SELF-ORGANIZING FEATURE MAPS AND SELECTED CONVENTIONAL NUMERICAL METHODS FOR ASSESSMENT OF ENVIRONMENTAL QUALITY

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ABSTRACT

The investigations concerned sites of *Acer platanoides* L. infected or not by *Rhytisma aceriniu* (Pers.) Fr. The aim of the study was to check the occurrence of *R. acerinium,* and whether it reflects the environmental status. Furthermore, an analysis was carried out to find out whether the applied SOFM offers additional advantages to solve problems in relation to conventional methods. Concentrations of selected elements in soils and leaves, and leaf and "tar-spot" morphometric traits were also measured. A significant differentiation was found between sites in relation to the analyzed traits. It appeared, that sites showing lower concentrations of chemical elements and proper developmental habitat conditions massive infections take place. The study showed that *R. acerinium* is a good biological indicator for assessment of environmental status. The applied, conventional statistical methods, SOFM and image techniques showed similar, but not identical results for assessment of environmental quality using *R. acerinium.* SOFM appeared to be more useful for ordination of results and ought to be taken into account as a proper tool of estimation of various plants and their biotopes.

KEY WORDS: SOFM, "neural networks", "tar-spot", pollution, bioindication, modelling.

INTRODUCTION

The degradation of natural ecosystems caused by pollution is one of the most important problems in many countries. According to Walker et al. (2006), the environment needs investigations on impact of pollution upon various organisms. Markert et al. (2003) consider, that plants having indicator or accumulator properties could potentially be used as biomonitors. On the basis of the commonly accessible literature one can say, that the most used indicators are lichens and mosses, but also shrubs and trees, and far less the fungi. But, according to Nordé and Appelqvist (2001) the introduction of new organisms into that type of investigations enables a more precise determination of toxicity, mechanisms of xenobiotics action, habitat requirements and life strategies of organisms.

Researchers use a lot of numerical methods, mainly classical (Sokal and Rohlf 2003), but also techniques of the "fourth generation" based on artificial intelligence (ANN's – artificial neural networks) (Ray and Klindworth 2000). Moreno-Sanches (2004) points to the use of various image and numerical techniques, which are more precise and practical in ecological studies.

Nowadays, particular attention is being paid to search of methods and solutions enabling higher verification possibilities of study results. One of the method is "data mining" (Sokołowski 2002). This author informs, that the classical statistics and methods of data exploration are often used, but procedures with "data mining" are rare. The typological conceptions of "data mining" are various: a) linear, neural networks, MARS (Multiple Adaptive Regression by Splines); b) automatic detection of clustering, classification trees, neural networks; c) supervised (regression, k-means algorithm, neural networks), and unsupervised ones (association principles, cluster analysis, SOFM-Self Organizing Feature Maps, principal components and classification analysis).

In case of ecological studies on global changes of environment and reactions of organisms affected by pollution, greater attention is drawn to artificial neural networks. The use of ANNs in modelling is widely recognized, because of their superiority to classical methods, e.g. Principal Component Analysis (PCA), Correspondence Analysis (CoA), Polar Ordination (PO) and hierarchical clustering analysis (Paruelo and Tomasel 1997; Giraudel and Lek 2001; Gevrey et al. 2003; Pastor-Bárcenas et al. 2005; Kosiba and Stankiewicz 2007; Samecka-Cymerman et al. 2007). According to Chon et al. (1996), Recknagel (2001) and Gevrey et al. (2003), Kohonen's ANN is routinely used for ordination and visualization of complex ecological data, and offers an attractive solution to lots of problems in many critical applications, presently used in many modelling tasks.

The aim of the present study, was to test selected numerical and image techniques for estimation of environmental properties. The ecological studies by means of various quantitative methods are seldom applied and are rare in bioindication investigations.

This paper is the second part of a bioindication study on occurrence of *Rhytisma acerinium* in different habitat conditions affected by pollution (Kosiba 2007). It presents the results of selected conventional numerical methods, SOFM and image techniques in modelling and assessment of environmental quality. Our working hypothesis was, that the occurrence of *R. acerinium* reflects the environmental status, and SOFM offer additional advantages to solve problems in ecological studies. The answer to that hypothesis was obtained by: determination of quantities of macro- and trace elements in soil and leaf of *Acer platanoides* sites, number and surface of "tar-spots". Next, to assess the environmental factors which affect the occurrence of "tar-spots", and to find out whether the applied methods are proper for assessing environmental status.

MATERIAL AND METHODS

Study area and object of interest

The investigations were carried out early summer and autumn. Thirty two sites were selected, and the study object was *Rhytisma acerinium* (Pers.) Fr., occurring or not on *Acer platanoides* L. leaves (Fig. 1). Additionally, the data of fifteen sites were taken from the paper by Kosiba (2007). Altogether forty seven sites were used.

All sites were selected according to kind and intensity of pollution, that is: industrialization sites (1 Bogatynia; 15, 24 Legnica; 25, 26, 44 Lubin; 27,28, 40, 41 Głogów; 30 Bełchatów; 42 Polkowice), urbanization sites (2, 3 Wrocław; 10 Tarnowskie Góry; 11 Chorzów; 21 Zielona Góra; 23 Wałbrzych; 29 Gliwice; 31 Sosnowiec; 43, 45 Zabrze; 46 Kraków; 47 Strzegom), traffic intensity sites (5 Łosiów; 6 Rozmierka; 7 Strzelce Opolskie; 8, 9 Płużniczka) and areas beyond direct influence of pollution-emission sources (4 Bardo Śląskie; 12 Libusza; 13 Osola; 14 Oborniki Śląskie; 16 Giżycko; 17 Pisz; 18 Olecko; 19 Mikołajki; 20 Sejny; 22 Miedzichowo; 32 Wejherowo; 33 Lubiatów; 34 Drezdenko; 35, 36 Kołobrzeg; 37 Świdwin; 38 Krzyż; 39 Lębork). The sites 1÷15 are objects which were studied by Kosiba (2007).

