Comparative study on *Plantago major* and *P. lanceolata* (*Plantaginaceae*) as bioindicators of the pollution in the region of the Asarel Copper Dressing Works*

Maya K. Kurteva

Institute of Botany, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: m_kurteva@yahoo.com

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Abstract. The aim of this study is by comparative investigation of microelement accumulation in *Plantago major* and *P. lanceolata* to assess the efficiency of use of these species as bioindicators of environmental pollution in the region of Asarel Copper Dressing Works (Bulgaria). Each species was studied within a period of three years: *P. lanceolata* (2003–2005) and *P. major* (2006–2008). The plant samples were collected from four experimental plots (EP) distanced differently from the Copper Dressing Works. Mt Lozenska (*P. lanceolata*) and Mt Vitosha (*P. major*) were used as control sites. Five elements (Cu, Zn, Pb, Mn, Fe) were determined by AAS – Perkin-Elmer 3030B. The species *P. major* is registered as a better accumulator, mainly of Cu, Fe and Pb within the entire period of investigation and in all EP, especially in EP_1. *P. lanceolata* accumulates greater amounts than *P. major,* preferentially of Zn, through all three years in EP_2 and EP_3 (2003, 2004), and Mn only in EP_2 (2003, 2004).

Key words: accumulation, bioindicators, microelements, *Plantago lanceolata*, *P. major*

Introduction

Biomonitoring can be defined as systematic use of the biological reaction to the environmental changes and application of this information for environment quality control (Mulgrew & Williams 2000). The plants have been successfully used as biomonitors or accumulators of pollunants, especially in ecological terms (Manning & Feder 1980; Kovács 1992). The plants responses to air pollution allow to detect the presence of pollutants and their identification on the base of their impact on the plant species, to determine the total effect of all environmental factors, including air pollution and climate, to measure up the concentration of pollutants, and to elucidate the use of plants as a sensitive system for early diagnosis of the environmenatal changes (Posthumus 1988; Sikora & Chappelka 2004). A range of advantages, such as an intergrated biological effect, low cost, comparatively easy conduct of the investigations and obtaining of the results makes the plant bioinducation to be a valuable source for characterization of air quality (Garrecc 1999).

The paper presents data from the applied passive phytomonitoring, as part of the long-term comprehensive ecological monitoring of environmental pollution in the Asarel-Medet region, town of Panagyurishte (Zyapkov 1995). The aim is by means of comparative investigation of the accumulation capacity of *P. major* L. and *P. lanceolata* L. for five microelements (Cu, Zn, Pb, Mn, Fe) to assess the efficiency of the species as indicators of pollution in the area around the Asarel open copper mine.

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Material and methods

The following two species were object of the investigation: *P. major* and *P. lanceolata* (*Plantaginaceae*). They have been used as indicator species in the phytomnitoring of pollution in the region of Asarel-Medet works. The investigation was carried out in the period 2003–2008 and each of the two species was studied in the course of three years: *P. lanceolata* in 2003–2005 and *P. major* in 2006–2008. *P. major* had been used as indicator since 2006, instead of the formerly used *P. lanceolata*. The wider distribution of *P. major* in the experimental plots explains its use as indicator and the start of a comparative investigation of the accumulation capacity of both species. The following four experimental plots (EP) were set for the purposes of monitoring (Zyapkov 1992), all situated at different distances from the Asarel mine (Fig. 1): EP_1 – near to the Administrative Office of Asarel-Medet AD, at a distance of 1575 m from the mine; EP_2 – at the road to Medet mine, eastwards of peak Lisets and at a distance of 4130 m from the mine: EP_3 – until 2007 within the Panagyurski Kolonii locality, 2660 m off the Medet foundation pit; since 2007 this EP removes to the area of Oborishte historical site, close to the old tourist chalet, 2870 m southwest of the Asarel mine. The selection of a new area for this EP_3 was necessitated by the halting of work at the Medet mine. So after its was closed down, the phytomonitoring investigations were wholly transferred to the area around the Asarel mine. The Oborishte historical site is an important historical and tourist highlight requiring control over the technogenic pollution of the environment. EP_4 – on a lawn in front the eastern embankment, about 2975 m off the mine. The selection of experimental plots was made in compliance with the direction of the actual and potential air flows in the region, and dispersal and deposition of the technogenic substances issued into the athmosphere during mining. Mt Lozenska (*P. lanceolata*) and Mt Vitosha (*P. major*) were used as control regions.

The plant samples, including the above-ground part, were collected at the end of the vegetation period (late September–early October). Samples of *P. major* from the closest to the mine experimental plot (EP_1), as well as from the conditionally clean area (K) have been collected twice during vegetation: in July and early in October, and in data extrapolation of the average values were used.

The content of the following five microelements was determined in the plant species – Cu, Zn, Pb, Mn, Fe (mg/кg dry weight) – by the method of wet ashing procedure with a mixture of concentrated acids (H_2SO_4, HNO_3, $HClO_4$ и HCl), and by atomic adsorption spectrometry (AAS) Perkin-Elmer 3030B.

