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Lichens as integrating air pollution monitors

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"Capsule": *An Index of Atmospheric Purity was calculated, using results from a survey with the lichen* Hypogymnia physodes.

Abstract

In this work an attempt to combine the results of lichen mapping with the quantitative levels of certain trace elements in *Hypogymnia physodes* (L.) Nyl. collected on a national scale is presented. An Index of Atmospheric Purity (IAP) was calculated using a simple method of mapping lichens based on the assessment of the cover and frequency of crustose, foliose and fruticose lichens on different tree species. For determination of trace elements in lichens k_0-instrumental neutron activation analysis was used. From the IAP results it can be concluded that the epiphytic lichen flora look quite poor with more than 70% of the territory in the fourth and third classes, which represent highly polluted and moderately polluted air. By comparing IAP results with elemental levels in *H. physodes* using multivariate statistical methods it was found that the elemental levels do not have a direct negative effect on the diversity of lichens but can help in identification of the type of possible pollution sources and their origin. © 2002 Published by Elsevier Science Ltd.

Keywords: Epiphytic lichens; Bioindication; IAP; Air pollution; Trace elements; *Hypogymnia physodes*

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1. Introduction

Epiphytic lichens can be used in different ways as air-quality bioindicators. The first group of methods are floristic, based mainly on lichen-specific sensitivity to gaseous pollutants, especially SO_2, fluorides and strongly oxidizing compounds such as ozone. The classical methods of this group are species distribution mapping and the phytosociological approach which studies different lichen communities (Wirth, 1988; Richardson, 1988). Using these methods changes in lichen species, especially the sensitive ones or community distribution, can be a measure of air pollution. However, nothing can be said about the origin and type of pollutants. One demand in the use of these methods is a well-trained lichenologist, and if possible, good historical records about the lichen flora of the area under consideration. By adding numerical values to the phytosociological characteristics of lichens and through a mathematical formula, the so-called Index of Atmospheric Purity (IAP) can be obtained for each site. Using this method which was pioneered by De Sloover and Le Blanc (1968) was then widely used in other investigations (Showman, 1988), the gradient of an effect can be obtained, before a species is completely eliminated because of severe pollution.

The second group of methods is based on the physical, chemical and biological properties of lichens which enable them to be used as monitors of metal deposition from the atmosphere, since they can accumulate trace elements to levels far greater than their expected physiological needs (Puckett, 1988). Passive as well as active biomonitoring using epiphytic lichens can be used on a national scale as well as around particular pollution sources to obtain information about the levels of particular trace elements in the atmosphere. Since the sources of elements that cause elevated levels in lichens are both natural (crustal material, marine aerosols) and anthropogenic (industry, traffic, etc.), there are at least two ways of identifying the contributions of elements

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from different source terms; (1) by calculating the enrichment factor (EF) for each individual element, and (2) by applying multivariate statistical techniques to the data set.

Both kinds of methods are used in Slovenia. Initial observations on the disappearance of lichens from urban and industrialized areas were recorded about 40 years ago by schoolteachers. Due to the lack of trained lichenologists, the first mapping of lichens around polluted areas was carried out in the late 1970s when comparisons were made in virgin forest reserves (Batič, 1984; Batič and Martinčič, 1982). As the lichen flora was poorly known, only lichen thalli types (crustose, foliose, fructicose) were mapped throughout Slovenia in several high school projects (Batič, 1991) in order to improve the overall ecological knowledge of students. Later, in the 1980s this method, the so-called IAP method, was adopted and modified for studies connected with forest decline inventories and since 1985 it was used regularly as an air quality indicator on more than 500 plots used for forest decline studies (Batič and Mayrhofer, 1996). The results of observations in 1991 showed that the epiphytic lichen flora look quite poor with more than 70% of the territory in the fourth and third classes, which represent highly polluted and moderately polluted air (Batič, 1991; Batič and Mayrhofer, 1996).

