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Chemical fingerprinting of plants

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The chemical fingerprinting of a plant demands the determination of a large number of elements at background level but also in polluted regions and in relation to other species. The most important stages of the development of plant fingerprints are mentioned in this paper and a short survey of the state of the art is presented. Examples on fingerprinting of lichens, mosses, *Taraxacum officinale* and *Populus nigra* 'Italica' are presented.

Key words: chemical fingerprinting; lichens; mosses; *Taraxacum officinale*; *Populus nigra* 'Italica'.

Introduction

The term 'fingerprinting' is usually connected with criminal law and forensic studies. In the broader sense of the word, however, it is already widely used in a number of sciences: medicine, biology, genetics (as 'DNA fingerprinting') and pharmaceutical studies (as fingerprinting of new substances in natural materials). In geology, archaeometry and environmental studies, chemical fingerprinting identifies the distribution of chemical elements within a matrix and thus defines its unique portrait in comparison to similar matrices. Fingerprinting implies the determination of as many elements as possible.

A number of environmental materials (rocks, soils, sediments) have already been fingerprinted by numerous investigations during many years. The genetic relationship between them, which began with the formation of the earth and followed the process from magma to igneous rocks to sediments and soils, makes the task relatively easy in comparison to biota. Thus, today the fingerprints of the basic types of rocks and sediments are well known and the variations within one type are amazingly small. Although there are many different types of soil and it is quite difficult to classify them, once the parent material is known certain observations can be done immediately (Pfeifer *et al.* 2000).

The possibility and wish to make fingerprints of plants in respect to the distribution of chemical elements is very natural since their composition depends to a great extent on the geochemical features of the environment where plants grow. Since it is possible to determine the inorganic components of this environment, it should also be possible to determine the composition of the plants growing there. Of course the biochemical processes within a living organism are very complicated and might not allow the definition of narrow intervals of chemical distribution within the organism.

The possibility of fingerprinting the chemical composition of a plant is supported by several facts. First, plants can exist under specific conditions determined by the nutrient supply and levels of toxic substances. The uptake of specific nutrients is dependant on their amount and availability but the plant can survive only within certain concentrations of nutrients otherwise there are deficiency symptoms and possibly death. As far as toxic elements are concerned no matter how toxicotolerant a plant is there is a certain limit of endurance after which the plant dies (Markert 1996). The fact that such 'limits' are necessary for the normal existence of plants indicates the existence of reasonable concentration intervals in a plant (at normal background levels).

Another very important fact is the Biological System of Elements (BSE) proposed by Markert in a series of papers which determines the position of an element according to: its correlation to other elements in the living organism (plant); the function of the element in the organism; and the uptake form of individual elements by living organisms (Markert 1987, 1993, 1994, 1996; Fraenzle & Markert 2002). Thus the BSE implies that elements are generally correlated in the organism

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according to their physiological role and the existence of an organism also depends on this correlation.

Fingerprinting of plants

Chemical fingerprints of plants may be considered as normal concentrations in a plant at background levels described with the respective interval of biological variation. The fingerprints of plants might facilitate research in a number of areas of environmental, biomonitoring and biological research. Some possible applications are: (i) better understanding of the accumulative, indicative and rejecter behavior of a plant and evaluation of the distribution pattern of the elements in the different compartments of the ecosystem by comparison of plant fingerprints; (ii) quick assessment of local pollution levels and quick risk assessment for ecosystems by direct comparison of the experimental results to the plant's fingerprints; (iii) the possibility to compare, type and systematize groups of plants.

The use of sophisticated methods of analysis during the past 20 years has led to new opportunities in environmental research (Djingova & Kuleff 2000). Methods, such as, inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA) and to a certain extent inductively coupled plasma atomic emission spectrometry (ICP-AES), graphite furnace atomic absorption spectrometry (GFAA) and X-ray methods permit the routine determination of more than 15 elements in plants with reasonable accuracy and overall certainty (Markert 1996; Djingova & Kuleff 2000). The possibility of determining a large number of elements within short time intervals facilitated the concept of using distribution patterns of chemical elements to produce fingerprints of environmental matrices. However, two extremely important conditions should always be kept in mind. The chemical characterization demands that the method used must have high accuracy and precision and this is only possible for specialized laboratories.

The production of 'correct' analytical data is meaningless if the intrinsic dynamics of the plant systems relative to location and time are overlooked. Therefore, representative sampling programs adapted to the needs of biological and environmental research should be developed. Otherwise the 'correct' analytical data will only be a 'snapshot' of a given element in a given plant at a given point in time at a given location and will be useless for comparison and further conclusions (Markert 1996).

A problem that resulted from multi-element analysis is how to make a presentation of the data obtained from the investigations of plants. One approach has been the graphic presentation of chemical fingerprints either individually or in respect to some basic concentrations or relationships. In that respect several approaches might be specifically mentioned.

Reference plant and fingerprint graphs

Markert proposes the reference plant where the concentrations for 80 elements are 'averaged' or calculated (Markert 1992, 1996). Fingerprints of various plants are done by graphically displaying positive or negative percentage deviations of the plant types from the normal values of the reference plant. Thus the fingerprint graph demonstrates characteristic distribution in relation to the reference plant. The fingerprint graphs of *Betula pendula*, *Pinus sylvestris*, *Vaccinium vitis-idaea*, *V. myrtillus*, *Deschampsia flexuosa*, *Molinia caerulea*, *Polytrichum formosum*, *Sphagnum* spp., *Galium aparine*, *Brassica oleracea*, and several certified plant reference materials are presented in Markert 1996.