The literature on *R. acerinium* and characterization its biological development, bioindication properties, and nomenclature are presented in the paper by Kosiba (2007).

Data collection

Every site (32 new ones) was represented by three *A. platanoides* area-samples including five tree specimens and soil samples in each site. Altogether, in July examined were 480 specimens of trees and 480 soil samples. Composite samples were made in order to decrease the field sample heterogeneity on sites. Data sets for 15 sites (Kosiba 2007), are represented by three samples for every site, i. e. minimum and maximum values, and third sample was calculated as arithmetic mean from five remaining ones.

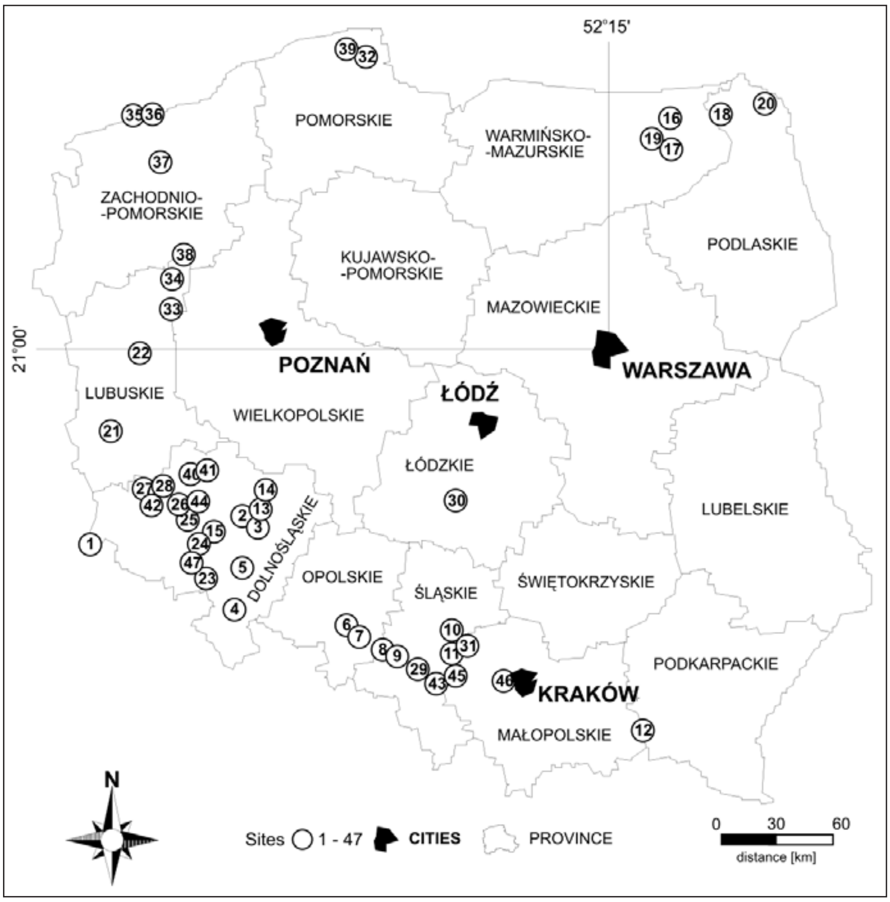

Fig. 1. Localization of sampling sites of *Acer platanoides* in Poland.

The morphological traits of tree specimens in 32 new sites were: height 11÷14 m, trunk circumference 0.80÷1.40 m measured at height of 1.80 m above ground surface. The distances between the sample areas ranged 40÷240 m, and fluctuated between the selected tree individuals from 30 to 75 m in communities, and from 17 to 50 m in alleyways. Singly growing individuals and those growing at community edges were omitted. From various sides of the outer zone of the tree crown collected were 37÷72 leaves, from eight branches of ca. 1 m length, from height of 2÷4 m above ground surface, thus from the lowest part of tree crown. The method for assessment of representative branch samples has been described in details by Kosiba (2007). Additionally, in November the dropped leaves were collected, from the same sample surfaces as in July, i.e. 32÷57 leaves from trees which grew in alleyways and 80÷112 leaves in communities.

Morphometric measurements

Fresh (summer) and dropped (autumn) leaves of *A. platanoides* specimens were segregated and used for morphometric measurements. Measured were: surface of fresh leaves (cm^2), number of "tar-spots" (n) on fresh leaves, and surface of "tar-spots" (cm^2) on dropped leaves, by means of the scanning technique in scale 1:1. For scanning and measurements of these traits the program for automatic morphometry digiShape (Cortex Nova 2005) was used.

The contour maps of "tar-spot" numbers and surfaces of *R. acerinium* were drawn using the coordinates of sampling sites. The maps were drawn by means of the isolines method by means of the Kriging method of gridding with the computer program Surfer 8.03 (Golden Software 2004).

