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DETERMINATION OF HEAVY METALS IN LEAVES AND BARK OF *POPULUS NIGRA L* BY ATOMIC ABSORPTION SPECTROMETRY

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Abstract. The aim of this paper was to study the metal accumulation in leaves and bark of *Populus nigra L* from atmosphere or soil. The heavy metals, including Cu, Ni, Zn, and Cd, concentrations were determined by atomic absorption spectrometry (AAS) in leaf, bark and soil samples collected from two polluted areas (*i.e.* Năvodari Camp and Năvodari Fertilizer Chemical Plant), in Romania. To determine the relationship between the metals concentration in leaves and bark and metals concentration in soil, the regression analysis has been performed and the values of the determined coefficient R^2 have been studied.

Key words: heavy metal, *Populus nigra L.*, regression analysis.

1. INTRODUCTION

Plants have been used intensively as biomonitors and bioindicators of environmental pollutants in urban, rural and in remote areas [1–8]. In many studies [1–11, 19] the *Populus nigra L.* is considered a good bioindicator for atmospheric pollution and has been recommended as a particularly suitable bioindicator of heavy metals in Europe [9]. Concentrations of major and trace metals in plants depend on root uptake or accumulation of dry and wet deposition on outer plant organs, such as foliage or bark. Several metals, which are essential as nutrients in lower concentration (*i.e.* Zn, Cu, Fe and Mn) can be considered harmful for the plant growing, if the levels of these elements in soil and atmosphere are increased [11].

It is well-known that the most important sources of anthropogenic major and trace element emissions are industrial production, the combustion of fossil fuels in vehicular traffic and energy production, sewage sludge dispersal and fertilizer production [14–18, 21].

The aim of this study was to investigate the heavy metals uptake from soil in leaves and bark from common poplar specie *(Populus nigra L.* - fam. Salicaceae). Leaves and bark samples from old trees together with soil samples from two historical polluted sites from Navodari area, Romania were analysed by using Atomic Absorbtion Spectrometry (AAS).

The dependence between the heavy metals concentration in soil and heavy metals concentration in leaves or bark was studied using the regression analysis as an interpretation tool of obtained data.

2. MATERIALS AND METHODS

Navodari area (**site 1** – the Chemical Fertilizer Plant – 44°18'21.37"N and 28°36'54.47"E, elevation 30 m; **site 2** – Northern Navodari Summer Camp – $44^0$19’27.13’’N and $28^0$36’23.58’’E, elevation 31 m) was chosen for this study because in the last 20 years, the number of population increased, due to the development of oil platform, chemical industry, shipyard and the tourists attraction on Romanian Black Sea Littoral. The distance between the studied sites is of 1 km (Fig. 1).

Fig. 1 – Navodari investigated area.

This investigation was achieved in the period May, 2012 – October, 2012. Samples of *Populus nigra L.* leaves were collected from twenty trees from **sites 1** and **2** (ten per site). Samples were collected from trees at a height of 2.5-3 m by evenly including all directions and cutting off at least four branches from all around the crown periphery. The bark samples were collected on both sites according with guideline for sampling [15], from the external surface of the trees, at a height of 1 m, from four different directions. All samples were washed with distilled water.

The leaf and bark samples were dried in oven at 70^0C for 24 hours. Approximately 0.2 g of the dried leaf and bark samples, respectively, were treated individually with 8 mL HNO_3 (65% Merck) and 10 mL H_2O_2 (30% Merck) and then were mineralized using a Berghof MWS-2 microwave digestion system. After 40 minutes of digestion the samples were cooled for 30 minutes and the clear solutions were filtered and brought at 50 mL with distilled deionized water. Metals concentrations in the final solutions were analysed by Flame Atomic Absorption Spectrometry (FAAS), using an AVANTA GBC spectrometer. Determination of elemental concentrations in leaves and barks samples were performed using the method of curve calibration according to the absorber concentration. The metal concentrations were reported as mg/kg dry weight. The method was verified by using the certified standard reference materials NCS DC 73350 Poplar Leaves. Average recoveries were greater than 94%. Twenty soil samples from both sites were collected from the root aria of each tree at 20 cm depth, according to the procedure of protocol sampling [15]. In each site, six sub-samples were collected within a circle of 3 m diameter and combined to make a composite soil sample per tree.

