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Conditions to obtain results analysing a small amount of plant material by EDXRF

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Topsoil and plant material from two plots are investigated. One of the plots was fertilised with wastewater treatment sludge. Normalised intensities ($\mathbf{I_n}$) of Na, Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Mo, Cr, Br obtained by EDXRF measuring, with or without a Mylar film, the pressed pellets prepared from plant material ashed at 240 °C using different dilution factors (DF) are compared. Plant material included mainly *Festuca rubra* and *Calamagrostis epigejos*. $\mathbf{I_n}$ values of Mg, Cr, Ni, Na, Al, P, S, Cl significantly decreased when measuring with the Mylar film, those of Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr usually significantly decreased with greater dilution of material. It is quite possible to use DF = 0.25 for the preparation of pellets, but it is better to measure without the Mylar film. Despite the influence of plant sample preparation and measurement ways on $\mathbf{I_n}$, the following group of elements with higher than 1.3 times content in the plants of the fertilised plot compared to that of the background plot was distinguished: Zn, Ni, Cu, S, P, Si, Rb, Sr. The contents of these elements, except Si and Rb, also increase in soil of this plot.

Keywords: EDXRF X-ray intensity, plant pressed pellets, dilution factor, Mylar film, wastewater treatment sludge

INTRODUCTION

Like neutron activation, the non-destructive X-ray fluorescence analysis (XRF) has advantages in comparison with analytical procedures which involve wet acid digestion with subsequent determination by ICP-MS, ICP-ES [1–3], AAS [4]. Simple, rapid sample preparation, relatively short analysis time, safety for environment and health are the main advantages of XRF. Meanwhile poor sensitivity for important trace elements is the main factor restricting its application for the analysis of plants, because there is a high degree of scattering of the X-ray source by organic matrices [5]. So XRF is usually used for the analysis of abiotic samples, e. g. for the study of corrosion of Zn and Cu metal plates [6].

However, in the modern energy dispersive XRF equipment the linearly polarised X-rays are used to reduce the background scattering and the secondary targets optimise the excitation conditions. An example of such instrument is a SPECTRO XLAB EDPXRF spectrometer which was demonstrated to give good analytical results of non-organic elements in foliage [5]. It is one of the energy dispersive XRF spectrometers which uses polarisation and secondary targets (Barkla Al_2O_3, Bragg HOPG and Mo secondary target) and therefore might be suitable for plant analysis. This instrument is equipped with the XLABPro software which has TurboQuant for the pressed pellets calibration method. The SPECTRO XEPOS EDPXRF spectrometer has the same targets and uses the same software. The advantages of the polarisation and TurboQuant calibration method were described by Schramm and Heckel [7].

The basic equation of calibration implies a linear relationship between the intensity and the concentration [8]:

$$C_i = (a_{i,0} + a_{i,1} * I_i) * M.$$

Here C_i is the concentration of the element of interest, expressed as mg/kg or the percentage of dry matter; $a_{i,0_i}$ is the offset of the calibration curve; $a_{i,1}$ is the slope of the calibration curve; I_i is the net intensity of the element of interest expressed as counts per second; M is the correction term due to the matrix effects. The measured intensity of incoherent scattering may be used directly to compensate for matrix effects or indirectly for the determination of the effective mass absorption coefficient μ to correct for matrix effects [8]. The TurboQuant calibration method combines different procedures: calculation of the mass attenuation coefficient, using the extended Compton model and final calibration based on fundamental parameters. The normalised intensities $\mathbf{I_n}$ are obtained after the first two procedures.

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The pressed pellets are prepared by mixing of sample material with a binder. The ratio of the mass of material to the mass of material plus a binder is the dilution factor (DF). A too little mass of some plant material causes a problem, because sometimes the standard mixture recommended by manufacturers for the preparation of pressed pellets cannot be used and therefore the researcher is obliged to use lower DF values. There is a lack of information about the influence of DF values in preparation of pressed pellets for XRF from biological samples on the sensitivity or quality of analysis. The European Standard for determination of the elemental composition of soil and wastes by XRF [8] indicates that different dilution factors can be used.

Owing to the poor sensitivity of XRF, the pre-concentration of plant material by dry ashing can be used. On the one hand, this procedure reduces the weight of available plant material even more, but on the other hand, dry ashed plant is powdery material, so the pressed pellet prepared from it is brittle and the broken material can fall down into the inner system of excitation and detection and cause measurement problems. Both problems can be solved in two ways: 1) using the Mylar film to protect the instrument; 2) adding more binder, i. e. reducing DF values. However, according to Dick et al. [9] the measured XRF intensity in the analysis of specimens in aqueous media is strongly affected by the Mylar film and there is an increasingly critical absorbance level with a decreasing atomic number of the element under examination. Sharma et al. [10] have shown that the Mylar film has stopping powers for heavy ions up to copper, Shiomi-Tsuda et al. [11] analysed its stopping powers for photons, Damache et al. [12] for protons. The changes of XRF sensitivity due to the influence of the mentioned factors, e. g. the percentage of proton transmittance when pellets are analysed with thin-films in comparison with measurements without them, was studied by Hall et al. [13].

