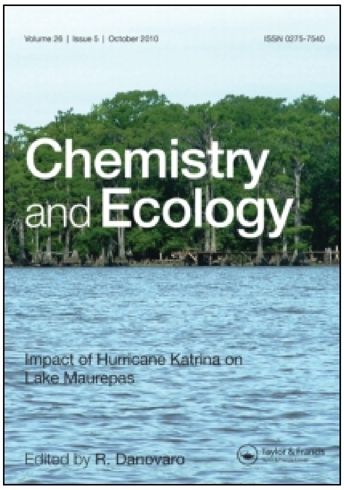

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Seasonal Variations in the Metal Concentration of *Taraxacum Officinale, Plantago Major* and *Plantago Lanceolata*

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SEASONAL VARIATIONS IN THE METAL CONCENTRATION OF *TARAXACUM OFFICINALE, PLANTAGO MAJOR* AND *PLANTAGO LANCEOLATA*

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The seasonal changes in the concentrations of aluminium (Al), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), magnesium (Mg), manganese (Mn), lead (Pb), and zinc (Zn) in the leaves of *Taraxacum officianale*, *Plantago lanceolata* and *Plantago major* are investigated. The most convenient periods for sampling are established when the elemental concentrations have minimal variation and are independent on the development of the plant itself. On this basis, the presumption is made that the most appropriate sampling period for herbaceous species for biomonitoring processes is the end of blossoming of the plant or immediately after that.

Keywords: Biomonitoring; *Taraxacum officianale*; *Plantago lanceolata*; *Plantago major*; sampling; seasonal variation

INTRODUCTION

Representative sampling of plants is a crucial step in biomonitoring studies which may introduce up to 1000% error in the final result thus rendering the subsequent analysis meaningless (Djingova and Kuleff, 1994; Markert, 1996; Sansoni, 1986; Wagner, 1995). The representative sampling of plants may be jeopardized if two vital questions are not considered: the heterogeneous distribution of plants in the total system and the intrinsic dynamics of living organisms in respect to the

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chemical composition in dependence on space and time (Markert, 1996). Seasonal changes in the element composition of plants belong to the second question and are considered to be even more significant to location (site-to-site) differences for shrubs and mosses reaching up to 160% (Markert, 1996) while for some trees this variation might be not so high (Capannesi *et al.*, 1993). The investigations in this field lead to the conclusion that each plant shows a different pattern of seasonal changes and before biomonitoring studies are undertaken investigations should be performed (Djingova and Kuleff, 1994) because the error at best exceeds 30% (Wagner, 1995) if the sampling time is not chosen properly. Usually, as an appropriate season (time) for sampling a period is defined when the concentrations are with lowest variability (Wagner, 1987). The representative sampling of plants in dependence on time is still more complicated if the results of Shtangeeva (1994, 1995) are considered. Investigating couch grass and plantain, she concludes that the concentrations of elements (essential, heavy and toxic, rare earths) pass through maxima and minima during the day which position (hour) changes in different sampling days.

Among the herbaceous species used for biomonitoring of integral pollution (atmospheric and soil) *Taraxacum officinale* has been standardized (Djingova *et al.*, 1986; Djingova and Kuleff, 1993; Kuleff and Djingova, 1984) and applied in a number of investigations (Djingova *et al.*, 1986, 1995; Djingova and Kuleff, 1993; Kuleff and Djingova, 1984; Kabata-Pendias and Dudka, 1989, 1990, 1991; Kabata-Pendias and Krakowiak, 1998; Rule, 1993, 1994; Toelgyessy *et al.*, 1993). In our earlier papers, we have proposed and used the period April-June for sampling depending on altitude and longitude of sampling locations. However, in other studies a variety of sampling times have been used: May (Kabata-Pendias and Krakowiak, 1998) summer months (Kabata-Pendias and Dudka, 1989, 1990, 1991) and even autumn months (Toelgyessy *et al.*, 1993).

