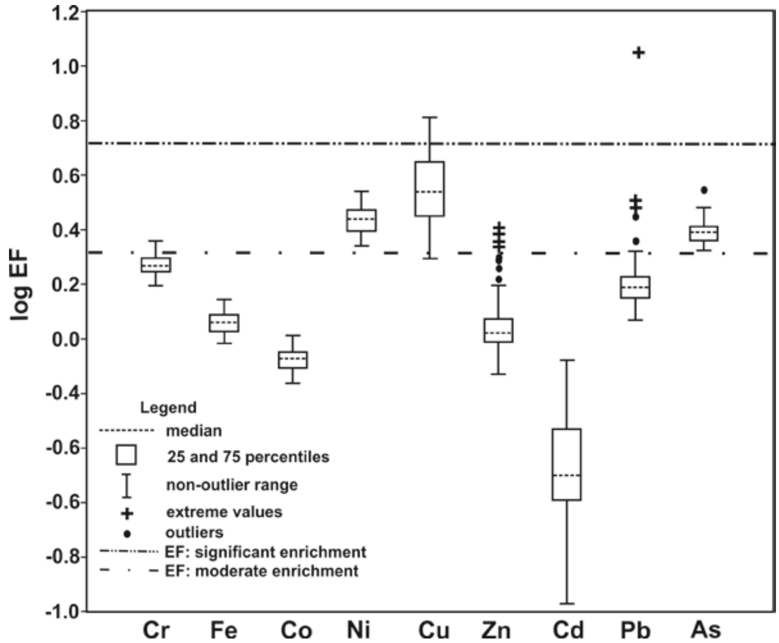

Figure 4. Boxplot EF for the heavy metals in the Botanical Garden soil

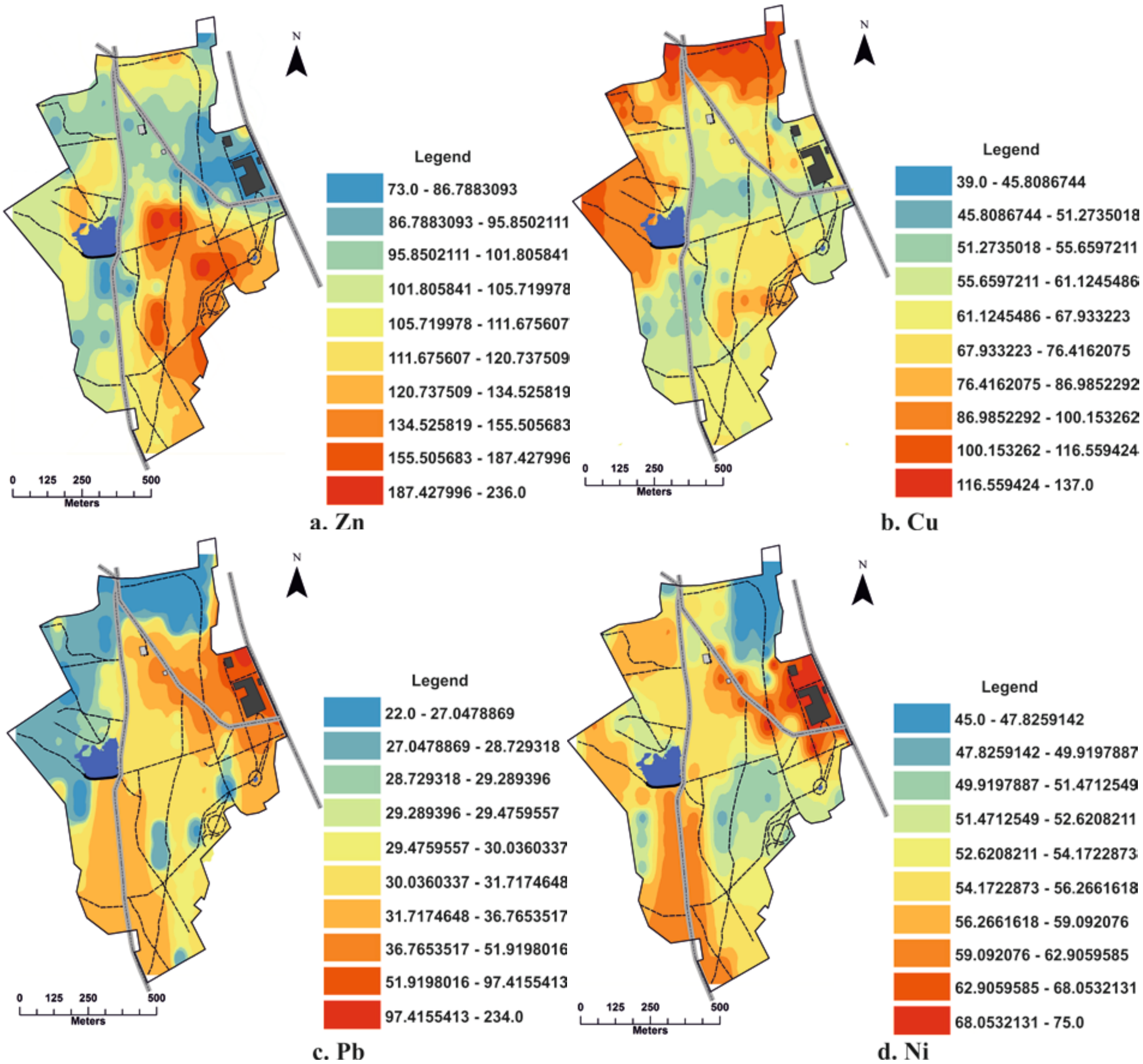

Figure 5. Spatial distribution of Zn, Cu, Pb and Ni contents (mg/kg) in the BGS

and fungicides, in time (Testa, 2004; Marcotullio et al., 2007) especially of those in which Cr acts as a fixing agent for Cu and As (Usman et al., 2012). In the case of Cr, CV value, the presence of bimodality, as well as the lower loading indicated by PCA, suggest the presence of a population that belongs to the natural geochemical background that is predominant in relation to the population generated by the anthropic input. The strong correlation of As with Cu, both elements being used as a pigment in paints, can also indicate the presence of an anthropic input generated by the urbanization phenomenon (La Rocca et al., 2010), which has also affected BGS. The landscape energy, as well as the capacity of migration suggests that a part of the Cu contents come from accumulations of copper sulphate used as fungicide (Herrero-Hernández et al., 2012) in vineyards neighboring BGI (Fig. 5b). In our opinion, Cu inputs due to car traffic are totally subordinate or absent, which is accounted for by the fact that Cu is not associated with Pb and Zn.

Through the supraunitary values of the ratios of mean contents against NVS and through EF values, the association of Ni with Pb, indicated by SCC, PCA and HCA, suggests the existence of anthropic inputs that vary from moderate (Ni) up to significant (Pb) levels and which are due to the use of fertilizers (Ni - Marcotullio et al., 2007), and especially to the use of fossil fuel combustion (Fuge, 2013), gasoline and/or diesel fuel, by cars and BG equipment (Figs. 5c-5d).

4. CONCLUSION