Chemical analyses of leaves and soils

The samples of leaves and soils were prepared to chemical analyses. The total contents of Cd, Cr, Cu, Fe, Mn, Mo, Pb, Zn were determined by atomic absorption spectrophotometer GBC Scientific Equipment Pty. Ltd. model AVANTA PM; Co, Ni by the electrothermal atomic absorption spectrophotometry using the attachment Model GF 3000 with AVANTA PM; B by colorimetric method with carminic acid using spectrophotometer Secommam S 250; N by Kjeldahl's method using the automatic aggregate for distillation WAPODEST 40 Gerhardt and titration method; S using the integrated analyzer CNS Carbo Erba NA-1500. All chemical analyses of leaf and soil samples were carried out in three measuring repetitions. The results were expressed in $mg*kg^{-1}$ dry weight of leaf and air-dried soil mass. The sample preparations and execution of chemical analyses has been described in details by Kosiba (2007).

Statistical analyses

The obtained results of chemical analyses of soil and leaves samples, as well as leaf and "tar-spot" traits were expressed by basic descriptive statistics: mean, standard deviation, minimum and maximum values.

On the basis of results the statistical analyses were performed. The fitting distribution of the empirical data was verified to the normal one by the Shapiro-Wilk's test, and the homogeneity of variances were checked out by Levene's test. The results showed compatibility with the normal distribution. Thus, the ANOVA with the F-test and Least Significance Difference (LSD) values were calculated. The relationships between elements were expressed by Pearson's coefficient (r). In respect of soil and leaf element concentrations the similarity of sites was analysed using the method of cluster analysis. The Ward's method of amalgamation was applied and the method of Euclidean distance was used as measure of similarity. The significance of clusters was defined by Sneath's index at 66.7 and 33.3% of maximal linkage distance, i.e. (Dlink/Dmax)*100 and on the basis of the graph of amalgamation schedule. The ordination of sites at fixed criteria of cluster analysis is presented by dendrograms.

For analysis of internal relations in sets of quantitative traits the factor analysis was used. For separation of common factors of the given sets of traits calculated were the matrix elements of their correlations. Next, the factor loadings were determined using the "normalized varimax rotation". By means of the slope plot diagram the number of factors was separated, the value of which is higher than 1. On the basis of that analysis selected were the traits highly correlated with principal components at load value >0.70, which explained most of the existing variability.

For the artificial neural network model the SOFM was used (Kohonen 2001), to classify the sites in respect of contents of chemical elements in soil and leaves, and "tar-spot" traits. The structure of the SOFM consists of two layers of neurons connected by weight (connection intensities). The input layer consisted of 47 input neurons (sites) and every neuron is represented by 26 chemical elements and two "tar-spot" traits (number and surface). The unsupervised training of the net was performed on the basis of dataset and the classical Kohonen's algorithm. The net was initiated by the random-Gaussian method. The learning phase has been broken down into 100 steps (EPOCHs) for the ordination phase and 1000 steps for the tuning phase. After the learning phase, it was found, that all the initial data are significant. Finally, the Kohonen's network has been created in the form of a two-dimensional map. Next, the Kohonen's topological map 5×5 has been designed. Its output layer consisted of 25 neurons (tetragons). The net was created according to the scheme "the winner takes all". The obtained Kohonen's topological map showed the neurons or groups of neurons activated by the particular investigated cases (sites).

The verification of the obtained results was carried out at significance level of 0.05 according to the statistical methods and principles given by Legendre and Legendre (1998), Zar (1999), Sokal and Rohlf (2003). For all calculations, the STATISTICA 8.0 program (StatSoft, Inc. 2007) was used. The numerical techniques applied in ecological investigations, i.e. collection of quantitative information on objects analysis and interpretation of results, as well as all numerical procedures, were based on principles given by Brower et al. (1998).

RESULTS AND DISCUSSION

Conventional numerical methods (descriptive statistics, ANOVA, relationships)

The examined *A. platanoides* sites differed significantly in respect of element concentrations in soil and leaf sam-

TABLE 1. Descriptive statistics of element concentrations (mg*kg^{-1}) in soil and leaf samples from *Acer platanoides* sites.

Element	Soil			Leaf		
	mean±SD	min.	max.	mean±SD	min.	max.
Be	0.92±0.74	0.10	2.60	0.37±0.33	0.02	1.50
Cd	0.83±1.13	0.10	6.10	0.85±0.88	0.01	3.04
Co	12.74±12.56	0.90	45.70	0.08±0.06	0.01	0.23
Cr	16.74±17.00	1.70	63.50	0.64±0.99	0.01	4.30
Cu	41.89±29.09	4.00	96.00	8.53±5.62	1.60	24.10
Fe	1192±1245	1160	7267	264±461	25.00	2975
Mn	259±103	113	624	120±161	23.00	853
Mo	1.17±1.06	0.20	6.80	1.57±0.96	0.21	4.10
N	2790±1233	1304	5142	37713±17301	6360	67581
Ni	9.13±10.01	1.40	45.70	2.98±2.76	0.20	10.20
Pb	242±274	12.10	847	9.28±8.50	0.30	34.50
S	1825±829	681	4846	2614±1749	647	6176
Zn	238±180	49.00	746	56.89±32.94	14.00	165

Explanation: SD – standard deviation

TABLE 2. Ranges of naturally occurring element concentrations (mg*kg^{-1}, ^{a}%) in soils and plants (Markert 1992).