In order to obtain information about atmospheric trace element levels in Slovenia, a monitoring survey was performed in 1992 at 86 sampling sites on the national scale using the epiphytic lichen *Hypogymnia physodes,* and by the application of multivariate statistical method possible pollution sources were identified (Jeran et al., 1996).

The main aim of this work is an attempt to combine the IAP results with the quantitative levels of certain trace elements in *H. physodes*.

2. Materials and methods

2.1. Determination of the IAP

In the period from July to September 1991 a lichen survey was performed on 546 inventory plots of a 4×4 km national grid as a part of a forest die-back inventory (Fig. 1). The lichen inventories were done by a well trained team under the guidance of professional staff from the Slovenian Forestry Institute. All lichen observations were performed exclusively on those plots which fall in forests and none of the plots was in the close vicinity of any emission source. It is worth mentioning that forests in Slovenia represent more than 50% of the territory.

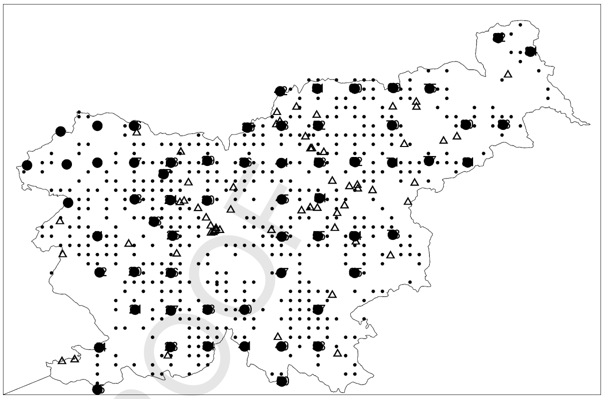

Fig. 1. A map of inventory plots (4×4 km grid) for IAP determination (small spots), 16×16 km grid for *Hypogymnia physodes* collection (large spots) and SO_2 air measurement sampling locations (open triangle).

At a given inventory plot in a group of six marked trees from 24 which were also used to assess forest tree damage (Batič, 1992), the abundance (a) and coverage (c) of three of the most important epiphytic lichen thallus types, crustose (C), foliose (F) and fructicose (R) were mapped at three heights (h; 1—from 0 to 0.5 m, 2—from 0.5 to 2.5, 3—above 2.5 m). If possible, climatozonal forest tree species were chosen for observation. Trees also had to meet other conditions necessary for lichen observation in pollution areas, such as the age of trees, position in the stand, light conditions, etc. The cover and abundance of all three thallus types of lichens were assessed on a very simple scale as follows: 0—no lichens, 1—thalli cover up to 10% of the observed trunk surface, 2—thalli cover between 10 and 50% of the observed surface, 3—thalli cover between 50 and 100% of the observed trunk surface. Abundance was assessed using the following scale: 0—no lichens, 1—very few lichen thalli (1–5 for observed surface), 2—moderately frequent thalli (6–10), 3—very frequent thalli (more than 10). The observed trunk surface was divided into three apparent squares whose sides were dependent on the diameter of the tree and the average data for the observed height were then written in the questionnaire for each tree. For lichen observation at the height above 2.5 m binoculars were used, as well as the observation of fallen tree branches. From the data collected an index of atmospheric purity was calculated for each inventory plot (IAP_t) for each stratum of observation separately (IAP_1 = observations on tree trunks up to 0.5 m, IAP_2 = 0.5–2.5 m; IAP_3 = above 2.5 m), using the following formulas:

$$IAP_{1,2,3} = C(a + c) + F(a + c) + R(a + c)$$

$$IAP_t = IAP_1 + IAP_2 + IAP_3$$

where the symbols represent the above mentioned observed lichen parameters. The IAP index has a span between 0 and 54 where the value 0 means a plot without lichens and very polluted air, and the value 54 means very rich lichen vegetation and very clean air. The details of the IAP method are given elsewhere (Batič, 1991, 1992).

