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Epiphytic lichens as indicators of atmospheric pollution from industrial sources

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Abstract. Industrial activities are important sources of atmospheric pollution worldwide. The elemental content in lichens was measured and analyzed with respect to the dominant wind direction and distance from pollution sources. In direction of the dominant winds, the content of Fe decreased with distance from the pollution source, which was a steel works situated in the flat country. Otherwise, in direction of the dominant winds, the content of Mn increased depending on the circumferences of trees found at various distances from the pollution sources. Older trees represented valuable indicators of elemental bioaccumulation over time. It was confirmed that geomorphology of the studied areas, dominant wind direction and older trees played an important role in the elemental bioaccumulation in lichens along the spatial gradient. Therefore, epiphytic lichens are regarded as valuable indicators in monitoring of environmental pollution in the study area.

Key words: distance from pollution sources, lichen, metals, monitoring, pollution sources, wind direction

Introduction

Lichens are valuable biomonitors of atmospheric pollution (Giordani 2007; Riddell & al. 2011; Stamenković & al. 2013a). Atmospheric pollution is widely responsible for human mortality (Cislaghi & Nimis 1997; Farkas & al. 2001); therefore, measurements of the improvement in air quality are needed (Estrabou & al. 2011). Application of lichens as monitors and indicators is potentially due to their capacity to accumulate chemical compounds and to their sensitivity to environmental stress (Frati & Brunialti 2006; Loppi & Frati 2006). Epiphytic lichens are important bioaccumulators because elemental concentrations in the lichen thalli reflect the concentration of pollutants in the environment (van Dobben & al. 2001). Furthermore, lichens accumulate pollutants in amounts that exceed their physiological needs; therefore, these organisms act as accumulators of air pollutants (Sujetovienė 2010). Lichens have been used on a different spatial scale to monitor atmospheric pollution and are not expensive as tools (Ite & al. 2014).

Industrial and urban areas are subject to anthropogenic pressure. Thus, different methods of assessing environmental quality have been developed, such as the lichen diversity value (Frati & Brunialti 2006; Svoboda 2007), index of lichen diversity (Loppi & Frati 2006), index of atmospheric purity (Gombert & al. 2004), and Verein Deutscher Ingeniure (Hierschläger & Türk 2012). It is well known that the *Xanthorion* community has greater resistance to air pollution in rural areas. Thus species that belong to that community are used in biomonitoring studies (Loppi & Frati 2006). In the transition zone between steel factories and forested areas, forests play a crucial role in reducing the atmospheric pollution (State & al. 2010).

The aim of this study was to assess atmospheric pollution as a consequence of industrial activities in the direction of and opposite to the dominant wind. The objectives of the study were: (i) to assess atmospheric pollution up to 90 km from a pollution source in direction of the dominant wind and up to 90 km from the same pollution source in direction opposite to that of the dominant wind (the spatial gradient of 90 km has been represented by spatial fragments of 30 km each); (ii) to assess metal concentrations detected in the central and peripheral parts of the lichen thalli according to the spatial gradient and direction of the dominant wind; and (iii) to assess the change in relationships between the metal concentrations in the central and peripheral parts of the lichen thalli with the distance from pollution sources, distance from roadways, altitude, and tree circumference. This study maintained the following hypothesis: along the distance gradient from the pollution sources, the lichens in direction opposite to the dominant wind would have lower concentrations of atmospheric pollutants in comparison to the enhanced concentrations of atmospheric pollutants in lichens in direction of the dominant wind.

Material and methods

Epiphytic lichen species were used to assess atmospheric pollution along a distance gradient from industrial pollution sources. Locations of the investigated pollution sources in Romania, geomorphological characterization of sites, dominant wind directions, mean wind speed, supposed pollutants, and the number of total sites are presented in Table 1. Seven sites were selected for the pollution sources of Galați Steel Works (GLSW), Ișalnița Power Station (IPS) and Oltchim works (OW) , while six sites were selected for Cernavodă Nuclear Power Plant (CNPP) and Cuprom Refiney (CR), because of the Black Sea in the case of CNPP and the northern border of Romania in the case of CR (Fig. 1). In the case of Copşa Mică Works (CMW), the distance gradient was partially overlapping with the distance gradient of OW; therefore, only five sites were selected (Fig. 1).

The pollution sources were represented by different industrial units situated in lowland (flatland and hilly zones) and highland (mountain zones) areas (Table 1). In the case of OW and CR, data were collected along a spatial gradient only in direction of the dominant wind (Fig. 1).Field data were collected

