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REGULAR ARTICLE

Multivariate statistical analysis of nutrients and trace elements in plants and soil from northwestern Russia

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Abstract Results are summarized of several field and greenhouse experiments designed to estimate differences in the ability of some plant species to take up from soil essential nutrients and various trace elements and transfer them from roots to upper plant parts. Instrumental neutron activation analysis was used to determine concentrations of 22 elements in plant and soil samples. Correlation and principal component analysis were applied for interpreting a large volume of experimental results. In many cases there was no statistically significant positive correlation between element concentrations in soil and concentrations of these elements in plants. Moreover, relationships between elements were often different in soil and in different plant parts, thereby suggesting quite different element behaviours in soil and in plants. Our experimental results and data published in the literature revealed that macro- and trace element concentrations might serve as a specific indicator of plant taxonomy, thus allowing for differentiation of the plants in accordance with concentrations of certain elements in roots or in leaves. Short-term variations in concentrations of elements typical for different plant species and factors affecting these variations indicated that diurnal dynamics of plant element concentrations were regular and species-specific.

Keywords Correlation and principal component analysis · Element uptake by different plant species · Relationships between elements in soil and in different plant parts · Short-term variations in plant element concentrations

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Introduction

During the last half-century, the amount and quality of analytical data on the distribution of various elements in different plants and in soils have increased greatly as new and improved analytical techniques have been developed and applied to environmental studies. Consequently, much new knowledge has emerged about the biological importance of more and more chemical elements. There is good reason to believe that many more elements, regardless of their concentrations, are necessary for plants. At the moment, there are insufficient experimental and analytical results to support this assumption.

Data on the concentration of just one or another element in a particular plant species does not provide sufficient information on the biogeochemistry of the element. It is more important to assess specific relationships between different macro- and trace elements typical for the plants. This requires an application of detailed statistical treatment of the experimental data. Until now there are different opinions on the use of multivariate statistical methods in environmental studies, varying from sceptical such as "complex and to some extent formal" (Hanson et al. 1993) to very optimistic like "the best tool for interpretation of environmental data and understanding the state of the environment" (Simeonov et al. 2000).

There is a wide variety of multivariate statistical methods that take account of, or specifically focus on, the various relationships between variables (Hand and Taylor 1987). Multivariate statistical methods have many applications in different environmental studies. They may be particularly useful when there is a large volume of experimental results and sometimes they provide insight into the multidimensional patterns in the data that would be overlooked with univariate analyses (Obuchowski 2005). The goal of such a statistical interpretation of the data is to try to make some assumptions on observed variations in the data and biogeochemical processes controlling the changes in element concentrations, thus raising the knowledge on the environmental behaviour of different elements. It should be noted, however, that interpretation of a large volume of the experimental results may represent certain problems.

The objectives of this paper are (1) to study the relationships between trace and macro-elements in soil and plants collected by one of the authors (I. Shtangeeva) during several years, (2) to summarize results of field and greenhouse experiments in order to estimate differences in the ability of some native and cultivated plant species to take up from soil essential nutrients and various trace elements and to transfer the elements from roots to upper plant parts, and (3) to assess short-term variations in plant element concentrations specific for particular plant species.

Materials and methods

Element analysis

Data used for this report were taken from our experimental studies. Plants and soils were collected over several years in northwestern Russia. In addition, greenhouse pot experiments were carried out to study the uptake of macro- and trace elements by different plant species. Instrumental neutron activation analysis (INAA) was used to determine element concentrations in the soil and plant material. This method provides a good opportunity to determine with high sensitivity and accuracy a wide range of elements in various environmental samples (Witkowska et al. 2005). INAA of plants and soils may be performed directly, without additional sample treatment, thus reducing the level of analytical errors that might arise during sample preparation for elemental analysis. The only necessary condition is to carefully wash plants just after sampling to remove from the plant surface dust and fine soil particles. The washing of plants is an essential step in the elemental analysis, especially for the elements that have lower concentrations in plants than in soil. In most cases it is quite enough to carefully rinse plants after sampling. However, sometimes analysts may go too far in this respect and use very strong reagents to wash the plant samples (Wyttenbach et al. 1994; Wyttenbach and Tobler 1998). As a result, cell walls of the plant may be destroyed and there will be no longer an ordinary plant, but the plant sample will represent something artificial. It is clear that the apoplast (an external layer of the plant cells) plays an important role in the exchangeable processes between plant roots and soil in the rooted zone, especially in the root-soil contact zone (rhizosphere). The lack of quantitative information about an element in the layer may lead to incorrect results.