2.2. Determination of trace elements in lichens

In the period from September to November 1992, the epiphytic lichen *Hypogymnia physodes* (L.) Nyl. was collected at 86 sampling locations of the national 16×16 km bioindication grid. This lichen species was selected due to its (1) high tolerance to elevated SO_2 levels, (2) it is a foliose lichen without rhizinae so it can be easily separated from the bark substrate, (3) it is widely dispersed in different geographical regions in Slovenia, and (4) it is often used as a biomonitor species in our country as well in some other countries (Jeran et al., 1995, 1996; Horvat et al., 2000; Herzig et al, 1989; Gartey and Ammann, 1987). In contrast to the previously mentioned biomonitoring survey in 1991, all sampling in 1992 was made on open habitats or in the nearest forest glade of the plot, sometimes a few hundred metres away from the well defined plots of the forest decline inventory.

In the laboratory, lichens were moistened with distilled water and then the adhering bark particles removed from the lichen using nylon tweezers. The samples were made brittle by immersion in liquid nitrogen and were then crushed and ground in a zirconium mortar with Zr ball in a Fritch vibration micro-pulverizer. About 100–200 mg of dry lichen powder was then used to make tablets for neutron activation analysis. In each lichen sample more than 30 elements, namely As, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hf, Hg, K, La, Mo, Na, Rb, Sb, Sc, Se, Sm, Sr, Th, U, W and Zn were determined using k_0-INAA (De Corte et al., 1993; Smodiš, 1992). Total concentrations of sulphur, P and Pb in lichen tablets were determined by X-ray fluorescence spectrometry (Philips PW 1410 with rhodium tube). The details of sampling, sample preparation and determination of trace elements were described previously (Jeran et al., 1996).

For routine quality control NIST SRM Citrus leaves (CL-1572) were analysed according to the same procedures as lichens (Smodiš et al., 1990).

3. Results and discussion

A direct comparison between IAP values obtained from forest decline inventories and trace element composition of *H. physodes* was possible for 61 of the 86 sampling plots of the 16×16 km bioindication grid. If at a certain plot of the 16×16 km bioindication grid the IAP value was missing, the average value of the surrounding plots of the 4×4 km bioindication grid was used for evaluation (Fig. 1). The IAP values at each inventory plot were calculated for different tree species and for each stratum of observation separately; however for further evaluations and comparisons a mean IAP value for each inventory plot which includes all trees regardless of species was used. We are aware that different tree species support different epiphytic lichen vegetation according to climatic influence. However, by using only the assessment of different lichen thalli types, their frequency and cover, this heterogeneity of lichens as bioindicators is to a certain extent overcome. This was also proved (not yet published) by comparing results of species mapping and this simplified technique.

In the first step the IAP results for 61 sampling locations from the observation of lichen flora in 1991 were ranked, similarly to damage classes of forest trees, in five classes (0; 1.3–13.5; 13.6–27.0; 27.1–40.5; 40.6–54.0). Then as presented in Table 1, a mean value with its standard deviation was calculated for 30 trace elements measured in *H. physodes* for each IAP class. In the last column of the table the background elemental levels obtained by analysis of *H. physodes* collected at remote places with abundant lichen vegetation was included. As evident from Table 1 (line 3), none of the 61 sampling points was in the first and fifth class according to the status of lichen vegetation; however 88.5% of sampling points were included in classes III and IV, suggesting that the air is moderately to highly polluted and only 11.5% of locations belong to class II of low pollution. As is further seen from Table 1, for a group of certain elements, namely Cr, Zn, Sr, Hf, As and Mo, there was a distinct tendency to lower values with increasing IAP values; however, the standard deviations were rather high. For some other elements like Pb, Sb, W and Cd the highest values were found in class IV.

An explanation for the rather high variability of trace element content in lichens collected within a certain pollution class might be that only a part of the total content could be attributed to pollution, the other part representing the natural origin of the elements, where the geology of the territory could not be neglected. Namely, Slovenia is known for its very heterogeneous geology, which is manifested by higher contents of certain elements in mineral particles suspended in air. And further, as described earlier, the sampling locations of both monitoring surveys were not exactly the same.

