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Searching for lichen indicator species: the application of self-organizing maps in air quality assessment—a case study from Balkan area (Serbia)

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Abstract The subject of this paper is the possibility of using self-organizing map (SOM) in the biomonitoring studies. We used lichens as biomonitors to indicate different degrees of air quality. This research included all of 88 lichen species that was collected at 75 investigated points. These lichen species showed the different responses to air pollution. The air quality was assessed by IAP (index of atmospheric pollution) values. The IAP values were calculated for all of investigated points on the territory of four natural and one urban ecosystem. Calculated IAP values were in range of 10 to 75. On the basis of the lichen data and IAP values, we have employed SOM analysis that distinguished three clusters (A, B, and C). It presented lichen indicator species for each cluster: 16 species for cluster A, 18 species for cluster B, and two species for cluster C. This paper presents a useful method to find indicator species.

Keywords Bioindication · Mapping · Zonification · SOM analysis · Clusters

Introduction

In the last two decades, the Kohonen artificial neural networks, also known as a self-organizing map (SOM) (Kohonen 2001), is considered a convenient tool for analyzing ecological data. Its effectiveness has been confirmed in several previous studies that considered various types of ecological data aiming to present distribution patterns of different biota (Park et al. 2005, 2006; Penczak et al. 2005; Kruk et al. 2007; Stojković et al. 2013) or emphasize main environmental factors that affect living organisms (Kruk et al. 2007; Park et al. 2005, 2006; Penczak 2007). Moreover, Samecka-Cymerman et al. (2009) applied the SOM analysis in biomonitoring studies. This analysis has been recently used in a moss biomonitoring survey (Deljanin et al. 2015). All these authors emphasized superiority of SOM method over traditionally used gradient (PCA, CCA) (Palmer 1993; Penczak et al. 2000, 2002) and cluster analysis (Penczak et al. 2000), in processing large and nonlinear datasets. Thus, SOM is considered a powerful visualization technique for presenting various patterns of ecological data (Kruk et al. 2007; Chon 2011).

Biomonitoring studies often use various types of organisms like lichens, bryophytes, and vascular plants as a tool

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for evaluating air quality (Wielgolaski 1975). Lichens are composed of two different organisms—algae and fungi—that are interdependent. Thanks to their structure, they may be used to study the spatial and temporal trends of atmospheric pollutants (Conti and Cecchetti 2001).

Lichens are used for bioindication studies, as well as biomonitoring studies (Boamponsem and de Freitas 2017; Chaparro et al. 2013; Kodnik et al. 2017; Marié et al. 2018; Lorenzini et al. 2003). The high sensitivity of different lichen species to atmospheric pollutants has been recorded by many authors (Conti and Cecchetti 2001).

In this paper, we start from the hypothesis that lichen function as an indicator of air quality will be more clearly outlined by using such a tool. Types of different levels of tolerance are expected to stand out as indicators. A wide range of different sensitivities has been established and single species, as well as combinations of species, have appeared to work well as bioindicators for monitoring the effects of air pollution (Van Haluwyn and Van Herk 2002). In multivariate analysis, species scores in an ordination diagram supply a measure of species sensitivity to air pollution.

The main goal of this study was to apply the SOM analysis for clustering the lichens data and to find the lichens indicator species for each of the clusters. Except that the important goal of this study is to estimate the degree of air quality in different environments in which it has not been done until now. To achieve this, three tasks were defined: (1) to pattern lichen data, (2) to define how many lichens can be distinguished as indicators, and (3) to present the importance of each lichen species as a numerical measure of its indicator value.

Materials and methods

Study area and sampling procedure

The Republic of Serbia is located in the central part of the Balkan Peninsula. The study area was located in Serbia, in the *Toplica* District (Fig. 1). The Toplica District is in southern Serbia. The study area is made up of four natural (Lukovska, Kuršumlijska and Prolom Spa, and Radan Mountain) and one urban ecosystem: the town of Kuršumlija.