The soil samples were dried at 60°C for 48 h and powdered. Approximately 0.5 g of sample was introduced in the digestion vessels (DAP-60K) of Berghof MWS-2 microwave digestion according to 3051 EPA standard. A mixture of 12 mL HNO_3 (65 % Merck), 6 mL HCl (37% Aldrich), 4 mL HF (40% analytical solution) and 1 mL H_2SO_4 (96% Aldrich) was added. After mineralization, the cooled samples were filtered and put into 50 mL bottles with distilled deionized water. Trace metals concentrations in the final solutions were analysed by Flame Atomic Absorption Spectrometry (FAAS). To check the analytical precision, randomly chosen samples (about 20% of the total numbers) were measured in triplicate according to International Standard Reference Material: NIST SRM 2709, 2710 and 2711 for soil. Average recoveries (n=5) were 83, 73, 76, 85, 104 and 99% for Zn, Cd, Cr, Cu, Pb and Ni, respectively [17, 20].

To determine the relationship between the metals concentration in leaves and bark and metals concentration in soil, the regression analysis has been performed and the values of the determined coefficient R^2 have been studied.

3. RESULTS AND DISCUSSION

The mean concentrations of Cu, Ni, Pb, Zn and, Cd in leaf and bark of *Populus nigra L.* and soil samples collected from both sites are presented in Tables 1 and 2.

Table 1

Mean concentration [mg/kg d.w.] of metals in leaf and bark of *Populus nigra L* and soil samples collected from site 1

Month	Sample (n=10)	Heavy metal concentrations [mg/kg d.w.]				
		Cu	Ni	Pb	Zn	Cd
May	Leaf	12.045±0.79	1.127±0.1	2.1413±0.5	29.9869±0.17	0.0090±0.19
	Bark	11.302±0.15	1.160±0.05	7.5550±1.17	35.7036±0.11	0.0209±0.01
	Soil	44.8911±2.3	22.08±0.56	47.279±1.75	328.965±1.75	1.5020±0.15
June	Leaf	13.012±1.95	1.571±0.08	8.2280±0.15	35.8271±0.68	0.0188±0.01
	Bark	11.887±0.96	1.915±0.12	9.5497±0.12	43.5997±0.18	0.0275±0.01
	Soil	46.362±1.67	28.66±0.12	49.572±2.50	369.235±2.5	1.9797±0.09
July	Leaf	15.821±4.58	1.735±0.05	11.989±0.11	38.3626±0.69	0.0226±0.01
	Bark	12.112±3.92	2.646±0.03	14.9658±0.1	43.9464±0.13	0.0287±0.01
	Soil	49.6082±1.5	31.413±0.4	51.445±0.74	376.892±3.44	1.9961±0.1
August	Leaf	17.133±2.13	1.833±0.89	10.877±0.12	41.265±0.33	0.0152±0.01
	Bark	15.500±4.45	1.9009±0.1	12.166±0.14	56.6343±0.7	0.031±0.015
	Soil	49.4651±1.1	3.2899±0.1	50.418±0.27	390.312±3.27	1.8958±0.15
September	Leaf	18.0939±0.9	1.898±0.01	7.4632±0.15	34.5479±1.0	0.0162±0.01
	Bark	16.9001±2.5	2.884±0.18	12.3371±0.1	48.2795±1.0	0.0325±0.13
	Soil	51.211±1.65	32.474±0.2	48.858±1.49	344.0714±4.5	1.8902±0.2
October	Leaf	14.1861±3.1	1.727±0.05	5.4701±0.1	32.4066±2.2	0.0066±0.001
	Bark	11.5906±1.5	2.049±0.55	9.2300±0.16	33.1049±1.8	0.0175±0.01
	Soil	48.241±1.35	28.93±0.35	47.422±0.33	338.203±4.33	1.5200±0.01

Table 2

Mean concentration [mg/kg d.w.] of metals in leaf and bark of *Populus nigra L* and soil samples collected from site 2