One of methodical aims of this experimental research was to find out the extent to which the Mylar film can decrease the detection possibility. The second aim was to test the influence of various DF on the XRF results without the Mylar film. To find out this influence, different plant species were analysed. Since normalised X-ray intensity ($\mathbf{I_n}$) is the primary information for the calculation of element concentrations and its higher values indicate better sensitivity of equipment [14], this research reveals the influence of the Mylar film and the dilution factor only on $\mathbf{I_n}$ values. Hence, there is no possibility to compare these results with the results of other researchers who present concentrations of elements in the same plants. In future, recalculations will be done using results obtained by analysis of respective certified reference materials (e. g. BCR-60, BCR-482) and samples of the WEPAL "Plant-analytical Exchange" (IPE) program. These samples will be mixed with the binder in the same proportion and analysed in the same way as the plant material for study.

Plant material was collected from two plots: one of them was fertilised with sewage sludge from the Vilnius Wastewater Treatment Plant; the second one was used as a background. So the third additional aim of this research was to analyse and characterize topsoil from these plots and reveal the most probable contaminants.

The sludge from the Vilnius Wastewater Treatment Plant was introduced in 1989 on part of the territory of the closed gravel quarry (coordinates are 54°47'25.53", 24°54'7.39") near Verkšionys. The first investigations of the contents of chemical elements in sludge and soil where it was introduced were done in 1989–1990 in the laboratory of the Institute of Geology using atomic emission spectrophotometry. This method has been successfully used in geochemical mapping of Lithuania until 2008–2009 [15, 16]. The contents of hazardous elements in sludge exceeded the background values of sandy soil of Lithuania [17].

In 2010 investigations of this territory were repeated. The present species composition and abundance of individuals of soil fauna (microarthropods) were also analysed in each of these plots. The analysed plant species can be subdivided into two groups: (1) representatives of *Poaceae* which have a long rhizome with two types of gemmae or which have long and strong truncal root favourable for wide spreading in sandy or gravelly soil; (2) cosmopolitan plants [18] with an abundant overground part which are found in all climatic zones and in various ecotopes. The main attention in this study is given to representatives of the first group *Festuca rubra* (FR), *Calamagrostis epigejos* (CE), *Elytrigia repens* (ER) (plant material collected from two plots was used). These plants are the first ones which appear in ecotopes that are disturbed due to some anthropogenic activity. They dominate in such habitats, help stabilise the substratum and form a favourable micro-climate for the establishment of other plants.

All these plants are perennial, have extensive distribution, and prefer sandy or gravelly soil. They can tolerate and accumulate chemical elements including contaminants [19]. Vascular plants can clean soil by involving the contaminants to material cycling [20–22].

EXPERIMENTAL

Measurement conditions

Measurement of pressed pellets prepared from both topsoil and plant samples was done by the same EDPXRF equipment SPECTRO XEPOS using the same TurboQuant for the pressed pellets method (X-LabPro software, version 4.5) and the same count time – 300 s. The excitation of all elements is always split into 3 single measurements using different targets. For chemical elements analysed in plants, the sequence was the following: (1) Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr were excited using a Mo secondary target (intense monochromatic nonpolarised X-rays) at 40 kV and 0.88 mA, (2) Mo was excited using the Barkla Al_2O_3 target (intense polychromatic polarised X-rays) at 49 kV and 0.70 mA, (3) Na, Mg, Al, Si, P, S, Cl, K, Ca were excited using the highly oriented pyrolytic graphite (HOPG) Bragg target (intense monochromatic polarised X-rays) at 17.5 kV and 2.00 mA. However, there are

differences between topsoil and plant sample preparation.

Topsoil

From each plot five composite topsoil samples were taken (the total number of samples was ten). Each composite sample consisted of five subsamples. Samples were air-dried, mixed and sieved through a 2 mm sieve. From each sample two 5 g subsamples were taken and milled by an MM 400 mill with zirconium oxide grinding jars and grinding balls (milling time 10 min, frequency 27 Hz). The ground material was mixed with a Licowax binder (4 g of material and 0.9 g of Licowax) and homogenised for 20 h. Then two pressed pellets of 32 mm diameter were prepared from each mixture.

The descending set of median relative standard deviations (RSD, %) calculated for pairs of pellets is the following: Cl and Mo – 18, Cu – 9.3, S – 7.1, Na – 6.8, Br – 5.6, Cr – 5.4, P – 5.0, Ca – 4.4, Mg – 3.8, Mn – 2.8, Ni and Sr – 2.5, K – 2.4, Al – 2.1, Si – 1.6, Rb – 1.5, Zn – 1.4, Fe – 1.0. Quality control has been performed since 2007 by participation in the International Soil-analytical Exchange (ISE) Program organised by Wageningen University [23, 24]. More than 40 ISE reference samples and other certified reference materials were used for the recalibration of results. The main geochemical characteristics of topsoil are in Table 1.

