Changes in the ionome of *Taraxacum officinale* under different anthropogenic influences

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Received: March 23, 2013 ▹ Accepted: November 19, 2014

Abstract. The concentrations of 60 elements in samples from *Taraxacum officinale* collected from 12 background and 10 polluted locations in Bulgaria were determined using ICP-MS. The results indicate that under natural unpolluted conditions the concentrations of the determined elements in the plants (the ionome) are relatively stable with time and region. The highest stability was established for essential elements. Under anthropogenic influence the ionome of the plant and of the plant parts changes drastically not only in respect to major polluting elements. Statistical interpretation of the results (cluster, factor and correlation analyses) revealed inter-element relations which are identified for the first time.

Key words: ICP-MS, multivariate statistics, plant ionome, *Taraxacum officinale*

Introduction

One of the challenges in ecotoxicology (Moore & al. 2002) may be defined as investigation of the plant (and animal) inorganic composition with the aim of establishing the mechanism of molecular and submolecular interactions with environmental pollutants. This is important not only for understanding the connection between effects in organisms and ecological changes, but for providing a possibility to predict the influence of new industrial processes, including biotechnology and nanotechnology on biota. It also helps developing an effective procedure for risk assessment at different levels – from genetic to ecosystem. Lahner & al. (2003) were the first to describe the ionome as a mineral nutrient and trace element composition (including all metals, metalloids and nonmetals) of an organism, representing the inorganic component of the cellular and organism systems. Study of the ionome, called ionomics, is defined as quantitative and simultaneous measurement of the element composition of living organisms and changes in this composition in response to physiological stimuli, development stage and genetic modifications (Salt & al. 2008). The need in understanding the regulation processes of elements in the organisms demands determination of as many elements out of the 92 in the Periodic Table as possible in the organism, tissue and cell (Baxter 2009). One of the potential perspectives for ionomics is environmental pollution, where enormous variety of conditions and pollutants exists, probably resulting in concentration and inter-element changes in the plant ionome. Changes in the chemical environment are expected to affect more than one element (Baxter 2009) and lead to respective changes in the genome. In spite of the limited number of investigated organisms, the existing information about the ionome poses the question of investigation of the "fingerprint" of an organism and its connection to various natural, physiological and genetic modifications. A serious perspective in ionomic research is the use of this information for evaluation of different environmental processes for ecological and biomonitoring studies, phytoremediation and elucidation of the mechanism of hyperaccumulation (Williams & Salt 2009; Lobinski & al. 2010).

Taraxacum officinale is a medical herb and a standardized biomonitoring species used in many investigations (Kuleff & Djingova 1984; Djingova & al. 1986; 1993; 1995; Djingova & Kuleff 1993; 1999; Winter & al. 1999; Charnovska & Milewska 2000; Georgieva & al. 2011; Keane & al. 2001; 2005). Preliminary information about its fingerprint has also been published (Djingova & al. 2004), although the sampling places, environmental and soil conditions have not been well determined and a concentration of 38 elements is available. Furthermore, genetic investigations have been performed for establishing a probable connection between the mutations and pollutants (e.g., Keane & al. 2005; Collier & al. 2010; Peycheva & al. 2012).

The aim of the present study was to determine the ionome of *T. officinale* at background level and to assess the influence of various anthropogenic pollutions on the ionome and on the element correlations in the plant.

Materials and methods

Samples, sampling sites and sample preparation

Samples of *T. officinale* were collected from 12 background sampling sites and 10 sites with anthropogenic pollution. Figure 1 presents the sampling sites on the map of Bulgaria. The background sampling sites (1–12) were situated mainly in mountainous regions without direct anthropogenic activities. Sites 1 and 2 are in the Rhodopi Mts, sites 3–5 in the Pirin Mts, sites 6–11 – in the Balkan Range, and site 12 – in Mt Zemen. Sampling sites 13 and 14 are in the vicinity of the two largest Pb-Zn smelters in Bulgaria. Site 15 is distanced 5 km from site 14. Sites 16 and 17 are around the two Bulgarian Fe metallurgical works. The one in Kremikovtsi (near Sofia – site 16) is out of operation for more than two years, while the other is still active. Sites 18 and 19 are situated in the vicinity of the biggest thermal power plants (TMP) in Bulgaria operating on local lignite coal. Site 18 is in the immediate vicinity of TMP Maritsa Iztok 1, while site 19 is 6 km away from TMP Maritza Iztok 3. Sites 20 and 21 are along the Trakia Motorway. Site 22 is near an abandoned uranium mine in Buhovo, near Sofia. The sample is taken from a recultivated corn field, where radioactive contamination has been established earlier (0.4–1.0 μSv/h) (Mihaylova & al. 2012a).