Statistical analysis

Data analysis was performed using STATISTICA for Windows 6.0 workpackage. To estimate the relationships between different elements we used correlation analysis. Additionally, principal component analysis was applied to experimental data to give a better insight into the bioaccumulation of various elements in different plant species and to assess the contribution of specific factors that may have an effect on soil/plant interactions.

Results

Correlation between elements in soil and in plants

The application of routine statistical methods is often limited by the fact that geochemical data rarely follow a normal distribution. The distribution of many elements in the environment is closer to lognormal (strong right-skewness). Moreover, statistical tests indicate that in most cases the data even does not pass a lognormal distribution (Reimann and Filzmoser 2000). Thus, to detect and exclude extreme (suspicious) values, as a first step of data analysis the raw data were carefully checked.

For our calculations we used only data on element concentrations in plants grown in soil. There are certain reasons for choosing such material. In fact, relationships between elements in soil-grown plants and in plants grown in various liquid media, including nutrient solutions, may be rather different. For example, in soil-grown plants, correlation between Ca in roots and leaves may be statistically significant and positive. In water-grown plants, the correlation may be statistically significant and negative (Shtangeeva 2005). This means we can hardly use results of experiments performed with plants grown in any artificial liquid medium to predict behaviour of elements in plants growing in natural conditions.

Among other statistical methods, the most common, straightforward, and of wide practical application for analysis of biogeochemical data is calculation of relationships among variables. Knowledge of relationships between two or several variables (generally, these are concentrations of macro- and trace elements in different samples) allows for preliminary assumptions on the biogeochemistry of these elements. For our calculations we used Pearson correlation coefficients that are less affected by normality of element distributions. Only statistically significant correlations with $P<0.05$ or better that are not affected by outlying values are discussed here.

Correlation analysis of element concentrations of two native plant species, couch-grass (*Elytrigia repens* L.) and plantain (*Plantago major*) collected from the same site showed that there was no statistically significant correlation between concentrations of 22 elements determined by NAA in the rhizosphere soil and concentrations of these elements in roots of plantain. On the other hand, correlation between concentrations of such elements as Fe, Sc, and Yb in soil and in roots of couch-grass was statistically significant and positive.

There were rather different relationships between the concentration of a particular element in roots and in leaves of couch-grass and plantain. Only Co exhibited a statistically significant and positive correlation in roots and leaves of both these plants. Therefore, this metal was easily transferred from roots to leaves of the plants. In the 'root-leaf' system of plantain statistically significant positive correlations were found for Ba, Rb, and Zn. In couch-grass, there was a positive ($P<0.05$) correlation between K content in roots and in leaves.

Differences in element uptake by different plant species

There are certain genotypic differences in the ability of different plants to take up and translocate various elements. Among the main macronutrients, K, Na and Ca have been the focus of numerous studies (Schlesinger 2005). These three elements are probably the most interesting plant nutrients. K^+ plays a unique role in all living cells. This macronutrient is required in high concentrations for many plant species; it is the most abundant inorganic cation in plant cells (Schachtman and Liu 1999). Compared to K, Na is essential only for certain plant species (Brownell 1979) but can also promote plant growth in relatively low concentrations (Marschner 1995). Na^+ toxicity is a principal component of the deleterious effects associated with salinity stress (Almansouri et al. 2001). On the other hand, sodium was classified as so-called "functional nutrient" that is needed for maximal biomass growth for many plants. It demonstrated ability to replace K in a number of ways, such as being an osmoticium for cell enlargement and as an accompanying cation for long-distance transport (Subbarao et al. 2003). Calcium is an essential plant nutrient which is required for various structural roles in the cell wall and membranes. It is a counter-cation for inorganic and organic anions in the vacuoles. Cytosolic Ca^{2+} concentration is an obligate intercellular messenger coordinating responses to numerous environmental stresses (White and Broadley 2003).

Table 1 shows concentrations of K, Na, and Ca in several plant species, both native and cultivated. Except for plantain, concentration of K in leaves of all these plants is higher compared to K content in roots. Interestingly, concentration of K in leaves of cultivated plants is higher than in leaves of native grasses.