Element concentration cadasters

The cadasters are a graphical representation of the chemical composition of a plant where the concentrations of individual elements are listed in classes of concentrations between 10^n and 10^{n-1} (Lieth & Markert 1985, 1986, 1988; Kovac *et al.* 1990; Markert 1996). Although very useful, the concentration cadasters have several shortcomings: plant-specific accumulation or rejection is not clearly emphasized; individual samples can be compared only with the help of the original data; concentration jumps from one concentration class to another are equivalent to an actual 10-fold difference in the rarest of cases (Markert 1996).

Conversion factors

An attempt to fingerprint the element distribution of a plant and its relationship to other plants are the conversion factors (Djingova *et al.* in press). These represent the concentration ratio of an element between a biomonitor species and any other plant in an ecosystem. Thus having the correct conversion factors together with concentrations of the biomonitor the behavior of all other plants in the specific location can be evaluated.

Basic problems in chemical fingerprinting of plants

Multi-element analysis of plants and attempts to generate the fingerprints of plants are continuously being done. Up till now, however, success has been achieved in a limited number of cases. The reasons are:

1 Unspecified or non-standardized sampling conditions concerning minimum plant quantity for

representative sample, sampling season (vegetative stage of the plant), sampling pre-treatment (cleaning, washing), which, in most cases, makes the results not representative enough for a fingerprint.
2 Lack of background investigations. Most of the species are only investigated in polluted regions and these results cannot be used for fingerprints or for evaluation of the behavior of the plant to pollution stress.
3 The limited number of elements that are determined (usually some nutrient and popular toxic elements) mainly due to the wide use of atomic absorption analysis for these purposes. In the majority of studies less than 10 elements are reported. Usually there is no evidence for quality assurance and control or estimation of the uncertainty, which makes any comparisons meaningless.
4 The lack of interspecies comparisons at background levels and in polluted levels as well as the lack of ecosystem approach hinders not only the fingerprinting of a single plant but makes it impossible to specify its position among other species in the plant kingdom.

State of the art in the fingerprinting of plants

Fingerprinting of lichens

There is an enormous variety of lichens – over 15 000 species. Depending on the growing mode they are classified as crustose, foliose or fruticose. Classification according to place of growth subdivides lichens into epigeic (growing on soil), epilithic (growing on rocks) or epiphytic (growing on trees; Tyler 1989). Additionally classifications according fungi partner in lichen symbiosis and algae partner (usually green alga) can be done. Lichens derive nutrients and mineral substances from rainwater, air, throughfall but also from the substrate, producing special acid substances (Tyler 1989). In this way lichens are considered among the best indicators of atmospheric pollution and are investigated in a number of studies aiming to monitor air pollution (Tyler 1989; Garty 1993, 2000). Therefore the construction of a fingerprint of lichen species should not be difficult. However, there are a number of problems:
1 Over 25 species have been used in different studies aiming to evaluate local pollution or regional distribution of heavy metals (Tyler 1989; Kubin 1990; Sloof & Wolterbeek 1991a,b, 1993; Reis *et al.* 1996; Freitas & Nobre 1997; Freitas *et al.* 1997; Garty 1993, 2000 Jeran *et al.* 1996a,b), and Cs-137 (Sawidis & Heinrich 1992) and there is not very high 'repeatability' in the species used. Different lichen types were used in most studies and fingerprint comparisons are impossible.
2 In comparison to the numerous data from polluted regions the data from background areas are rather limited (Garty *et al.* 1977; Gough *et al.* 1988a,b; Frenzel *et al.* 1990; Crete *et al.* 1992; Geiser *et al.* 1994; Chiarenzelli *et al.* 1997).
3 Sampling parameters are also not comparable. In some of the studies lichens are washed before sampling, and/or after sampling (Garty *et al.* 1977), in other studies lichen samples are mechanically cleaned (Chiarenzelli *et al.* 1997) and in many cases the samples are not treated at all. Therefore concentration differences in many lithophilic elements are difficult to be interpreted. Are they species specific or springing from dust?
4 Comparisons between species from the different groups epigeic (growing on soil), epilithic (growing on rocks), or epiphytic (growing on trees) are also impossible due to the role of the substrate. There is also some evidence of within one group (epiphytic) concentration differences depending on which tree the lichen grows.

The investigations of Chiarenzelli *et al.* (1997) and Gough *et al.* (1988a,b) might be considered good examples of studies aiming to fingerprint lichens. Gough *et al.* (1988a,b) investigated *Parmelia sulcata* (and sporadically *P. chlorochroa*), *Hypogymnia enteromorpha* and *Usnea* spp. from national parks in the USA.

Chiarenzelli *et al.* (1997) reported on a background study of 12 lichen species collected in remote arctic ecosystems and subdivided into seven substrate types. The data obtained for As, Cd, Cr, Cu, Ni, Pb, Sb, V and Zn indicate that substrate rocks do not significantly influence the element concentration of the lichen. The concentrations are comparable to earlier results for Canada, Finland and the USA. Crustose lichens are characterized by higher metal concentrations than foliose lichens and the lowest concentrations were found in fruticose lichens.

Table 1 presents chemical fingerprints for several lichen species (Garty *et al.* 1977; Gough *et al.* 1988a,b; Frenzel *et al.* 1990; Chiarenzelli *et al.* 1997). It is difficult to discuss the concentration differences especially between *Caloplaca aurantia* and the rest of the lichens.