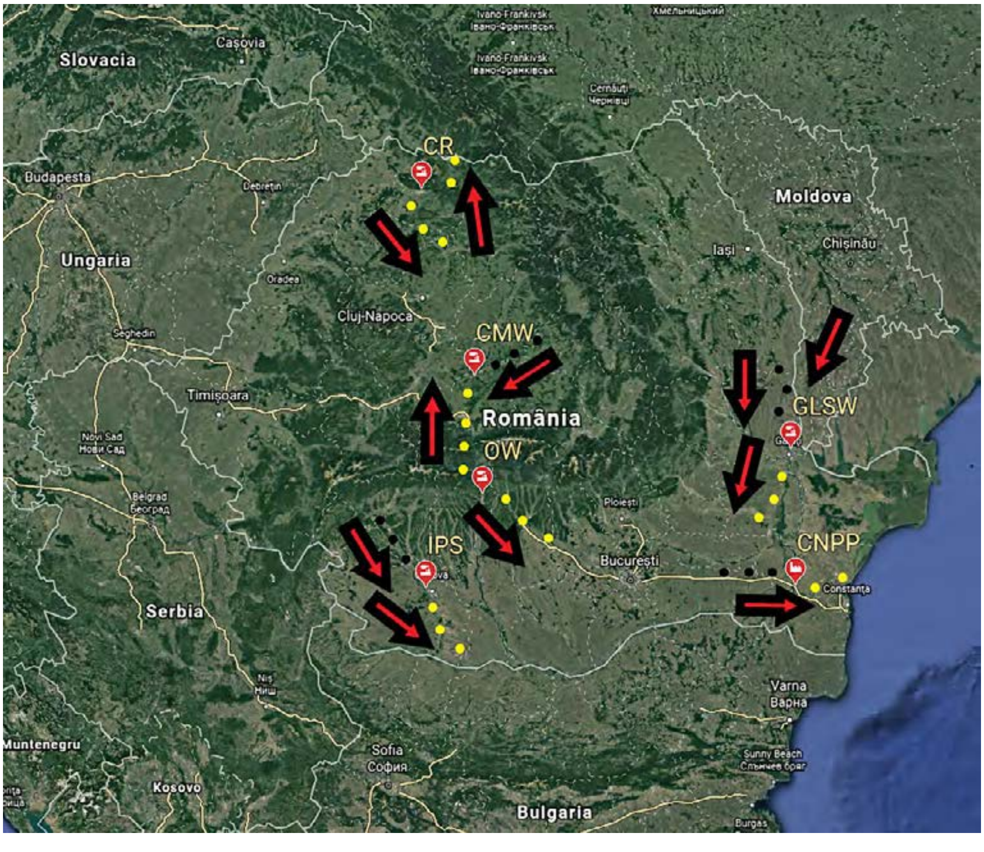

FIg. 1. The locations of the investigated pollution sources – CMW-Copşa Mică Works, CNPP-Cernavodă Nuclear Power Plant, CR-Cuprom Refinery, GLSW-Galaţi Steel Works, IPS-Işalniţa Power Station, and OW-Oltchim Works – are presented by red pictograms. The yellow points show direction of the dominant wind, while the black points show direction opposite from the dominant wind (Sources: Google Earth Pro V 9.3.105.0; (2009); Romania; 48°27'24"N; 12°50'30"E; Eye; alt.: 702 m; Google Data SIO, NOAA, U.S. Navy, NGA, GEBCO GeoBasis-DE/BKG (2009) Landsat/Copernicus; http://www.earth.google.com [February 02, 2020]. The map was based on information from Vespremeanu-Stroe & al. 2012.

Table 1. Pollution sources and their locations in Romania: information on geomorphological characteristics, winds, released pollutants and number of studied sites.

Abbreviation of pollutions source	Pollution source	Location in Romania	Geomorphological characterization of sites	Prevailing wind directions[1]	Average wind speed (m/s)[2]	Supposed pollutants	Number of total sites
CMW	Copşa Mică Works	Sibiu County	highland	South, northeast	1.70	Heavy metals[3]	5
CNPP	Cernavodă Nuclear Power Plant	Constanța County	lowland	West	3.30	Heavy metals, radioactive elements[4; 5]	6
CR	Cuprom Refinery	Maramureș County	lowland	Northwest, south	1.59	Heavy metals[3], nonferrous elements[6]	6
GLSW	Galați Steel Works	Galați County	lowland	Northeast	4.26	Heavy metals[7]	7
IPS	Işalnița Power Station	Dolj County	lowland	Northwest	2.63	SO_2, NO_2, CO, dust[8], heavy metals[9]	7
OW	Oltchim Works	Vâlcea County	lowland	Northwest, south	2.24	Heavy metals, organic compounds[9; 10]	7
Total number of sites							**38**

Legend: numbers in superscript represent the reference sources for the prevailing wind direction, average wind speed, and supposed pollutants (1-Bălteanu & al. 2006; 2-Vespremeanu-Stroe & al. 2012; 3-Alpopi & Colesca 2010; 4-Tudorache & Marin 2013; 5-Magnusson & al. 2004; 6-Oros & al. 2011; 7- Ene & al. 2010; 8-Racoceanu 2011; 9-Mladin & al. 2010; 10-Florescu & al. 2011).

along a spatial gradient both in direction of the dominant wind and in opposite direction from the dominant wind in the case of CMW, CNPP, GLSW, and IPS (Fig. 1). The general pattern of sampling was as follows:

- Site 1: 90 km off the pollution sources in direction of the dominant wind.
- Site 2: 60 km off the pollution sources in direction of the dominant wind.
- Site 3: 30 km off the pollution sources in direction of the dominant wind.
- Site 4: within 1 km of the pollution sources in direction of the dominant wind.
- Site 5: 30 km off the pollution sources in opposite direction from the dominant wind direction.
- Site 6: 60 km off the pollution sources in opposite direction from the dominant wind direction.
- Site 7: 90 km off the pollution sources in opposite direction from the dominant wind direction.