Element distributions in different plant parts

Generally, concentrations of many elements, especially micro-nutrients in plant roots are higher than

Table 1 Mean concentrations of K, Na, and Ca in roots and leaves of native and cultivated plants

	K, %		Na, %		Ca, %	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Plantain	5.92±0.84	3.71±0.76	0.14±0.04	0.06±0.03	0.65±0.07	1.86±0.45
Couch-grass	1.45±0.59	3.38±0.65	0.23±0.05	0.06±0.02	0.40±0.11	0.47±0.08
Wheat	2.93±0.96	6.68±1.55	0.77±0.26	0.06±0.01	0.78±0.20	0.86±0.31
Oats	2.87±1.53	6.68±1.58	0.88±0.59	0.39±0.45	0.54±0.36	0.41±0.09
Barley	2.72±1.23	6.82±2.46	0.81±0.50	0.55±0.43	0.30±0.11	0.47±0.14
Rye	2.13±0.81	5.54±1.88	0.91±0.24	0.13±0.02	0.49±0.14	0.78±0.23

those in upper plant parts (Cardwell et al. 2002; Fritioff 2005; Shtangeeva 2008). It is assumed that plants can keep the elemental composition of leaves at a sufficiently stable level (Granato et al. 2004). Different plant species differ, however, in their abilities to take up and accumulate various elements (Rai et al. 1995). In particular, our results showed that concentrations of many elements in leaves of native plants (couch-grass and plantain) collected simultaneously from the same place differed significantly. For example, the concentration of U was lower and concentrations of K, Ca, Cr, Fe, Co, Sr, Sb, Ba, La, Sm, Eu, and Hf were higher in leaves of plantain than in leaves of couch-grass. The same was observed in the plant roots. Compared to plantain, roots of couch-grass had larger amounts of Cs, Eu, Na, Sb, Yb, and Zn, but lower amounts of Ca, K, and Sr (Table 2).

Table 2 Mean concentrations of elements (mg kg^{-1}) in couch-grass and plantain

Element	Couch-grass		Plantain	
	Roots	Leaves	Roots	Leaves
Na,%	0.23±0.05[a]	0.06±0.02	0.14±0.04*	0.06±0.03
K,%	1.4±0.6[a]	3.4±0.6	5.9±0.8* [b]	3.7±0.8*
Ca,%	0.40±0.11	0.47±0.08	0.65±0.07* [b]	1.9±0.4*
Sc	0.34±0.01[a]	0.06±0.01	0.36±0.08	0.13±0.07
Cr	8.0±2.3[a]	3.5±0.6	5.8±1.2	5.3±0.9*
Fe	1700±237[a]	307±28	1870±840[b]	562±239*
Co	0.96±0.27[a]	0.19±0.07	0.77±0.10[b]	0.39±0.13*
Zn	80±10[a]	49±3	48±5*	49±3
Br	3.8±0.9[a]	1.4±1.3	4.3±3.1	4.6±6.1
Rb	4.4±0.8	4.1±2.4	6.7±2.8	3.9±2.3
Sr	16±6	11±4	42±7*	40±9*
Sb	0.29±0.08[a]	0.14±0.01	0.19±0.03*	0.26±0.05*
Cs	0.67±0.30[a]	0.09±0.02	0.26±0.06* [b]	0.09±0.07
Ba	31±11[a]	14±4	56±18*	51±9*
La	1.7±0.2[a]	0.58±0.13	1.6±0.3	1.1±0.5*
Sm	0.27±0.06[a]	0.07±0.01	0.20±0.07	0.15±0.07*
Eu	0.021±0.011[a]	0.0019±0.0005	0.0064±0.0014* [b]	0.0031±0.0007*
Yb	0.18±0.08[a]	0.08±0.02	0.06±0.02*	0.14±0.06
Hf	0.60±0.04	0.53±0.10	0.56±0.21	0.76±0.15*
Ta	0.09±0.03[a]	0.03±0.01	0.04±0.01*	0.04±0.02
Th	0.43±0.04[a]	0.14±0.01	0.38±0.08	0.25±0.13
U	0.16±0.19	0.14±0.08	0.11±0.08	0.09±0.01*

*differences between species are statistically significant ($P<0.05$); [a] differences between roots and leaves of couch-grass are statistically significant ($P<0.05$), [b] differences between roots and leaves of plantain are statistically significant ($P<0.05$)

Discussion

Element behaviours in soil and in plants

Comparison of data on the correlation between different elements showed that there were both similarities and differences in element relationships in soil and in plants growing in the soil. On the one hand, it is clear that there may be similar relationships between element concentrations in plants and in soil collected from the same place. On the other hand, concentration of an element in different plant parts is not a simple and direct function of the element content in soil.