Month	Sample (n=10)	Heavy metal concentrations [mg/kg d.w.]				
		Cu	Ni	Pb	Zn	Cd
May	Leaf	8.552±1.5	1.316±0.2	5.656±0.8	27.145±2.4	0.0162±0.01
	Bark	8.007±2.0	1.549±0.3	7.794±1.1	32.308±2.8	0.0292±0.02
	Soil	35.882±3.2	23.222±1.4	35.082±2.3	396.745±3.6	1.2990±0.05
June	Leaf	7.634±1.3	2.173±0.3	7.833±1.0	13.847±1.9	0.0294±0.01
	Bark	9.547±1.7	2.396±0.4	1.549±0.5	18.510±2.1	0.0362±0.02
	Soil	34.279±2.9	28.395±1.5	40.171±2.1	336.875±2.9	1.5658±0.06

Table 2 (continued)

July	Leaf	10.391±1.2	2.022±0.5	7.208±1.1	14.389±1.2	0.0238±0.01
	Bark	11.027±1.6	2.467±0.4	8.661±1.3	25.606±2.2	0.0308±0.02
	Soil	41.081±3.6	26.334±1.7	39.763±2.7	334.266±2.9	1.4284±0.04
Aug	Leaf	10.611±1.3	2.234±0.5	6.282±1.2	24.193±1.5	0.0134±0.01
	Bark	11.360±1.5	2.617±0.6	8.492±1.1	27.663±1.8	0.0241±0.01
	Soil	42.080±2.8	27.578±1.2	37.286±2.4	383.915±2.7	1.3374±0.05
Sept	Leaf	8.904±1.2	1.8907±0.2	6.436±0.9	22.6802±1.4	0.0139±0.01
	Bark	9.349±1.4	2.110±0.4	8.092±1.1	23.1998±1.3	0.0242±0.01
	Soil	39.458±3.1	26.397±1.4	37.497±2.3	376.4829±2.8	1.2918±0.03
Oct	Leaf	9.638±1.3	1.7535±0.3	6.714±0.8	15.5988±1.3	0.0186±0.01
	Bark	9.716±1.4	1.627±0.2	8.627±1.5	18.5360±1.2	0.0223±0.01
	Soil	39.849±3.2	25.881±1.7	37.362±2.2	358.5705±3.2	1.3677±0.04

The obtained heavy metals concentrations in the analysed soil samples were compared with the normal values according to the Romanian legislation, such as OM 756/1997 (Fig. 2). It can be seen the exceeding values for Cu, Ni, Pb, Zn and Cd in all the analysed soil samples. A higher concentration of Ni, Pb, Zn, and Cd in the soil samples (n=10) from site 1 with respect to those from site 2 was observed as well, that can be explained by the presence of Fertilizer Chemical plant landfill. The highest level of Pb was observed on site 1 in summer, especially in July and August, such as 49.6082 mg/kg, respectively 49.4651 mg/kg when the summer traffic is intensified. These values are approximately equal with 50 mg/kg, which is the alert threshold value provided in OM 756/1997.

A linear dependence between heavy metal concentrations in leaves - soil and bark – soil was obtained by regression analysis. The coefficients in the regression models have been checked for significance, using the t-test, at the significance level of 0.05. Also, the significance of the entire linear models has been tested by the F-test, at the same significance level. All of them were significant. The charts are presented in figures 3 and 4.

Analyzing the R^2 whose values, ranged between 0.9022 and 0.9470, it was concluded that there is a strong linear dependence between the metal concentrations in leaves and soil for both analysed sites. These values show the heavy metals uptake from soil in leaves. A medium correlation was obtained between Ni, Pb, and Cd concentration in bark and those in soil (R^2 ranged between 0.6197 and 0.7580). The values of R^2 lead to the conclusion that the heavy metal uptake in bark can be due to heavy metals content of both soil and air. This can be explained considering the porous structure of bark which can collect air borne particles.