Element	Be	Cd	Co	Cr	Cu	Fe	Mn	Mo	N	Ni	Pb	S	Zn
Soils	0.1-5	0.01-3	1-40	2-100	1-80	0.7-42^{a}	20-3000	0.2-5	0.2^{a}	2-50	0.1-200	0.02-0.2^{a}	3-300
Plants	0.001-0.4	0.03-0.5	0.02-0.5	0.2-1	2-20	5-200	1-700	0.03-5	1.2-7.5^{a}	0.4-4	0.1-5	0.06-1^{a}	15-150

ples ($F=24.7\div187.3 > F_{0.05;\ df1=46,\ df2=94}=1.50$) and show a wide range between the sites (Table 1).

Average values of element concentrations in soil and leaves from sites examined are similar in majority of cases in relation to data of Markert (1992), except lower value of Fe in soils and higher value of Pb in leaves (Table 2).

It has been stated connection between element in soil and leaf samples. The elements on the X axes are ordinated according to the criterion of the arithmetic mean and values of element concentrations on the Y axes are showed in logarithmic scale (Fig. 2).

The comparison of Figure 2a and b allows to conclude that the sequence of positions of N, S, Fe, Mn, Cu, Be is the same, while in case of Pb and Zn it is shifted by one position. The remaining elements do not show any ordination sequence. Such a sequence allows to conclude that higher values of element concentrations in soils correspond with higher concentrations in leaves.

The counted relationships revealed, that the contents of metals in soils correspond with higher concentrations in leaves. All relationships are positive and statistically significant ($r_{0.05;\ df=45}=0.29$), and ranged from r=0.29 to r=0.89 for Mn, Cd, Co, Cu, Ni, Pb, Be, Zn, S, N, Fe, respectively, except of Cr (r=0.22) and Mo (r=0.07). Kabata-Pendias (2001), Brej and Fabiszewski (2003), Walker et al. (2006) mention, that aerosols of the elements pollute the soil finally affect the plants. Further, these authors state, that the environmental deposition, mainly heavy metals, taken up from soil by plants through their root system are transported to other parts of the plant. That influence of pollution and the transport of ions is conditioned by various factors, namely by: size and mass of particles, kind of pollution, climatic conditions, wind strength and humidity level (Harrison and Chirgawi 1989; Markert 1992; Marschner 1995; Mengel and Kirkby 2001). All statements of the above mentioned authors are also reflected in the relationships fo-

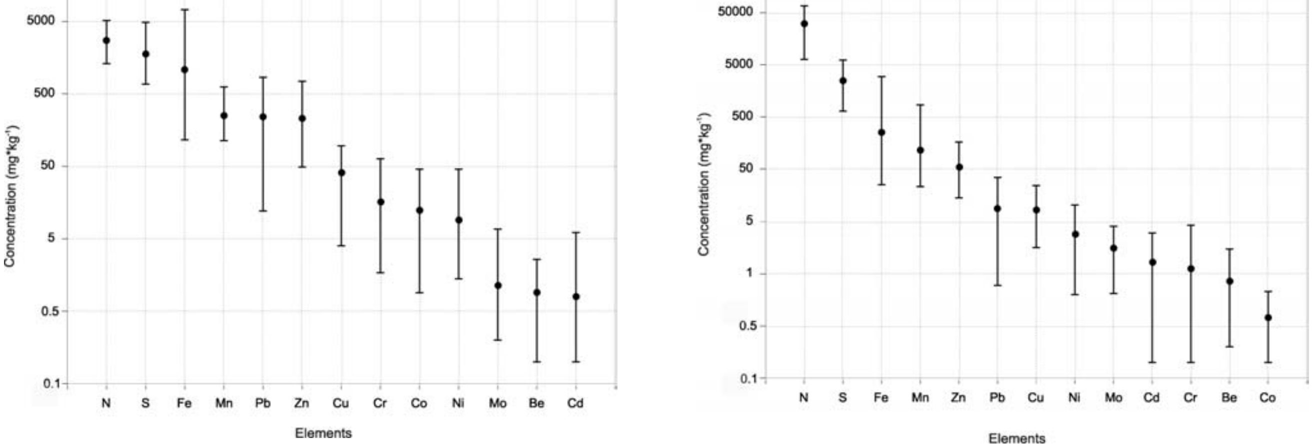

Fig. 2. The range of element concentrations in soil (a) and leaf (b) samples from examined sites of *Acer platanoides*.
Explanations: ● mean; ⟂ min., max. values (Y axes are expressed in logarithmic scale).