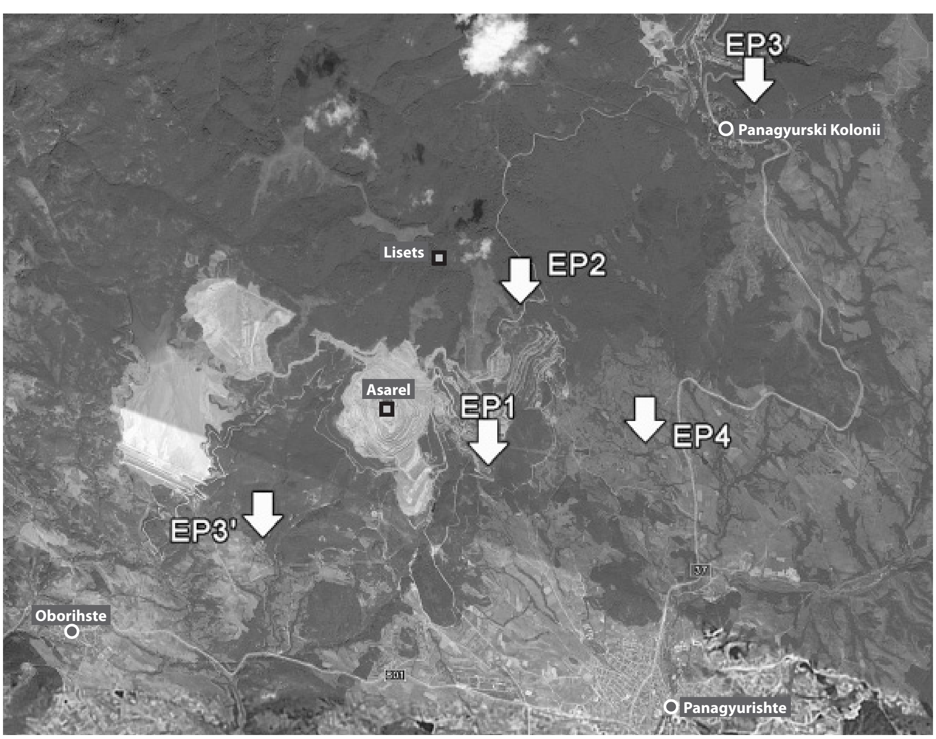

Fig. 1. Map of the region of the Asarel Copper Dressing Works, with experimental plots.
Legend: EP_1, EP_2, EP_3, $EP_{3'}$, EP_4 – experimental plots.

Results

The average values of the accumulated amounts of microelements in the plant species in the region of Asarel are presented in Table 1, Fig. 2A for *P. lanceolata* and Table 2, Fig. 2B for *P. major*. Data from the experimental plots have shown a considerably higher accumulation of three of the investigated elements: Cu, Fe and Pb (for both biomonitors), as well as of Zn (*P. major*) from EP_1, the closest to the mine plot (Tables 1 & 2).

The comparison of data for the accumulated Cu in the *P. major* and *P. lanceolata* has shown *P. major* as a better accumulator in all EP, and especially in EP_1, where the average value (167.42 mg/kg) was 10.6 times higher than the one measured for *P. lanceolata* (15.78 mg/kg); more limited in EP_2 and EP_3 – 3.3 and 2.8 times respectively; and relatively lowest in EP_4 –1.9 times higher Cu concentration (Table 2) as compared to the *P. lanceolata* (Table 1). Most limited levels of Cu for both species were found in the individuals from EP_3, but there again *P. major* accumulated 2.8 times higher amonts (15.29 mg/kg) than the *P. lanceolata* (5.53 mg/kg). Both species accumulated the maximum amounts of copper in the first year of their investigation: 2006 for *P. major* and 2003 for *P. lanceolata*, with 6.6 times higher accumulation for *P. major* (72.19 mg/kg) as compared to *P. lanceolata* (10.99 mg/kg) (Fig. 2-B & A). During the three-year period of investigation, the *P. major* in Asarel has demonstrated on the average 5.9 times higher accumulation capacity for Cu (56.23 mg/kg) as compared to the *P. lanceolata* (9.53 mg/kg) (Tables 2 & 1). The control version of *P. major* during the three years have shown a twice higher level of Cu than the control values of *P. lanceolata*, but the coefficient of deviation of average value from the control has been twice higher

Table 1. Average values of microelement contents in *P. lanceolata* (2003–2005).

Experimental plots (EP)	Years/ Values	Microelements (mg/kg dry weight)				
		Cu	Zn	Pb	Mn	Fe
EP_1	2003	21.25	23.39	2.62	30.52	71.35
	2004	12.31	26.18	1.01	15.36	74.76
	2005	13.77	21.18	3.70	19.00	77.80
	Average	**15.78**	**23.58**	**2.44**	**21.63**	**74.64**
	St. dev.	4.80	2.51	1.35	7.91	3.23
EP_2	2003	5.52	41.63	1.84	63.20	45.11
	2004	3.85	28.07	2.57	57.74	49.30
	2005	11.79	26.01	1.83	28.80	78.08
	Average	**7.05**	**31.90**	**2.08**	**49.91**	**57.50**
	St. dev.	4.19	8.49	0.42	18.49	17.95
EP_3	2003	6.54	47.50	1.75	59.99	32.76
	2004	5.05	63.48	1.68	71.21	45.83
	2005	5.00	27.10	1.80	23.31	98.61
	Average	**5.53**	**46.03**	**1.74**	**51.50**	**59.07**
	St. dev.	0.88	18.23	0.06	25.05	34.86
EP_4	2003	10.66	34.86	1.81	69.59	40.63
	2004	6.46	52.80	0.99	72.17	71.64
	2005	12.17	23.51	0.94	55.54	103.60
	Average	**9.76**	**37.06**	**1.25**	**65.77**	**71.96**
	St. dev.	2.96	14.77	0.49	8.95	31.49
$EP_1 - EP_4 = C_S$	2003–2005	**9.53**	**34.64**	**1.88**	**47.20**	**65.79**
K	2003	3.81	57.15	1.89	112.36	36.10
	2004	4.57	75.71	0.31	103.66	30.52
	2005	4.12	65.06	1.82	85.29	56.68
	Average	**4.17**	**65.97**	**1.34**	**100.44**	**41.10**
C_K	St. dev.	0.38	9.31	0.89	13.82	13.78
Coefficient of deviation from K	$C_{dk}=C_s/C_k$	2.29	0.52	1.40	0.47	1.60

Table 2. Average values of microelement contents in *P. major* (2006–2008).