Table 1. **Geochemical characteristics of topsoil in background and fertilised plots**

Chemical elements	Background plot		Fertilised plot		European values	
	M_B	VK_B	M_F	VK_F	M_{NE}	M_{TE}
Al	28498	6.2	12668	38	47319	58219
Br	2.3	15.9	3.4	23	–	2.5*
Ca	13805	3.8	56075	45	9934	6590
Cl	226	13	403	23	80	180**
Cr	19	24	284	47	32	60
Cu	5.5	11	198	48	11	13
Fe	10042	4.3	21590	32	17065	24550
K	16449	2.9	10547	27	15605	15939
Mg	5296	7.6	5223	15	3437	4644
Mn	527	4.8	401	10	426	503
Mo	0.60	7.2	0.77	16	<2	0.62
Na	6338	3.6	3829	20	8977	5935
Ni	6.0	3.5	86	45	9.9	18
P	655	13	6639	46	820	559
Rb	53	0.9	35	17	67	86.8
S	311	22	1851	44	253	435
Si	370422	1.1	261293	24	331387	316457
Sr	71	1.4	104	22	110	130
Zn	23	16	548	48	43	52

Explanation. Geochemical characteristics of plots according to five sampling sites in each of them: M_B and M_F are median values (mg/kg), VK_B and VK_F are coefficients of variation (%) in the background plot and the fertilised plot, respectively. European values: M_{NE} are medians (mg/kg) in agricultural soil of Northern Europe [28], M_{TE} are medians in all European soil [29], except Br and Cl; * is average abundance in crust [30]; ** is average crust [31].

Plants

Plants were air-dried and primarily crushed in agate mortars. Then the plant material was pre-concentrated by dry ashing. Though according to Isaac and Benton Jones [25] only insignificant part of some chemical elements is lost during ashing of plants at higher than 400 °C temperature, other researchers indicate appreciable loss of some elements, e. g. according to Koch et al. [26] there is decrease of Cl, Br, Cr at higher than 200 °C temperature. Hence, our plant samples were ashed at experimental temperatures 200, 240 and 300 °C, but finally 240 °C temperature was chosen taking into account that the ignition temperature of peat is 240 °C [27].

The ashed plant material was milled by the MM 400 mill with zirconium oxide grinding jars and grinding balls (milling time 6 min, frequency 27 Hz). Then it was mixed with the Licowax binder in three main proportions: 1.5 g of material and 0.5 g of binder (DF = 0.75); 1 g of material and 1 g of binder (DF = 0.50); 0.5 g of material and 1.5 g of binder (DF = 0.25). Additional proportions were used for the analysis of *Festuca rubra* (FR) and *Calamagrostis epigejos* (CE): 2.0 g of material and 0.5 g of binder (DF = 0.80); 0.25 g of material and 1.5 g of binder (DF = 0.17). Then two pressed pellets of 20 mm diameter were prepared from each mixture. As a result, there were 45 pairs of identical pressed pellets prepared from plant material ashed at 240 °C.

The measurement of pellets prepared from plant material with different DF was done in two different ways: 1) in plastic cups with Mylar film protection; 2) in plastic cups without Mylar film protection. The thickness of the Mylar film was 2.5 μm. All samples were analysed for the determination of normalised intensities $\mathbf{I_n}$ of Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Mo, Br. The total number of measurements of pressed pellets was 180.

Then the mean value and relative standard deviation of $\mathbf{I_n}$ were calculated for each pair of identical pressed pellets measured in an identical way (either with or without the Mylar film). The descending sequence of median relative standard deviations (RSD, %) calculated for $\mathbf{I_n}$ values obtained for the pairs of pressed pellets measured without the Mylar film was the following: Br(7.4), Cr(7.3), Cu(6.5), Ni, Mo(6.1), Na(5.0), Si(3.9), Al(3.6), Zn(3.5), Cl(2.8), Fe(2.7), Sr, Rb, P(2.6), Mn(2.5), K(2.3), Mg, S(2.2), Ca(1.9). The respective sequence according to measurements with the Mylar film was as follows: Cr(16.9), Ni(11.8), Br(8.5), Mo(8.0), Na(5.8), Cu(5.6), Al(5.5), Fe(2.4), Mn(2.1), Cl(1.8), Mg, S(1.4), Si(1.3), P(1.2), Rb, Sr(1.1), Zn(0.9), Ca(0.8), K(0.7).

The 0.5 g amount of plant used for pressed pellets was considered as lack of material. $\mathbf{I_n}$ values when measuring the pressed pellets with DF = 0.25 without the Mylar film which were the basis for comparison are given in Table 2.

Table 2. **Normalised intensities of different plant material from different plots obtained measuring pellets with a dilution factor of 0.25 without the Mylar film**

Elements	Background plot		Fertilised plot	
	FR	CE	FR	CE
Cl	1342	998	1542	1926
S	605	720	1118	1390
P	406	502	587	730
Ca	523	405	621	381
K	325	292	475	462
Si	1565	1231	3288	2661
Sr	102	72	149	138
Zn	46	65	117	144
Rb	56	63	107	96
Fe	87	63	122	56
Mg	26	21	28	33
Mn	62	120	20	18
Cu	8.1	8.3	13	12
Br	32	36	20	18
Al	14	12	17	13
Na	10	9.8	9.7	9.6
Ni	7.2	7.0	11	7.8
Cr	5.9	6.0	6.1	5.4
Mo	5.6	5.1	3.8	4.8

Abbreviations: FR is *Festuca rubra*, CE is *Calamagrostis epigejos*.