Nevertheless, comparing our results with the results of the more sophisticated study of Herzig et al. (1989), similar trends (decreasing contents with decreasing total pollution) of environmentally significant elements (Cr, Zn, Pb, Cd) were found. Also, the mean values of common elements in *H. physodes* in corresponding pollution zones agreed well in both studies, as well as with the background levels.

On the basis of observations of lichen vegetation on different tree species combined together, a lichen map

Table 1
Mean elemental levels with their standard deviations (Φg g^{-1}) in *Hypogymnia physodes* collected at 61 sampling locations of the 16×16 km bio-indication grid calculated for five IAP classes

Class	5	4		3		2		1	Background	
IAP range		1.0–13.5		13.6–27		27.1–40		40.6–54		
%	0	38		51		11		0		
Elements		Mean	SD	Mean	SD	Mean	SD		Mean	SD
IAP91		9.38	2.91	19.31	3.58	31.64	2.47			
As		1.43	0.53	1.21	0.57	1.14	0.42		0.51	0.17
Ba		38.5	26.1	30.9	37.6	14.8	3.57		23.7	8.21
Br		13.3	5.39	17.0	6.46	14.5	3.26		10.1	2.58
Ca		23 257	14 562	24 172	12 179	16 693	14 739		22 266	3745
Cd		1.26	1.04	0.97	0.43	1.09	0.44		0.76	0.19
Ce		2.45	1.24	2.75	1.25	2.06	0.86		1.53	0.43
Co		0.55	0.23	0.63	0.32	0.43	0.13		0.28	0.16
Cr		6.69	4.19	5.83	3.95	4.82	2.04		2.62	0.82
Cs		0.33	0.17	0.36	0.24	0.26	0.09		0.17	0.03
Fe		1175	567	1363	634	1220	749		676	231
Ga		0.59	0.28	0.66	0.43	0.42	0.14		0.3	0.1
Hf		0.19	0.10	0.17	0.09	0.12	0.05		1.38	2.75
Hg		0.10	0.04	0.10	0.05	0.11	0.04		0.08	0.03
K		4158	1191	4138	1366	3469	947		3205	893
La		1.12	0.53	1.25	0.59	0.89	0.38		0.67	0.17
Mo		1.03	1.86	0.57	0.23	0.43	0.22		0.26	0.17
Na		182	91	190	95	126	48		101	27
P		1298	83	1264	157	1236	48		1200	
Pb		33.4	50.8	27.3	7.77	28.2	12.3		26.5	9.19
Rb		13.3	6.45	16.8	12.5	12.7	7.61		20.5	12.2
S		1865	492	1902	559	1730	510		980	14.1
Sb		0.50	0.67	0.31	0.13	0.35	0.22		0.19	0.04
Sc		0.36	0.19	0.38	0.21	0.29	0.10		0.2	0.07
Se		0.27	0.11	0.29	0.14	0.27	0.11		0.21	0.02
Sm		0.18	0.09	0.21	0.10	0.14	0.06		0.11	0.03
Sr		26.5	19.3	22.6	15.9	12.0	5.6		31.6	10.6
Th		0.28	0.15	0.31	0.15	0.24	0.09		0.16	0.06
U		0.12	0.05	0.13	0.07	0.09	0.03		0.07	0.04
W		0.23	0.21	0.15	0.11	0.17	0.11		0.1	0.04
Zn		104	23.8	92.6	28.4	85.1	24.0		57.3	12.1

IAP, Index of Atmospheric Purity.