The interpretation of the results of statistical analysis, corroborated with the results provided by the Spearman correlation analysis and the results of multivariate statistical techniques, has supplied information on the origin of heavy metals in BGS. Comparing BGS heavy metal contents with NVS in Romania has enabled the classification of the chemical elements studied in two groups, according to the sources of provenance, i.e. natural and anthropic. PCA, HCA, SCC and the EF analysis have provided further information on the sources of provenance of heavy metals in BGS and on the intensity of the anthropic inputs. We have thus identified two anthropic sources that can, potentially, generate heavy metals, and whose contribution is hard to estimate due to the lack of information on their evolution in time. Thus, Fe, Mn, Co, Zn and Cd are attributed to a main origin in soils, and in the case of Zn, there is also a slight anthropic input due to agricultural chemicals. In the case of Cr, As and Cu, anthropic inputs are mainly generated by the accumulation of heavy metals which are found as minor elements in agricultural chemicals and, as a totally subordinate factor, by urbanization. The impact of car traffic, of the use of maintenance-specific equipment, and partially of the use of agricultural chemicals on BGS, is reflected in the values of Ni and Pb contents.

Acknowledgements

Our special thanks go to Professor Dr. Cătălin Tănase (Faculty of Biology) for granting us the permission to sample the soils of the Botanical Garden, and to Professor Dr. Nicolae Buzgar (Faculty of Geography and Geology), for his analyses of soil samples.

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******* *Law 756/1997*. The Official Journal of Romania, 303 bis.

Received at: 08. 03. 2016
Revised at: 26. 04. 2016
Accepted for publication at: 14. 06. 2016
Published online at: 16. 06. 2016