The investigated area is mountainous. The altitude ranges from 300 to 1703 m. The climate is moderate-continental. The mean air temperature is 11.4 °C, ranging from 1.8 to 32.8 °C (mean monthly minimum and maximum, respectively). The most frequent wind is the south-west (RHSS 2013).

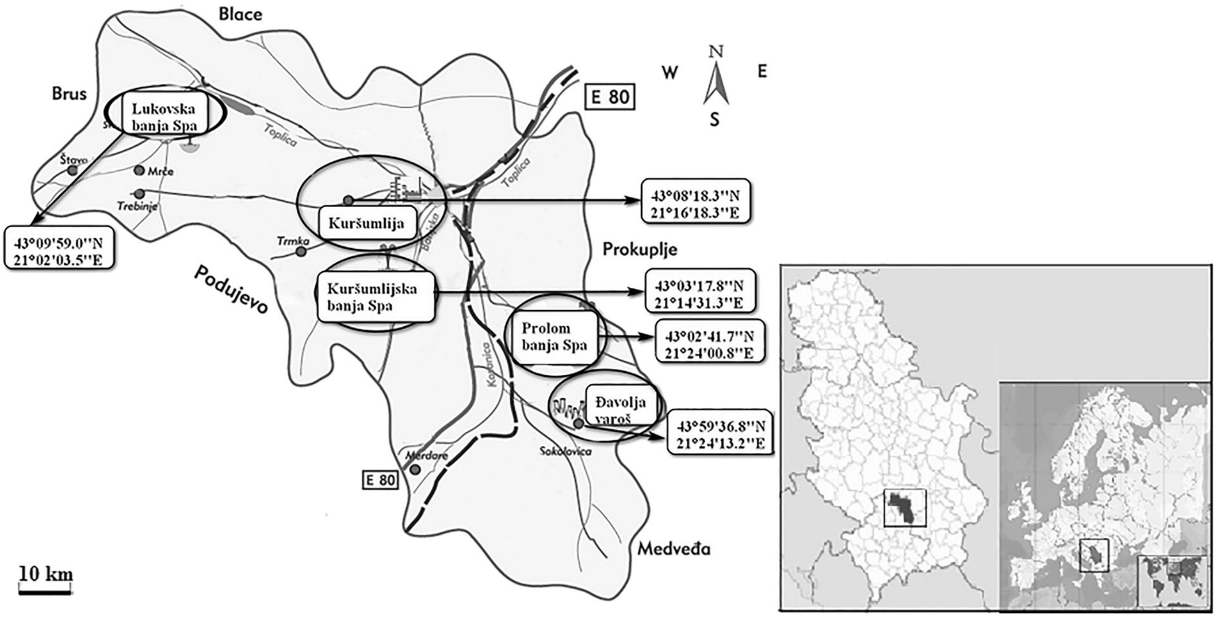

Fig. 1 Study areas

Epiphytic lichens were sampled at 75 sampling points in the year of 2015. The research included 50 sampling points in natural (15 sampling points of each selected area) and 15 sampling points in urban ecosystem.

The only urban ecosystem analyzed in this paper is characterized by relatively well-developed motor traffic, which is a possible cause of pollution, with a certain deficiency of the vegetation, which is the basis for the development of lichens. Potential pollution can also be caused by individual household heating. On the other hand, natural ecosystems that include spas are characterized by a much lower intensity of motor traffic. Another important feature is that hot water from natural thermal springs is used in spas as a source of heating, thus reducing the number of vines being the source of pollution caused by combustion. Natural ecosystems include the Radan Mountain range. This area is covered with dense forests and sparsely populated. There are no potential air pollutants on Radan Mountain.

The lichens were collected from the bark of species of trees possessing the same or similar properties (pH, water storing capacity, nutrient content), exclusively from trunks angled no higher than 5°. The sampling ladder (having five 10 × 10 cm contiguous quadrates) was attached to the trunk at the cardinal points so that the upper edge of the ladder was 1.5 m above the highest point of the ground. All lichen species in the ladder and their frequency in the 5 quadrates of the ladder were recorded. The list of species with their frequency values per single ladder constitutes a releve of lichen vegetation (Nimis et al. 2002). Voucher specimens of the lichens were determined and deposited in the lichenological herbarium at the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Serbia.