Experimental plots (EP)	Years/ Values	Microelements (mg/kg dry weight)				
		Cu	Zn	Pb	Mn	Fe
EP_1	2006	253.80	61.54	5.35	55.38	247.00
	2007	98.34	73.65	1.91	34.95	613.17
	2008	150.12	88.76	14.02	58.79	167.65
	Average	**167.42**	**74.65**	**7.09**	**49.71**	**342.61**
	St. dev.	79.16	13.64	6.24	12.89	237.65
EP_2	2006	13.84	22.57	1.55	47.24	225.47
	2007	22.27	15.58	2.29	28.68	311.85
	2008	34.24	23.87	2.83	58.87	156.13
	Average	**23.45**	**20.67**	**2.22**	**44.93**	**231.15**
	St. dev.	10.25	4.46	0.64	15.23	78.02
EP_3	2006	8.73	27.12	0.20	25.17	181.20
	2007	8.60	14.13	1.68	48.22	389.33
	2008	28.54	44.14	6.34	147.83	121.23
	Average	**15.29**	**28.46**	**2.74**	**73.74**	**230.59**
	St. dev.	11.48	15.05	3.20	65.19	140.71
EP_4	2006	12.35	52.90	0.90	125.82	238.09
	2007	19.21	25.40	4.40	44.88	343.81
	2008	24.69	35.74	4.03	277.48	172.11
	Average	**18.75**	**38.01**	**3.11**	**149.39**	**251.34**
	St. dev.	6.18	13.89	1.92	118.08	86.61
$EP_1 - EP_4 = C_S$	2006–2008	**56.23**	**40.45**	**3.79**	**79.44**	**263.92**
K	2006	9.83	61.82	3.37	52.39	250.09
	2007	9.20	21.57	3.81	116.60	367.14
	2008	15.97	23.03	2.48	57.07	136.40
	Average	**11.67**	**35.47**	**3.22**	**75.35**	**251.21**
C_K	St dev.	3.74	22.83	0.68	35.80	115.37
Coefficient of deviation from K	$C_{dk}=C_s/C_k$	4.82	1.14	1.18	1.05	1.05

Legend: K – control; St. dev. – standard deviation; $\mathbf{C_s}$ – average value for $EP_1 - EP_4$; $\mathbf{C_k}$ – average value for control.

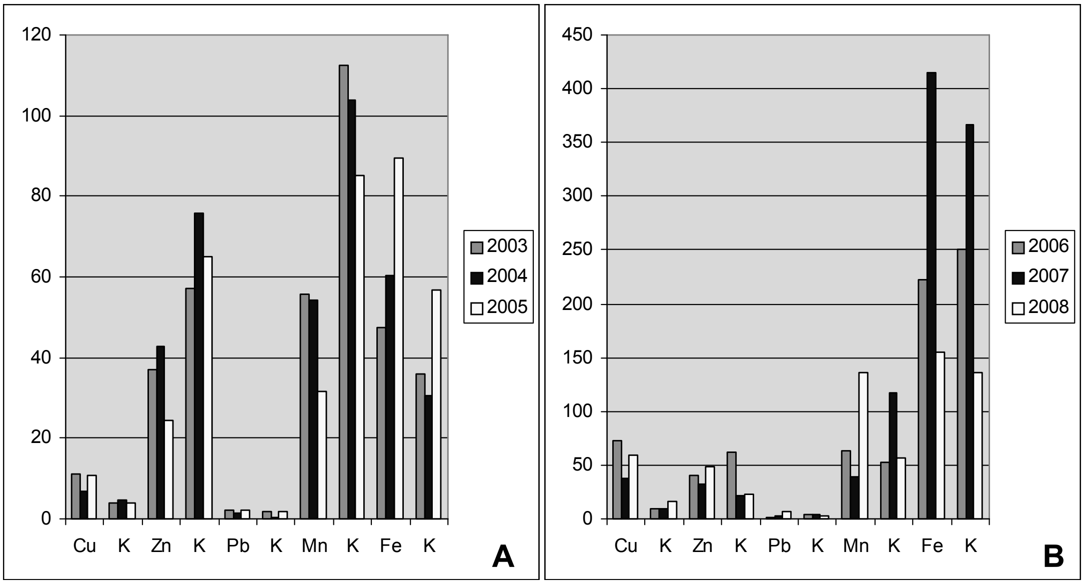

Fig. 2. Annual microelement concentrations in *Plantago*: **A**, *P. lanceolata*; **B**, *P. major*; **K,** control.

(4.82) from the one established for *P. lanceolata* (2.29) (Tables 2 & 1). The high value of the above-mentioned coefficient for both species, especially for the *P. major*, proves indirectly the high level of environment contamination with Cu in the region of Asarel-Medet. The average annual levels of Cu in the experimental plots in the area of Asarel in both biomonitors exceed the control values, with a very wide range for *P. major*: 3.7 times (2008) – 7.3 times (2006) and a much smaller range for *P. lanceolata:* 1.5 times (2004) – 2.9 times (2003) (Fig. 2-B & A).