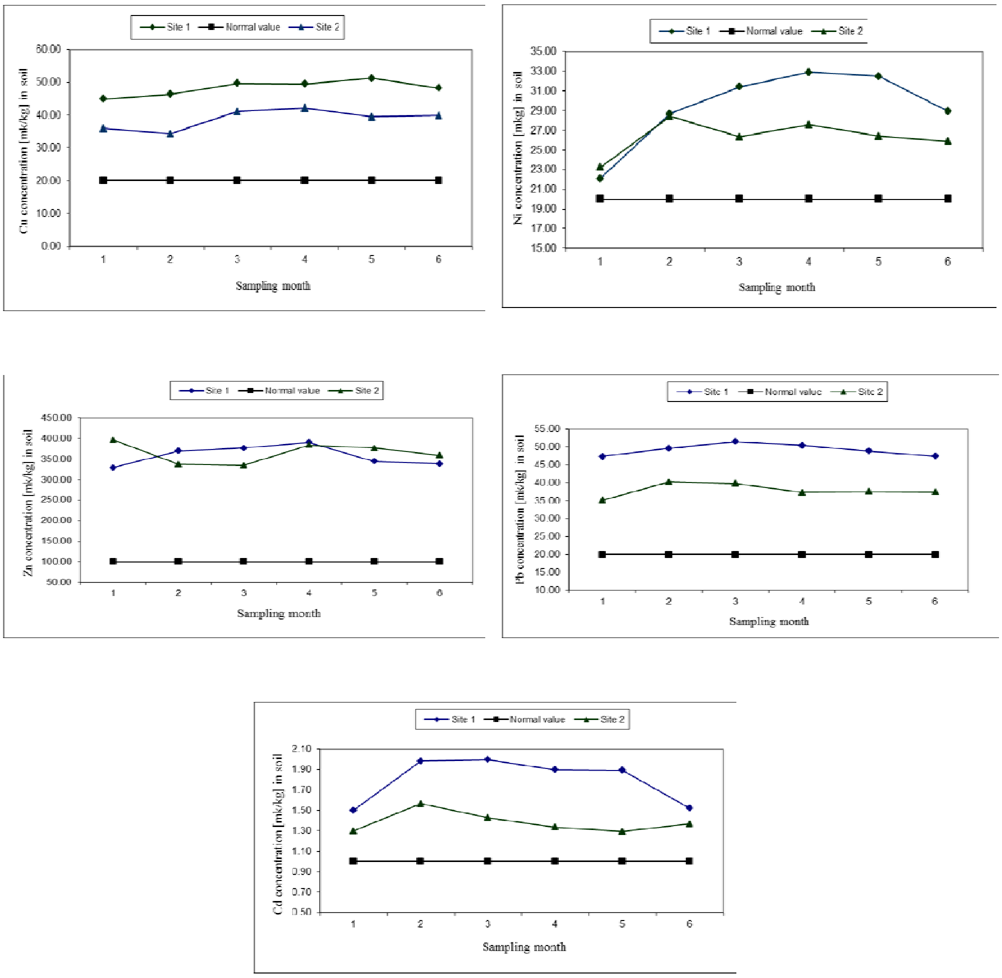

Fig. 2 – Comparison between heavy metals concentration in soil and normal value according to Romanian legislation (OM 756/1997).