Therefore the goal of the present paper is to report the results from the study of the seasonal variation of metals in the leaves of *Taraxacum officinale* and two other herbaceous species used as bioindicators such as *Plantago major* (Kabata-Pendias and Krakowiak, 1998; Toelgyessy *et al.*, 1993) and *Plantago lanceolata* (Kovacs *et* *al.*, 1993) so as to specify the most appropriate period for sampling for biomonitoring purposes irrespective of the geographic location.

EXPERIMENTAL

Sampling and Sample Preparation

Samples of *Taraxacum officinale*, *Plantago major*, and *Plantago lanceolata* have been collected in the period 23. April-26. November from two sampling stations in Sofia (residential area "Losenetz" and the city park "Borisova gradina") which are not subjected to extreme anthropogenic pollution. Sampling dates and climatic conditions are presented in Table III.

Depending on the plant on two consecutive days (5.06 and 6.06 for *Taraxacum officinale* and 18.09 and 19.09 for *Plantago major*) samples were taken on different hours during the day.

The sampling stations are having dimensions 10×10 m and each time, leaves of a minimum 10 plants per species (preferably 25) were collected, which has been shown to be enough to overcome individual biological differences (Djingova and Kuleff, 1994). The leaves from a sampling date (or hour) are combined in a bulk sample. Quantity of the bulk sample is not less than 10 g. The leaves were washed first with tap and then with twice distilled water and dried in an oven at 80°C for 4 h. They were ground and homogenized in a teflon ball mill for 12 h.

Method of Analysis

The samples (of about 0.25 g) were digested in 5 cm^3 nitric acid/hydrochloric acid (4 : 1) at 160°C and after dilution to 10 cm^3 are subjected to ICP-AES using Varian-Liberty 110/220 spectrometer. The concentrations of aluminium, calcium, cadmium, chromium, copper, magnesium, manganese, lead, and zinc were determined. The accuracy of the method was checked by analysing a number of certified reference materials (Djingova *et al.*, 1998) and is illustrated in Table I where results from the analysis of NIST-SRM-1572 (Citrus leaves) are presented together with the achieved detection limits. In all cases but

TABLE I Results from the analysis of NIST-SRM-1572 (Citrus leaves) [mg/kg]

Element	*Experimental result*	*RSD* [%]	*Experimental detection limit*	*Concentration certified*
Al	95 ± 4	4.2	1	92
Ca%	3.12 ± 0.15	4.8	2*	3.15
Cd	< 0.08	–	0.08	0.03
Cr	0.90 ± 0.06	6.7	0.1	(0.8)
Cu	17 ± 1	5.9	1	16.5
Mg%	0.56 ± 0.02	3.6	1*	0.58
Mn	23.8 ± 0.9	3.8	1	23
Pb	12.7 ± 0.9	7.1	0.1	13.3
Zn	28.2 ± 1.1	3.9	2	29

*detection limit in mg kg^{-1}.

for cadmium (and two samples for lead) the quantitative determination of the investigated elements has been possible.

RESULTS AND DISCUSSION

Table II presents as an example the results for some essential and one toxic element from the analysis of leaf samples from *Taraxacum officinale* and *Plantago major* collected on June 5th and September 19th at different hours. Each result is a mean of three independent determinations and the respective standard deviation is presented. The overall mean value and its standard deviation are representative of the daily variation on the one hand and on the between-sampling variation on the other. The RSD (relative standard deviation) for the determined elements for both species is between 4 and 11% which is commensurable to the analytical error presented in Table I, and in Djingova *et al.* (1998). Besides no well defined maxima or minima can be detected during the day as Shtangeeva (1994, 1995) has reported. The same results were obtained on the next sampling days. The between day variation (see also Tables III and V) can be estimated as very low within 2–3 days.