During the second year, a drop in the accumulation of Cu has been observed in both species, especially in *P. major*, with a subsequent increase of the amount of this element at the end of the investigation, again more pronounced in the *P. major*. The concentrations of Cu measured up in *P. lanceolata* in EP_2 and EP_3 for the different years and averagely for the entire period were below the minimum background count of this element for the region (Doncheva-Boneva 2006). In EP_1 and EP_4 the accumulation of Cu was within the limits of the normal background count, with only the average value in EP_1 being at its optimum for this element throughout the three years, and equal to its amount in the leaves of *Fagus sylvatica* in the region (Doncheva-Boneva 2006). The pattern differred for *P. major,* insofar that in the samples from EP_2, EP_4 (2006) and EP_3 (2006, 2007) the amounts of Cu were within the limits of the nomal background count for the region, while in 2008 (in all EP) and the average values in the different EP for the entire period of investigation, were above the optimum values of the element for the region, especially in EP_1: 11.2 times above the optimum value.

According to the classification of Kabata-Pendias & Pendias (2001) and after data provided by other researchers (Shkolnik 1974; Kovalskiy 1971; Beckett & Davis 1977), concentrations of Cu in the *P. major* in EP_1, especially in 2006 (253.80 mg/kg) and in 2008 (150.12 mg/kg), and the average value for this experimental plot within the three-year period (167.42 mg/kg) (Table 2), exceeded the top critical level of the element (Beckett & Davis 1977), and was respectively 12.7, 7.5 and 8.4 times above the permissible limited concentration (PLC) for medicinal plants (Nazdrjuhina, Grinkevich 1980), adopted as a standard for Poland (Wierzchowska-Renke 1994). It also exceeded by 8.5, 5.0 and 5.6 times the upper limit for normal concentration, and according to Szentmihalyi & al. (2006), the average concentration, as well as by 6.3, 3.7 and 4.2 times the threshold concentration of Cu in livestock food (Kovalskiy 1971). The above-cited values for Cu, especially in EP_1 (2006), as well as the average value for this EP throughout the three-year period of research exceeded the maximum concentration of this element and are strongly toxic for plants. In the other EP the content of Cu in plantain was within the norm, at the lower limit of the maximal threshold concentration in the livestock food, and was not toxic for plants.

The maximum concentration of Fe in both species was registered in the samples from EP_1, where the average value for the three-year period in *P. major* (342.61 mg/kg) exceeded by 4.6 the value recorded for *P. lanceolata* (74.64 mg/kg) (Tables 2 & 1). Second, with larger accumulations of this element come the samples from EP_4, and the most limited quantities of Fe were recorded in EP_3 (*P. major)* and EP_2 (*P. lanceolata*). The maximum content of Fe in the *P. major* was found in EP_1 (2007), and in the *P. lanceolata* in EP_4 (2005) (Tables 2 & 1). With the exception of *P. major* (2006), all annual values in both species from the region of Asarel were higher than the control ones, with a relatively great difference in *P. lanceolata,* especially in 2004 (Fig. 2-A & B). Averagely for the three-year period, *P. major* in the region of Asarel has accumulated four times more Fe (263.92 mg/kg) than *P. lanceolata* (65.79 mg/kg) (Tables 2 & 1). In our opinion, the reduction of the average annual level of Fe in the *P. major* in 2008 depended to a great extent on the distinct antagonism of Fe/Mn (2007, 2008) (Fig. 2-B). The coefficient of deviation of the average value of element from the control one is higher for *P. lanceolata* (1.60) as compared to *P. major* (1.05) (Tables 1 & 2) and indicateed a lower degree of pollution (as compared to Cu) in the region of Asarel. All amounts of Fe measured in *P. lanceolata* were under the minimum background count of the element for the studied region (Doncheva-Boneva 2006). For *P. major,* the values of Fe were within the normal limits (2008) and above the optimum for the element, especially in 2007. Accumulation of iron in this *Plantago* species in EP_1 (2008) corresponded to the level of the element in the beech leaves in the region (Doncheva-Boneva 2006). The average values for Fe in *P. major* in the investigated region have kept within the range of the average concentrations after Kabata-Pendias & Pendias (2001), considering the fact that the amounts of this element in *P. major* in 2007, and especially in EP_1 (613.17 mg/kg), as well as the average value of Fe in this plot for the entire period, especially in 2007, exceeded the upper limit (2.0 times) of the average value, according to Baker & Chesnin (1975). All values of Fe in the *P. major* from Asarel were by far below as the maximum concentration of the element as the toxic one for the plants.