RESULTS AND DISCUSSION

Geochemical variability of topsoil of study plots

The median values of K, Mo, Na, P, S in the background plot are similar to European median values [28] for Northern Europe and for all European topsoil [29] (Table 1). The values of Ca, Mg and Si are respectively higher; those of Al, Cr, Cu, Fe, Ni, Rb, Sr and Zn are lower. The contents of Br and Cl are similar to average abundance in the crust [30, 31]. Mann–Whitney U test revealed that the contents of Br, Ca, Cl, Cr, Cu, Fe, Mo, Ni, P, S, Sr and Zn in the topsoil of the fertilised plot are significantly ($p < 0.05$) higher than in the background plot, meanwhile the contents of Al, K, Mn, Na and Rb are significantly lower. According to the descending sequence of ratios of the content in topsoil of the fertilised plot in comparison with that of the background plot chemical elements can be arranged as follows: Cu(36)>Zn (24)>Cr(15)>Ni(14)>P(10)>S(6.0)>Ca(4.1)>Fe(2.2)>Cl(1.8)> Br, Sr(1.5)>Mo(1.3)>Mg(1.0)>Mn(0.76)>Si(0.71)>Rb(0.66)> K(0.64)>Na(0.60)>Al(0.44).

The comparison of the average contents in the topsoil of the fertilised plot determined in 2010 with the content of metals in sludge of the Vilnius Wastewater Treatment Plant determined in 1989–1990 [32] showed that the content of Cr decreased 1.16 times, that of Cu decreased 2.47 times, Mn 1.6 times, Mo 5.7 times, Ni 2.5 times. The content of Zn, on the contrary, increased 1.25 times. This disagreement can be explained not only by washout from fertilised topsoil but also by high geochemical variability of sludge reflected by much higher coefficients of variation of chemical elements in the fertilised plot VK_F in comparison with the respective coefficients of variation in the background plot VK_B (Table 1).

Influence of Mylar film on intensity $\mathbf{I_n}$

Matched pairs of mean $\mathbf{I_n}$ values obtained in a different measurement way were studied. The set of ratios of the mean $\mathbf{I_n}$ value without the Mylar film to the respective mean $\mathbf{I_n}$ value with the Mylar film was calculated. Medians of ratios at each DF as well as overall medians of ratios were found (Table 3). The nonparametric Wilcoxon test was performed for comparison of $\mathbf{I_n}$ values with and without the Mylar film. It showed that only for Si the differences in $\mathbf{I_n}$ values obtained by different measurement ways are insignificant ($p > 0.05$), meanwhile for other chemical elements they are significant ($p < 0.05$). The following was observed at all different DF values: 1) the $\mathbf{I_n}$ values of Mg, Cr, Na, Al are significantly higher when measuring without the Mylar film in comparison with measurements with the Mylar film; 2) the $\mathbf{I_n}$ values of Sr, Fe, Ca, K are significantly lower when measuring without the Mylar film in comparison with measurements with the Mylar film. Higher than 1 ratios in Table 3 indicate how many times $\mathbf{I_n}$ values can be lower during measurements with the Mylar film compared to measurements without the Mylar film.

The arrangement of chemical elements according to the descending overall median ratio reveals the group Mg>Cr>Ni>Na>Al>P>S>Cl the measurement of which can be disturbed by the presence of the Mylar film. A great part of these elements has a low atomic number: Na(11), Mg(12), Al(13), P(15), S(16), Cl(17). This is in accordance with the statement of Dick et al. [9]. Hall et al. [13] have also observed a great effect of a thin film on photon transmittance for light elements. Besides, the $\mathbf{I_n}$ values of Mg, Cr, Ni, Na, Al with the Mylar film are significantly lower than without this film at different DF ≥ 0.25, meanwhile for P, S, Cl the $\mathbf{I_n}$ values with the Mylar film are significantly lower at different DF < 0.80, but not at DF = 0.80 when the differences are insignificant (this DF value is the closest to the recommended value by the manufacturer – 0.816).

The sequence of elements for which the Mylar film increases $\mathbf{I_n}$ values is the following: Cu>Br>Rb>Sr>Zn>Mo>Fe (arrangement is according to descending values of this increase). When analysing plant material pellets with 0.25≤DF≤0.80 in plastic cups with the Mylar film, the $\mathbf{I_n}$ values of K, Ca, Fe, Zn, Sr, Rb, Cu were significantly higher than without this film. For Mn and Br the significant increase of $\mathbf{I_n}$ was found at 0.50≤DF≤0.80 values. It can be presumed that the chemical composition of the Mylar film is one of the reasons of this increase.

So different EDXRF measurement methods, i. e. with the Mylar film or without it, provide significantly different results, except Si. The arrangement of the elements according

Table 3. **Range of normalised intensities obtained in different measurement ways and their median ratios**