Taraxacum officinale

Table III presents the results from the analysis of *Taraxacum officinale* leaves collected at the sampling station "Losenetz". Each value is a mean of three independent determinations and the respective standard

TABLE II Results from the analysis of leaves of *Taraxacum officinale* and *Plantago major* collected in different hours within one day [$mg\,kg^{-1}$ DW*]

Sampling hour/ date	*Taraxacum officinale* 5.06				*Plantago major* 19.09			
	Cu	*Mg%*	*Mn*	*Pb*	*Cu*	*Mg%*	*Mn*	*Pb*
8	20.8 ± 0.7	0.25 ± 0.01	27.2 ± 0.9	1.58 ± 0.09	12.7 ± 0.7	0.25 ± 0.01	16.6 ± 0.7	1.1 ± 0.1
11	21.7 ± 0.9	0.26 ± 0.01	26.3 ± 1.0	1.64 ± 0.11	12.3 ± 0.9	0.27 ± 0.02	16.9 ± 0.8	1.0 ± 0.1
13	19.3 ± 0.6	0.26 ± 0.02	28.0 ± 0.7	1.55 ± 0.10	13.1 ± 1.0	0.27 ± 0.01	19.2 ± 0.9	1.2 ± 0.2
14	19.6 ± 0.5	0.25 ± 0.01	26.7 ± 1.1	1.70 ± 0.07	11.6 ± 1.0	0.29 ± 0.02	15.8 ± 0.7	1.0 ± 0.1
15	21.4 ± 0.5	0.25 ± 0.01	28.1 ± 1.0	1.62 ± 0.06	13.9 ± 0.8	0.28 ± 0.02	18.8 ± 0.8	1.3 ± 0.2
17	20.0 ± 0.5	0.26 ± 0.02	27.9 ± 0.8	1.52 ± 0.09	13.8 ± 1.0	0.27 ± 0.01	18.0 ± 0.8	1.2 ± 0.1
M ± SD	20.5 ± 1.0	0.26 ± 0.01	27.4 ± 0.8	1.59 ± 0.12	13.0 ± 1.4	0.27 ± 0.01	17.3 ± 1.2	1.18 ± 0.14
(RSD %)	5	4	4	8	7	4	7	11

M = mean value; SD = standard deviation; RSD = relative standard deviation.
* = dry weight.

TABLE III Concentrations in the leaves of *Taraxacum officinale* collected on different days at station Losenetz [$mg\,kg^{-1}$ dry weight*]

Date	*Al*	*Ca* [%]	*Cd*	*Cr*	*Cu*	*Mg* [%]	*Mn*	*Pb*	*Zn*	*Weather conditions before sampling day*
23.4	215 ± 11	0.81 ± 0.05	< 0.1	0.97 ± 0.08	4.0 ± 0.1	0.08 ± 0.01	15.0 ± 0.9	2.2 ± 0.2	17 ± 1	10 d dry period
8.5	242 ± 10	0.89 ± 0.03	< 0.1	0.50 ± 0.02	11.9 ± 0.2	0.18 ± 0.01	19.5 ± 0.5	0.80 ± 0.06	43 ± 3	1 d raining
23.5	280 ± 32	1.21 ± 0.06	0.74 ± 0.02	0.60 ± 0.02	12.6 ± 0.4	0.27 ± 0.02	21.4 ± 0.6	0.90 ± 0.07	41 ± 1	7 d dry period
1.6	290 ± 24	1.20 ± 0.04	0.72 ± 0.03	1.17 ± 0.05	22.0 ± 0.7	0.27 ± 0.02	27.9 ± 1.1	1.65 ± 0.11	72 ± 5	7 d dry period
6.6	297 ± 19	1.23 ± 0.05	0.65 ± 0.03	1.20 ± 0.10	21.0 ± 0.5	0.26 ± 0.01	29.0 ± 1.0	1.60 ± 0.25	77 ± 4	1 d raining
20.6	197 ± 30	1.22 ± 0.07	0.69 ± 0.03	1.10 ± 0.07	21.1 ± 0.3	0.26 ± 0.02	28.1 ± 1.1	1.62 ± 0.20	74 ± 3	1 d raining
4.7	249 ± 26	1.60 ± 0.09	1.30 ± 0.10	2.20 ± 0.15	15.0 ± 0.4	0.28 ± 0.04	20.2 ± 0.9	1.40 ± 0.30	74 ± 5	1 d raining
20.7	225 ± 10	1.50 ± 0.03	0.19 ± 0.02	1.20 ± 0.20	16.1 ± 0.3	0.28 ± 0.02	17.9 ± 0.8	1.22 ± 0.15	72 ± 4	5 d dry period
7.8	322 ± 25	1.69 ± 0.05	0.40 ± 0.03	1.20 ± 0.10	17.0 ± 0.6	0.34 ± 0.03	33.0 ± 2.0	1.20 ± 0.10	90 ± 5	3 d raining
27.8	193 ± 18	1.03 ± 0.04	1.20 ± 0.10	1.10 ± 0.15	4.6 ± 0.1	0.12 ± 0.01	12.0 ± 0.9	< 0.1	37 ± 2	7 d dry period
18.9	65 ± 3	0.80 ± 0.04	< 0.1	0.40 ± 0.03	13.3 ± 0.3	0.16 ± 0.02	4.4 ± 0.3	< 0.1	50 ± 2	2 d raining
7.10	113 ± 13	1.22 ± 0.03	< 0.1	0.70 ± 0.07	7.0 ± 0.4	0.14 ± 0.01	17.0 ± 1.0	0.60 ± 0.04	41 ± 2	4 d raining
5.11	2612 ± 140	1.47 ± 0.04	< 0.1	0.60 ± 0.08	6.9 ± 0.2	0.27 ± 0.03	26.9 ± 1.1	2.70 ± 0.40	32 ± 1	frost
26.11	2222 ± 89	1.23 ± 0.03	0.22 ± 0.02	0.70 ± 0.09	10.1 ± 0.2	0.29 ± 0.03	26.4 ± 1.5	5.20 ± 0.40	67 ± 3	2 d snow and rain
M ± SD	570 ± 830	1.20 ± 0.30	0.45 ± 0.43	0.95 ± 0.50	12.0 ± 5.7	0.23 ± 0.07	21 ± 8	1.50 ± 1.30	56 ± 23	
RSD (%)	146	25	96	53	48	30	38	87	41	