High concentrations of Pb were registered in the samples of *P. major* from EP_1 in 2008 and 2006 and for the *P. lanceolata* in 2005, 2003 (Tables 2 & 1). *Plantago major* registered maximum concentration in 2008 (14.02 mg/kg), and *P. lanceolata* in 2005 (3.70 mg/kg). The average value of lead in the *P. major* in EP_1 for the entire three-year period (7.09 mg/kg) was three times higher than the accumulation of that element in the *P. lanceolata* (2.44 mg/kg), and the antagonistic Pb/Mn relationship was manifest in both species in that plot. The average concentration of the element in *P. major* from all EP (3.79 mg/kg) was twice higher than the one established for *P. lanceolata* (1.88 mg/kg) (Tables 2 & 1). The average annual concentration of lead in *P. lanceolata* throughout the three years were higher than the control values, especially in 2004, while in *P. major* in the first two years (2006, 2007) the control values were higher (Fig. 2-A & B). On the contrary, during the last year of study the amount of Pb in the background sample of *P. major* registered a triple drop, as compared to the annual value from Asarel (Fig. 2-B). The coefficient of deviation of the average concentration of Pb from the control one was higher (1.40) for *P. lanceolata* as compared to *P. major* (1.18). The concentrations of lead measured up in the samples of *P. lanceolata* were lower than the minimum background count of that element for the region (Doncheva-Boneva 2006). In *P. major*, with the exception of the amounts of Pb in EP_1, especially in 2008, and for the entire three-year period too, which were within the optimum level limits, all other values were below the minimum for that element in the region. The established amounts of Pb in both species were below the established norm in Bulgaria for the concentration of the element in edible plants (Regulation 5 1984). The values of *P. major* in EP_1 (2008, 2006) make an exception, 14.02 mg/kg and 5.35 mg/kg respectively, as well as the average level of the element for the entire period (7.09 mg/kg), but they also fall within the range of normal values of Pb in plants. All values were within the permissible limits for Pb in the herbaceous species (Kabata-Pendias & Pendias 2001), below the upper limit of the background count accepted for Europe and much lower than the toxic range for plants.

Contrary to elements considered above, Mn showed the highest concentrations in the samples from EP_4, throughout the three years for *P. lanceolata*, and for *P. major* in the years 2006, 2008, while in 2007 its concentrations were the highest in EP_3 (Tables 1 & 2). The maximum value of Mn in *P. major* for the entire period of investigation was in the samples from EP_4 (2008) – 277.48 mg/kg, and for *P. lanceolata* – again in EP_4, but in 2004 – 72.17 mg/kg (Tables 2 & 1). The average value of the element in the *P. major* from EP_4 (149.34 mg/kg) was twice higher than the value registered in the *P. lanceolata* (65.77 mg/kg) (Tables 2 & 1). *Plantago major* proved a better accumulator of manganese in three of the experimental plots, especially in EP_1 and EP_4, respectively with twice and three times greater amounts of Mn as compared to the *P. lanceolata*. The latter species has accumulated more of that element only in EP_2 – 1.1 times more Mn as compared to *P. major*. According to the average annual levels, *P. major* has accumulated 1.7 times more Mn (79.44 mg/kg) as compared to *P. lanceolata* (47.20 mg/kg) (Tables 2 & 1). The average annual values of Mn in *P. lanceolata* from the region of Asarel throughout the three years of investigation were lower than the control ones, especially in 2005, while in *P. major* in 2007 alone the accumulation of manganese in the region exceeded thrice the control value. This very high accumulation of Mn in EP_4 was due to its interaction with other elements, in this case the Cu/Mn antagonism (Tables 1 & 2). The low values of the coefficient of deviation of the average level of Mn in relation to the control value (1.05) and the value in the *P. lanceolata* (0.47) indicated a lower contamination of the region with manganese, commensurable with Zn (*P. lanceolata*) and Fe (*P. major*), much lower than the one recorded for Cu (Tables 2 & 1). The amounts of Mn measured up in the above-ground parts of both biomonitoring species, especially in *P. lanceolata*, were by far below the minimum background values of the elements for the region, but it should be taken under consideration that the investigated region was strognly influenced by the mining and ore-dressing activities, which set up higher background levels for the microelements (Doncheva-Boneva 2006). According to the classification of Kabata-Pendias & Pendias (2001), the level of Mn in both *Plantago* species and in all EP in the region of Asarel was within the normal limits for plants, within the range of the average normal value after Baker & Chesnin (1975), Szentmihalyi & al. (2006), and only in EP_4 *P. major* hit the upper limit. All values were under the maximum of the element for plants.

Data on Zn in both *Plantago* species were not synonymous. Thus, while the highest concentrations of this element in the different years in *P. major* were in the samples from EP_1, for *P. lanceolata* they were in EP_3. The year 2008 witnessed the maximum accumulation of zinc in the *P. major*, and for the *P. lanceolata* this was the year 2004 (Fig. 2-B & A). The average values in the different EP showed greater accumulation of Zn in *P. major* (3.2 times) as compared to *P. lanceolata* only in EP_1, 74.65 mg/kg and 23.58 mg/kg respectively (Tables 2 & 1). The element showed equal values in both biomonitors in EP_4. In the two other plots *P. lanceolata* accumulated a greater amount of Zn, with one-time greater accumulation in EP_3 (46.03 mg/kg) as compared to *P. major* (28.46) (Tables 1 & 2). Despite of the more pronounced accumulation of zinc in the *P. lanceolata* from the above-mentioned EP, the average concentration of the element in all plots in Asarel was 1.2 times higher in *P. major* (40.45 mg/kg) as compared to the *P. lanceolata* (34.64 mg/kg) (Tables 2 & 1). The average annual levels of Zn were under the control values throughout the three years for *P. lanceolata* and for *P. major* only in 2006 (Fig. 2-A & B). There was antagonism traced out between Zn and the other elements: Zn/Mn in EP_1 (*P. major*) and Zn/Cu in EP_1 and EP_3 (*P. lanceolata)*. The deviation coefficient of the average value from the control one was twice higher for the *P. major* (1.14) as compared to the *P. lanceolata* (0.52) and indicated a lesser contamination of the environment than the one recorded for Cu and Mn. The levels of Zn in *P. major* were under the minimum level (EP_2), and for the same species in EP_3, as well as for *P. lanceolata* (EP_1, EP_2) – within the range of the normal background count of the element for the region. The average concentrations of Zn in EP_1 and EP_4, especially in EP_1 (*P. major*), as well as in EP_3, EP_4 (*P. lanceolata*) were above the optimum amounts of the element for the region (Doncheva-Boneva 2006). The amounts of Zn established in the *P. lanceolata* from EP_1 (2004) and EP_2 (2005) corresponded to the amounts of that element in the beech leaves in the region (Doncheva-Boneva 2006). According to the classification of Kabata-Pendias & Pendias (2001) and after data reported by Kovalskiy (1971); Nazdrjuhina & Grinkevich (1980) etc., the concentrations of Zn in the *P. major* in 2006–2008 (EP_1), as well as the average value for the element in that plot exceeded by 1.2 to 1.8 times, and in EP_3 (*P. lanceolata*) by 1.3 times, or were close to the values in EP_4 in 2006 (*P. major*) to the maximum permissible concentration for medicinal plants (Nazdrjuhina & Grinkevich 1980). In the other plots the amounts of Zn in both species were lower than LPC and fell into the normal range under Kovalskiy (1971). All values for Zn were within the limit of the normal, average concentration, according to Szentmihalyi & al. (2006) and by far under the toxic level for plants (Beckett & Davis 1977).