Species identification

Two different kinds of microscopes (stereomicroscope from 20- to 60-fold and microscope 100-fold) were used for identification of lichen species in a laboratory. In order to distinguish different lichen species, the following reagents and chemicals were used: potassium hydroxide 10% (K), sodium hypochlorite (C), and paraphenylenediamine (P). A number of different sources (Dobson 2005; Wirth 1995) were used as well.

Determination of the plant species used as substrates for growth of lichens was made according to the Flora of Serbia (Josifović 1970-1976; Josifović ed. 1977) and nomenclature of the plant species followed the Euro-Med Plant Base (http://www.emplantbase.org).

Methodology

The assessment of air quality is determined by quantitative method for calculation of index of atmospheric purity (IAP) (Kricke and Loppi 2002):

$$IAP = \sum f$$

where f is the coefficient which represents the frequency and coverage of each species within the study area.

The coverage of each recorded lichen species is expressed in values from 0 to 10, on a scale characterizing the cover: Values 9–10 were assigned to species with very high frequency of finding and a very high degree of coverage (80% to 100%); values 7–8 were assigned to species with either high frequency or high coverage (60% to 80%); values 5–6 are assigned to species that are not often found or have a low degree of coverage (40% to 60%); values 3–4 are assigned to rarely encountered species or the ones that have low coverage (20% to 40%); values 0–2 are assigned to species that are very rare and with very low coverage (0% to 20%) (Byazrov 2002).

The index of atmospheric purity was calculated for each of the 75 sampling points. Higher index values indicate better air quality, while lower values indicate that the air quality is low. A scale was used to estimate the degree of air pollution and determine the indication zone (Conti and Cecchetti 2001) (Table 1).

Based on the IAP values, three basic air quality zones were distinguished:

1. “Normal zone” occupies areas where the air is clean or there is no significant air pollution ($IAP > 37.5$).
2. “Struggle” zone includes a surface with a moderate level of pollution ($12.5 \leq IAP \leq 37.5$).
3. “Lichen desert” occupies the territory with the highest degree of air pollution ($0 < IAP \leq 12.5$) (Conti and Cecchetti 2001).

The universal kriging interpolation method with a linear variogram was applied to construct maps displaying formed air quality indication zones based

Table 1 The scale of air quality assessment on the basis of the degree of pollution and the IAP value (Conti and Cecchetti 2001)

Degree of pollution	***Extremely high***	***Very high***	***High***	***Moderate***	***Low***	***Very low***
Color	🟥	🟥	🟧 🟧	🟨 🟨	🟩 🟩	🟩 🟦
IAP	0		12.5	25.0	37.5	50.0
Lichen indication zones	*Lichen desert*		*Struggle zone*		*Normal zone*	

on IAP values. In order to graphically display the distribution of points with different IAP values, percentile maps were used where different colors show different IAP values.

Statistical analyses

The sites were classified based on lichen data using a Kohonen self-organizing map (SOM) (Kohonen 2001). In comparison with other multivariate methods, SOM analysis is a powerful tool for the clustering and visualization of a large dataset and has already been successfully applied in many ecological studies (Chon et al. 1996, 2000; Chon 2011).

The SOM network consists of two layers: an input or Kohonen layer composed of neurons whose number depends on the number of variables used in the analysis and an output layer represented as a hexagonal lattice (Kohonen 2001). The input matrix in our study contains 88 rows (sampling sites) and 75 columns (taxa). The values of species coverage were log transformed (log (x + 1)) and then normalized. As soon as input layer receives data from input data matrix, the training of the network begins, which considers the sequential inclusion of each input vector (a row of the input matrix) in the SOM network. When the input vector passed through the network, it is mapped to a specific neuron of the output layer. The each output layer contains a specific model of a data. The similarity of models carried by neurons is proportional to their mutual distance (dissimilarity increases when increasing its mutual distance).

Based on the rules proposed by Vesanto et al. (2000) and Park et al. (2003), an optimal number of output neurons (in our study 7 × 6) was chosen. Finally, the *k*-means clustering method was used to divide the SOM cells into several clusters (Jain and Dubes 1988).