Fingerprinting of mosses

Bryophytes are among the most commonly used mosses as bioindicators for environmental pollution since the works of Rühling and Tyler (1968, 1969, 1970, 1971, 1973). They represent the simplest form of terrestrial plants. Mosses are subdivided into endohydric, ectohydric and mixohydric. Ectohydric mosses have no internal conductive system or cuticula (e.g. *Hylocomium splendens* and *Hypnum cupressiforme*). Endohydric species (e.g. *P. formosum*) possess

Table 1 Baseline concentrations (mg kg^{-1}) of several lichen species

Element	*Hypogymnia enteromorpha*	*Usnea* spp	*Parmelia sulcata*	*Caloplaca stellaris*	*Caloplaca nivalis*	*Parmelia mougoetti*	*Usnea polyphylla*	*Caloplaca aurantia*
Al	1100	260	2000	–	–	–	–	–
As	–	–	0.97	0.19	0.19	0.48	0.54	–
Ba	24	16	79	–	–	–	–	–
Cd	<0.08	<0.05	–	0.07	0.24	0.24	0.19	1.92
Ca	3800	3300	4400	–	–	–	–	–
Ce	0.55	0.22	–	–	–	–	–	–
Cr	4.9	1.0	7.3	1.24	1.18	12.4	3	45
Co	0.32	0.19	–	–	–	–	–	–
Cu	3.7	2.6	24	1.39	1.98	5.08	5.21	25.5
Ga	0.31	0.09	–	–	–	–	–	–
Fe	850	170	2700					11537
K	1800	1800	–	–	–	–	–	–
La	0.37	0.11	–	–	–	–	–	–
Li	0.33	0.097	–	–	–	–	–	–
Mg	1300	1600	730	–	–	–	–	–
Mn	89	97	72	–	–	–	–	162
Mo	<0.09	<0.05	–	–	–	–	–	–
Na	320	300	–	–	–	–	–	–
Nd	0.30	<0.09	–	–	–	–	–	–
Ni	11	6	6.6	1.83	1.92	6.4	2.42	36
P	670	420	790	–	–	–	–	–
Pb	12	7.5	26	0.75	3.23	12.5	10.4	46
S	470	18	1200	–	–	–	–	–
Sb	–	–	–	0.023	0.020	0.041	0.024	–
Sc	0.26	0.08	–	–	–	–	–	–
Sn	<0.9	<0.5	–	–	–	–	–	–
Sr	18	18	28	–	–	–	–	–
Ti	51	16	16	–	–	–	–	–
V	2.5	0.53	4.4	1.14	0.92	19.3	5.73	–
Y	0.22	0.10	2.5	–	–	–	–	–
Zn	25	21	95	15.6	25.3	24	22.2	304

an internal conductive system and water uptake from the soil is possible while the cuticle layer on the leaves of some permit absorption of water via the leaf surface (Bell *et al.* 1992; Markert 1996). Ectohydric mosses are favorites in the biomonitoring studies proposed by Rühling and Tyler although studies with endohydric mosses have been carried out by others and baseline concentrations for Europe have been derived (Markert 1996). A European project used mosses to determine, 'Atmospheric heavy metal deposition in Europe – estimations based on moss analysis' (Herpin *et al.* 1995; Wolterbeek *et al.* 1995; Markert *et al.* 1996).

In contrast to lichens several moss species have been investigated: *H. splendens, Pleurozium schreberi, H. cupressiforme, Scleropodium purum* and *P. formosum*. Sampling strategies are unified and as a rule of thumb the samples are not being washed. Intercomparisons between several moss taxa have been carried out and calibration factors for some elements have been derived (Steinnes 1993; Wolterbeek *et al.* 1995). However, the conclusions about the possibility of using one species instead of another (although often done) within one study are still contradictory.

Table 2 presents the fingerprints of several moss taxa used in different local and spatial studies. Considerable differences can be established between *P. formosum* and the rest of the mosses, which is to be expected considering the differences between the types of mosses (endohydric and ectohydric).

Fingerprinting of higher plants

Although lichens and mosses have been used in a large number of monitoring investigations and large scale screenings higher plants are acknowledged to have a lot of advantages as monitors. Generally higher plants are much more widely distributed, have a better morphology than lower plants, most of them are toxicotolerant and are the basis of human alimentation (Wittig 1993). This means that studying higher plants leads to much easier standardization of exposure methods, sampling

Table 2 Fingerprint of several moss species (mg kg^{-1})

Element	*Pleurozium schreberi*	*Hylocomium splendens*	*Sphagnum* spp.	*Polytrichum formosum*
Al	–	322	385	400 (305)[†]
As	0.2	–	–	–
Ba	–	–	–	5 (7.8)
Br	1.6	7.4	12.6	–
Ca	–	–	2003	1000 (2710)
Cd	0.2	0.3	0.27	0.2
Ce	0.6	–	1.41	–
Co	0.2	–	–	–
Cr	0.9	1.07	1.24	3 (1)
Cs	0.12	–	–	–
Cu	4.5	4.9	2.5	12 (16.6)
Dy	–	–	0.1	–
Eu	–	–	0.023	–
Er	–	–	0.066	–
Fe	150	210	352	400 (165)
Gd	–	–	0.1	–
Ho	–	–	0.025	–
K	–	–	5227	7500 (15390)
La	0.32	–	0.72	–
Mg	–	–	1088	500 (240)
Mn	110	235	101	–
Na	130	322	760	–
Ni	0.6	1.4	1.5	15
Pb	5.7	9.1	9.4	10 (2.7)
Pr	–	–	0.16	–
Rb	12	–	–	–
Sb	0.34	–	–	–
Sc	0.062	–	–	–
Se	0.34	–	–	–
Sm	0.04	–	0.11	–
Sr	–	–	–	4 (4.8)
Tb	–	–	0.026	–
Th	0.069	–	–	–
Ti	4	4.5	–	8
Tm	–	–	0.011	–
V	1.4	1.75	2.36	–
Yb	–	–	0.065	–
Zn	25	26.5	26	40 (46)