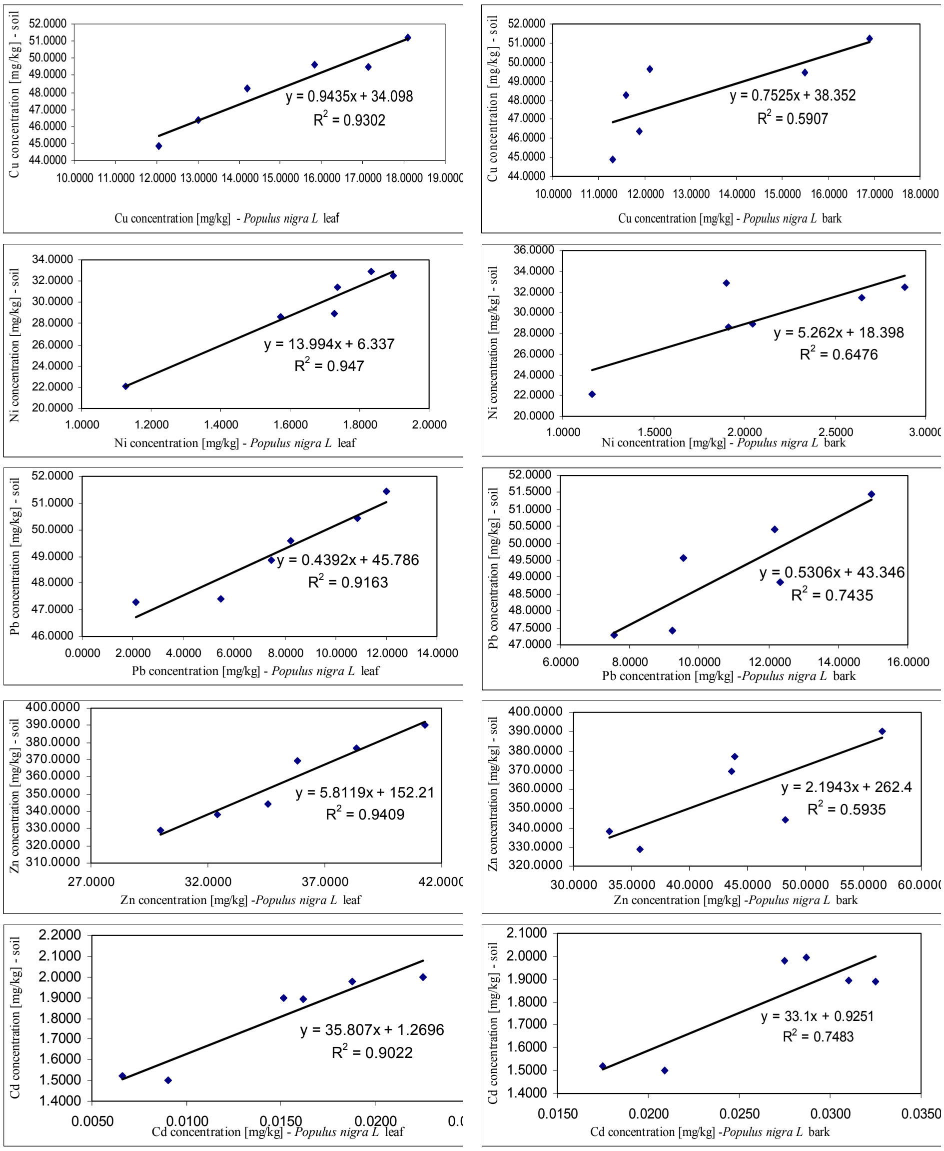

Fig. 3 – The linear dependences between heavy metal concentrations in leaves – soil and bark-soil, obtained by regression analysis (site 1).