The plants were collected as described in Djingova & Kuleff (1994), at the end of the flowering period, by cutting with plastic scissors 1 cm above the ground. Immediately after sampling, the foliage was washed thoroughly under tap water and rinsed with distilled water. The plant samples were air dried in a clean room for 4–5 days. After air drying, the samples were put in an oven for 4 h at 85 °C, ground in a polytetrafluoroethylene (PTFE) ball mill to fine powder and subjected to digestion. The samples were stored at 4 °C.

Instrumentation

For microwave digestion of the samples, Microwave Reaction System (Anton Paar, Multiwave 3000) was used.

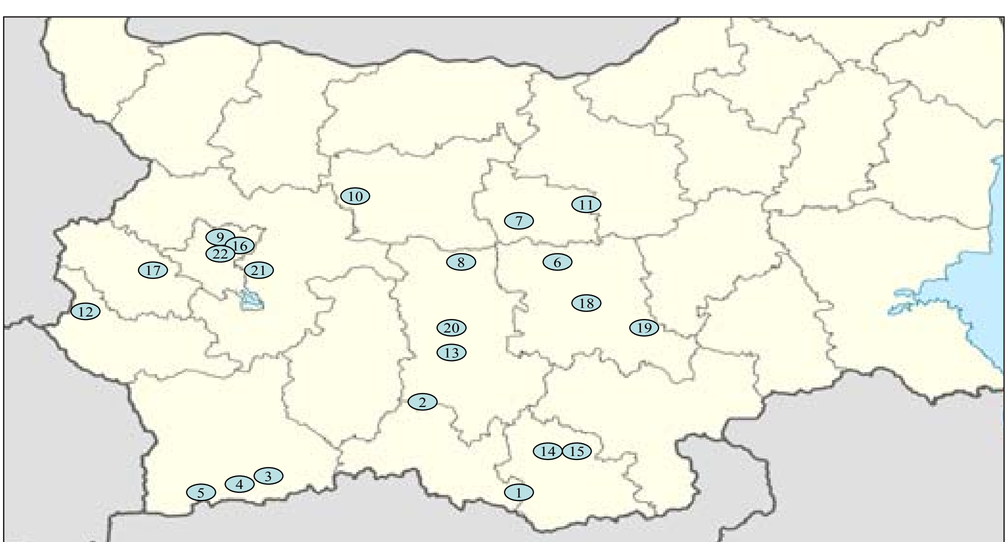

Fig. 1. Map of Bulgaria with sampling locations.

Analysis of the samples was carried out by using a Perkin Elmer SCIEX DRC-e ICP-MS system with cross-flow nebulizer. The spectrometer was optimized to provide minimal values of the ratios CeO^{+}/Ce^{+} and Ba^{2+}/Ba^{+} and optimal intensity of the analytes. External calibration, both by certified reference materials (NIST 1547, CRM DC 73348 and CRM DC 73349) and multi-element standard solution, was performed. The calibration coefficients for all calibration curves were at least 0.99. The spectrometer was equipped with a dynamic reaction cell (DRC) for removal of multi-element interferences, using methane as a reaction gas. Depending on the analyte, two different strategies for interferences removal were adopted, including reaction gas and/or correction equations.

Analytical procedure

ICP-MS analysis of the plant samples was performed, as described in Mihaylova & al. (2012b).

Statistical interpretation of the results

Cluster, factor and correlation analyses were performed with STATISTICA 7.0 software package.

Results and discussion

Characterization of the ionome of T. officinale under background conditions

Table 1 presents the average concentrations and concentration intervals (minimum and maximum values) for the determined elements. A comparison with

Table 1. Average concentrations and concentration intervals of *T. officinale* from background regions.