was drawn (Fig. 2) which visualizes through lichen vegetation the regions where air quality was bad. Very scarce lichen vegetation was evidenced mainly in the northern and central part of the country which is the most populated region with major pollution sources, and in the western and southern part where most probably transboundary pollution takes place. A direct comparison of lichen results with measured atmospheric SO_2 levels as obtained from the National Air-Pollution Monitoring Network (Hrček et al., 1992) was not possible since,the basic Monitoring Network in Slovenia comprised 49 sampling locations, which are located mainly in urban centres or close to the main pollution sources (Fig. 1). It must be stressed again that both biomonitoring surveys were performed mostly at remote forest locations. This was the reason that the SO_2 results were not used in statistical evaluation of lichen results (see later). In spite of this, the results of measured atmospheric SO_2 showed the highest pollution at the central and northern parts of Slovenia and since SO_2 can be transported far away from its emission, it is true to say that this gaseous phytotoxic pollutant is one of the most important factors which has negative effects on epiphytic vegetation even at remote sites (Van Dobben et al., 2001). It is also interesting to note that there were relatively similar mean S concentrations in *H. physodes* (Table 1) with a range between 1780 and 1900 Φg g^{-1}, dry weight in all IAP classes which was about twice the background level (980 Φg g^{-1}).

It was found earlier (Jeran et al., 1996) that using factor analysis the concentration levels of 28 selected elements in lichens collected at 86 sampling locations can be explained by nine factors or source types in Slovenia. To find out how the two biomonitoring surveys (IAP and quantitative determination of trace elements in lichens) can explain the pollution situation in the country, a slightly modified data set compared to the earlier mentioned study was prepared since some additional trace elements (P, Pb, S) were included in the data set. All together 30 elements (As, Ba, Br, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hf, Hg, K, La, Mo, Na, P, Pb, Rb, S, Sb, Sc, Ser, Sm, Sr, Th, U, W and Zn) together with IAP values for 61 sampling locations were used in factor analysis. By using the Statistica for Windows 5.0 software package in this study, seven factors were extracted which explain 78.6% of the total variance of the data set. In Table 2, those loadings after varimax rotation that were equal or greater than 0.3 are presented in bold print. Although using a slightly different data set for trace elements than that published before (Jeran et al., 1996), similar factors were extracted. There were three factors, namely F4, F2 and F7 in which IAP values were included with loadings greater than 0.3; however in the other factors, loadings for IAP were much lower, with the exception of F6 where a loading of 0.2 was found for IAP. Again, as in previous work (Jeran et al., 1996), the so-called soil factor (F1) explained the highest percentage (37%) of the total variance in the data set, and from its composition it is evident (Table 2) that the IAP was not included.

A detailed insight into the composition of F4 which has the highest loadings of IAP among the factors showed that it contains the elements W, Cr, Hg, As and Zn which are likely to represent the metal-producing or steel industry since its geographical distribution also supports this origin (Fig. 3; Jeran et al., 1996). The

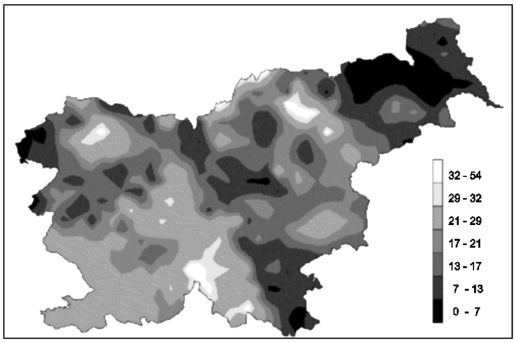

Fig. 2. Lichen map drawn on the basis of the epiphytic lichen vegetation assessment in the forest decline inventory of 1991 calculated for 546 observation plots. Observations were on all tree species. IAP values have a range between 0 and 54, where the value 0 means a plot without lichens (dark areas) and presumably highly polluted air and the value 54 means very reach lichen vegetation and very clean air.

Table 2
Factor loading matrix after varimax rotation for 61 samples of *Hypogymnia physodes*