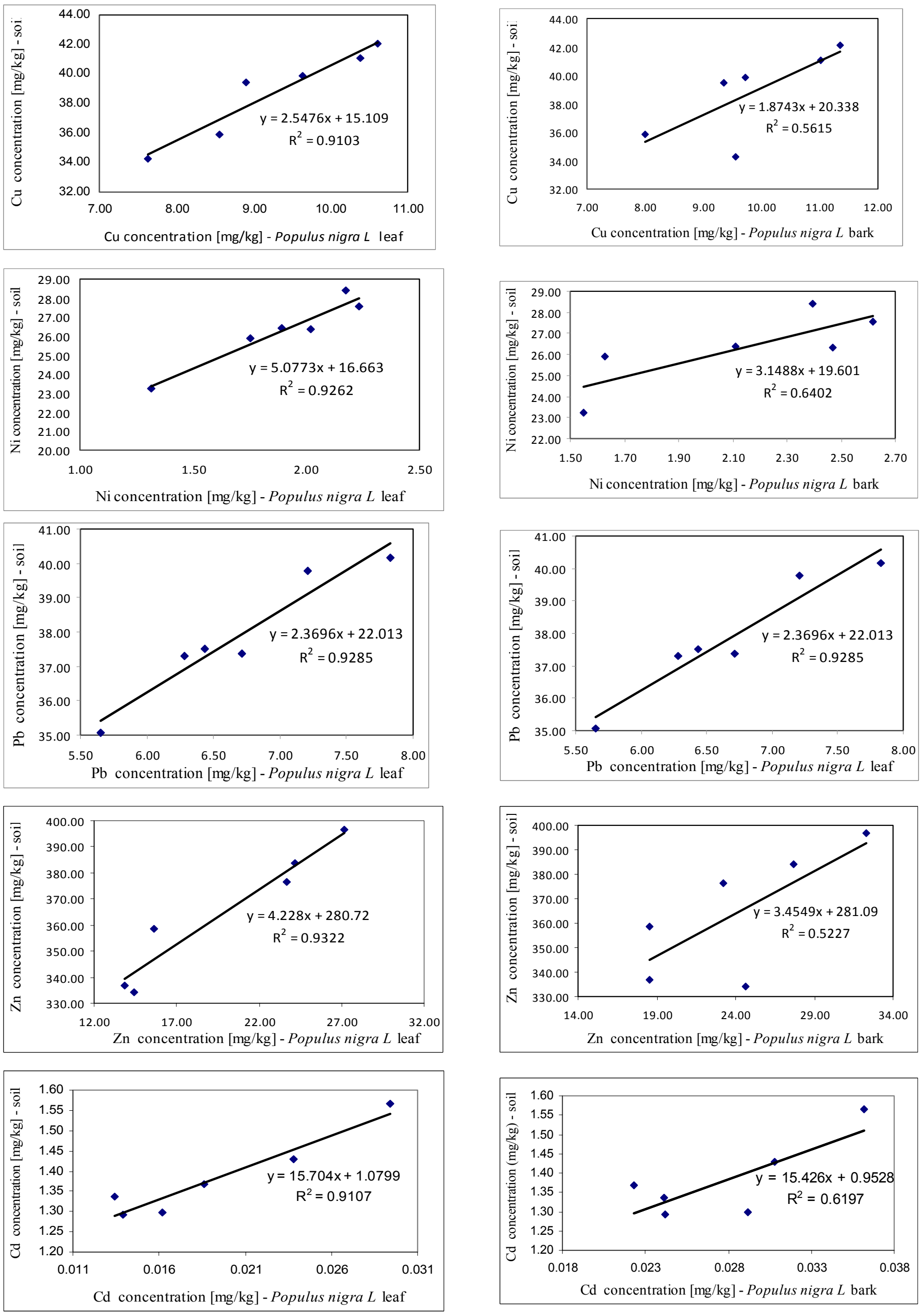

Fig. 4 – The linear dependences between heavy metal concentrations in leaves – soil and bark-soil, obtained by regression analysis (site 2).

4. CONCLUSIONS

This study shows that the leaves of *Populus nigra L*. can be used as suitable bioindicators for soil pollution with Cu, Ni, Pb, Zn, and Cd. Hence it can conclude that the bark of *Populus nigra L* is an excluder for Cu and Zn concerning the uptake of these metals from soil. Certainly for Ni, Pb and Cd the bark of poplar can be considered a passive monitor of atmospheric pollution with these metals. Due to the insufficient knowledge about element mobility in poplar bark, a heavy metals transfer from bark to leaves cannot be established.