El. [µg g-1]	Mean values and intervals	Literature data*	El.	Mean values and intervals	Literature data*	El.	Mean values and intervals	Literature data*
Ag	0.13 (0.023-0.35)		Gd	0.05 (0.003-0.90)		Sb	0.01 (0.001-0.03)	
Al	237 (96-511)		Hf	0.07 (0.02-0.3)		Sc	0.09 (0.004-0.30)	0.05-0.1
As	0.18 (0.03-0.52)	0.1-0.4	Hg	0.04 (0.004-0.096)	<0.1-0.2	Se	0.08 (0.01 -0.17)	0.05-0.2
B	28 (17-40)		Ho	0.007 (0.0007-0.01)		Si [%]	0.18 (0.10-0.50)	
Ba	20 (12-40)	14-80	In	0.002 (0.001-0.003)		Sm	0.05 (0.005-0.10)	0.05-0.2
Be	0.05 (0.015-0.12)		K [%]	1.10 (0.75-1.80)	2.1-4	Sn	0.17 (0.02-0.50)	
Bi	0.06 (0.0002-0.23)		La	0.54 (0.11-1.30)	0.2-0.8	Sr	27.5 (10-50)	
Ca [%]	1.37 (1.0-2.4)	1.1-2.0	Li	0.46 (0.17-1.4)		Tb	0.007 (0.002-0.013)	
Cd	0.13 (0.04-0.30)	0.03-0.20	Lu	0.002 (0.001-0.005)		Th	0.06 (0.01-0.20)	
Ce	0.50 (0.20-0.74)	0.3-0.6	Mg [%]	0.20 (0.10-0.30)	0.2-3.0	Ti	14.7 (1.60-30)	
Co	0.18 (0.09-0.32)	0.1-0.2	Mn	36 (16-90)	15-200	Tl	0.02 (0.001-0.10)	
Cr	1.90 (0.62-3.0)	0.24-1.12	Mo	1.2 (0.30-4.0)	0.6-2.9	U	0.04 (0.01-0.17)	
Cs	0.05 (0.016-0.12)	0.04-0.2	Na	230 (17-500)	5-400	V	0.78 (0.10-2.0)	
Cu	11.4 (8-14)	9-19	Nd	0.32 (0.15-0.60)		W	0.06 (0.01-0.20)	
Dy	0.03 (0.005-0.06)		Ni	1.20 (0.50-3.0)	0.3-4	Y	0.15 (0.04-0.30)	
Er	0.02 (0.002-0.024)		P [%]	0.26 (0.03-0.70)	0.2-0.4	Yb	0.015 (0.004-0.030)	
Eu	0.015 (0.003-0.030)	<0.005-0.03	Pb	1.55 (0.40-3.6)	0.3-4	Zn	32.5 (10-60)	30-60
Fe	90 (32-330)	60-500	Pr	0.06 (0.002-0.10)		Zr	1.50 (0.70-2.5)	
Ga	0.19 (0.04-0.50)		Rb	12.5 (4.0-20)	24-160			

* – Djingova & Kuleff (1993).

the results for *T. officinale* from other background regions in Bulgaria obtained 20 years ago by instrumental neutron activation analysis and atomic absorption spectrometry (Djingova & Kuleff 1993) is also presented.

Data in Table 1 show relatively narrow concentration ranges in the various background regions. Especially narrow are the intervals of the essential elements. A comparison with the data obtained 20 years ago from other background regions in Bulgaria show very good agreement. These facts indicate that at this stage of study it can be assumed that the presented average values and intervals characterize the ionome of *T. officinale* under natural conditions, without anthropogenic influence. Furthermore, the comparatively small variation of data obtained for different background regions is an indication of the relative independence of the ionome of *T. officinale* from differences in soil characteristics, when no pollution is present.

Assessment of the anthropogenic influence on the ionome

Figures 2.1–2.4 present the accumulation factors (AF – concentration of the element in the polluted region to its concentration in the background region) of *T. officinale* in some of the polluted regions. Only elements with accumulation factor higher than 2 are given. Concentrations of the remaining elements are not significantly different from the background values.

The sample from the vicinity of the Pb-Zn smelter in Plovdiv (No 13) had higher concentrations of 28 elements in comparison to the background regions (Fig. 2.1). Besides the typical pollutants (Pb, Zn, As, Cd, Cu, Hg, Sn), extremely high accumulation factors have been identified for In and Sb (over 1000), platinum metals, Tl, Se, and Bi. Although the yearly production of Pb exceeds 70 000 t and of Zn – 100 000 t, accumulation in the plant, especially of Zn, was not so high (around 14), while concentration in the adjacent soil was 0.5 % (to be published). A similar effect (low accumulation of Zn) has been established for lettuce in the vicinity of the same smelter (Dinev 2011), as well as in other studies (Rosseli & al. 2006). Figure 2.2 confirms this result for the samples from the other Pb-Zn smelter (sample 14), where accumulation for Zn amounted to 10. Apparently, *T. officinale* regulates the uptake of Zn (and probably other essential elements) (Kabata-Pendias 2004; Rosseli & al. 2006). In both locations, accumulation of Pb was around 100. The concentrations of 40 elements were significantly higher in sample 14 than the background values. The degree of accumulation was less than in sample 13, which corre-