In order to quantify importance of species on which the cluster division was based on, the indicator value analysis (IndVal) (Dufrêne and Legendre 1997) was employed. Dufrêne and Legendre (1997) assumed that the maximum indicator value (100%) occurs when all individuals of some species are present at all sites of one particular cluster. In addition, a threshold level of 25% (IndVal >25) indicates that the species occurs in at least 50% of the sites from a specific cluster and that this cluster contains at least 50% of the total abundance of the species. Indicator values were calculated for each species and were attributed to the clusters distinguished by the SOM. The Monte Carlo test with 1000 permutations was applied to select species with a statistically significant indicator value. To calculate the IndVal values, PC-ORD 4.0 for Windows software (McCune and Mefford 1999) was used. Finally, we compared the component planes produced by the SOM to establish which species were shown to be important by means of their indicator values.

Results and discussion

At 75 sampling points, the total number of recorded lichen species with different responses to pollution was 88. The species showing the highest frequency values (100%) were the following: *Hypogymnia physodes*, *Parmelia sulcata*, *Physcia adscendens*, *Evernia prunastri*, *Flavoparmelia caperata*, *Lecidella elaeochroma*, *Melanelixia subaurifera*, *Phaeophyscia orbicularis*, *Physcia aipolia*, *Physcia tenella*, *Physcia stellaris*, *Physconia grisea*, and *Pseudevernia furfuracea*. A very low frequency (6.67%) is assigned to some species which were found at only one sampling point: *Graphis elegans*, *Lecanora expallens*, *Lecanora chlarotera*, *Lecanora glabrata*, *Lecidella carpathica*, *Melanohalea olivacea*, *Peltigera collina*, *Physcia clementei*, *Physconia detersa*, *Ramalina canariensis*, *Rinodina colobina*, and *Xanthoparmelia conspersa*.

The values of IAP ranged between 10 and 75 (Table S1, S2, S3, S4, and S5). These values are closely related to the number of lichen species and the level of atmospheric pollution. For estimation and determination of the degree of air pollution and the indication zone, Conti and Cecchetti's scale was used (Conti and Cecchetti 2001) (Table 1). Pursuant to the scale, the investigated area includes three air quality zones: "lichen desert zone," "struggle zone," and "normal zone" (Fig. 2).

The normal zone (A) indicates good air quality, and it occupies a significant part of almost all of the areas investigated. The exception in this study is the area of the town of Kuršumlija, where the presence of the normal zone was not recorded. This zone is characterized by IAP values ≥ 37.5.

The struggle zone (B) occupies the narrow central part of Kuršumlija Spa and a large part of the area investigated in Kuršumlija town. Compared with the normal zone, this zone indicates lower air quality, and so the IAP values are significantly lower, ranging from 14 to 37.

No lichen desert zones (C) were recorded in rural, scarcely inhabited parts of the areas investigated. The presence of this zone was recorded only in the urban ecosystem, i.e., in the town of Kuršumlija. Namely, this zone covers the narrow center of the city, and, in this study, it is characterized by values of 10 and 11.

Based on the SOM analysis, three cluster,: A (31 points), B (18 points), and C (26 points), were distinguished (Fig. 3). In comparison with the a priori defined zones, cluster A consisted of 31 sampling points, and 100% (31 out of 31) of present points in cluster A belong to the normal zone indications of air quality. Cluster B consisted of 18 sampling points. Similar to cluster A, all of the sampling points that made up cluster B belong to the "normal zone" indications of air quality. Unlike clusters A and B, all of the sampling points in cluster C were located in the town of Kuršumlija which was the only urban study area. The majority of sampling points in cluster C belonged to the "struggle" zone. These points make up 53.85% of