M = mean value; SD = standard deviation; RSD = relative standard deviation.
* = dry weight.

TABLE IV Concentrations in the leaves of *Plantago lanceolata* collected on different days at station Losenetz [$mg\,kg^{-1}$ dry weight*]

Date	*Al*	*Ca* [%]	*Cd*	*Cr*	*Cu*	*Mg*	*Mn*	*Pb*	*Zn*
23.4	2191 ± 121	1.99 ± 0.01	< 0.08	0.83 ± 0.03	13.9 ± 0.9	0.40 ± 0.01	23.9 ± 0.7	3.7 ± 0.4	32 ± 1
8.5	2419 ± 113	1.89 ± 0.02	0.18 ± 0.02	0.90 ± 0.06	15.7 ± 1.1	0.38 ± 0.03	21.0 ± 0.9	3.0 ± 0.4	40 ± 2
23.5	2259 ± 142	2.58 ± 0.05	< 0.08	0.61 ± 0.01	5.7 ± 0.5	0.30 ± 0.01	23.7 ± 0.8	2.1 ± 0.3	28 ± 1
6.6	2254 ± 105	1.47 ± 0.04	0.26 ± 0.03	0.65 ± 0.08	4.1 ± 0.2	0.19 ± 0.01	11.2 ± 0.3	1.3 ± 0.2	18 ± 1
20.6	1998 ± 100	1.64 ± 0.03	0.33 ± 0.04	0.63 ± 0.04	2.0 ± 0.2	0.12 ± 0.01	11.6 ± 0.2	3.9 ± 0.3	16 ± 1
4.7	1282 ± 98	1.71 ± 0.02	< 0.08	0.53 ± 0.03	5.5 ± 0.2	0.22 ± 0.02	7.7 ± 0.1	2.0 ± 0.3	33 ± 2
20.7	2900 ± 190	1.22 ± 0.03	< 0.08	0.92 ± 0.04	3.1 ± 0.1	0.18 ± 0.01	18.5 ± 0.6	1.2 ± 0.2	21 ± 1
7.8	3559 ± 200	2.00 ± 0.06	0.24 ± 0.02	0.73 ± 0.04	9.4 ± 0.2	0.32 ± 0.01	24.0 ± 1.0	2.9 ± 0.2	44 ± 3
27.8	2308 ± 78	2.10 ± 0.05	0.13 ± 0.02	0.19 ± 0.02	8.9 ± 0.5	0.25 ± 0.02	16.1 ± 1.0	3.8 ± 0.4	48 ± 1
18.9	2899 ± 126	2.17 ± 0.02	< 0.08	0.90 ± 0.07	16.4 ± 0.4	0.58 ± 0.04	29.2 ± 0.9	1.6 ± 0.2	49 ± 2
7.10	2100 ± 100	1.58 ± 0.07	0.17 ± 0.01	0.64 ± 0.05	8.4 ± 0.3	0.25 ± 0.02	25.0 ± 0.7	1.5 ± 0.1	34 ± 1
5.11	2114 ± 92	1.60 ± 0.10	0.17 ± 0.01	0.62 ± 0.07	7.9 ± 0.1	0.21 ± 0.01	24.1 ± 1.0	1.4 ± 0.2	33 ± 1
26.11	2981 ± 130	2.00 ± 0.10	0.16 ± 0.01	0.60 ± 0.08	5.8 ± 0.4	0.22 ± 0.01	12.4 ± 0.4	5.9 ± 0.4	29 ± 1
M ± SD	2328 ± 1000	1.59 ± 0.4	0.16 ± 0.08	0.64 ± 0.36	8.2 ± 4.6	0.27 ± 0.13	18 ± 8	2.6 ± 1.3	32 ± 11
RSD %	43	19	50	56	55	48	45	49	35