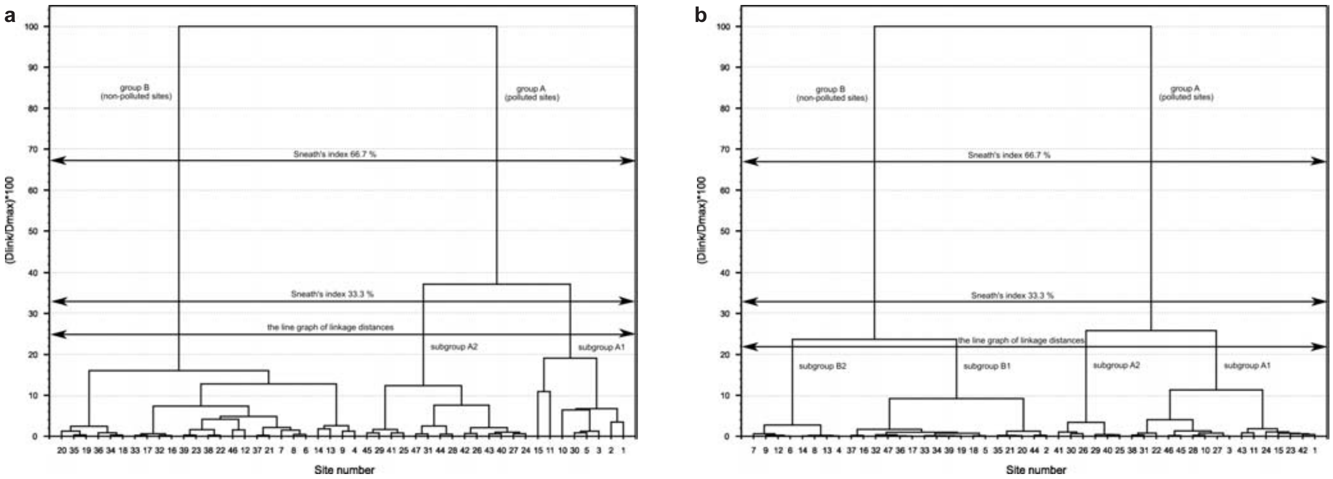

Fig. 3. Hierarchical tree plot of *Acer platanoides* sites in respect of soil (a) and leaf (b) chemical properties.

und in the present study, and show the actual chemical habitat conditions.

Multivariate exploratory techniques and "neural networks" (cluster, factor analyses and SOFM)

The cluster analysis determined the similarity of sites in respect of element concentrations in soils and leaves. The clustering method enabled to determine the proper structure of data, which divided them into two groups of sites A and B, using the Sneath's index 66.7%, and revealed their differentiated similarity (Fig. 3).

Group A includes sites of higher element concentrations in soils and group B with lower ones, thus polluted and non-polluted sites. But, the Sneath's index 33.3% differentiated additionally group A into two subgroups A1 and A2, similarly as the line graph of linkage distances. The lower similarity of subgroups A1 and A2 is probably caused by the different participation of elements in soils of the sites analysed quantitatively and qualitatively. Subgroup A1 connects sites from areas polluted by emissions of mining and energetic, and copper industry (sites 1, 30 and 15), urban agglomerations (sites 2, 3, 10, 11), and one traffic (site 5). Subgroup A2 includes mainly sites localized in areas polluted by emissions of copper industry (sites 24÷28, 40÷42, 44) and sites influenced by agglomeration emissions (sites 29, 31, 43, 45, 47). The remaining sites with lower element concentrations in soil are beyond direct influence of pollution, and forming group B in the dendrogram.

The similarity of sites in respect of element concentrations in leaves revealed two groups of sites A and B (Sneath's index 66.7 and 33.3%), and showed the least similarity of sites forming group A in relation to group B. That difference is caused by higher concentrations of elements in leaves from group A sites, and lower ones from sites of group B. The line graph of amalgamation schedule differentiated groups A and B into two subgroups (A1, A2 and B1, B2). Subgroups A1 and A2 clustering sites situated in areas at mining and energetic (sites 1, 30), copper industry (sites 15, 24÷28, 40÷42), and also urban (sites 3, 10, 11, 23, 29, 31, 43, 45, 46). Most of sites of subgroups B1 and B2 are not subjected directly with the mentioned above emission sources, but exist here some sites which are influenced by copper industry (site 44), urban (sites 2, 21) and motor transport pollution (sites 5÷9).

The two dendrograms are similar in relation to the site ordination of groups A and B. They show similarity of the examined sites in respect of element concentrations in soils and leaves. Thus, the different similarity is probably conditioned by type of emission, not only quantitatively, but also by its quality. The more so, that the sites, mainly from group A are situated in vicinity of emission sources, and are therefore under direct influence of their pollution. Sites of group A are affected by coal and energetic industry, copper industry, as well as by the complex of urban pollutions. Sites in group B are not directly connected with the mentioned above emission sources, and therefore these sites are recognized as non-polluted.

The present study shows, that the pollution of sites depends on character of the emission sources, which influence the environment and metal concentrations in plants. The same influence of various types of pollution on plants is confirmed by Kwapuliński and Sarosiek (1988), Dahmani-Müller et al. (2000), Kabata-Pendias (2001), Spencer (2001) and Salemaa et al. (2004).

It is supposed, that the factor conditioning the differentiation of sites' pollution is the chemism of pollution, both quantitative and qualitative. This is also confirmed by Kabata-Pendias (2001), Sawicka-Kapusta et al. (2003) and Salemaa et al. (2004). These authors found, that the quantitative and qualitative composition of pollutions depend on the type of emission sources, which condition the environmental chemism. Moreover, according to Helios-Rybicka (1996), Market et al. (1996) and the EMEP Report (2004), the sources of pollution are also, though in a lesser degree, pollutions of trans-boundary type, which modify the real pollution coming from local sources.

Factor analysis was used to reduce variables influencing the chemical composition of sites (in respect of soil and leaf chemical properties) (Table 3).

In case of soils, the first factor explained 57.2%, the second and third factor only 18.8% and 8.7%, respectively, of the total variance, and show positive values. Factor 1 differentiates the sites in respect of Cd, Fe, Mo, whereas factors 2 and 3 concern Cr, Cu and Be, N, Pb, S, respectively. In case of leaves, the first factor reflects 44.0%, the second and third factor only 24.9% and 8.8%, respectively, of the total variance, and have positive values. Factor 1 differentiates the sites in respect of Cd, Cu, Mo Ni, Pb, whereas factors 2 and 3 concern Cr, Mn, Zn and Be, N, S, respectively.

The comparison of factor analysis results for soils and leaves in respect of element concentrations of *A. platanoides*

TABLE 3. Factor loading values.