Accumulation dynamics of the microelements in *P. major* from EP_1 and the control version was established. In most cases the microelements showed maximum concentration at the end of the vegetation period (October). There was an exception only in Fe and Zn in EP_1 (2006, 2007) and in the conrol values (2008), as well as in Pb in the control site (2008), which showed higher values in July (Table 2). A higher variation of the values in October-July in EP_1 was registered in relation to Cu (2006), Pb (2008) and Mn (2006, 2008), and in the control sites for Mn and Fe (2007), Pb and Zn (2006) (Table 2).

A change in the accumulation of microelements in the new area of EP_3 was observed in 2007–2008 (Table 2). Thus, in the first year of study in the region of the Oborishte historical site *P. major* showed lower levels of Zn and Cu, especially of Zn (twice lower) (14.13 mg/kg) as compared to the former region of Panagyrski Kolonii (27.12 mg/kg). The remaining elements were in greater amounts as compared to 2006, especially of Pb (8.4 times), and lower amounts of Fe and Mn, 2.0 and 1.9 times respectively. In 2008 *P. major* showed higher accumulation of four of the investigated elements, especially of Pb (3.8 times) and Cu (3.3 times), and more limited amounts of Zn and Mn (3.1 times), as compared to 2007. Only Fe has shown a reduced level 3.2 times. Mn/Fe antagonism was traced out, with a trend for increase of Mn at a drop in Fe. Although these are only reference data, they have proved the contamination of the environment, chiefly with Cu and Pb, even in this more distanced plot from the mine.

Discussion

Environmental pollution with heavy metals poses a grave problem to the contemporary world. The ions of many heavy metals, such as Cu, Zn, Mn, Fe, and Ni are important microelements in the mineral nutrition of plants, but at high levels and jointly with other metals, such as Cd and Pb, could become extremely toxic.

Owing to their ability to effectively capture and accumulate chemical substances from the environment, the plants are widely used as passive biomonitors in the urbanized and natural environment (Jones & al.1992; Markert 1993; Monaci & Bargagli 1997).

The two plantain species investigated in this study are representatives of the ruderals in natural vegetation in the region of Asarel-Medet. Their selection as monitors complies with the recommendation of Kovács (1992) about the use of ruderal species as bioaccumulating indicators, owing to their ability to accumulate metals in large quantities, without visible damage. Ruderals are widely spread, which makes possible their comparative investigation in different regions.

Plantago lanceolata is determined as a highly tolerant species of Zn (Schwanitz & Hahn 1954) and Pb (Horak & Huber 1974; Wu & Antonovics 1976). This species is still an object of study under various types of pollution (Yurukova 2004; Dimitrova & Yurukova 2005; Tamás & Kovács 2005). Horak & al. (2006) maintain that *P. lanceolata* could be used as an indicator for metals (Zn, Cd, Pb) in the leaves and soil. Tamás & Kovács (2005) have established in this plantain species growing on the slag from a mine for Zn and Pb, considerable accumulation of Pb, Cu and Zn.

Data on the accumulation of Cu in *P. lanceolata* from EP_2, EP_3 (2003, 2004) and EP_4 (2004) are below the concentration of this element in the same species from the industrial zone of the town of Plovdiv – 10 mg/kg (2001) (Dimitrova & Yurukova 2005) and in Sofia – 9.7 mg/kg (Yurukova 2004). The greater amount of Cu in EP_1 (2004) equalled the level of this element in Plovdiv, both in the industrial zone in 1998 (12 mg/kg) (Dimitrova & Yurukova 2005), and in other points of the city (12.3 mg/kg) (Yurukova unpubl.), and is commensurable with the amount recorded in *Achillea millefolium* from transport pollution in Sofia (12.50 mg/kg) (Wierzchowska-Renke & al. 1997). The annual values of Cu (2003, 2005) are comparable with the level of the elements in the same plantain species from the industrial zone of Plovdiv – 10 mg/kg) (Dimitrova & Yurukova 2005), but within the three years of investigation are considerably lower (8–14 times) than the accumulated Cu in *Achillea millefolium* (96.98 mg/kg) and 1.4 lower than in *Tanacetum vulgare* (16.97 mg/kg) in the region of the Pirdop Copper Works (Wierzchowska-Renke & al. 1997).