The fact that some pairs of elements are well correlated may indicate a similar ability of the elements to enter into the plant (Kment et al. 2005). This might be explained by similar chemical characteristics of these elements. For example, correlation between Fe and Co (chemically similar elements) in soil was positive ($P<0.001$). In roots and leaves of couch-grass, correlation between these two metals was also positive and statistically significant (Fig. 1). However, there was no correlation between Co and Fe in different parts of plantain (Fig. 2). This may serve as a good illustration of the different ability of these plant species, which belong to different classes, to transfer similar metals. Correlation between Sc and Rb in soil and in roots of both couch-grass and plantain was positive ($P<0.01$). On the other hand, in leaves there was no correlation between Sc and Rb, thus, probably suggesting different ways of translocating these two elements from roots to leaves.

Although plant samples were carefully washed just after sampling until they were free from any visible soil particles, there was a certain probability that similar relationship between a pair of elements in soil and in plant roots might result from surface contamination of the roots by fine soil particles. However, in this case we could expect similar behaviour of all or at least the most part of elements both in soil and roots, and this does not always happen. The relationships between elements in soil and in different plant parts may differ significantly. A good example of such differences might be the relationship between Na and Sc. There was a positive ($P<0.05$) correlation between Na and Sc in soil. Correlation between these two elements in roots of wheat seedlings grown in the soil was statistically significant and negative, and there was no correlation between Sc and Na in the plant leaves.

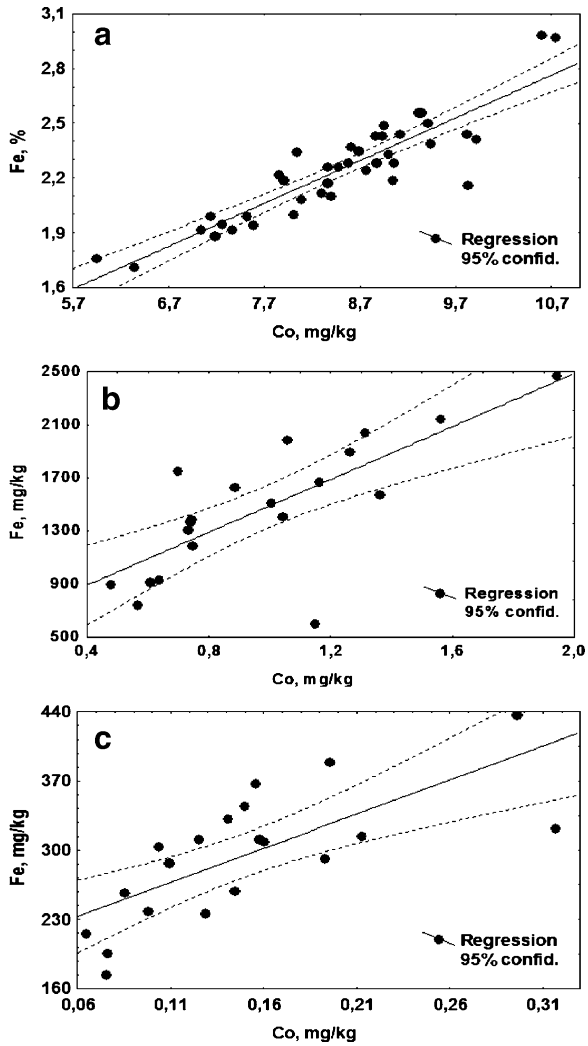

Fig. 1 Correlations between Fe and Co in soil (**a**), roots (**b**) and leaves (**c**) of couch-grass are $r^2=0.9$, $r^2=0.75$, and $r^2=0.72$, respectively ($P<0.001$)

Comparison of relationships between K and Na in soil and in plants growing in the soil showed that there was a significant positive correlation between K and Na in soil, while in plant roots and leaves this correlation was negative ($P<0.01$). This may be explained by different behaviour of these two metals in soil and in plants. We may assume that positive correlation between K and Na in soil is based on similar chemical characteristics of K^+ and Na^+ ions. On the other hand, numerous studies demonstrated clear selectivity in K^+–Na^+ uptake by plants (Schachtman and Liu 1999; Santa-Maria and Epstein 2001; Cuin and Shabala 2006). Plants favour influx, net uptake and