M = mean value; SD = standard deviation; RSD = relative standard deviation.
* = dry weight.

TABLE V Concentrations in the leaves of *Plantago major* collected on different days in station Losenetz [$mg\,kg^{-1}$ dry weight*]

Date	*Al*	*Ca* [%]	*Cd*	*Cr*	*Cu*	*Mg* [%]	*Mn*	*Pb*	*Zn*
23.4	1827 ± 90	1.45 ± 0.03	< 0.08	1.54 ± 0.03	14.7 ± 0.6	0.30 ± 0.01	10.2 ± 0.9	0.23 ± 0.03	40 ± 1
8.5	2200 ± 98	1.65 ± 0.01	< 0.08	1.40 ± 0.11	18.0 ± 1.0	0.35 ± 0.01	12.8 ± 0.7	0.30 ± 0.03	50 ± 3
23.5	3412 ± 169	2.11 ± 0.01	0.17 ± 0.02	1.50 ± 0.06	27.5 ± 1.1	0.47 ± 0.03	20.2 ± 1.1	0.20 ± 0.02	80 ± 2
6.6	3400 ± 140	1.90 ± 0.01	< 0.08	0.57 ± 0.05	25.0 ± 0.7	0.48 ± 0.01	19.0 ± 1.0	1.40 ± 0.12	72 ± 6
20.6	5793 ± 210	2.45 ± 0.02	0.28 ± 0.01	1.57 ± 0.12	26.8 ± 1.0	0.52 ± 0.02	24.0 ± 0.8	0.60 ± 0.04	86 ± 3
4.7	5000 ± 200	2.20 ± 0.02	0.29 ± 0.02	1.50 ± 0.07	12.8 ± 0.5	0.48 ± 0.01	18.1 ± 0.3	0.50 ± 0.06	80 ± 3
20.7	4290 ± 320	2.32 ± 0.03	0.21 ± 0.02	1.40 ± 0.10	10.9 ± 0.8	0.41 ± 0.02	17.3 ± 0.6	0.31 ± 0.02	62 ± 2
7.8	4300 ± 220	2.20 ± 0.01	0.15 ± 0.02	0.90 ± 0.07	11.8 ± 0.9	0.28 ± 0.01	16.0 ± 0.6	0.24 ± 0.03	60 ± 1
27.8	4408 ± 172	2.29 ± 0.01	0.22 ± 0.02	1.10 ± 0.10	15.7 ± 0.9	0.30 ± 0.01	19.3 ± 0.9	1.10 ± 0.05	51 ± 3
18.9	4670 ± 120	2.27 ± 0.01	0.26 ± 0.01	1.10 ± 0.07	15.2 ± 0.6	0.31 ± 0.01	20.3 ± 1.2	1.22 ± 0.05	54 ± 2
7.10	3508 ± 97	2.19 ± 0.02	0.26 ± 0.01	0.73 ± 0.03	13.2 ± 0.4	0.30 ± 0.02	14.6 ± 0.5	1.20 ± 0.04	52 ± 2
5.11	5774 ± 245	2.20 ± 0.01	< 0.08	0.50 ± 0.08	11.3 ± 0.4	0.36 ± 0.01	20.3 ± 1.0	0.57 ± 0.02	55 ± 4
26.11	5138 ± 300	2.29 ± 0.01	< 0.08	2.70 ± 0.12	9.3 ± 0.3	0.35 ± 0.01	29.0 ± 1.0	4.70 ± 0.06	71 ± 5
M ± SD	4390 ± 1390	2.12 ± 0.29	0.17 ± 0.22	1.20 ± 0.14	14.9 ± 5.3	0.37 ± 0.09	19.3 ± 5.7	0.86 ± 1.26	62 ± 16
RSD	32	14	130	34	35	25	30	146	25