Elements	Range of normalised intensities		Rm	Median ratios at different DF (N – number of matched pairs of mean I_n)				
	Without Mylar film	With Mylar film		0.80	0.75	0.50	0.25	0.17
				N = 4	N = 12	N = 12	N = 11	N = 4
Mg	16–421	9.1–218	**1.9**	**1.7**	**1.8**	**1.9**	**1.9**	**1.8**
Cr	2.8–7.4	1.8–7.5	**1.6**	**1.8**	**1.6**	**1.6**	**1.5**	**1.4**
Ni	3.8–13	1.3–14	**1.4**	**1.4**	**1.6**	**1.4**	**1.3**	1.2
Na	7.5–12	4.5–10	**1.3**	**1.5**	**1.4**	**1.4**	**1.3**	**1.2**
Al	7.2–40	7.3–32	**1.3**	**1.3**	**1.3**	**1.3**	**1.1**	**1.2**
P	295–4511	276–3981	**1.1**	1.0	**1.1**	**1.2**	**1.1**	**1.1**
S	251–4589	221–4191	**1.1**	1.0	**1.1**	**1.1**	**1.1**	**1.1**
Cl	293–8334	272–7672	**1.0**	0.9	**1.0**	**1.1**	**1.0**	**1.0**
K	205–2754	206–2724	**1.0**	**0.9**	**1.0**	**1.0**	**1.0**	**1.0**
Ca	270–3624	290–3651	**1.0**	**0.8**	**0.9**	**1.0**	**1.0**	**1.0**
Mn	6.8–24	5.5–292	**0.9**	**0.8**	**0.9**	**0.9**	1.0	1.0
Si	17–8591	344–7153	0.9	1.0	0.8	0.8	0.6	1.0
Fe	29–207	29–232	**0.9**	**0.8**	**0.9**	**0.9**	**0.9**	**0.9**
Mo	2.6–8.7	2.7–12	**0.9**	0.9	**0.9**	**0.8**	**0.9**	1.3
Zn	35–764	37–911	**0.9**	**0.7**	**0.9**	**0.9**	**0.9**	0.9
Sr	66–463	42–603	**0.8**	**0.8**	**0.8**	**0.9**	**0.9**	**1.1**
Rb	45–1461	46–1753	**0.8**	**0.7**	**0.8**	**0.8**	**0.9**	1.0
Br	1.3–364	1.3–449	**0.8**	**0.8**	**0.8**	**0.7**	0.9	**2.3**
Cu	4.0–90	4.7–110	**0.8**	**0.7**	**0.8**	**0.8**	**0.8**	0.8

Notes. Abbreviation: Rm is the overall median ratio. The ratios are the mean $\mathbf{I_n}$ value in two identical pressed pellets obtained without the Mylar film to the mean $\mathbf{I_n}$ value obtained in the same pressed pellets with the Mylar film. The median ratios are in bold if the respective Wilcoxon matched pair test is significant ($p < 0.05$).

to coefficients indicating the decrease might be useful for selection of the way of measurement with or without the Mylar film. Recently Hall et al. [13] have revealed the pronounced differences in transmittance for Mg, Al, Si, S, K and Ca for pellets lined with Mylar versus Prolene, the greater influence of the thickness of the Mylar film on transmittance as well as trace contamination levels of S, P and Ca in Mylar.

Influence of dilution factor DF on intensity $\mathbf{I_n}$

Calamagrostis epigejos and *Festuca rubra* were chosen aiming to reveal the influence of DF on $\mathbf{I_n}$. The $\mathbf{I_n}$ values of the greater part of chemical elements decreased with reduction of DF values. To study this tendency in detail, the ratios of $\mathbf{I_n}$ obtained at various DF with $\mathbf{I_n}$ obtained at DF = 0.17, i. e. ratios with $\mathbf{I_n}(0.17)$, were calculated (Fig. 1). For most chemical elements the following can be seen from charts: 1) closeness of ratios $\mathbf{I_n}(0.80)/\mathbf{I_n}(0.17)$ and $\mathbf{I_n}(0.75)/\mathbf{I_n}(0.17)$; 2) lower ratios $\mathbf{I_n}(0.50)/\mathbf{I_n}$ (0.17) in comparison with the above-mentioned two ratios but higher than the ratios $\mathbf{I_n}(0.25)/\mathbf{I_n}(0.17)$ which are usually lower than 1.5 and for most elements higher than 1, i. e. than the ratio $\mathbf{I_n}(0.17)/\mathbf{I_n}(0.17)$. It was possible to form eight matched pairs of $\mathbf{I_n}$ values corresponding to ten different pairs of dilution factors and the Wilcoxon test was performed according to them (Table 4). As a result, two groups of chemical elements were distinguished. The first one includes Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr (14 elements) for which there is usually a significant ($p < 0.05$) difference between $\mathbf{I_n}$ values obtained measuring pressed pellets produced with different DF values. The only exception is when DF = 0.80 and DF = 0.75, i. e. when DF are high and do not much differ.

Table 4. **Chemical elements with insignificant ($p > 0.05$) differences between normalised intensities obtained at ten different pairs of dilution factors**

First group of pellets	Second group of pellets			
	DF = 0.75	DF = 0.50	DF = 0.25	DF = 0.17
DF = 0.80	**Na, Cr,** *Ni, Cu,* Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr	**Na, Cr,** *Ni, Cu*	**Na, Cr,** *Ni*	**Na, Cr,** *Ni*
DF = 0.75		**Na, Cr,** *Ni*	**Na, Cr,** *Ni*	**Na, Cr,** *Ni, Mo*
DF = 0.50			**Na, Cr,** *Ni*	**Na, Cr,** *Ni, Mo*
DF = 0.25				**Na, Cr,** *Cu*

Notes. The search of differences was done according to $\mathbf{I_n}$ values of only two species *Festuca rubra* and *Calamagrostis epigejos* sampled from the background and the fertilised plot. The number of samples for the Wilcoxon test was eight. For the elements in bold the differences are always insignificant, for the elements in italic they are often insignificant, for the underlined elements they are usually significant.