procedures, large scale screenings, and comparative studies between places with very different degrees of anthropogenic influence. In the attempt to look for plants suitable for biomonitoring investigations several species have been tested and their reactions studied. Among them are the trees *Populus nigra* '*Italica*' (Wagner 1987), *Picea abies* (Wagner & Müller 1979; Krivan *et al.* 1987; Krivan & Schaefer 1989), *P. silvestris* (Laaksovirta & Olkkonen 1977), and *Fagus sylvatica* (Zimmermann 1989). Two *Vaccinium* species are relatively well-investigated: *V. angustifolium* (Scheppard & Evenden 1990) and *V. myrtillus* (Markert 1989). Among grasses *Lolium multiflorum* has gained popularity for active biomonitoring (Scholl 1987). Various studies have reported that many weeds reflect environmental pollution, however, these species are not well studied and the snapshot of the chemical composition of a plant at a certain location (usually only very few elements are reported) does not permit us to proclaim it as a biomonitor (as is often the case) or to obtain real information about its chemical fingerprint and characteristic behavior. Two plants that have been well-studied in respect to biomonitoring investigations and are among the few higher plants used for large scale screening of the distribution of a large number of elements (over 35) will be given as examples in the fingerprinting of plants.

Taraxacum officinale

Taraxacum officinale has been introduced as a biomonitor of environmental heavy and toxic element pollution

(Kuleff & Djingova 1984; Djingova *et al.* 1986, 1993, Djingova & Kuleff 1993, 1999; Djingova *et al.* 1999a) and used in a number of regional and large scale distribution studies in Poland (Kabata-Pendias & Dudka 1991), Germany (Winter *et al.* 1999), USA (Rule 1994; Keane *et al.* 2001), Canada (Marr *et al.* 1999), and Hungary (Bacso *et al.* 1984; Kovac *et al.* 1993). All of these studies have contributed to the information obtained about the fingerprinting of chemical composition and reactions of *T. officinale*.

Sampling

Leaves of *T. officinale* are collected from at least 10 (preferably 25) individual plants, and immediately after sampling are washed with water. Sampling should be carried out in spring or autumn, immediately after the plant blossoms (Djingova & Kuleff 1994, 1999). This procedure was followed in most studies.

Chemical composition at background level

Table 3 presents the concentration intervals for 39 chemical elements at background level. These data were collected from all the studies and may be considered as representative for the plant. For some elements (e.g. Au, Mn, Cs, Sc) the intervals are larger and this is especially typical for elements whose concentration is highly dependent on the type and composition of soil.

Table 3 Fingerprint of *Taraxacum officinale* (mg kg^{-1})

Element	Concentration interval for background regions in Europe and USA	Element	Concentration interval for background regions in Europe and USA
Al	60–300	Mn	15–200
As	0.1–0.4	Mo	0.6–2.9
Au	0.004–0.03	N%	2.2–3.3
Ba	14–80	Na	50–400
Br	7–30	Ni	0.3–4
Ca%	1.1–2.0	P	2000–4000
Cd	0.2–0.8	Pb	0.3–6
Ce	0.3–0.6	Rb	24–160
Co	0.1–0.2	S	2200–5000
Cr	0.1–0.5	Sc	0.05–0.1
Cs	0.04–0.2	Se	0.05–0.2
Cu	5–20	Si	70–500
Eu	<0.005–0.02	Sm	0.05–0.2
Fe	60–500	Sr	10–45
Ga	0.16	Ti	5.6
Hg	<0.1–0.2	Th	<0.3–0.5
K%	2.1–4	Tl	0.025
La	0.2–0.8	V	0.18
Mg%	2.0–3.0	Zn	30–100

Inter-element correlations

Investigations of the inter-element correlations in *T. officinale* indicate statistically significant correlations among: Al/Fe; Mn/Al; Mn/Fe; Ca/Mg; Pb/Zn; Cu/Zn and Cu/Pb in practically all studies carried out in relatively unpolluted regions. In polluted regions the correlations sometimes change depending on the type and source of pollution (Kuleff & Djingova 1984).

Reaction to environmental pollution with heavy and toxic elements

Taraxacum officinale has been proven to react quantitatively to air and soil pollution with As, Br, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Pt, Pd, Rh and Zn (Kuleff & Djingova 1984; Kabata-Pendias & Dudka 1991; Djingova *et al.* 1993; Rule 1994; Djingova *et al.* 1995b, 1999b; Djingova *et al.* 2003). The correspondence between the air–soil pollution and the concentration in the plant is usually not simply linear (Rule 1994; Keane *et al.* 2001) but is described with a log normal or more complicated relationship (Kuleff & Djingova 1984; Djingova & Kuleff 1993; Djingova *et al.* 1993).