REFERENCES

1. M. Ataabadi, M. Hoodaji, P. Najafi, F. Adib, *Evaluation of airborne heavy metal contamination by plants growing under industrial emissions*, Environmental Engineering and Management Journal, **9**, *7*, 903–908 (2010).
2. J.O. Olowoyo, E. van Heerden, J.L. Fischer, C. Baker, *Trace metals in soil and leaves of Jacaranda mimosifolia in Tshwane area. South Africa*, Atmospheric Environment, **44**, 1826–1830 (2010).
3. J. Sardans, F. Montes, J. Peñuelas, *Determination of As, Cd, Cu, Hg and Pb in biological samples by modern electrothermal atomic absorption spectrometry*, Spectrochimica Acta Part B., **65**, 97–112 (2010).
4. S. Rossini Oliva, A.J. Fernandez Espinosa, *Monitoring of heavy metals in topsoils*, *atmospheric particles and plant leaves to identify possible contamination sources*, Microchemical Journal, **86**, *1*, 131–139 (2007).
5. B. Markert, A.M. Breure, H.G. Zechmeister, *Review of Bioindicators and Biomonitors: Principles. Concepts and Applications*, Marine Pollution Bulletin, **50**, *3*, 360 – 361 (2005).
6. M.A. Reis, *Biomonitoring and assessment of atmospheric trace elements in Portugal: methods. response modelling and nuclear analytical techniques*, PhD Thesis, Delft University of Technology, Delft, The Netherlands, 2001.
7. B. Wolterbeek, *Biomonitoring of trace element air pollution: principles. possibilities and perspectives*, Environmental Pollution, **120**, *1*, 11–21 (2002)
8. C. Radulescu, C. Stihi, G. Busuioc, I.V. Popescu, A. Gheboianu, Gh.V. Cimpoca, *Evaluation of essential elements and heavy metal levels in fruiting bodies of wild mushrooms and their substrate by EDXRF spectrometry and FAA spectrometry*, Romanian Biotechnological Letters, **15**, *4*, 5444–5456 (2010).
9. M. Kovács (Ed.), *Biology Indicators in Environmental Protection*, Ellis Horwood, New York, 1992.
10. C. Stihi, C. Radulescu, G. Busuioc, I.V. Popescu, A. Gheboianu, A. Ene, *Studies on accumulation of heavy metals from substrate to edible wild mushrooms*, Romanian Journal of Physics, **56**, *1–2*, 257–264, (2011).
11. M. Miclean, M. Senila, C. Roman, T. Frentiu, E. Cordos, *Plant uptake factors for 55 elements in a rural mining area. NW Romania*, Metal Elements in Environment, Medicine and Biology, 162–165 (2009).
12. C. Radulescu, C. Stihi, G. Busuioc, A. Gheboianu, I.V. Popescu, *Studies Concerning Heavy Metals Bioaccumulation of Wild Edible Mushrooms from Industrial Area by Using Spectrometric Techniques*, Bulletin of Environmental Contamination and Toxicology, **84**, *5*, 641–646 (2010).
13. G. State, I.V. Popescu, C. Radulescu, C. Macris, C. Stihi, A. Gheboianu, I. Dulama, O. Nițescu, *Comparative Studies of Metal Air Pollution by Atomic Spectrometry Techniques and*

Biomonitoring with Moss and Lichens, Bulletin of Environmental Contamination and Toxicology, **89**, *3*, 580–586 (2012).
14. B. Thomson, M. Rose, *Environmental contaminants in foods and feeds in the light of climate change*, Quality Assurance and Safety of Crops and Foods, **3**, *1*, 2–11 (2011).
15. L. Gratani, M.F. Crescente, L. Varone, *Long - term monitoring of metal pollution by urban trees*. *Atmospheric*, Environment, **42**, 8273–8277 (2008).
16. E.E. Ukpebor, J.E. Ukpebor, E. Aigbokhan, I. Goji, A.O. Onojeghuo, A.C. Okonkwo, *Delonix regia and Casuarina equisetifolia as passive biomonitors and as bioaccumulators of atmospheric trace metals*, Journal of Environmental Sciences, **22**, *7*, 1073–1079 (2010).
17. G. State, I.V. Popescu, A. Gheboianu, C. Radulescu, I. Dulama, I. Bancuta, R. Stirbescu, *Identification of air pollution elements in lichens used as bioindicators by the XRF and AAS methods*, Romanian Journal of Physics, **56**, 240–249 (2011).
18. I. Dulama, I.V. Popescu, C. Stihi, C. Radulescu, G.V. Cimpoca, L.G. Toma, R. Stirbescu, O. Nitescu, *Studies on accumulation of heavy metals in Acacia leaf by EDXRF*, Romanian Reports in Physics, **64**, 1063–1071 (2012).
19. G. Wagner, *Richtlinie zur Probenahme und Probenbearbeitung Pyramidenpappel (Populus nigra 'Italica')*, in: *Umweltbundesamt (Hrsg.): Umweltprobenbank des Bundes – Verfahrensrichtlinien für Probenahme, Transport, Lagerung und chemische Charakterisierung von Umwelt- und Human-Organproben*, Erich Schmidt Verlag, Berlin, 1996.
20. I.V. Popescu, M. Frontasyeva, C. Stihi, G.V. Cimpoca, C. Radulescu, G. State, A. Gheboianu, C. Oros, O. Cilicov, I. Bancuta, I. Dulama, *Atomic and nuclear methods applied in the study of heavy polluting elements*, Romanian Reports in Physics, **63**, 1205–1214 (2011).
21. S. Landau, B.S. Everitt, *A Handbook of Statistical Analyses using SPSS*, Chapmann and Hall/CRC, Boca Raton, 2004.