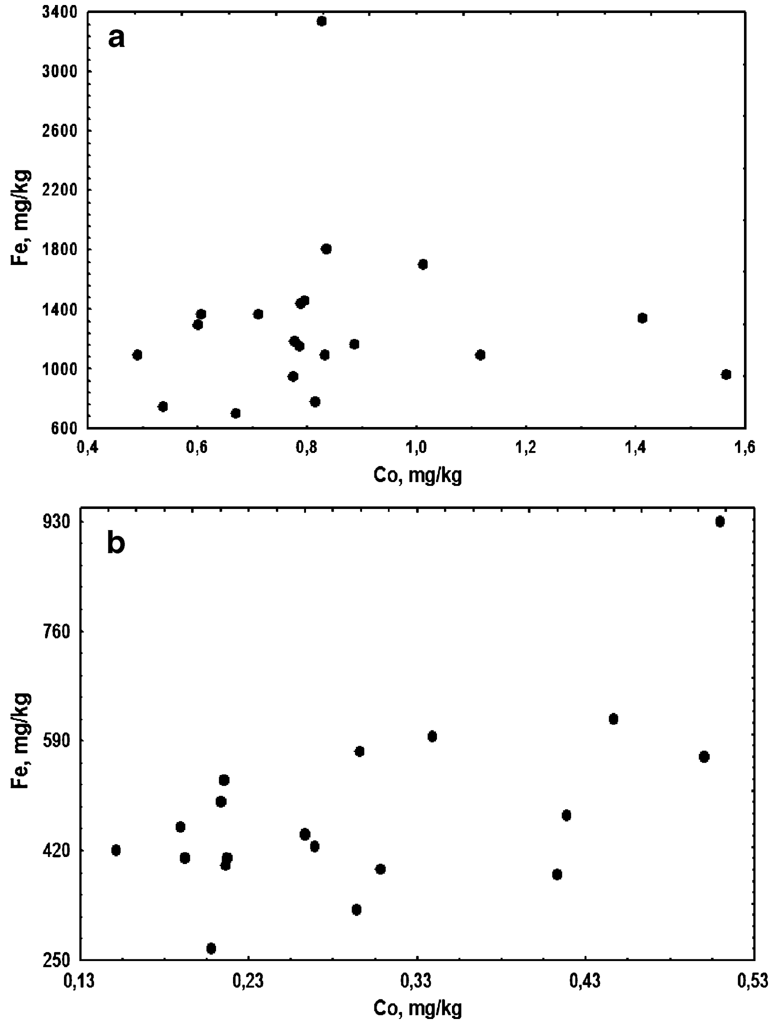

Fig. 2 Relationships between Fe and Co in roots (**a**) and leaves (**b**) of plantain

translocation of K^+ over that of Na^+ at low (normal) external concentrations.

When grown under identical conditions, Ca concentration of various plant species can differ markedly (Table 1). This reflects contrasting Ca requirements of different plants and different abilities of the plants to translocate Ca from roots to leaves. A large proportion of these differences may be attributed to the phylogenetic division between Monocotyledoneae and Dicotyledoneae plants. Dicotyledoneae usually have a higher $[Ca]_{leaves}$ than Monocotyledoneae (Thompson et al. 1997; Broadley et al. 2003). This is in a good agreement with our results.

Numerous studies have shown that plant nutrient and trace element concentrations may be different in different plant species (Fismes et al. 2005; Burgos et al. 2008). It appears that the elemental composition of plants may be used as a specific indicator of plant taxonomy. A.P. Vinogradov (1953) was probably the first researcher to make such a conclusion, although at that time the level of analytical techniques used for determination of plant element concentrations was rather poor. Recently this finding was confirmed by other researchers (Shtangeeva 1994; Broadley et al. 1999; Willey and Fawcett 2006).