M = mean value; SD = standard deviation; RSD = relative standard deviation.
* = dry weight.

deviations are given. The sampling dates and weather conditions are presented as well. The standard deviation of the mean values may be considered as a measure of the seasonal changes over the investigated six months period.

The concentration levels in *Taraxacum officinale* are within the intervals already established for this plant in residential areas of Sofia, and is lower than or commensurable to the concentrations in the leaves of the same plant in some other European cities and regions (Djingova *et al.*, 1995; Djingova and Kuleff, 1993; Kabata-Pendias and Dudka, 1989, 1990, 1991; Kabata-Pendias and Krakowiak, 1998; Toelgyessy *et al.*, 1993).

The seasonal changes in the leaves of *Taraxacum officinale* collected from the second sampling station are illustrated in Figures 1 and 2.

The results for *Taraxacum officinale* show considerable seasonal variation. It is lowest for the nutrient elements – calcium and magnesium (30%) and relatively moderate for the rest of the essential elements – copper, zinc, manganese (below 50%). The highest seasonal variations are to the toxic elements – aluminium, cadmium, and lead (up to 147%). Similar high seasonal variation for some elements, including aluminium, has been reported in Markert (1989).

The seasonal trend for all elements is similar. Generally after increase in the concentrations in April – May, a relatively stable period

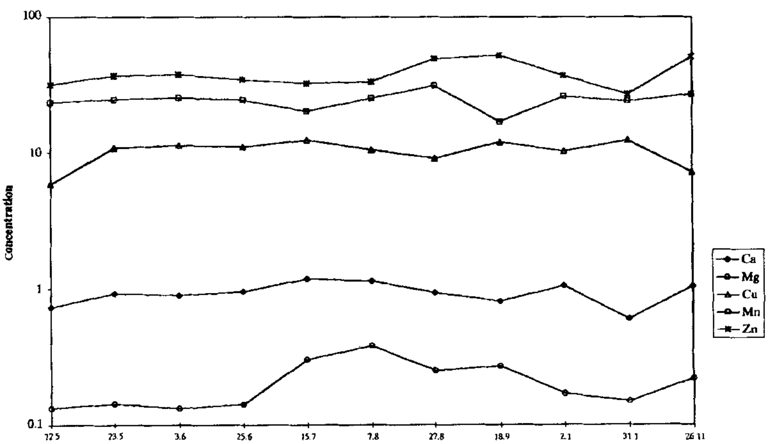

FIGURE 1 Seasonal changes in the concentration of essential elements in the leaves of *Taraxacum officinale*.