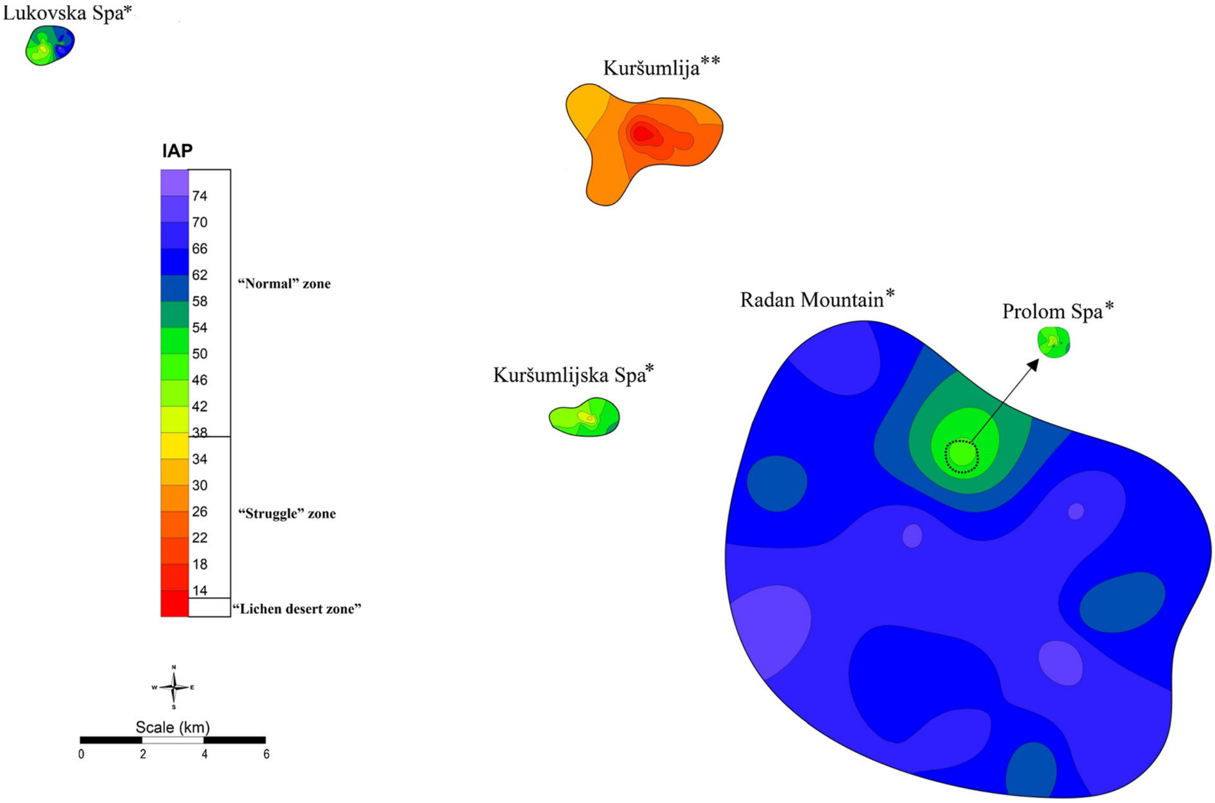

Fig. 2 General map of the spatial distribution of different zones to indicate the air quality for all of the study areas (*natural ecosystem, **urban ecosystem)