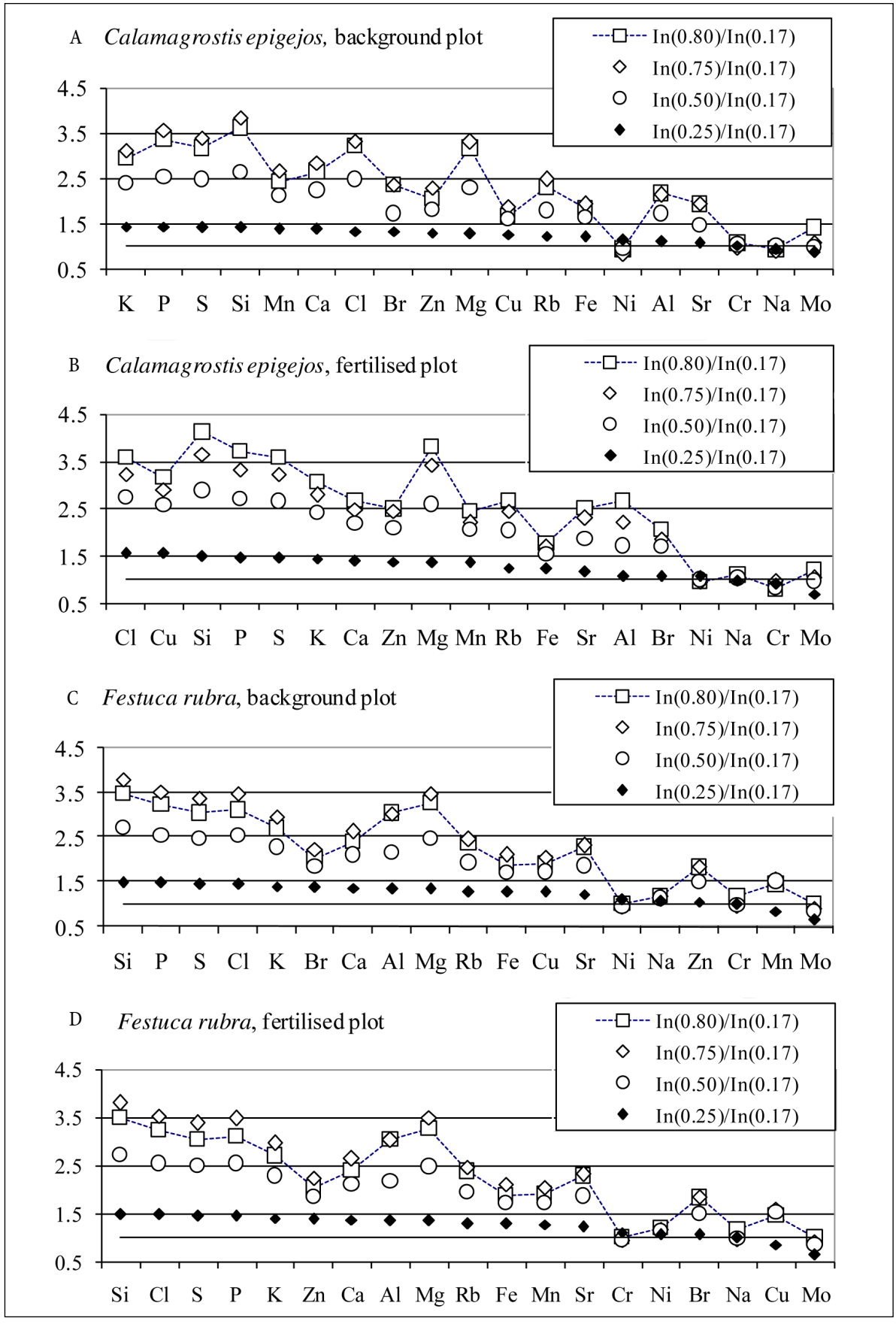

Fig. 1. The influence of dilution factor on normalised intensities

Notes. A is *Calamagrostis epigejos*, background plot, B is *Calamagrostis epigejos*, fertilised plot, C is *Festuca rubra*, background plot, D is *Festuca rubra*, fertilised plot.

For another group of seven chemical elements, the differences between $\mathbf{I_n}$ values at different DF are always (Na and Cr) or often (Ni, Cu, Mo) insignificant ($p > 0.05$). Unlike most chemical elements, for Mo the difference between $\mathbf{I_n}$ values obtained at DF = 0.80 and at DF = 0.75 is significant.

Chemical elements from the first group are usually at the beginning of four charts in Fig. 1, meanwhile those from the second group are often at the end of them. A greater part of elements from the first group have $\mathbf{I_n} > 10$, except Al, Mn, Br, while most of elements from the second group have $\mathbf{I_n} < 10$ (Table 2). So the tendency of the influence of DF is clearly seen on those chemical elements which have higher $\mathbf{I_n}$. This is the tendency of $\mathbf{I_n}$ decrease when DF values become lower, i. e. when material is more diluted with wax. On the contrary, the decreasing tendency is not seen for Na, Cr and Ni (Fig. 1). This may be influenced by various X-ray spectral peculiarities including those which caused low $\mathbf{I_n}$ values. Therefore preparation of pressed pellets with lower DF for these elements is maybe not reasonable.

While the problem of lack of plant material can arise, it might be necessary to make some changes in the recommended sample preparation for EDXRF, i. e. to increase the dilution with the binder. However, it should be remembered that the intensities usually decrease with increase of dilution, so too high dilution, i. e. DF = 0.17, is also undesirable. On our opinion, when there is lack of material, DF = 0.25 value is acceptable.