The average annual levels of Zn recorded in *P. lanceolata* for the region of Asarel, within the rage 24.45 mg/kg (2005) – 42.63 mg/kg (2004) are considerably lower than those measured in the same species in the industrial zone of Plovdiv – 119 mg/kg (2001) – 128 mg/kg (1998) (Dimitrova & Yurukova 2005), as well as much lower than the content of Zn in the leaves of the same species growing around a former lead and zinc smelter in Austria – 580 mg/kg (Horak & al. 2006). However, the average annual concentration in Asarel (2003) of 36.85 mg/kg is very close to the level of this element in the same species in Sofia: 35 mg/kg (Yurukova 2004). The annual values of Zn during all three years of the investigation are lower than the ones recorded in *Achillea millefolium* and *Tanacetum vulgare* in the region of Pirdop, 58.31 mg/kg and 50.04 mg/kg respectively (Wierzchowska-Renke & al. 1997), as well as than the level of this element in different regions of Pliovdiv (51.02 mg/kg) (Yurukova unpubl.). The accumulation of Zn in the *P. lanceolata* is higher only in EP_3 in 2004 (63.48 mg/kg.)

The annual concentrations of Pb in *P. lanceolata* from Asarel, with the range 1.56 mg/kg (2004) – 2.07 mg/kg (2005), are much lower than those measured in the industrial pollution zone in Plovdiv: 6.8 mg/kg (2001) – 7.0 mg/kg (1998) (Dimitrova & Yurukova 2005), as well as in the different regions of Plovdiv (6.5 mg/kg) (Yurukova unpubl.), much below the level of this element in Sofia (16 mg/kg)(Yurukova 2004), but commensurable with the concentration of Pb in the same species (2.00 mg/kg) growing on lime-treated soils in the vicinities of a former lead and zinc smelter in Austria (Horak & al. 2006).

The average annual amounts of Mn in the *P. lanceolata* are higher (2003, 2004) than the levels of the same element in the same species in different regions of Plovdiv (41.9 mg/kg) (Yurukova unpubl.), but by half lower than the concentrations of Mn in Sofia (97.0 mg/kg) (Yurukova 2004). The annual levels of the element in the region of Asarel are twice higher than the amount of Mn in *Achillea millefolium* from Pirdop (27.72 mg/kg), while the values for the *Plantago* from Asarel (2004) and *Achillea millefolium* in the Parangalitsa Reserve (Rhodopi Mts) are almost equal, 54.12 mg/kg and 55.40 mg/kg repectively (Wierzchowska-Renke & al. 1997).

The annual values of Fe in the *P. lanceolata*, within the range 47.46 mg/kg (2003) – 89.52 mg/kg (2005), are much lower than the level of this element in the same species in various regions of Plovdiv (330 mg/kg) (Yurukova unpubl.), and especially than the concentrations recorded in Sofia (1679 mg/kg) (Yurukova 2004).

In nature, *P. major* is used as an indicationr for overgrazing of pasturelands (Czeglėdi & Radácsi 2005). This species falls with the ruderals from natural vegetation in the industrial centres of the Ural Mts (Shilova 1989). In a techogenic environment *P. major* is a valuable bioindicator species of SO_2 (Schubert 1985), O_3 (Davison & Reiling 1993), as well as of a number of microelements in different types of environmental pollution (Stolarska & al. 2004; Akram & Al-Homaidan 2007; Doncheva-Boneva 2000, 2003). *Plantago major* has turned out (Stolarska & al. 2004) a better accumulator of Cd, Pb, Ni, and Co as compared to *Taraxacum officinale*. The leaves of this plantain species accumulate more Pb from contaminated waters as compared to the leaves of *Phaseolus vulgare* (Akram & Al-Homaidan 2007).

A comparison of the data on microelement accumulation by *P. major* from Asarel with those measured in Pirdop and Bunovo (two sites under the impact of the air pollution by the Copper smelter in Pirdop) (Doncheva-Boneva 2003) has shown equal values for Cu and Zn in Pirdop and EP_1 in Asarel (2008): for Cu – 149.0 mg/kg (Pirdop) and 150.12 mg/kg (Asarel), and for Zn – 87.8 mg/kg (Pirdop) and 88.76 mg/kg (Asarel). In Asarel, the accumulation of Cu is higher again in EP_1, but in 2006 (253.80 mg/kg). All values of Cu in this plantain species in the region of Asarel (with the exception of EP_1) are considerably lower than the values recorded for the regions of Pirdop and Bunovo above. The concentration of Zn in EP_1 in 2007 (73.65 mg/kg) is the same as the amount measured up in Buhovo (under the influence of the contamination from the Kremikovtsi Metallurgical Works): 74.0 mg/kg) (Doncheva-Boneva 2000). The concentrations of Pb in *P. major* in Buhovo and Pirdop are higher, 19.8 mg/kg and 17.8 mg/kg respectively, from the maximum value of the element for Asarel in EP_1 (2008): 14.02 mg/kg. The value from Asarel is commensurable with the levels of Pb in Bunovo (14.3 mg/kg) and Svoge (13.3 mg/kg) (Doncheva-Boneva 2000). The highest levels of Mn accumulated in *P. major* in EP_4 and EP_3 (2008) are above (277.48 mg/kg) (EP_4) and below (147.83 mg/kg) (EP_3) the level of the element in Buhovo (184.0 mg/kg). Fe showed high concentrations both in Pirdop and Bunovo: 588 mg/kg and 568.8 mg/kg respectively. This element recorded considerably lower levels in the *Plantago* from the region of Asarel, with the exception of its accumulation in EP_1 in 2007 (613.17 mg/kg). The level of Fe in *P. major* depends on the presence of the element in the habitat (Stolarska & al. 2004), which supports our view of a greater accumulation of the element in 2007, following a higher environment pollution level. The drop in the accumulation of iron in *P. major* in 2008 relates to a great extent to the distinct Fe/Mn antagonism.