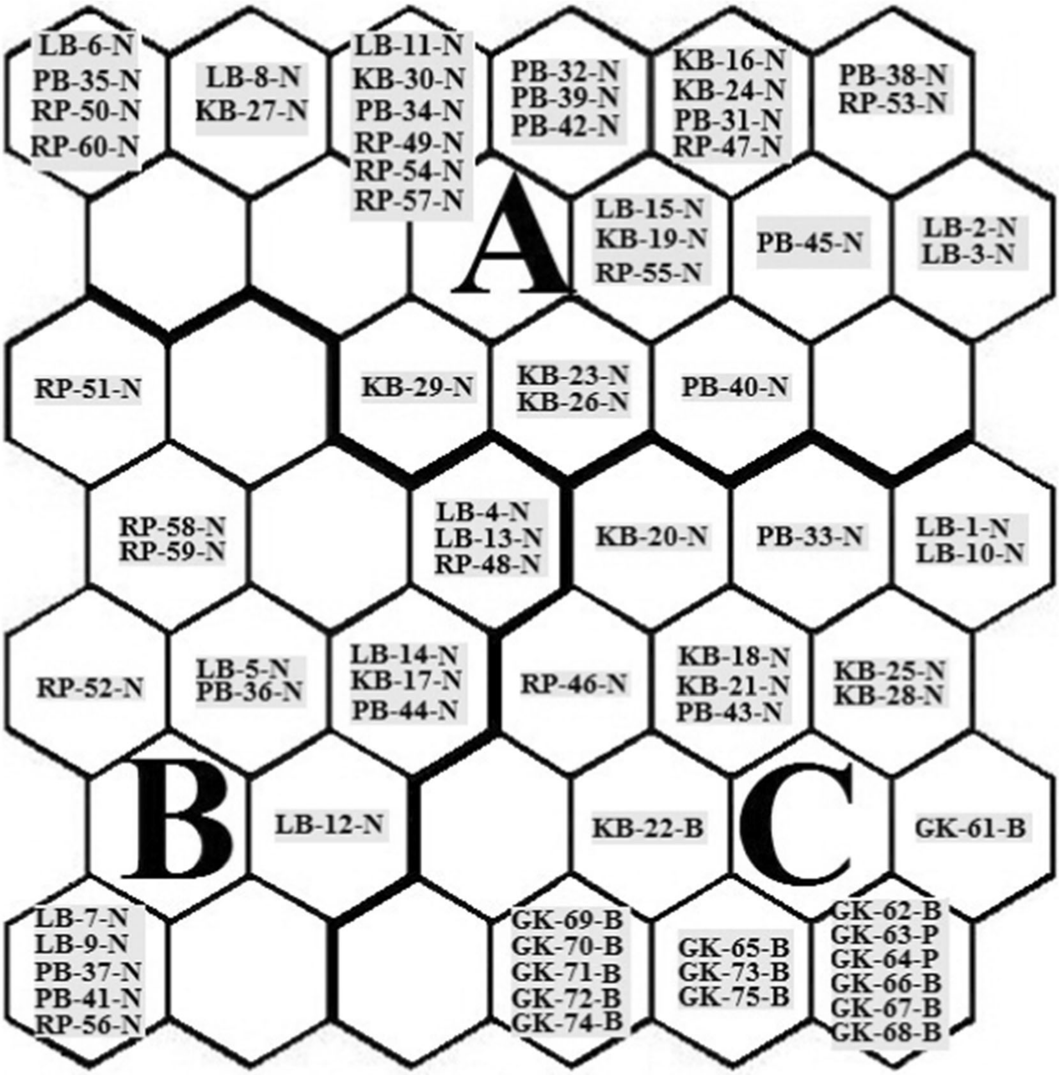

Fig. 3 Distribution of the 75 sampling points on the SOM. Symbols within the neurons represent different sampling points and different zone of lichen indication of air quality (marked with symbols N, B, P)

cluster C. Cluster C is made up of two sampling points (7.69%) in the "lichen desert" zone, while 10 of the 26 sampling points in cluster C (38.46%) belong to the "normal zone" of lichen indications.

Finally, differences in the species richness and lichen cover were examined between the urban and natural environment. The results demonstrated that the species number and the percentage of cover were higher in the natural ecosystem than the urban one. The highest IAP values were calculated for the sampling points on Radan Mountain (natural ecosystem). Also, at the sampling points in Kuršumlija (urban ecosystem), the IAP values were the lowest.

The IndVal analysis produced indicator species for each cluster. The IndVal analysis selected 16 representative species and one taxa with IndVal lower than 25% for cluster A (Table 2). Species that have high and significant IndVal values greater than 25% are listed as representatives of cluster B. However, three species with indicator values lower than 25% were also considered to be important and significant (Table 2). Finally, the IndVal analysis revealed two representative species for cluster C.