Populus nigra *'Italica'*

Populus nigra 'Italica' has been standardized as a biomonitor by Wagner (1987) in respect to three elements Cd, Pb and Zn and is among the tree species (together with *Picea abies*) collected within the Federal Environmental Specimen Bank of Germany (Klein & Paulus 1995). *Populus nigra* 'Italica' has been used to evaluate the spatial distribution and large scale screening of 40 elements in the territory of Bulgaria (Djingova *et al.* 1995a, 1996, 1999b, 2001) and Cd, Pb and Zn in Germany and some cities in Poland (Wagner 1987). For rare earth elements (REE) and elements like U, Th, and Be it has been proven that the elemental content depends on the type of soil (Djingova *et al.* 2001). *Populus nigra* 'Italica' is the only plant in which the concentration of elements like Be, Te, Tl, U, Th and the REE has been determined over a large territory and in more than 200 samples. This information permits the fingerprint of this plant to include such rarely determined elements.

Sampling

Sampling is done according the standardized procedure of Wagner (1987) in early autumn before senescence at tree height of 4–6 m. After sampling leaves are washed with tap and distilled water.

Chemical composition at background level

Table 4 presents the background concentration intervals for 40 elements in *P. nigra* 'Italica'. For REE, Tl,

Table 4 Fingerprint of *Populus nigra* 'Italica' (mg kg^{-1})

Element	Background concentration interval	Element	Background concentration interval
As	0.2–0.4	La	0.1–0.54†
Be	0.0047–0.008†	Mn	26–126†
Bi	0.0016–0.019†	Na	64–300
Br	5.6–8.1	Nd	0.084–0.35†
Ca%	1.73–2.38	Ni	0.35–3.2
Cd	0.1–0.3	Pb	0.5–2
Ce	0.18–0.89†	Pr	0.02–0.26
Co	0.6–0.8	Rb	2.8–10.5
Cr	0.12–0.67	Sb	0.02–0.06
Cs	0.05–0.15	Sc	0.03–0.05
Cu	1.9–8.2	Sm	0.016–0.08†
Dy	0.011–0.056†	Sr	67–169
Er	0.0056–0.021†	Tb	0.002–0.027†
Eu	0.0066–0.028†	Te	0.0065–0.20†
Ga	0.61–1.19†	Tl	0.0083–0.061†
Gd	0.016–0.06†	U	0.015–0.083†
Ho	0.0019–0.0066†	V	0.15–0.79†
Fe	62–130	Y	0.067–0.36†
K%	0.66–1.35	Yb	0.0045–0.0145†

†Dependent on soil concentration.

Th, and U highest background concentrations have been established in trees growing on rendzina soils while lowest concentrations of the soil dependant elements are established for carbonate chernozem soils. (Table 4; Djingova *et al.* 2001).

Inter-element correlations

The investigation showed statistically significant correlations between *P. nigra* 'Italica' leaves collected from regions with low or intermediate level of pollution. Significant correlations were established between all REE and between Be and Sb; Ca; Co; Th; U; Bi and also between Cd, Co; Cu, Ni, Zn and all REE; Th and Be, Cr; U, Tl and As; Cr; Fe; Mn; U; U and Au; Be; Fe; Sc; Tl and Zn. It is impossible to determine whether these correlations are typical only for *P. nigra* 'Italica' because these elements are not among those usually determined in plants. Our investigations of several vegetables, however, did not indicate such correlations.

Reaction to environmental pollution with heavy and toxic elements

Populus nigra 'Italica' has been proven to react to air and soil pollution other than Cd, Pb and Zn and also to most other toxic metals.

Cross examination of Taraxacum officinale and Populus nigra *'Italica'*

The comparison between the fingerprints of *T. officinale* and *P. nigra* 'Italica' indicates that at background levels *T. officinale* has higher concentrations of Br, Cd, Cu, K, Rb and Sm, and *P. nigra* has higher concentrations of Ca, Co and Sr. The concentrations of the rest of the elements (determined in both species) are similar. Neither of the plants can be described as natural accumulators or rejecters.

Both plants have been collected from more than 40 sampling sites in the territory of Bulgaria and the different anthropogenic pollution and toxic elements have been determined using several analytical methods. The results indicate very constant conversion factors for all elements irrespective of the sampling site (Djingova *et al.* in press). This is a good indication that both plants are successful indicators of environmental pollution reacting adequately to stress.

Conclusion

Fingerprinting of the chemical composition of plants might be very helpful for a number of studies: botanical, physiological and environmental. However, several prerequisites are necessary to attain a successful fingerprint: standardized sampling procedures; accurate and precise analytical methods; determination of as many elements as possible; investigation of a large number of samples from background regions as well as from regions with different types of pollution and soils; and the determination of the relationship between the investigated plant and other plant species in similar conditions (within the same ecosystems). For lichens and mosses such investigations have been more common but for higher plants the scope of these investigations should definitely be enlarged.

References

Bacso J., Kis-Varga M., Kovacs P. & Kalinka G. (1984) Investigation on the accumulation of lead and other traffic metals in plants caused by motor traffic and smelting. *Journal Radioanalytical and Nuclear Chemistry* **81**: 59–65.

Bell S., Asheden T. W. & Rafarel C. R. (1992) Effects of rural roadside levels of nitrogen dioxide on *Polytrychum formosum Hedw*. *Environmental Pollution* **76**: 11–14.