As an example, Table 2 illustrates the distribution of different elements in couch-grass and plantain collected simultaneously from the same sites. As can be seen, concentrations of most elements (Ba, Br, Co, Cr, Cs, Eu, Fe, La, Na, Sb, Sc, Sm, Ta, Th, Yb, and Zn) in roots of couch-grass are statistically significantly higher than those in leaves. The only exception is K. Its concentration in leaves of this plant is higher ($P<0.01$) than the K content in roots. On the other hand, in leaves of plantain, only concentrations of Co, Cs, Eu, Fe, and K are higher ($P<0.01$) and concentration of Ca is statistically significantly lower than those in roots.

Figure 3 shows results of a principal component analysis (PCA) performed on the basis of element concentrations in plantain and couch-grass. Roots of the two plant species are well separated from each other (Fig. 3a). PC1 explaining 27% of the total variance is responsible for this separation. The main loading values in the PC1 are obtained for K, Ca, Na, Cs, Rb, Zn, Eu, and Ba. As shown in Table 2, concentrations of many of these elements in roots of plantain and couch-grass are rather different. Although concentrations of many elements in leaves of these plants also differ significantly, no similar good separation is found between leaves of couch-grass and plantain (Fig. 3b). Rb, Cs, U, Zn, and Th are highly correlated with the first PC that is responsible for the separation. Among these elements, only the concentration of U is higher ($P<0.05$) in leaves of couch-grass compared to U content in leaves of plantain.

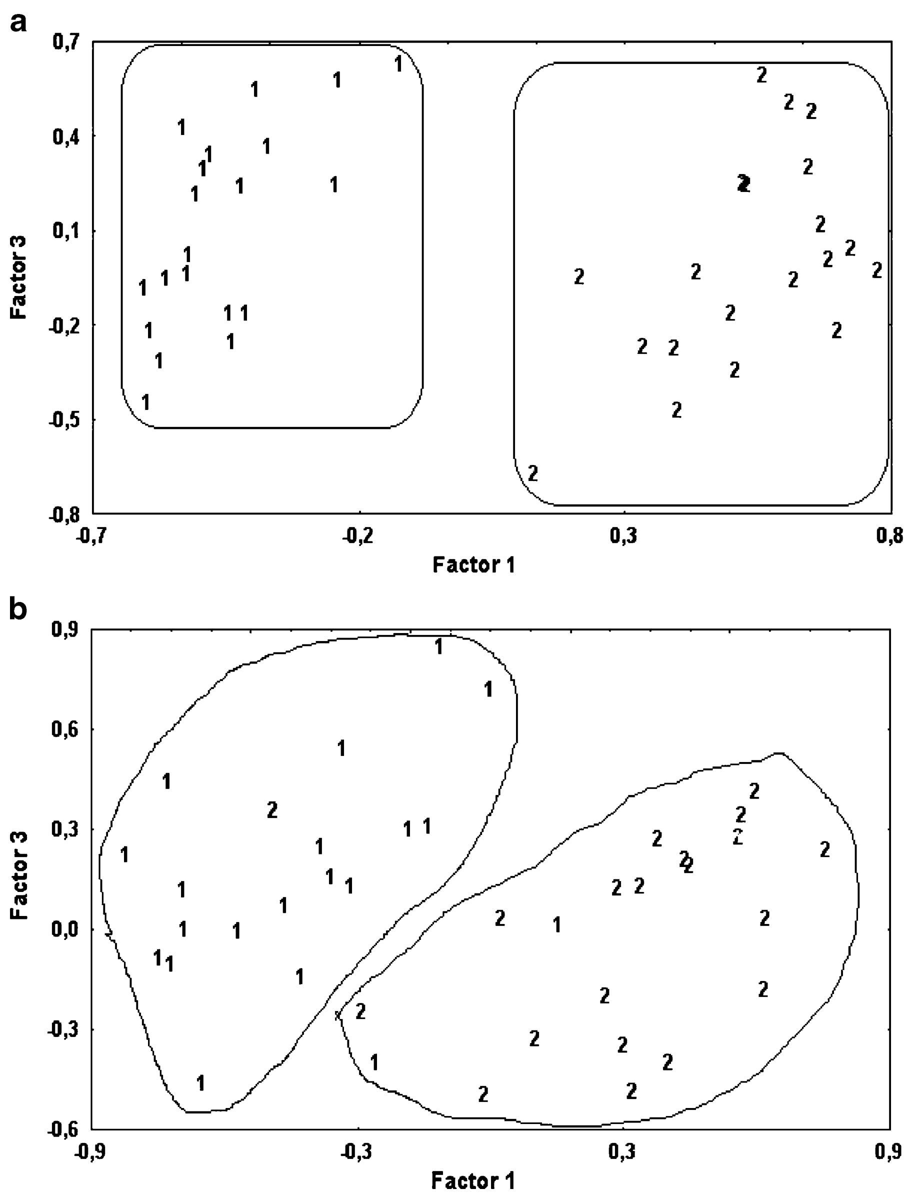

Fig. 3 Score plot of the first and third principal components of PCA of roots (**a**) and leaves (**b**) of couch-grass (1) and plantain (2)