Our investigation has shown maximum accumulation of Mn, Pb and Zn in *P. major* in 2008. There are two known types of pollution with Pb: from the soil through the roots (Finster & al. 2004; Del Rio-Celestino & al. 2006), with accumulation of the element mainly in the underground parts of the plants (Huang & Cunningham 1996), with subsequent translocation in the aboveground organs of the plants, even in fruits (Yaman & al. 2000; Toth & al. 2006); and from the air via the leaves (Aydinalp & Marinova 2004). According to Jaffer & al. (1999), accumulation from the air seemes to be the main pollution source for plants with lead. The accumulation of microelements in plants, besides by the type and degree of pollution, depends to a very great extent on the influence of other environmental factors: pH of the soil solution, climate, as well as on the microelement itself and the biological characteristics of the plant species. The soils in the region of Asarel are mainly cinnamon forest soils, strongly leached and eroded, and brown forest soils occur only in single patches in the beech forests. According to the data from the soil analyses in the region (Malinova 2006), the soil pH in the surface layer (0–5cm) is 4.88 at a low content of humus, N, P, Ca and Zn, high content of Fe, Pb and Cu within the limits of the average values, according to ICP-Forest criteria. The average values of soil pH in the experimental plots, according to our data (Kurteva unpubl.) for the entire period have been within the range 6.03 (EP_3) – 6.71 (EP_1), with strong increase in 2008 up to 7.17 (EP_2), 6.82 (EP_4), 6.78 (EP_1), which obstructed the mobility of microelements and their translocation to the aboveground organs of the plants. The great increase of the level of Pb in *P. major* in 2008, especially in EP_1, took place against the background of a slight increase in its concentration in the soil (Kurteva unpubl.), while the maximum of Mn in EP_4 (2008) was recorded at twice lower accumulation of this element in the soil, as compared to the previous year. Zn also registered a maximum for the three-year period in 2008 and in EP_1, although its accumulation in plantain was strongly limited against the background of the very great accumulation of this element in the soil. Thus our study corroborates the findings of Doncheva-Boneva (2000) that there is no direct relationship between the content of microelements in the soil and in the plants. The slighter accumulation of Zn in the *P. major* from the soil probably corresponds chiefly with the biological characteristics of the plant species, as well as the interrelation of Zn with the other elements. The increased accumulation of Zn, Pb and Mn in *P. major* in 2008 in Asarel, in our opinion, besides to pollution, should be related in a considerable extent to the influence of the climatic factors – drought, wind-blown dust from the mine and accumulation of microlements mainly from the air. Considering the climatic conditions in Asarel in 2008, as well as the reaction of the soil solution, we think that there are grounds to relate the greater accumulation of Cu, Pb and Mn by *P. major* in 2008 to the accumulation of the above-mentioned elements chiefly from the air. For the plants in EP_1, the more intensive automobile transport is also important, for it additionally increases the amount and accumulation of Pb. A similar situation for Asarel has been registered earlier too (Doncheva-Boneva & Bezlova 2000).

Data on the investigated elements in the samples of *P. major* from the new EP_3, especially in 2008, have shown greater accumulation of Cu (double), Pb and Mn in the same degree (1.5 times), and Zn (1.4 times) as compared to the recordings in the leaves of *Quercus cerris* there (Doncheva-Boneva & Bezlova 2000).

The degree of accumulation in both Plantane species, besides by pollution and other environmental factors, especially the climatic and soil pH, depends to a greater extent on the antagonistic relationships between the elements: Fe/Mn in *P. major* (2007, 2008), *P. lanceolata* (2005), Zn/Fe in *P. lanceolata* (2005), and Cu, Zn/Fe in *P. major* (2007).

Conclusions

The results of our investigation have shown maximum accumulation of Cu, Fe, Pb (for both biomonitors) and Zn (*P. major*) in EP_1, Mn (in both species) in EP_4, and Zn (*P. lanceolata*) in EP_3.

In EP_1, which is closest to the Asarel mine, *P. major* accumulates more Cu (10.6 times), Fe (4.6 times), Zn (3.2 times) and Pb (2.9 times) as compared to *P. lanceolata*.

The average values of the investgated elements from the region of Asarel are higher in *P. major*, namely, Cu (5.9 times), Fe (4.0 times), Pb (2.0 times), Mn (1.7 times), and Zn (1.2 times), as compared to those found in *P. lanceolata*.

Plantago lanceolata has proved a better accumulator, chiefly of Zn in EP_3 and EP_2, 1.6 and 1.5 times respectively, as well as of Mn in EP_2 by 1.1 times higher than in *P. major*.

Dynamics of the accumulation of microelements was registered in *P. major* in EP_1 and in the control region. Microelements showed maximum concentration mostly at the end of vegetation, with greater variation in the period October-July for Cu, Pb, Mn (EP_1), and for Mn, Fe, Pb, and Zn also in the control region.

The higher level of elements in the new EP_3, especially of Pb and Cu, illustrates the stronger technogenic pollution in that more distanced from the mine plot too.

The obtained results prove that *P. major* could be successfully used as bioaccumulating indicator, especially of Cu, Fe and Pb, under the conditions of technogenic pollution in the region of the Asarel Copper Dressing Works.

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