The distribution patterns of each species individually, including indicator lichen species selected by IndVal analysis, were presented on component plans on the

Table 2 Species distinguished by the IndVal index for each cluster (A, B, and C)

A		B		C	
Taxa	IndVal	Taxa	IndVal	Taxa	IndVal
Lepra amara	69.3***	***Pleurosticta acetabulum***	80.2***	***Xanthoria parietina***	42.8**
Ramalina fraxinea	69.2***	***Melanelixia glabra***	45.3**	***Candelariella xanthostigma***	34.1*
Lepraria incana	68.1***	***Lecanora pulicaris***	44.9**		
Ramalina fastigiata	64.1***	***Platismatia glauca***	44.7***		
Flavoparmelia caperata	62.3***	***Graphis scripta***	42.8**		
Physcia semipinnata	59.5***	***Physcia stellaris***	40.1*		
Usnea hirta	58.9***	***Cladonia fimbriata***	39.9**		
Ramalina farinacea	52.4***	***Ochrolechia pallescens***	39.8*		
Pseudevernia furfuracea	51.6***	***Phaeophyscia orbicularis***	39.7*		
Evernia prunastri	46.2***	***Peltigera canina***	39.5**		
Usnea subfloridana	43.8***	***Lecidella elaeochroma***	38.8*		
Hypogymnia tubulosa	41.4*	***Cladonia rangiformis***	34.9**		
Hypogymnia physodes	39.3*	***Melanohalea exasperata***	34.2*		
Cladonia subulata	29.4**	***Melanelixia fuliginosa***	33.9**		
Cladonia foliacea	28.1*	***Xanthoparmelia somloensis***	30.1**		
Lepra albescens	26.5*	***Physconia enteroxantha***	30*		
Anaptychia ciliaris	24*	***Buellia punctata***	27.1*		
		Cladonia ramulosa	26.5***		
		Candelariella aurella	21*		
		Cladonia pyxidata	19.2**		
		Parmelina quercina	16.6*		

* Significance level: < 0.05

** Significance level: < 0.01

*** Significance level: < 0.001

Those in bold indicate the representative species for the SOM clusters with IndVal values of more than 25%. The species without significant IndVal values are included at the end of the columns

SOM (Figure S1). This visualization technique provides important information on the importance of each species in the SOM units on a gray scale.

The indication and zonation of different degrees of air pollution using lichens as bioindicators are presented in the form of basic zones. Figure 2 clearly shows a comprehensive general presentation of the differences in air quality among the different sampling areas.

It should be noted that these zones describing varying degrees of air quality reflect the conditions in a given space over an extended period of time (Mayer et al. 2013; Sujetovienė 2015). In this respect, one of the earliest and most notable estimations of this condition is the scale used to measure the qualitative rating of the sensitivity of epiphytic lichens in the area of England and Wales (Hawksworth and Rose 1970).

As part of their research, Conti and Cecchetti (2001) showed a scale of different levels of air pollution based on variations in IAP values. The authors distinguish five levels of pollution: very high, high, moderate, low, and very low. The same scale was applied in this study (Fig. S1).

On the basis of this investigation, it can be concluded that the worst air quality is in Kuršumlija town (Fig. 2). On the Kuršumlija town map, two basic zones indicating the presence of lichen can be seen. The desert zone occupies a small part of the area, primarily the socially active center of the town. This is expected since it is under more intensive anthropogenic influence. Within the zone, some of the sampling points have characteristic, relatively low IAP values. However, this zone shows tendencies towards a possible transition into the struggle zone because the presence of species that are moderately tolerant to air pollution, such as *Lecidella elaeochroma* (Van Dobben and Ter Braak 1999), is also recorded. The struggle zone includes points which have higher IAP values, so the conclusion is that this part of the area is characterized by good air quality.

On the basis of these results, conducted starting hypothesis is confirmed. Lichen species of different levels of tolerance are highlighted as indicators.

The biggest problem for the air quality is pollution from the production facilities owned by the Kopaonik Company, where fuel oil and wood waste are used for heating the workspace and in the steam boilers that are part of the production process. The combustion of these materials constantly releases smoke containing a large amount of inert dust. In addition to the Kopaonik Company, other potential sources of aerial contamination in the city are high furnaces in other companies and institutions located mostly in the center of the city. Households also use wood and coal for heating.