Chiarenzelli J., Aspler L., Ozarko D., Hall G. E. M., Powis K. & Donaldson J. (1997) Heavy metals in lichens, southern district of Keewatin, Northwest territories, Canada. *Chemosphere* **35**: 1329–1341.

Crete M., Lefebvre M. A., Zikovsky L. & Walsh P. (1992) Cadmium, lead, mercury and cesium-137 in fruticose lichens of northern Quebec. *Science of the Total Environment* **121**: 217–230.

Djingova R., Ivanova Y., Wagner W., Korhammer S. & Markert B. (2001) Distribution of lanthanoids, Be, Bi, Ga, Te, Tl, Th and U on the territory of Bulgaria using *Populus nigra* 'Italica' as an indicator. *Science of the Total Environment* **280**: 85–91.

Djingova R., Kovacheva P. & Kuleff I. (1999a) On the use of *Taraxacum officinale* Web. and *Populus nigra* 'Italica' for the evaluation of heavy metal pollution risk in urban ecosystems. *Phytologia Balcanica* **5**: 123–129.

Djingova R., Kovacheva P. & Kuleff I. (2003) Risk characterization for ecosystems using conversion factors. *Annuaire de L'universite de Sofia*. **96**.

Djingova R., Kovacheva P., Wagner G. & Markert B. (2003) Distribution of platinum group elements and other traffic related elements among different plants along some highways in Germany. *Science of the Total Environment* **308**: 235–246.

Djingova R. & Kuleff I. (1993) Monitoring of heavy metal pollution by *Taraxacum officinale*. In: *Plants as Biomonitors* (ed. B. Markert) pp. 435–460. VCH, Weinheim.

Djingova R. & Kuleff I. (1994) On the sampling of vascular plants for monitoring of heavy metal pollution. In: *Environmental Sampling for Trace Analysis*. (ed. B. Markert) pp. 395–414. VCH, Weinheim.

Djingova R. & Kuleff I. (1999) Seasonal variations in the metal concentration of *Taraxacum officinale*, *Plantago major* and *Plantago lanceolata*. *Chemistry and Ecology* **16**: 239–253.

Djingova R. & Kuleff I. (2000) Instrumental techniques for trace analysis. In: *Trace Elements – Their Distribution and Effects in the Environment* (eds B. Markert & K. Friese) pp. 137–185. Elsevier Science BV, Amsterdam.

Djingova R., Kuleff I. & Andreev N. (1993) Comparison of the ability of several vascular plants to reflect environmental pollution. *Chemosphere* **27**: 1385–1396.

Djingova R., Kuleff I., Penev I. & Sansoni B. (1986) Br, Cu, Mn and Pb content of the leaves of *Taraxacum officinale*. *Science of the Total Environment* **50**: 197–208.

Djingova R., Kuleff I. & Voulgaropoulus A. (1995a) Heavy metal pollution in Sofia (Bulgaria) and Thessaloniki (Greece) using *Taraxacum officinale* as biomonitor. *Journal Environmental Chemistry* **4**: 241–245.

Djingova R., Wagner G. & Kuleff I. (1999b) Screening of heavy metal pollution in Bulgaria using *Populus nigra* 'Italica'. *Science of the Total Environment* **234**: 175–184.

Djingova R., Wagner G. & Kuleff I. & Peshev D. (1996) Investigation of the time-dependant variations in metal concentration in the leaves of *Populus nigra* 'Italica'. *Science of the Total Environment* **184**: 197–202.

Djingova R., Wagner G. & Peshev D. (1995b) Heavy metal distribution in Bulgaria using *Populus nigra* 'Italica' as a biomonitor. *Science of the Total Environment* **172**: 151–158.

Fraenzle S. & Markert B. (2002) The Biological system of the elements (BSE) – a brief introduction into historical and applied aspects with special reference on 'ecotoxicological identity cards' for different element species (e.g. As and Sn). *Environmental Pollution* **120**: 27–45.

Freitas M. C. & Nobre A. S. (1997) Bioaccumulation of heavy metals using *Parmelia sulcata* and *Parmelia caperata* for air pollution studies. *Journal Radioanalytical and Nuclear Chemistry* **217**: 17–20.

Freitas M. C., Reis M. A., Alves A. C., Wolterbeeck H. Th, Verburg T. & Gouveia A. (1997) Biomonitoring of trace element metal pollution in Portugal: qualitative survey. *Journal Radioanalytical and Nuclear Chemistry* **217**: 21–30.

Frenzel R., Witmer G. & Starky E. (1990) Heavy metal concentrations in lichen of Mt. Rainier and Olympic national parks, Washington, USA. *Bulletin Environmental Contamination and Toxicology* **44**: 158–164.

Garty J. (1993) Lichens as biomonitors for heavy metal pollution. In: *Plants as Biomonitors*. (ed. B. Markert) pp. 193–263. VCH, Weinheim.

Garty J. (2000) Environmental and elemental content of lichens. In: *Trace Elements. Their Distribution and Effects in the Environment* (eds B. Markert & K. Friese) pp. 245–276. Elsevier Science Publication, Amsterdam.

Garty J., Galun M., Fuchs C. & Zisapel N. (1977) Heavy metals in the lichen *Caloplaca aurantia* from urban, suburban and rural regions in Israel (a comparative study). *Water, Air and Soil Pollution* **8**: 71–188.

Geiser L. H., Derr C. C. & Dillman K. L. (1994) Air quality monitoring on the Tongass National Forest, Appendices A1–C1 pp. 1–85. USDA-Forest Service, Petersburg.