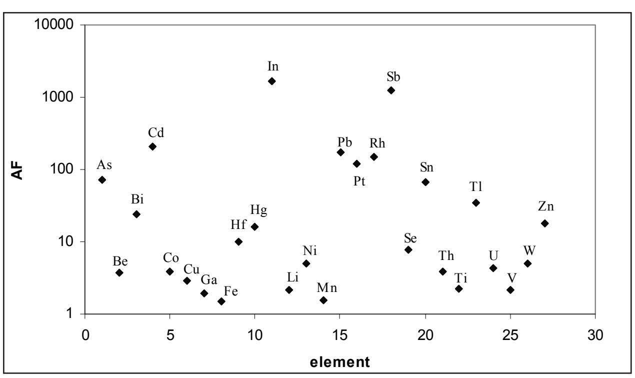

Fig. 2.1. Accumulation factor of *T. officinale* from sampling site 13.

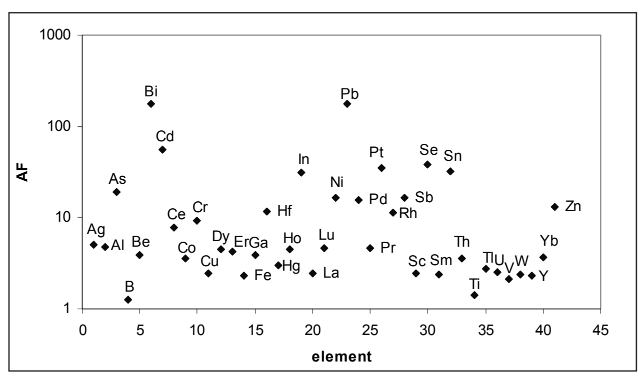

Fig. 2.2. Accumulation factor of *T. officinale* from sampling site 14.

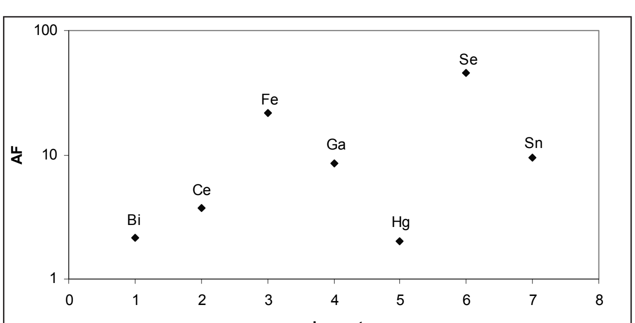

Fig. 2.3. Accumulation factor of *T. officinale* from sampling site 16.

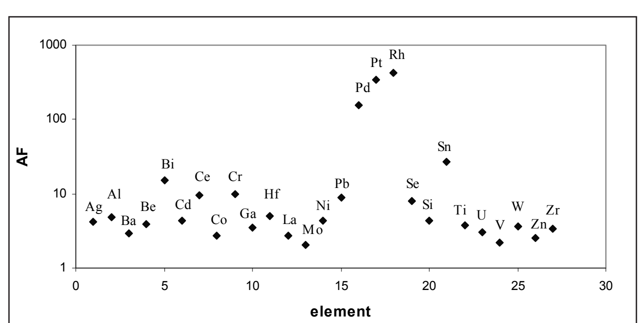

Fig. 2.4. Accumulation factor of *T. officinale* from sampling site 21.