Element	Soil			Leaf		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Be	0.1803	0.4463	**0.7338**	-0.2105	0.4683	**0.7206**
Cd	**0.8778**	0.3316	0.2813	**0.9298**	0.0389	0.2103
Co	0.2927	0.4402	0.6217	0.0221	0.1488	0.6921
Cr	0.3385	**0.7757**	0.3473	0.0120	**0.7927**	0.3281
Cu	-0.0052	**0.8710**	0.1504	0.1059	**0.9634**	-0.0396
Fe	**0.9048**	0.0522	0.1140	0.0693	0.6816	0.4729
Mn	0.6687	0.0383	0.6371	0.0065	**0.8423**	-0.0849
Mo	**0.8812**	0.1327	0.0436	**0.9387**	0.1203	-0.1146
N	0.1913	0.3750	**0.8472**	0.6031	0.0047	**0.7062**
Ni	0.6794	0.5984	0.3043	**0.9241**	0.0226	0.0592
Pb	-0.3384	0.5423	**0.7133**	**0.8581**	-0.0096	0.3460
S	0.5547	-0.1000	**0.7108**	0.4033	0.2791	**0.7136**
Zn	0.5446	0.6720	0.3294	0.3043	**0.7058**	0.3940
% of total variance	57.2	18.8	8.7	44.0	24.9	8.8

Explanation: bold type indicates significance at >0.70

sites enables to show the conformity in respect of number of factors (three factors) and the same loadings (Cd, Cr, Cu, Mo, N, S).

Factors and loading values represent various quality pollution. According to Kabata-Pendias (2001), various elements present the type of industrial pollution. One can say, that the factor 1 represents assorted pollutions mainly metallurgy works, factor 2 represents industrial sources of copper works dust emissions, whereas factor 3 shows gaseous-dust pollution which are connected with energetic and urban sources.

The SOFM was used to identify groups of similar sites consisting of two types of units: the input layer is connected to each vector of the dataset (e.g. all soil, leaf chemical elements and "tar-spot" traits of 47 sites), and the output layer forms a two-dimensional array of nodes. In the output layer, the units of the grid give a representation of distribution of sample units in an ordered way. The topological map shows clearly group of sites with the highest and qualitatively different pollution, and with out infections. Group of sites with the lowest and low pollution have massive and sporadically infection, respectively (Fig. 4).

The examined sites within one neuron on the SOFM are the most similar, while sampling sites in neighboring neurons are more similar than sites in more distant ones.

In this study, SOFM mapping was demonstrated in patternizing environment by means of ecological data. The grouping of sites is possible through conventional analyses.

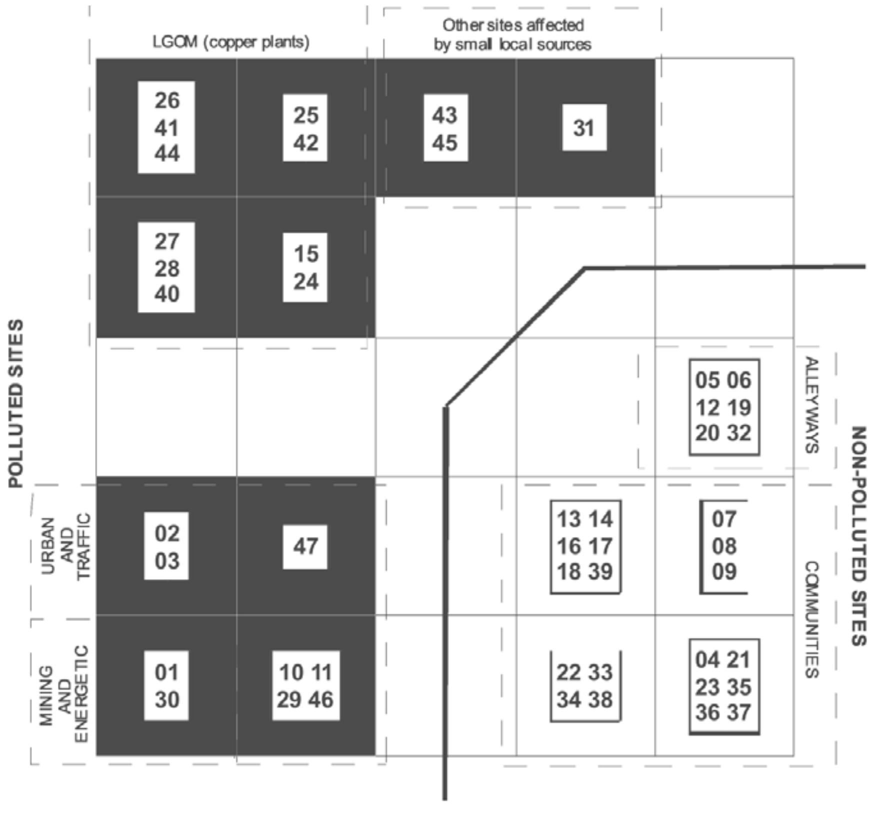

Fig. 4. SOFM of concentrations of elements in soil, leaf and "tar-spot" traits from *Acer platanoides* sites based on a 5×5 Kohonen layer. Explanation: 1÷47 sampling site numbers.