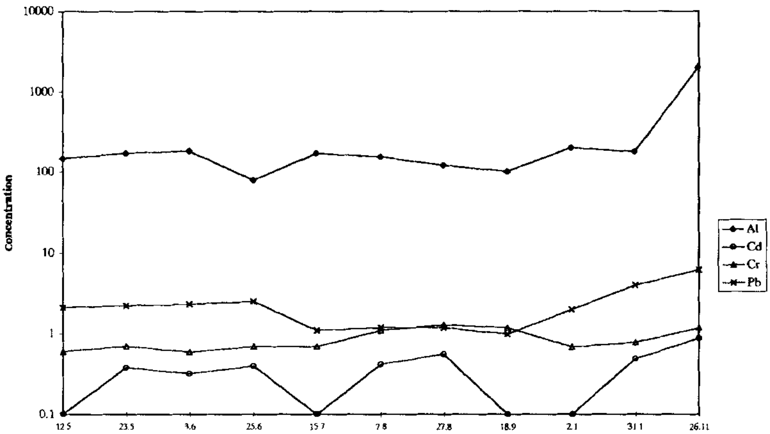

FIGURE 2 Seasonal changes in the concentration of toxic elements in the leaves of *Taraxacum officinale*.

comes that resembles the seasonal changes in deciduous trees. A minimum in early autumn is followed by increase in the concentrations in late autumn for most of the elements but the detected quick changes of the concentrations do not favour this time as convenient for representative sampling.

The period June 1st – June 20th is characterized by the lowest variation in the concentrations for the majority of the investigated elements (see Tab. III). Sampling in this period ensures reproducible results for all elements investigated in this study. If the sampling period is extended for example backwards (to 23 May) then the error in the determination of chromium, copper, zinc and lead will be increased up to 40% (Pb) not due to contamination but to an unfavourable sampling time factor.

Plantago lanceolata

Table IV presents the results from the analysis of *Plantago lanceolata* collected at the sampling station "Losenetz". The mean values from three independent determinations together with the respective standard deviations are presented. The weather conditions are the same as indicated in Table III.

The seasonal changes are between 35 and 60% with the exception of calcium (19%). After a period with relatively high concentrations and low variation in April to beginning of May a relative decrease in the concentrations might be detected in June. The concentrations of most of the elements have a maximum in August. Relatively lowest variation in the concentrations are established in October – beginning of November, and the variation coefficient decreases significantly which indicates it as the most suitable for representative sampling. In November, before senescence, the concentrations of most elements (especially lead) increase drastically, which resembles the behaviour of deciduous trees and bushes (*e.g.*, Wagner, 1987) However, the period (7 October – 5 November) is rather late for the present latitude and at unfavourable conditions (drought or early frost) the species might not be available for sampling. A second best possibility is at the end of April – beginning of May. The variation is slightly worse for some elements than in October but still this might be a good possibility for sampling though this time should be characterized with the most intensive shoot development.

Plantago major

Table V presents the results from the analysis of *Plantago major* leaves at the sampling station Losenetz while Figures 3 and 4 indicate the trend of the changes for sampling station 2.

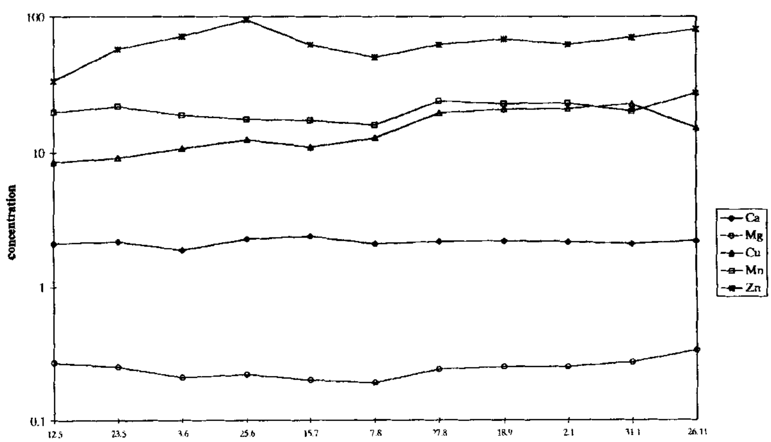

FIGURE 3 Seasonal changes in the concentration of essential elements in the leaves of *Plantago major*.