sponded to the smaller output of the smelter. Instead of In and Sb, the highest accumulation has been found for Bi and Pb. Another difference between the plant ionomes in the two smelter locations was the accumulation of REE and elements like B, Ag and Al in *T. officinale* from site 14. These elements were also accumulated in sample 15 collected at a distance of 5 km from sample 14, along the most probable wind direction. Although accumulation was lower, the same elements have been identified as in sample 14, which confirms their presence in *T. officinale*. The differences in concentrations of the plants from both smelter locations could be attributed to the difference in raw materials. The smelter in Plovdiv (site 13) works with Bulgarian polymetallic ores from the Rhodopi basin, while the smelter in Kardzhali (site 14) is using imported raw materials practically from all over the world, including Canada, Peru, Australia, etc. Sample 16 (Fe Metallurgical Works in Kremikovtsi near Sofia, Fig. 2.3) was characterized with high accumulation of Se (AF 45), Fe (AF 22), Sn and Ga (AF around 10), Hg and Bi. It has been found out earlier that the main reason for higher concentrations of Se and Hg in the air of Sofia was due to coal combustion attributed to the Metallurgical Works (Djingova & Kuleff 1993). Since the Metallurgical Works is already out of operation for several years, the still higher concentrations in *T. officinale* were obviously due to residual soil pollution. Accumulation of Se and Bi has been identified in *T. officinale* collected in the vicinity of the other Fe metallurgical works (sample 17). However, many other elements have exhibited an accumulation factor over 2. Anthropogenic influence on samples 18 and 19 was also due to coal combustion, similarly to samples 16 and 17. However, the type of accumulation was rather different. Sample 18 was collected in the immediate vicinity of TMP Maritsa Iztok 1, where Cd, Pb and Sn have shown the highest accumulation, followed by Se, Bi, As, Hg Tl and Zn. Sample 19 collected at about 6 km from the other TMP Maritsa Istok 3 showed higher accumulation of Sn, Hg, Se and Bi. Apparently, coal combustion was the cause for emission and accumulation of Se, Bi and Hg in all four samples, while differences in the other elements may be due to differences in the raw materials and distance from the source. Se, and especially Hg, are typical volatile pollutants from coal combustion; however, even their emission in the gaseous phase depends on the chemical composition of the used coal (e.g. Esenlic & al. 2006). Samples 20 and 21 (Fig. 2.4) were collected along a motorway, in two places at different distances from Sofia. Sample 21 was taken at the roadside and sample 20 from a petrol station 2–3 m away from the motorway. In both cases, the three traffic-emitted pollutants, Pt, Pd and Rh, showed the highest accumulation, although the accumulation factors were different in accordance with the distance from the road. For the first time pollution with platinum metals has been established in vegetation along the Bulgarian roads. In a study performed 10 years ago, the concentration of platinum metals in *T. officinale* was below the detection limit of the method (DL > 0.016 ng g^{-1}). This result, along with the low accumulation of Pb, comes from the use of unleaded petrol during the last 10 years. The accumulation of Al, Cd, Zn, and Sn could be also attributed to traffic.

Sample 22 (near a uranium mine) did not show very high accumulation. Factors between 6 and 12 have been established for Se, U, As, Bi; and between 2 and 4 for Cd, Ni, Sb, Sb, Mo, Mn, and Ba.

The results clearly indicate serious changes in the plant ionome. Mostly typical pollutants are accumulated in the plant; however, many other elements have been established, which may be due to raw materials or to inter-element relations in the plant, as Baxter (2009) has established for *Arabidopsis thaliana*. Therefore, in an attempt to elucidate further these relationships, multivariate statistics was applied.

Cluster and factor analysis of the plant samples from the background regions

According to Baxter (2009), understanding regulation of the ionome is dependant on the elucidation of relationships between the elements, which may exist regardless of tissue, species or network, but may be associated and vary with combination of all these factors.

Cluster, factor and correlation analysis were performed in an attempt to discover the inter-element relations in the leaves of *T. officinale* from the background regions. Since there was a high degree of correlation of rare earth elements (REE) in the plants from the background regions and it was earlier established that accumulation in the absence of pollution is dependant only on the soil (Djingova & al. 2001), the concentrations of REE were not considered as multipliers of the same information. Only Ce and La as representatives of the group were included. Cluster analysis has led to formation of five statistically significant clusters:

- Cluster 1: In, V, Fe, Zr, Co, K, Sr, Mn, Li, Ga, U, Ni, Cr, Ce, Be;
- Cluster 2: Pb, Na, Se, Ca, Hf, La, Hg, Sb, Bi, Sn, As, Al;
- Cluster 3: Sc, Tl, Rb, Cs, Cd;
- Cluster 4: Th, W, Cu;
- Cluster 5: Ti, Mg, Ba, Zn, P, B, Mo, Ag.

Factor analysis confirmed the results from the cluster analysis. Five factors were found to be responsible for 45.2 % of the total variance. The first factor was loaded with Be, Ce, Co, Cr, Ga, Li, Mn, Ni, U, and Zr, all members of Cluster 1. Al, As, Bi, Hf, Hg, La, Sb, Sn (members of Cluster 2) had the highest positive loadings in the second factor, and negative contribution was established for K. Factor 3 included Ag, Mo and Ti (members of Cluster 5). Cd, Cs, Rb, Sc, Tl (all members of Cluster 3) had the highest loadings in factor 4, factor 5 registered the highest loadings of Cu, Th, W (members of Cluster 4).

A parallel correlation analysis was performed to reveal the further dependences between elements in the plant organism.

The three statistical procedures have agreed to a great extent. Mention deserve the positive correlations between Ca and Na and, respectively, the negative correlations K-Ca and K-Na, positive correlation B-P and negative Fe-B and Fe-P, and positive correlation Mg-Ba which did not appear in the cluster and factor analysis. The use of cluster analysis only provides results for the grouping of elements; however, the nature of grouping (positive or negative relationship) could be possibly revealed after using factor and correlation analysis. Furthermore, correlation analysis provides information on the "individual" relations between elements, which obviously remain hidden when group relationships are looked for. Therefore, statistical interpretation of such analytical data should include more than one approach, so as to establish similarity in the behavior of elements.