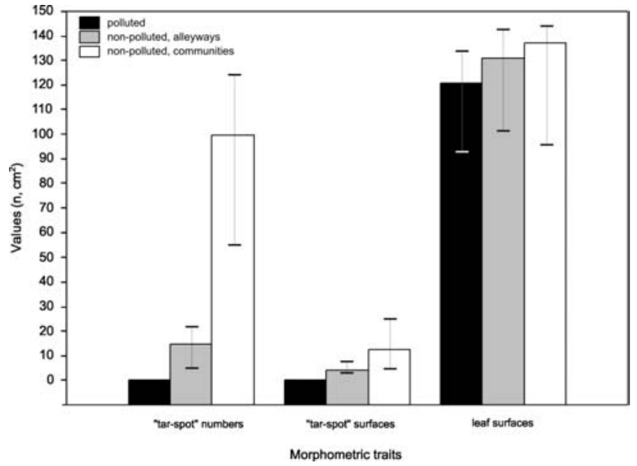

Fig. 5. The values of morphometric traits of *Acer platanoides* leaves and "tar-spots" of *Rhytisma acerinium*.
Explanations: ⊓ mean, ⌶ min., max. values.

However, it only represents the degree of intra-environmental association. The SOFM allows not only the grouping, but enables to patternize new data by assigning a new component. Chon et al. (1996) show, that this may be especially helpful in comprehensive understanding of ecological data.

Similar comparative techniques with use of conventional statistics, advanced methods and SOFM were applied by Giraudel and Lek (2001) for ordination of ecological communities, Lee and Scholz (2006) for assessment of SOFM as an alternative method, performance indicators, for constructed treatment of wetlands with respect to k-nearest neighbors (KNN) and support vector machine (SVM), Kosiba and Stankiewicz (2007) used SOFM and PCA for modelling *Utricularia* species microhabitats, Samecka-Cymerman et al. (2007) for classification of the relation between chemical compositions of aquatic bryophytes and streambeds, and by Stankiewicz and Kosiba (2009) for modelling of soil properties. These authors and Tadeusiewicz (2000) proved the importance of SOFM and recommend it as a good tool in ecological modelling, which can be used in various fields of applied ecology.

Image methods (morphometry and interpolation of leaf and "tar-spot" traits)

The examined leaf samples of *A. platanoides* differ significantly between sites in respect of "tar-spot" numbers on fresh leaves and the size of their surfaces on fallen leaves (F=84.3 and 7.8 $>F_{0.05;\ df1=46,\ df2=94}=1.50$, respectively). No significant differentiation was found in leaf surfaces (F=1.17 $<F_{0.05;\ df1=46,\ df2=94=}1.50$). The used post-hoc LSD test allowed to compare sites in respect of the *R. acerinium* traits examined. Found were three different groups of sites which statistically differ in number of "tar-spots" and in surface of stomata on fallen leaves. First group is represented by leaves without infection (polluted sites), second group of leaves was infected, but sporadically (not polluted alleyways sites) (mean: 14.9 and 4.2 cm^2, respectively), and third group of leaves has massive infections (non polluted communities sites) (mean: 99.8 and 12.7 cm^2, respectively) (Fig. 5).

Moreover, the relationship between the surface of the stomata on fallen leaves and the number of infections on fresh leaves collected in July, was calculated. The relationship revealed, that the surface of "tar-spots" on fallen leaves corresponds with number of infections on fresh leaves. It was found to be positive and statistically significant (r=0.72 $>r_{0.05;\ df=45}=0.29$). That relationship can be explained by increase in number of "tar-spots" closely situated, with initially small infection spots, which connect into bigger ones in autumn on fallen leaves. No significant relationship was found between number of infections and leaf surfaces (r=0.23 $<r_{0.05;\ df=45}=0.29$).

The use of morphometric traits of "tar-spots" appears to be an evident tool for presentation of the environmental status in bioindication. Klein and Paulus (1997), Niinemets and Kull (2003), Tretyakova and Noskova (2004), were used morphometrical techniques for assessment of variability of leaves and pollens of trees and shrubs in relation to pollution. According to these authors the usability of morphometrical techniques for various plant traits offer additional information on environmental quality.

The distribution maps "tar-spot" traits designed were by means of data interpolation (Fig. 6).