Traffic is another polluter, too. According to the data from the Statistical Office of the Republic of Serbia, gathered in 2015 when this research was conducted on the territory of the Kursumlija municipality (SORS 2016), a total of 4890 motor vehicles were registered, which was a relatively large number since Kursumlija is the city with 12,000 inhabitants (SORS 2014). Vehicles emit not only gases and particulate matter by combustion but also harmful particles by abrasion of the brake system and other different parts.

A possible reason for this distribution of air pollution zones relates to certain limitations in the process of recording the presence of lichens and collecting samples, which is reflected in the inability to visit inaccessible terrains, as well as the existence of young and underdeveloped trees and deforestation in certain parts of the area, which has led to many potential substrates losing their predisposition to be settled by the lichens.

Slaby and Lisowska (2012) record identical results in their research conducted in Krakow. The subject of their research was the recolonization of epiphytic lichens in the urban ecosystem as a result of air quality improvement. According to Llop et al. (2012), the intensity of traffic and firewood used in individual households has a great negative impact on the air quality, and they result in a reduction in the diversity of the lichen. Similar results were also reported in earlier studies (Conti and Cecchetti 2001; Loppi et al. 2002; Loppi and Corsini 2003; Washburn and Cullen 2006; Perlmutter 2010).

According to the results of the SOM analysis, based on the distribution of the sampling points and zones expressing the different degrees of air quality on the SOM map, three groups were formed—A, B, and C (Fig. 3). Group A is characterized by the largest number of sampling points that feature very good air quality, while Group C is characterized by points with very low IAP values.

In previous studies, some types of lichen have been presented as being very good indicators. The *Flavoparmelia caperata* indicator was useful in identifying different air quality zones (Nimis et al. 1990). On the basis of several quantitative results, Will-Wolf and associates (Will-Wolf et al. 2015) included it as a relatively vulnerable indicator of air pollution. Our study highlighted *Flavoparmelia caperata* as an indicator of good air quality (an A cluster indicator), thanks to the fact that our study has found its very high IndVal value. In their earlier publication, Will-Wolf et al. (2015) presented the species *Usnea hirta*, *Usnea subfloridana*, *Hypogymnia tubulosa*, *Hypogymnia physodes*, *Flavoparmelia caperata*, *Platismatia glauca*, and *Melanelixia fuliginosa* as being sensitive to air pollution, the finding of whom was confirmed by our results. The species *Lepra amara*, *Ramalina fraxinea*, and *Lepraria incana* had the highest IndVal values, and these types are considered to be the best indicators of cluster A (Table 2), although the values of the species that have been singled out show a clear uniformity. The IndVal value lower than 25% was met only in one species in cluster A (*Anaptychia ciliaris*), indicating that this species is important, but not its representative.

In group B, all the sampling points are characterized by relatively good air quality based on IAP values, too. IndVal analysis indicated 21 species that describe this group and have high and statistically significant values. With this we can conclude that the *Pleurosticta acetabulum* species describes this group best, showing an extremely high indicator value (80.2).

Almost all of the sampling points in group C are characterized by the poorest air quality (Fig. 3). The indicators of this group are two representative species—*Xanthoria parietina* and *Candelariella xanthostigma* (Table 2)—which have also been characterized as tolerant or moderately tolerant to air pollution in earlier studies (Saipunkaew et al. 2005; Davies et al. 2007).

As the technique of applying self-organizing maps is considered particularly suitable for use with large (a 75 × 88 data matrix) datasets (Chon 2011), the selection of the method is fully justified since the amount of data in this study is extensive.

Conclusion

Self-organizing maps, as an important instrument for detecting and confirming the role of large groups of data, have proved to be very useful in examining the relationship between the state of the environment and the function of lichens as indicators. Using this tool is recommendable for obtaining relevant results in bioindication and biomonitoring studies.

Since there is no information from previous studies of this type conducted in the study area, it is important and necessary for research to continue, which would give rise to new knowledge about the possibilities of Kohonen's neural networks in the field of bioindication as the basis of biomonitoring.

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