It is assumed that significant differences in element concentrations and relationships between elements found in roots and leaves of these plant species may be associated with differential active transport of ions (or metal-organic complexes) into the xylem of the plants. Analysis of recent literature shows that plants growing in the same place can take up major and minor nutrients to different concentrations and that element uptake by each species is regulated by the plant genotype (Willey and Fawcett 2006). Couch-grass and plantain belong to two classes (couch-grass is Monocotyledoneae, family Graminaceae and plantain is Dicotyledoneae, family Plantaginaceae). Therefore, such differences might be expected. Plants not just reflect soil nutrient availability, but the uptake process is mainly dependent upon the genetic capability of particular plant species for maintaining a certain level of element concentrations in different plant parts.

Short-term variations in element concentrations of different plant species

Long-term (seasonal) variations in plant element concentrations are well-known and described in the literature (Hagemeyer et al. 1992; Mohamed 1999; Leblond et al. 2004). Much less is known about short-term variations (e.g., hours) in plant element concentrations.

It has been reported (Walter and Schurr 2005) that, in spite of certain inter-species differences, diurnal changes in the plant growth rate are generally significantly larger than the changes in mean growth rate of the plant from day to day. This shows that processes controlling the plant growth variations within 24 h are stronger than processes acting on a day-to-day scale.

Some publications have reported that uptake of K by plants is regulated by light (Lowen and Satter 1989; Kim et al. 1992; Suh et al. 2000) and the circadian clock (Kim et al. 1993). We may assume that uptake of other elements by plants is also controlled by light and the biological clock and these variations are species-specific. Plant growth and development, including uptake and translocation of macro- and micronutrients depend on various factors. Among abiotic factors, temperature and level of lighting are probably the most important.

Figure 4 shows diurnal variations in Rb concentrations in wheat seedlings grown in pots. The

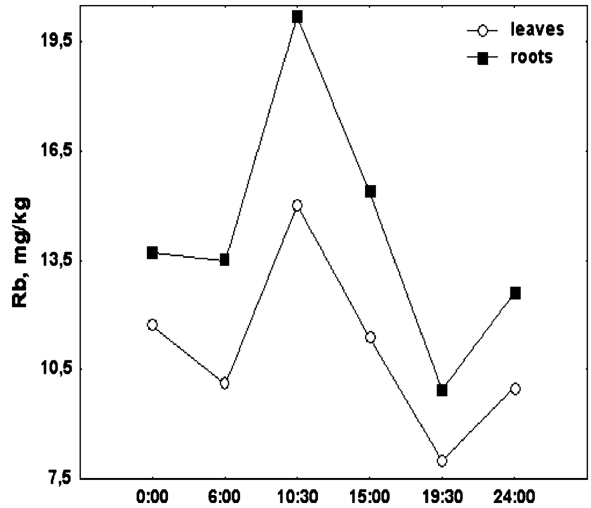

Fig. 4 Diurnal dynamics of Rb concentrations in roots and leaves of wheat seedlings

variations are rather significant and very similar for both roots and leaves with a maximum at 10:30. Figure 5 illustrates diurnal dynamics in soil temperature and concentration of U in roots and leaves of couch-grass and plantain growing in U contaminated soil. There is a clear maximum in U uptake by couch-grass, which correlates well with soil temperature. However, the highest concentration of U in roots and leaves of plantain collected simultaneously is observed 4 h later. Similar significant and regular variations in concentrations of many other elements in roots and leaves of plants collected in the field have also been observed previously (Shtangeeva 1995).

Different plants have different sensitivity to temperature and photoperiod. The differences in diurnal dynamics of element concentrations in plants therefore may be expected.