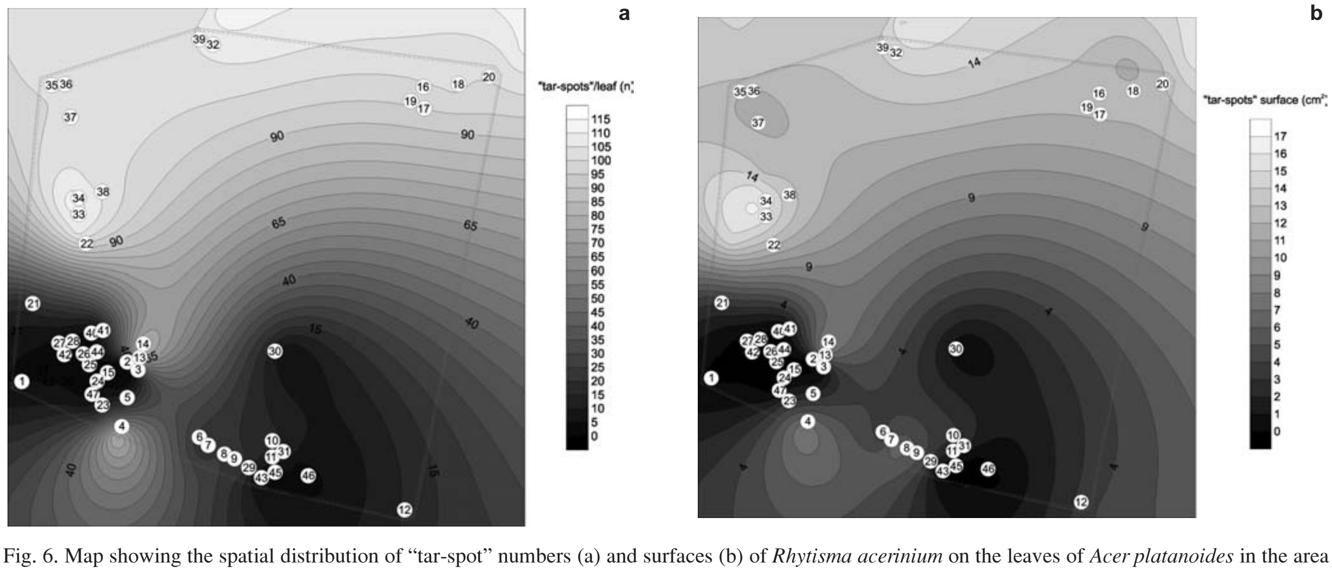

Fig. 6. Map showing the spatial distribution of "tar-spot" numbers (a) and surfaces (b) of *Rhytisma acerinium* on the leaves of *Acer platanoides* in the area of Poland.
Explanations: ○ sampling sites, □□□□ range of data.

The first map shows, that on sites being within the reach of pollution no *R. acerinium* infections have been found. Few infections occurred in sites, which were situated closely to thoroughfares or in territories situated beyond the direct influence of pollution. Numerous infections correspond to sites localized in forest communities, far away from emission sources. The second map shows a similar spread of stomata surfaces in relation to pollution level and habitat conditions of the sites examined.

The visualization of results by means of numerical techniques of imaging appeared to be convenient for presentation and interpretation. However, in that case the use statistic-mathematical methods is essential for a full verification of study results.

"Tar-spot" traits show the impact of pollution. In sites of higher element concentrations in soils and leaves no infections of *A. platanoides* by the fungus *R. acerinium* were recorded. On the contrary, massive infections and their large surfaces on fallen leaves collected in autumn, occur on sites of the lowest element concentrations. Worthy of notice is the fact, that *A. platanoides* trees growing in alleyways show a low or sporadic number of infections and their small surfaces, even though the sites are characterized by lower concentrations of the analysed elements in soils and leaves, i.e. they are polluted in a small degree. Here, *R. acerinium* does not find optimal developmental conditions. The habitat conditions differ in relation to sites where *A. platanoides* growth in communities. Alleyways are characterized by high insolation, windy, and of relatively low air humidity, as compared with sites occurring in mixed forest communities of wet and shady conditions. This suggests a significant impact of different microclimatic conditions upon the occurrence of *R. acerinium* (Kosiba 2007). Moreover, other factors influencing maple leaves infected by that fungus are substances neutralizing the negative influence of some elements included in the composition of pollution. Such a situation takes place on sites situated in urban areas and near thoroughfares, thus contaminated, but in a different degree. Here, the contents of most of the elements in soils and leaves point to the situation, in which infections should not occur. But they do occur here, though sporadically. It seems, that in this case the significant factor is the influence of cement and limestone dusts from the neighboring plants (Strzelce Opolskie and Opole) (sites 6÷9). This type of dusts acts as a neutralizer of gaseous pollutions (NO_X, SO_2) and heavy metals (Marschner 1995; Siedlecka et al. 2001). This is related with their basic properties through increase of soil reaction and water vapour in air, immobilizing in effect the heavy metals in soil and their assimilability by plants, and thus reducing their toxicity (Kabata-Pendias 2001), as well as mechanisms of tolerance to metals (Baranowska-Morek 2003). Sites 6÷9 are likewise affected by dusts from cement and limestone sources. Because of the considerable distance of the mentioned sites from the cement and limestone sources, the influence of neutralizing dusts on xenobiotics seems to be small. Nevertheless, the infections of leaves by the fungus are massive. It is supposed, that the massive occurrence of *R. acerinium,* as compared with the remaining sites, is affected by low contents of the elements in soils and leaves. But, probably the habitat conditions are also of importance in development of leaf infections. One can conclude, that the variability and differentiation between sites in respect of chemical elements and "tar-spot" traits on leaves is caused by the level of pollution.

CONCLUSIONS

The occurrence of *R. acerinium* on *A. platanoides* leaves depends strongly on complex of pollution (quality and quantity) of the environment. Of significance is the fact, that these infections do not occur in sites affected by various pollution as regards its quality. This is connected with the complex of gaseous-dust pollutions. In non polluted sites with proper developmental habitat conditions for *R. acerinium* numerous infections take place in the summer period, which correspond to larger stomata surfaces on dropped leaves in autumn. The easiness and accessibility of using the traits of that fungus twice a year considerably rises its rank as a bioindicator.

The use of various methods of ecological objects is an important means for presentation of environmental status. The comparison of results obtained by means of conventional statistical methods and image techniques with results of SOFM displayed the differences of *A. platanoides* sites in respect of pollution level, but different in degree of precision. Hence, SOFM appeared to be more effective, more proper/fit for phenomena and processes taking place in natural environment. SOFM, in relation to the remaining numerical and image methods is more useful in ecology, and ought to be taken into account as a possible tool of estimation of various plants and their biotopes. Moreover, the model of SOFM "write down" to the file could be used in future, to prognose and the simulation across introduction of a new data and components, and a good tool in ecological modelling, which can be used in various fields of applied ecology.

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