Component	F1	F2	F3	F4	F5	F6	F7
IAP91	−0.039	**0.407**	−0.104	**−0.508**	0.016	**−0.222**	**−0.391**
As	**0.680**	0.035	**0.370**	**0.327**	**0.366**	0.101	−0.015
Ba	−0.011	**−0.639**	0.056	0.168	0.006	**−0.400**	−0.103
Br	**0.604**	**0.551**	0.072	−0.133	0.153	−0.158	0.132
Cd	0.015	−0.134	0.210	−0.124	0.009	0.059	**0.786**
Ce	**0.961**	0.060	0.026	−0.043	0.042	−0.040	0.087
Co	**0.783**	0.074	0.016	0.028	0.265	0.014	0.192
Cr	**0.362**	−0.009	−0.014	**0.643**	0.255	−0.036	0.145
Cs	**0.372**	0.126	−0.008	0.178	**0.593**	−0.200	0.026
Fe	**0.870**	0.098	0.035	0.103	0.290	−0.043	0.029
Ga	**0.850**	−0.046	0.050	0.173	0.179	0.037	−0.134
Hf	**0.723**	−0.151	−0.040	0.080	0.162	0.189	0.155
Hg	0.190	**0.328**	0.103	**0.349**	**0.620**	−0.236	−0.071
K	**0.442**	**−0.409**	−0.064	−0.097	**0.459**	**0.392**	0.036
La	**0.962**	0.061	0.023	−0.029	0.057	−0.062	0.082
Mo	0.027	0.042	0.076	0.065	−0.022	**0.810**	0.024
Na	**0.892**	−0.170	−0.045	0.052	0.181	0.030	0.066
P	**0.418**	−0.232	−0.015	−0.002	**0.712**	**0.343**	−0.030
Pb	0.045	0.005	**0.960**	−0.069	−0.101	−0.045	0.130
Rb	0.187	0.104	−0.084	−0.155	**0.740**	0.035	0.237
S	**0.723**	0.108	−0.050	0.165	**0.366**	0.190	−0.101
Sb	0.094	−0.003	**0.968**	0.087	0.041	0.115	0.097
Sc	**0.931**	−0.004	0.048	0.102	0.291	0.032	−0.002
Se	**0.733**	**0.375**	0.177	−0.048	0.164	0.024	0.163
Sm	**0.961**	0.080	0.023	−0.037	−0.054	−0.058	0.063
Sr	−0.107	**−0.843**	−0.013	−0.120	−0.107	0.063	0.123
Th	**0.956**	0.104	0.060	0.085	0.177	−0.035	0.010
U	**0.862**	0.119	0.063	0.149	0.086	0.119	−0.110
W	−0.017	0.026	−0.007	**0.836**	−0.075	0.017	−0.130
Zn	0.218	0.292	0.009	0.294	**0.320**	−0.068	**0.632**
Explained variance (%)	38	8	7	7	10	5	5

IAP, Index of Atmospheric Purity.