Conclusions

Multivariate statistical methods applied to a large volume of experimental data on concentrations of macro- and trace elements in soil and in different plants showed that relationships between many elements in soil and in plants growing in the soil may be quite different. In particular, correlation between pairs of elements can differ significantly in soil and in different plant parts. There are certain genotypic differences in the ability of different plant

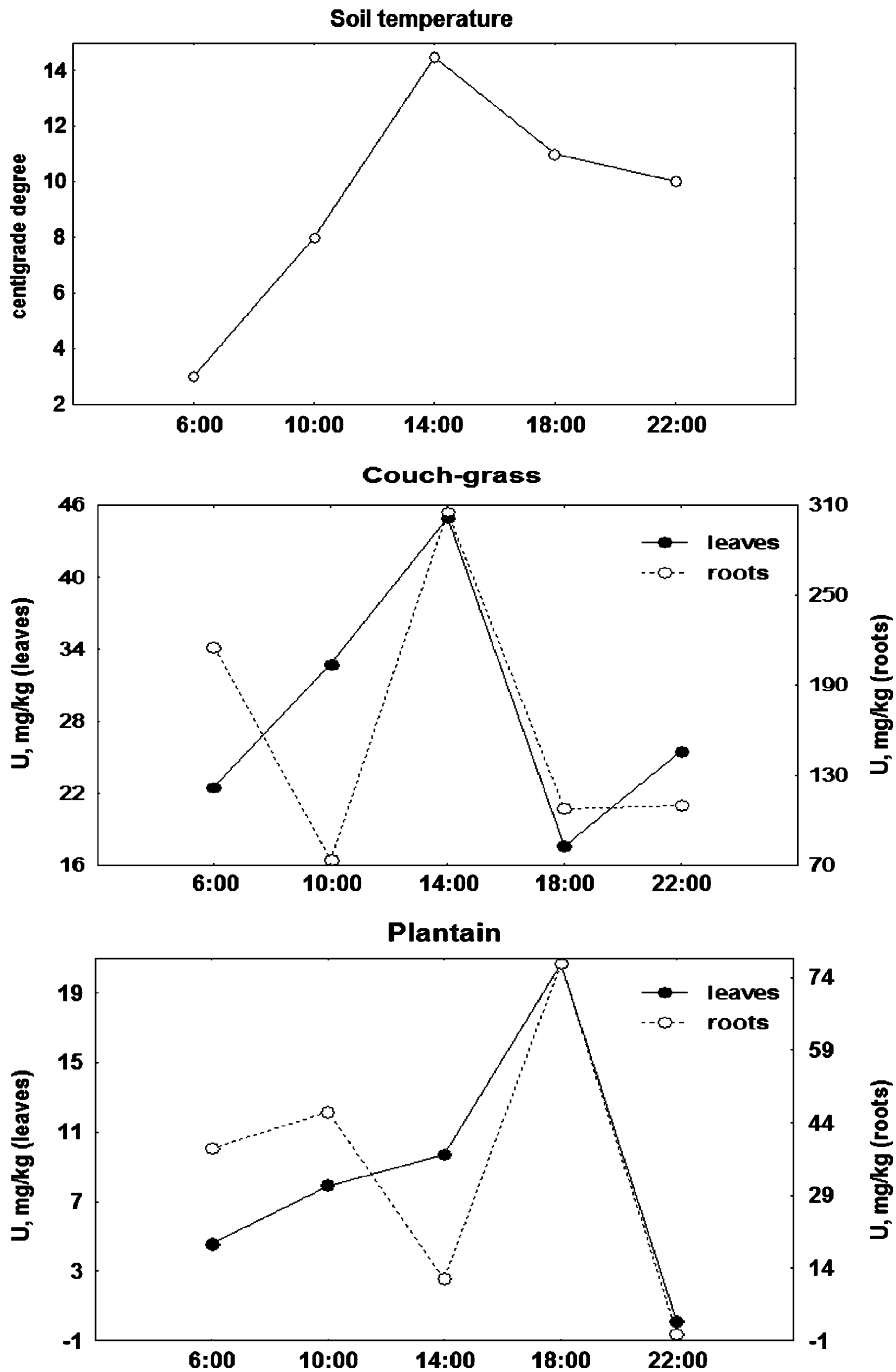

Fig. 5 Diurnal dynamics of soil temperature and U concentration in roots and leaves of couch-grass and plantain

species to take up and translocate various elements. Short-term (several hours) variations in concentrations of elements in roots and leaves may be significant, regular and quite different for different plants growing on the same site.

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