Cluster and factor analysis of the plant samples from the polluted regions

Cluster analysis of samples from the polluted regions resulted in the formation of five statistically significant clusters:

- Cluster 1 : Rh, Pt and Pd;
- Cluster 2 : Mo, Hg, Mg, U, Ca, ;
- Cluster 3 comprises two subclusters: Se, Sc, Ga, Fe and Sr, Rb, Na, Li, P, and B;
- Cluster 4: Ce, Cr, Be, Zr, Ti, Mn, Ba, Th, La, V, Hf, Al;
- Cluster 5: W, Ni, Cs, Co, Bi, Sn, In, Cd, As, Zn, Cu, Tl, Pb and Ag.

Factor analysis has also defined five factors responsible for 45 % of the total variance. The first factor accounting for 15 % of the total variance coincided exactly with Cluster 5 from the cluster analysis. This may be defined as an anthropogenic factor and the presence of W and Cs indicates inter-element correlations, which are not exactly due to anthropogenic pollution. Factor 2 was loaded with Ce, Cr, Be, Zr, Th, La, V, Hf, Al (all members of Cluster 4) and Sr and Li (members of Cluster 3). Obviously, this factor reflected soil conditions. Factor 3 was loaded with Pt, Pd and Rh, coinciding with Cluster 1 and reflected traffic pollution. Factor 4 was loaded with Ba, Ca, Mn, Ca, and U, which is a combination of members of Clusters 4 and 2. Factor 5 included Fe, Ga and Mo.

Correlation analysis has established the following statistically significant correlations (at $P<0.05$): As, Ag, Bi, Cd, Co, Cs, Cu, In, Ni, Pb, Sn, Tl, Zn, W, which perfectly coincide with factor 1 and Cluster 5; Ce, Be, Cr, La, Th, Zr, all members of Cluster 4 and factor 2; Se, Sc, Ga, Fe (part of Cluster 3) correlated negatively with Mo; Ba, Ca, Mn, U, Hf ,Al, Be, Li, La, Pt-Pd-Rh, members of Cluster 1 and factor 3. K did not correlate with any other element, P correlated with B.

Comparison of the results from the background and polluted regions

A comparison of the results has clearly showed regrouping of the elements in the plants, obviously as a result of environmental conditions. As predicted by Baxter (2009), redistribution affected not only the major polluting elements but other indirectly related to the type of pollution. After all statistical treatments, several relationships appeared both in polluted and in background regions: B-P; Cs-Tl, Fe-Ga; Cu-W; Be-Cr-Li-Zr. While for some of them (B-P) and (Cs-Tl) physiological or chemical explanation could be sought, the others were difficult to explain at this stage. The results need further confirmation after an analysis of additional samples and sampling sites. Comparison with literature data for *T. officinale* was rather difficult, since in most papers a very limited number of elements was determined, or correlations among the elements have not been investigated.

Changes in the ionome of different plant parts of *T. officinale*

There is already sufficient information that the ionome in different plant parts and tissues differs, as well as that most probably the reaction to environmental conditions is also different. Many investigations have studied the distribution of a limited number of elements among the roots and leaves (shoots) of *T. officinale* under different anthropogenic conditions. Most results indicate that the investigated elements (usually Cd, Zn, Fe, Pb) accumulate to a higher extent in the leaves of the plant in the presence of environmental pollution (Kabata-Pendias & Dudka 1991; Gjorgieva & al. 2011; Bini & al. 2012). Therefore, *T. officinale* has been qualified as a hyperaccumulator and a candidate for phytoremediation (Bini & al. 2012). However, depending on the element, quite opposite results were also reported (e.g. Vaculík & al. 2013). Discrepancy in the results agrees with the statement of Keane & al. (2001) that factors affecting metal absorption by the Dandelion are complex. In an earlier study (Djingova & Kuleff 1994), roots, stems, leaves, and blossoms of *T. officinale* collected from background and polluted regions in Bulgaria were investigated and the results indicated accumulation of most elements either in the roots or in the leaves. In the present study, leaves, stems and seeds of *T. officinale* from three of the investigated regions were collected: background site No 9 and two of the polluted sites No 16 and 22. The choice of these two sites was determined by the fact that they were polluted with a smaller number of elements in comparison to the other investigated regions, which at this initial stage gives better opportunities for evaluation of the distribution of elements. Figure 3.1 presents the results from the analysis of samples from the background site.