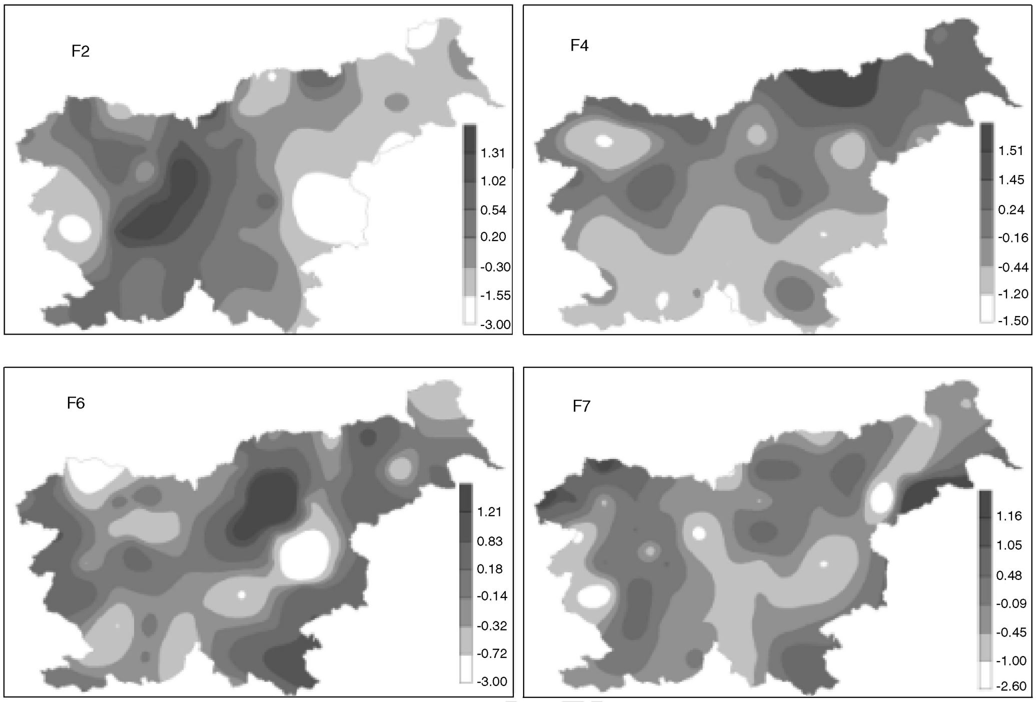

Fig. 3. Geographical distribution patterns of the factors F2, F4, F6 and F7. Increasing depth of shading represent higher factor scores as shown on the scale.

highest contribution of this factor as presented in Fig. 3 coincides with the lower IAP levels in the northern, northeastern and western parts (near the Italian border) of Slovenia (Fig. 3).

Factor 2 is characterized by the elements Sr, Ba, Br, K, Hg and Se, with relatively high loadings (0.4) for IAP. It can be considered as a complex factor, representing a marine component (high loadings for Br) on the one hand, while on the other hand the high loadings for Ba and Sr could suggest a Ba-source. Namely, the geographical distribution of F2 is very similar to F5 (Jeran et al., 1996) defined as a Ba-source and was explained by formation of insoluble (Ba and Sr) compounds in the atmosphere due to elevated levels of SO_2. It appears rather surprising that S is not included in F2. But it is questionable how total S in *H. physodes* reflects atmospheric SO_2 (Monaci et al., 1997). Namely, higher loadings of S (0.4) were found in factor 5 with elements such as Rb, P, Hg, K, Cs, and in the soil factor (0.7) where no correlation with IAP was found.

The third factor with higher loadings for IAP is factor 7, which contains Cd and Zn, and as follows from its geographical distribution (Fig. 3) and also from a comparison of the composition of this factor with that of F2 from the previous work (Jeran et al., 1996), reflects the steel industry. The higher loadings of this factor coincide with the location of a steel factory at Jesenice (northwestern Slovenia), the industrial region around Trieste (Italy), the steel factory at Ravne (northern Slovenia) and some other metal industry in the eastern part. The higher scores of F7 are also in agreement with the poor lichen vegetation in northwestern and central parts of Slovenia (Fig. 3).

Factor 6 has relatively low loadings (0.2) of IAP; however its composition and especially its high loadings for Mo could suggest emissions from coal fired power plants (Nriagu, 1989) since the highest contribution of F6 (Fig. 3) coincides with the location of the largest Slovenian power plant (central part of Slovenia), while in the western part the influence of atmospheric transport from Italy can be recognized. Again, this kind of pollution can be responsible for lower IAP values in these regions (Fig. 2).

The evidence of our work is that the relatively low loadings for IAP in factor analysis indicated that there are different effects which cause poor lichen vegetation and only part can be explained by trace elements. The elements (As, Cr, Cd, Mo, Zn) which defined particular factors could also have negative influence on lichen vegetation, especially Zn (Nash, 1990; Folkeson and Andersson-Bringmark, 1988). Nevertheless, it can be said that the elemental levels in lichens more reflect the types of possible pollution sources and their origin and not the direct cause for reduced diversity.

4. Conclusion

In this study, two different biomonitoring surveys were compared which present lichens as an integrating air pollution monitors. Namely, by observation of lichen vegetation using very simple IAP method, based on observation of coverage and abundance of lichen thallus types, but where mistakes are possible due to subjective judgement and also since the estimation of crustose thallus types at heights above 2.5 m is sometimes difficult, reasonably good results on the average pollution status of the Slovenian territory were obtained. By adding multi-elemental analysis of *H. physodes* and incorporating factor analysis an attempt to find the possible pollution sources which cause lichen degradation was made.

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