Element sequences according to increase in the fertilised plot compared to the background plot at different dilution factors

How to justify that $\mathbf{I_n}$ at different DF values is suitable? As a rule, the higher the element content in soil, the more probable that it will be higher in certain plant species. In our case, we compared the fertilised plot (higher contents of most chemical elements) with the background plot (lower contents). If comparing $\mathbf{I_n}$ values at different DF a similar group of chemical elements with a noticeable increase in the fertilised plot in comparison with the background plot is revealed, this serves as an indirect proof that these DF values can be used for sample preparation.

The groups of elements with higher than 1.2 coefficients of increase (**CI**) in the fertilised plot in comparison with the background plot were revealed according to the ratio of $\mathbf{I_n}$ values obtained during the measurement of pellets with different dilution factors (Fig. 2). The **CI** values at DF = 0.25 were the basis for comparison. Despite some differences in element arrangement according to **CI** for each plant material at different DF, the groups of elements with an increasing content were rather similar.

For *Calamagrostis epigejos,* the common group of 11 chemical elements with **CI** > 1.2 is as follows: Zn, S, P, Si, Rb, Sr, K, Mg, Cl, Ni, Cu (only at DF = 0.25 **CI** of Ni is slightly lower and equals to 1.1). However, with an increase of dilution

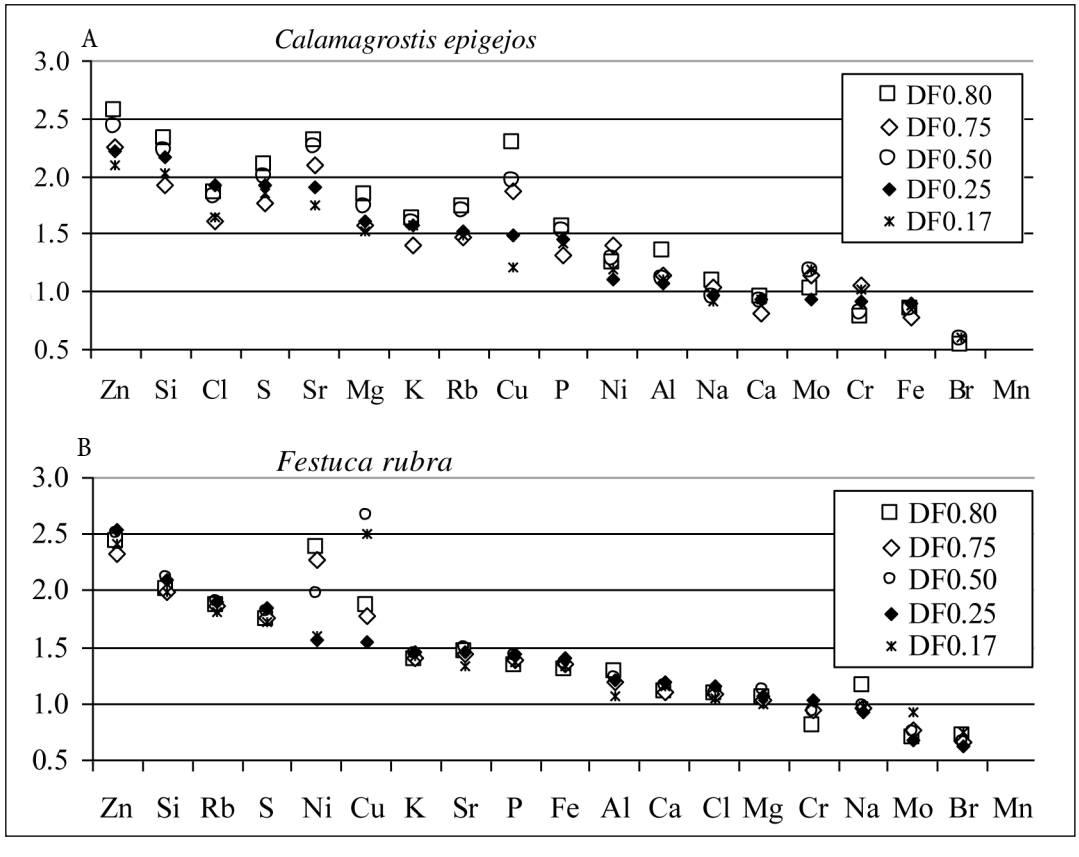

Fig. 2. The influence of dilution factor on the estimates of increase coefficients in the fertilised plot in comparison with the background plot

Notes. A is *Calamagrostis epigejos*, B is *Festuca rubra*

the estimate of **CI** for Cu decreases: when DF = 0.8, it is 2.3, when DF = 0.75, it is 1.9, when DF = 0.50, it is 2.0, when DF = 0.25, it is 1.5 and when DF = 0.17, it is 1.2. At all DF values the set of elements with CI > 1.2 in *Festuca rubra* includes the same 10 chemical elements: Zn, Ni, Cu, S, P, Si, Rb, Sr, K, Fe. Besides, when DF ≥ 0.25, Al and sometimes Ca are added. An analogous study of other species has shown that despite great influence of DF on $\mathbf{I}_n$, the group of elements which usually increase in the fertilised plot compared to the background plot (**CI** > 1.3) is the following: Zn, Ni, Cu, S, P, Sr, Si, Rb. These elements, except Si and Rb, are also enriched in soil of this plot (Table 1) indicating that the influence of wastewater treatment sludge still exists. Though Zn, Ni, Cu, S, P are essential chemical elements [33], higher contents of Zn, Ni, Cu might be dangerous to plants [34]. However, not all pollutants from sludge are bioavailable, e. g. despite high content in soil (Table 1), Cr content is not high in plants of the fertilised plot. The sludge also serves as a fertiliser, because Mg and K are depleted from soil, but both are high in *Calamagrostis epigejos* and K in *Festuca rubra*.