The results show that most elements were predominantly present in the leaves, while B, Cu, Fe, Mg, Ni, Rb, and P were in higher concentrations in the seeds.

The potentially toxic As, Ag, Se, Hg, Sn, and Tl were identified only in the leaves, while Be, Pb, Th, Hf, U, W, and Zr were found mainly in the leaves and only small amounts in the seeds. This indicates serious differences in the ionomes of the plant parts of *T. officinale.*

Figures 3.2 and 3.3 present the results from analysis of samples from the two polluted sites. The results indicate that B, Cu, Fe, Mg, Ni, Rb, and P have remained accumulated mainly in the seeds, as in the background region, but the highest concentrations of Ca, Mn, Zn, Co, Cd, Sb, Th, and W were also identified in the reproductive organs of *T. officinale* from sampling site No 16. The potentially toxic Hg and Sn were identified only in the leaves in both polluted samples, as in the background region. The other determined elements were concentrated mainly in the leaves. In the samples from site 22, the concentrations of B, Rb, Th, and REE have considerably increased in the seeds, as compared to the background region. In sample No 22, U was expected to be the major pollutant and indeed its concentration in all plant parts was 10 to 25 times higher than the concentrations in the samples from the other two locations. U did not accumulate specifically in any plant part and was practically evenly distributed in the samples from site 22.

The above results permitted us to make the following conclusions:

- In the polluted region, there are changes not only in the ionome of the whole plant, but also in the ionome of the different plant parts.
- Redistribution among the plant parts has been identified not only for the major pollutants, but for the other elements too.

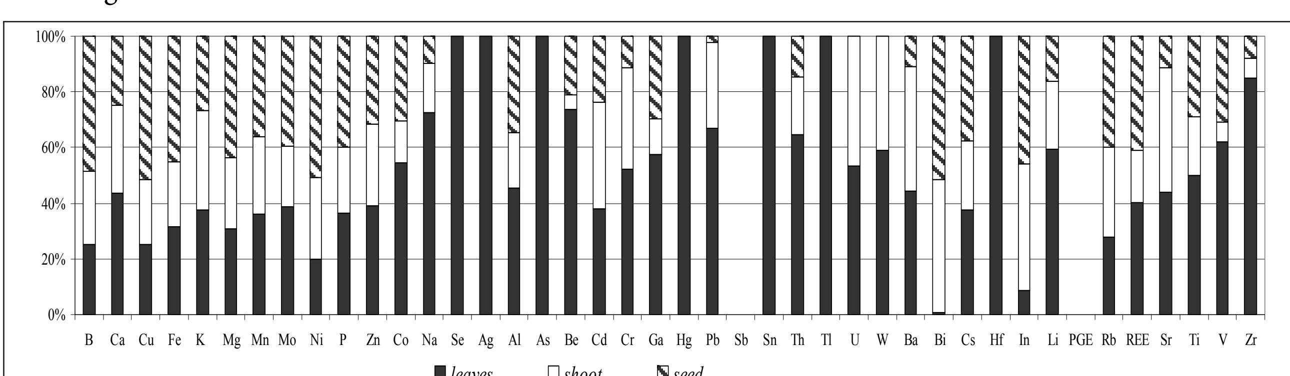

Fig. 3.1. Distribution of chemical elements in the leaves, stems and seeds of *T. officinale* collected from the background region No 9.