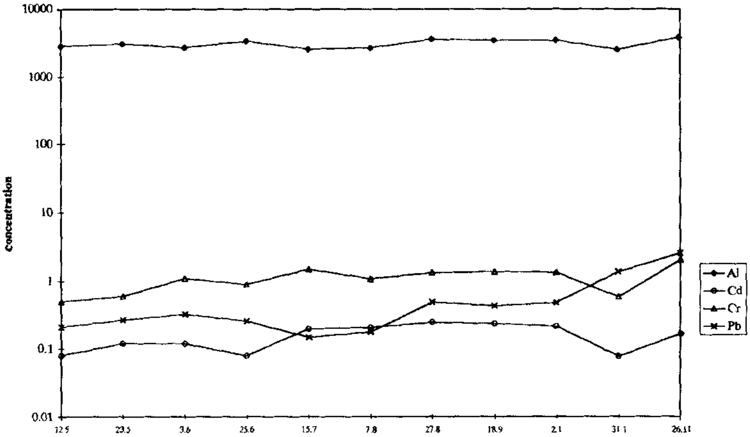

FIGURE 4 Seasonal changes in the concentration of toxic elements in the leaves of *Plantago major*.

The data in Table V indicate the lowest seasonal variation for most of the elements in comparison to *Taraxacum officinale* and *Plantago lanceolata*. However, the highest variation of cadmium and lead (over 100%) are established for this plant. A maximum in the concentrations around the 20th of June may be detected, but as a period with relatively low variation, September may be determined (see also Figs. 3 and 4). The coefficients of variations for all elements decrease significantly during that time.

Climatic Conditions

In the sampling period April–November, the climatic conditions have been recorded daily and special attention has been paid to the duration, type and intensity of rainfalls and their possible influence on the results. It is known that usually lowest concentrations in the leaves are recorded after intensive rainfalls (Ho and Tai, 1979). Additionally with grasses and herbs other than washing out, splashing may occur leading to the opposite effect. When sampling over such a long period (6 months) is performed, it is rather difficult to have always the same weather conditions (*e.g.*, a week without rain as recommended by Ho and Tai, 1979). Therefore, the method for sample pretreatment and cleaning is extremely important for neutralizing the influence of climatic conditions (Djingova and Kuleff, 1994). The comparison between the results for the three plants and the respective weather conditions given in Table I shows that concentration maxima and minima have been recorded both in rainy periods and in dry ones. This is an indication that sample preparation (washing of the samples) has been enough to neutralize the effects of rain and snowfalls and to ensure representative results (Djingova and Kuleff, 1994).

Additional indication about the correct conclusions we have reached is the inter-element correlation between calcium/magnesium (R = 0.8009), manganese/iron (R = 0.9117), and copper/zinc (R = 0.8712) established statistically significant for all species over the whole period (at $p = 0.05$). A significant correlation iron/aluminium (R = 0.9267) was also found which is connected not so much to similar physiological functions (as in the case of the essential elements; Garten, 1976) but to the oxidizing state of the cations of these elements and the similar radii of the hydrated ions of aluminium (Al^{3+}) and iron (Fe^{+3}) (Markert, 1996). These inter-element correlations independent of the species prove that the trend of the development of the three investigated species has been followed correctly.

CONCLUSIONS

Taraxacum officinale, *Plantago major*, and *Plantago lanceolata* are herbaceous perennial plants growing up to 2900 m altitude (Andreev *et al.*, 1992; Delipavlov *et al.*, 1983). Their blossoming period is different (not to mention all physiological and morphological inter-species differences).

On the territory of Bulgaria, *Taraxacum officinale* blossoms in the period April–June, *Plantago lanceolata* in May–October, and *Plantago major* in June–August (Andreev *et al.*, 1992; Delipavlov *et al.*, 1983) (of course in the mountains the blossoming of the species is later). The comparison of the blossoming periods with the intervals where element concentrations have lowest variations, established in this work, indicates that sampling must be performed at the end of the blossoming or immediately after that (June for *Taraxacum officinale*, September for *Plantago major*, and October for *Plantago lanceolata*). Physiologically the plant has finished the development of its vegetative and reproductive organs and has reached maturity.

The results we have obtained encourage us to presume that suitable time for representative sampling of herbacious species, characterized by low variation of the elemental concentrations is the end of the blossoming or immediately after that. Thus, the stage of development of the plant may be standardized irrespective when it has reached it at different altitudes and latitudes.

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