Many metals exhibit chalcophilic tendencies [35]. Sufficiently high $\mathbf{I}_n$ values of S at low dilution factor (DF = 0.25) enable to search for sulphide mineral deposits even in those cases when there is a low amount of plant material. Zinc and copper which are characteristic of chalcophile ores [29] can also be used for this aim. When there is a small amount of plant material, Cl, P, Mg, Si, Al, K, Ca, Rb, Sr, Br, Mn, Fe and Mo can also be used as more distinct biogeochemical tracers in search of particular mineral deposits. The methodical results of this research were useful for the implementation of the project "Changes in Biotic and Abiotic Ecosystem Components Induced by an Invasive Species: Case Study of Cormorants". They will be useful in studies of phytoremediation. Two scientific publications are based on the results using DF = 0.25: about the possible influence of biogeochemical changes of environment on forest lichens [36] and about the spreading of rare myxomycetes [37].

CONCLUSIONS

The following was revealed during the investigation of X-ray intensities $\mathbf{I}_n$.

1. Application of the Mylar film significantly reduced the sensitivity of 8 chemical elements: Mg, Cr, Ni, Na, Al, P, S, Cl. The reduction for most of them was observed at all dilution factor DF values used in the experiment: 0.80, 0.75, 0.50, 0.25, 0.17. When the reference dilution factor 0.25 is chosen, the elements according to the growing loss of sensitivity are arranged as follows: Cl<Al<S<P<Na<Ni<Cr<Mg. So it is better, if possible, to analyse plant material without the Mylar film.

2. The analysis of *Calamagrostis epigejos* and *Festuca rubra* in the background and fertilised plots has shown a significant decrease of $\mathbf{I}_n$ values of Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr with lowering of DF values. The only exception was when DF = 0.80 and DF = 0.75, i. e. when DF are high and similar. For Na, Cr, Ni, Cu, Mo, this decrease is not so obvious and the differences in $\mathbf{I}_n$ obtained at different DF values are usually insignificant. These elements have often $\mathbf{I}_n$ < 10 values, so high uncertainty of analysis might be increased due to dilution of material. There is higher sensitivity at the reference dilution value DF = 0.25 in comparison with sensitivity at DF = 0.17 which can be estimated according to the ratio of $\mathbf{I}_n$ at DF = 0.25 to $\mathbf{I}_n$ at DF = 0.17. According to higher than 1.10 descending median values of such ratios calculated for *Calamagrostis epigejos* and *Festuca rubra* in background and fertilised plots, the elements are arranged as follows: Si(1.45), P, S(1.43), Cl(1.42), K(1.41), Ca(1.37), Mn, Zn(1.34), Cu, Mg(1.31), Rb(1.24), Fe(1.22), Br(1.16), Al(1.15), Sr(1.14). So it is better to use DF = 0.25 than DF = 0.17, meanwhile DF = 0.25 is suitable for preparing of pressed pellets having lack of plant material for XRF analysis.

3. The analysis of chemical elements accumulating in different plants species of the fertilised plot in comparison with the background plot has shown that despite different DF, the sets of higher than 1.3 increase coefficients are similar, i. e. include common chemical elements for the same species.

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AUGALŲ MAŽŲ KIEKIŲ ANALIZĖS GALIMYBĖS TAIKANT ENERGIJOS DISPERSIJOS RENTGENO FLUORESCENCIJĄ

S a n t r a u k a

Tirtas dviejų sklypų paviršinis dirvožemis ir augalai, kurių pagrindinę dalį sudaro *Festuca rubra* ir *Calamagrostis epigejos*. Vienas sklypas patręštas nuotekų dumblu. Lyginami energijos dispersijos rentgeno fluorescencinės analizės metodu nustatyti Na, Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, Sr, Mo, Cr, Br normalizuoti intensyvumai ($\mathbf{I_n}$), gauti matuojant tabletes, pagamintas iš augalų, juos išdeginus 240 °C temperatūroje, gautą medžiagą skirtingomis proporcijomis, t. y. esant įvairiems skiedimo koeficientams (DF), sumaišius su rišikliu ir įdėjus į indelio dugną, padengtą Mylar plėvele arba be šios plėvelės. Matuojant su plėvele, Mg, Cr, Ni, Na, Al, P, S, Cl intensyvumai reikšmingai sumažėdavo, be to, Mg, Al, Si, P, S, Cl, K, Ca, Mn, Fe, Zn, Br, Rb, Sr intensyvumai dažniausiai reikšmingai sumažėdavo esant didesniam skiedimo koeficientui. Visiškai leistina tablečių gamybai naudoti DF = 0,25, tačiau geriau matuoti be Mylar plėvelės. Nepaisant augalų mėginių paruošimo ir matavimo būdo įtakos $\mathbf{I_n}$ reikšmėms, išskirta grupė elementų, kurių koncentracijos patręšto sklypo augaluose daugiau nei 1,3 karto didesnės, palyginti su foniniu sklypu: Zn, Ni, Cu, S, P, Si, Rb, Sr. Šių elementų kiekiai, išskyrus Si ir Rb, taip pat padidėja ir šio sklypo dirvožemyje.