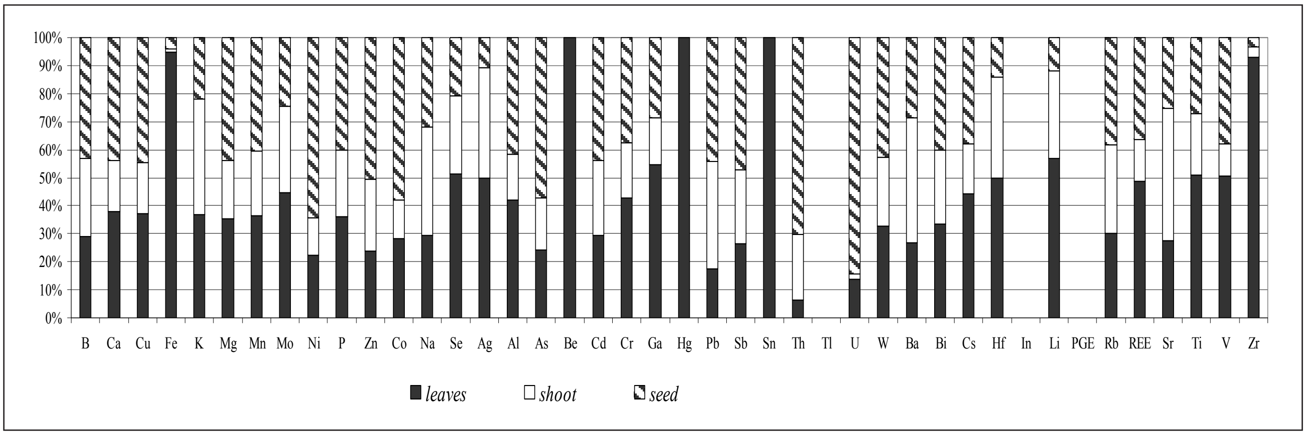

Fig. 3.2. Distribution of chemical elements in the leaves, stems and seeds of *T. officinale* collected from the polluted region No 16.

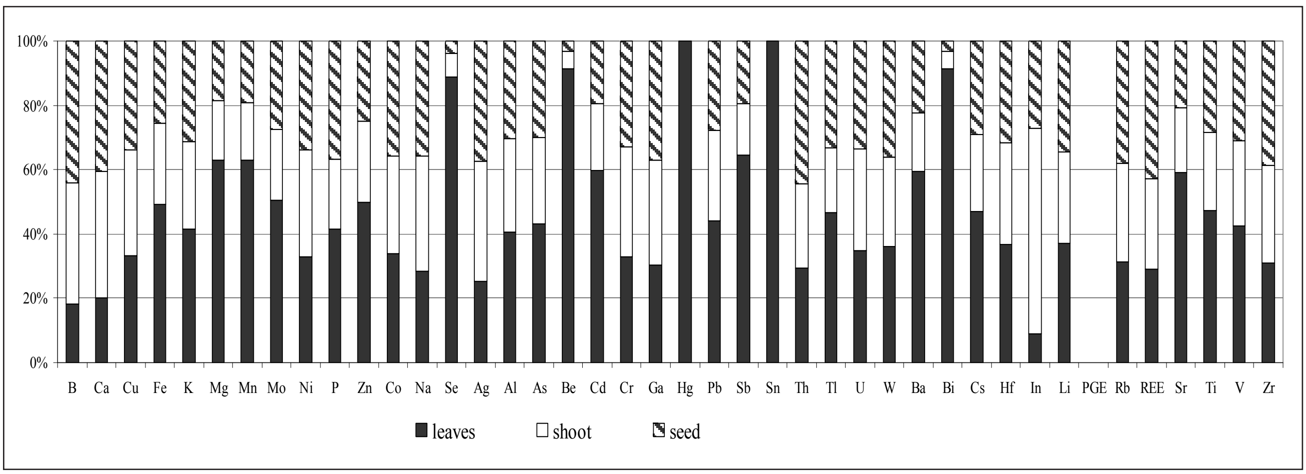

Fig. 3.3. Distribution of chemical elements in the leaves, stems and seeds of *T. officinale* collected from the polluted region No 22.

- There is strong indication that the highest concentrations of B, Cu, Mg, Ni, and P have been usually found in the seeds of *T. officinale,* while Hg and Sn have been typical for the leaves. Distribution and redistributions of the other elements depend on the environmental conditions of the sampling site.
- Another strong indication is that anthropogenic pollution leads to accumulation of potentially toxic elements in the reproductive organs of *T. officinale*, and this change in the ionome may lead to a respective change in the genome.

Genome investigation

Preliminary results of the genome investigations of *T. officinale* from sites No 9, 16 and 17 were published by Peycheva & al. 2012. Differences in DNA content of the plants from the polluted regions were established, suggesting different ploidy. The response of plants to metal pollution resulted in gross genomic rearrangements in the plants from the site, regardless of the absence of DNA damage. Further investigations are in progress for the remaining sampling sites and with use of complementary methods too.

Conclusion

The present study provides characterization of the ionome of *T. officinale* growing in background regions. The results indicate that under natural unpolluted conditions concentration of the determined elements in the plants are relatively stable with time and region. The highest stability was found for essential elements. Statistical interpretation of the results revealed inter-element relations established for the first time. Under anthropogenic influence, the ionome of the plant and in the plant parts changes drastically not only in respect to major polluting elements. The established inter-element correlations differ significantly in the background and in the polluted regions.

Acknowledgements. The authors gratefully acknowledge the financial support of the National Science Fund, Ministry of Education, Youth and Science of R Bulgaria, Contract No DDVU 02-61/2010 and the Operational Program "*Human resources development*" financed by the European Social Fund of the European Union, Project No BG051PO001-3.3.06-0048.

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