Gough L., Jackson L. & Sacklin J. (1988a) Determination of baseline element composition of lichens. II. *Hypogymnia enteromorpha* and *Usnea* spp. at Redwood National Park, California. *Water, Air, and Soil Pollution* **38**: 169–180.

Gough L., Severson R. & Jackson L. (1988b) Determination of baseline element composition of lichens. I. *Parmelia sulcata* at Theodore Roosevelt National park, North Dacota. *Water, Air, and Soil Pollution* **38**: 157–167.

Herpin U., Markert B., Siewers U. & Lieth H. (1995) *Monitoring der Schwermetallbelastung in der Bundesrepublik Deutschland mit Hilfe von Moosanalysen*. Forschungsbericht 108, 02, 087, UBA – FB–125,. Berlin.

Jeran Z., Jacimovich R., Batic F., Smodis B. & Wolterbeek H. Th (1996b) Atmospheric heavy metal pollution in Slovenia derived from results for epiphytic lichens. *Fresenius Journal Analytical Chemistry* **354**: 681–687.

Jeran Z., Jacimovich R., Smodis B. & Batic F. (1996a) The use of lichens in atmospheric trace element deposition studies in Slovenia. *Phyton* **36**: 91–94.

Kabata-Pendias A. & Dudka S. (1991) Trace metal contents of *Taraxacum officinale* (dandelion) as a conventional environmental indicator. *Environmental and Geochemical Health* **13**: 108–113.

Keane B., Collier M. H., Shann J. R. & Rogstad S. H. (2001) Metal content of dandelion (*Taraxacum officinale*) leaves in relation to soil contamination and airborne particulate matter. *Science of the Total Environment* **281**: 63–78.

Klein R. & Paulus M. (1995) *Umweltproben für die Schadstoffanalytik Im Biomonitoring*. Gustav Fischer Verlag, Jena.

Kovac M., Turcsanyi G., Nagy L., Koltay A., Kaszab L. & Szoeke P. (1990) Element concentration cadasters in a *Quercetum petraeae-cerris* forest. In: *Element Concentration Cadasters in Ecosystems* (eds H. Lieth & B. Markert) pp. 255–265. VCH, Weinheim.

Kovac P., Turcsanyi G., Penksza K., Kaszab L. & Szöke P. (1993) Heavy metal accumulation by ruderal and cultivated plants in a heavily polluted district of Budapest. In: *Plants as Biomonitors*. (ed. B. Markert) pp. 495–505. VCH, Weinheim.

Krivan V., Hausbeck R. & Schaldach G. (1987) Deposition of heavy metals on the surface of spruce needles in Southwest Germany. *Fresenius Zeitschrift für Analytische Chemie* **327**: 19.

Krivan V. & Schaefer F. (1989) Surface deposition on spruce needles as a possible indicator for the degree of heavy metal pollution of the atmosphere. *Fresenius Zeitschrift für Analytische Chemie* **333**: 726.

Kubin E. (1990) A survey of element concentrations in the epiphytic lichen *Hypogymnia physodes*. In: *Acidification of Finnland* (eds P. Kuppi, P. Antilla & K. Kenttamies) pp. 421–446. Springer-Verlag, Berlin.

Kuleff I. & Djingova R. (1984) The dandelion (*Taraxacum*

officinale) – A monitor for environmental pollution? *Water, Air and Soil Pollution* **21**: 77–85.

Laaksovirta K. & Olkkonen H. (1977) Epiphytic lichen vegetation and element contents of *Hypogymnia physodes* and pine needles examined as indicators for air pollution at Kokkola, W. Finland. *Annale Botanika Fennici* **14**: 122–130.

Lieth H. & Markert B. (1985) Concentration cadasters of chemical elements in contrasting ecosystems. *Naturwissenschaften* **72**: 322–324.

Lieth H. & Markert B. (1986) Element-Konzentrations-Kataster für Böden und einige Pflanze in Walchsee/Österreich, Veröffentlichung der ETH-Zürich. Stiftung Rübel. *Festschrift Landolt* **70**: 326–342.

Lieth H. & Markert B. (1988) The establishment of chemical concentration cadasters for ecosystems (ECCE) in the different vegetation zones of the earth. *Biology International* **16**: 7–11.

Markert B. (1987) Interelement correlations in plants. *Fresenius Zeitschrift für Analytische Chemie* **329**: 462–465.

Markert B. (1989) Distribution of chemical elements in *Vaccinium myrtillus* (blueberry) – basic problems for representative sampling of plants for multi-element analysis in ecosystems. *Fresenius Zeitschrift für Analytische Chemie* **333**: 11–14.

Markert B. (1992) Establishing a 'reference plant' for inorganic characterization of different plant species by chemical fingerprinting. *Water Air and Soil Pollution* **64**: 533–538.

Markert B. (1993) Occurrence and distribution of chemical elements in plants, outlook and further research plans. *Toxicological and Environmental Chemistry* **40**: 31–41.

Markert B. (1994) The Biological System of the Elements (BSE) for terrestrial plants (glycophytes). *Science of the Total Environment* **155**: 221–228.

Markert B. (1996) *Instrumental Element and Multi-Element Analysis of Plants*. John Wiley and Sons, Chichester.

Markert B., Herpin U., Siewers U., Berlekamp J. & Lieth H. (1996) The German heavy metal survey by means of mosses. *Science of the Total Environment* **182**: 159–168.

Marr K., Fyles H. & Hendershot W. (1999) Trace metals in Montreal urban soils and the leaves of *Taraxacum officinale*. *Canadian Journal Soil Science* **79**: 385–387.

Percy K. S. & Borland A. (1985) Multivariate analysis of element concentrations in *Sphagnum magellanicum* brid. in the maritime provinces, Canada. *Water, Air and Soil Pollution* **25**: 331–338.

Pfeifer H. R., Derron M. H., Rey D., Schlegel C., Atteia O., Dalla Piazza R., Dubois J. P. & Mandia Y. (2000) Natural trace element input to soil-sediment-water-plant system: examples of background and contaminated situations in Switzerland, Eastern France and North Italy. In: *Trace Elements Their Distribution and Effects in the Environment* (eds B. Markert & K. Frieze) pp. 33–86. Elsevier Science Publishers, Amsterdam.

Reis M. A., Alves A. C., Wolterbeeck H. Th, Verburg T., Freitas M. C. & Gouveia A. (1996) Main atmospheric heavy metal sources in Portugal by biomonitor analysis. *Nuclear Instruments and Methods in Physical Research* **B109/110**: 493–497.

Rühling A. & Tyler G. (1968) An ecological approach to the lead problem. *Botanika Notiser* **121**: 321–342.

Rühling A. & Tyler G. (1969) Ecology of heavy metals – a regional and historical study. *Botanika Notiser* **122**: 248–259.

Rühling A. & Tyler G. (1970) Sorption and retention of heavy metals in the woodland moss – *Hylocomium splendens* (Hedw). *Oikos* **21**: 92–97.

Rühling A. & Tyler G. (1971) Regional differences in the deposition of heavy metals over Scandinavia. *Journal Applied Ecology* **8**: 497–507.

Rühling A. & Tyler G. (1973) Heavy metal deposition in Scandinavia. *Water, Air, and Soil Pollution* **2**: 445–455.

Rule J. (1994) Use of small plants as trace element phytomonitors, with emphasis on the common dandelion, *Taraxacum officinale*. In: *Biogeochemistry of Trace Elements* (ed. D. Adriano, Z-S. Cheng & S-S. Yang) pp. 627–654. Science and Technology Letters, Bradford.

Sawidis T. & Heinrich G. (1992) Cesium-137 monitoring using lichens and mosses from Northern Greece. *Canadian Journal of Botany* **70**: 140–144.

Scheppard S. C. & Evenden W. G. (1990) Characteristics of plant concentration ratios assessed in a 64-site field survey of 23 elements. *Journal Environmental Radioactivity* **11**: 15–36.

Scholl G. (1987) Grundlagen des Verfahrens der standartisierten Graskultur zur Messung der Wirkdosis von Luftverunreinigungen. *VDI-Bericht* **605**: 287–299.

Sloof J. E. & Wolterbeek H. Th (1991a) National trace element air pollution monitoring survey using epiphytic lichens. *Lichenologist* **23**: 139–165.

Sloof J. E. & Wolterbeek H. Th (1991b) Pattern in trace elements in lichens. *Water, Air, and Soil Pollution* **57/58**: 785–791.

Sloof J. E. & Wolterbeek H. Th (1993) Interspecies comparisons of lichens as biomonitors of trace element air pollution. *Environmental Monitoring Assessment* **25**: 149–157.

Steinnes E. (1993) Some aspects of biomonitoring of air pollutants using mosses, as illustrated by the 1976 Norwegian survey. In: *Plants as Biomonitors*. (ed. B. Markert) pp. 381–394. VCH, Weinheim.

Tyler G. (1989) Uptake, retention and toxicity of heavy metals in lichens. *Water, Air, and Soil Pollution* **47**: 321–333.

Wagner G. (1987) Entwicklung einer Methode zur grossraeumigen Überwachung mittels standartiesierte Pappelnblattproben von Pyramidenpappeln (*Populus nigra* 'Italica') am Beispiel von Blei, Cadmiun und Zink. In: *Beiträge Zur Umweltprobebank*, No.5 (eds M. Stoeppler & H. W. Duerbeck) p. 412. Juel. Spez.

Wagner G. & Müller P. (1979) Fichten als Bioindikatoren für die Immissionsbelastung urbaner Ökosysteme unter besonderer Berücksichtigung von Schwermetallen. *Verhandlunge der Geselschaft für Oekologie* **7**: 307–314.

Winter S., Wappelhorst O., Leffler U. S., Korhammer S. & Markert B. (1999) Biomonitoring in Städten mit *Taraxacum officinale* Web. (Loewenzahn) – Eine Studie in der Stadt Zittau, Sachsen. In: *Ökotoxicologie/ Ökosystemare Ansätze und Methoden* (eds J. Oehlmann & B. Markert) pp. 238–247. ECOMED-Verlaggesellschaft, Landsberg.

Wittig R. (1993) General aspects of biomonitoring heavy metals by plants. In: *Plants as Biomonitors*. (ed. B. Markert) pp. 3–27. VCH, Weinheim.

Wolterbeek H. Th, Kuik P., Verburg T., Herpin U., Markert B. & Thoeni L. (1995) Moss interspecies comparisons in trace element concentrations. *Environmental Monitoring Assessment* **35**: 263–286.

Zimmermann R. D. (1989) Erste Ergebnisse einer Ringanalyse zur Erstellung eines internen Buchenblatt-Referenzmaterialien für Ökosystemuntersuchungen. *Fresenius Zeitschrift für Analytische Chemie* **334**: 323–325.