Distances between the sampling sites were represented by spatial segments of 30 km each. The distance around each pollution source was 1 km. In the case of GLSW and IPS, sampling was performed from the pollution source up to 90 km in direction of the dominant wind, and from the same pollution source up to 90 km in opposite direction from the dominant wind (Fig. 1). In the case of OW, sampling was performed from the pollution source up to 90 km towards the northern part of the country, and from OW up to 90 km towards the southeast of the country, only in direction of the dominant wind. Similarly, for CR sampling was performed from CR up to 90 km towards the northeast of the country and up to 60 km towards the north of Romania (along this spatial gradient the sampling was limited by the northern border of Romania), only in direction of the dominant wind (Fig. 1). For the other two pollution sources, CNPP and CMW, sampling was performed from the two of them up to 90 km in opposite direction from the dominant wind, while in direction of the dominant wind, sampling was performed up to 60 km for CNPP (limited by the Black Sea) and up to 30 km for CMW, due to overlapping of the spatial gradient of CMW and OW (Fig. 1).

A total of 38 sites were sampled (Table 1). Within each site, three replicates (situated approximately 1 km from each other) were selected; each replicate was represented by one tree so that three trees per site were sampled. A total of 114 trees were sampled, and five lichen species were identified as follows: *Physcia adscendens* (Fr.) H. Olivier, *Xanthoria parietina* (L.) Beltr., *Flavoparmelia caperata* (L.) Hale, *Cladonia pyxidata* (L.) Hoffm., and *Cladonia glauca* Flörke (Tables 2-5). In respect to pollution sources, the sites were selected to cover a large part of Romania's territory (Table 1; Fig. 1).

The following data were recorded for each pollution source: distance from the main pollution source, distance from roadways, altitude, and circumferences of the host trees. The tree circumferences were used to assess the accumulation time of metals.

The sizes of collected lichen thalli were approximately 3.5-4 cm (3.5 cm in the central parts with apothecia, and 0.5 cm in the peripheral parts without apothecia).

The lichen samples were cleaned of impurities, and the thalli were divided into central and peripheral parts. The samples were ground with pestle in a porcelain mortar into fine powder. Chemical analysis of the metals was performed by a X-ray fluorescence system (XRF Rigaku ZSX100e, Supermini Model). Approximately 5 g of the powdered material of each sample was used for XRF analysis. A quantitative assessment of metals in mg kg^{-1} was obtained by calculating the percentage of mass in dry weight of the analyzed samples.

The Shapiro-Wilk (W) test indicated abnormally distributed dataset ($p < 0.05$) (Hammer & al. 2001). Thus, the Kendall rank order correlation coefficient (τ) was used to identify the significant relationships among the response variables (the content of metals accumulated in central and peripheral parts of *X. parietina*) and environmental variables (Dytham 2011). The statistical test was carried out with PAST software (Hammer & al. 2001).

Results and discussion

A significant negative relationship was obtained between the accumulation of Fe in the central part of the *X. parietina* thallus and the distance from the pollution source in direction of the dominant winds ($\tau = -0.46$; $p = 0.04$). The main pollution source in that case was the Galați Steel Works situated in a plain with no depressions and at a lower altitude in Southeast Romania. Thus, the dominant winds made an important contribution to the dispersion of atmospheric pollutants. The obtained result was contrary to the hypothesis of this study. Presumably, lower Fe concentrations in the older parts of *X. parietina* in direction of the dominant winds should be attributed to geomorphology of the studied area. Thus, the Romanian Plain, which is an open geomorphological lowland area, helped dispersion of the air masses and induced air cleaning. Observations have shown that *X. parietina* accumulated higher concentrations of Fe than *Hypogymnia physodes* (L.) Nyl. and *Parmelia sulcata* Taylor (Parzych & al. 2016) and, therefore, was suitable for monitoring of that element (Parzych & al. 2016). As in the present study, industrial activities were responsible for the heavy metal pollution in the study of Parzych & al. (2016). Studies carried out in the field in the southeastern part of Serbia indicated that higher concentrations of Fe accumulated in the central parts of *F. caperata* (Mitrović & al. 2012). Within this study, no significant relationships were obtained with regard to elemental accumulation in the peripheral and central parts of *F. caperata*. Otherwise, Fe, as a sedimentary element, could be released into the atmosphere by roadway erosion, vehicular traffic (Stamenković & al. 2013b) and military operations (Rosamilia & al. 2004). In the southern part of Poland, Fe emissions were released into the atmosphere by metallurgical plants, domestic heating and car traffic (Kapusta & al. 2004). The average values of Fe accumulated in the central and peripheral parts of *X. parietina* were well presented in the case of Galați Steel Works, as compared to the other elements from all pollution sources (Table 2). Similarly, *P. adscendens* accumulated considerably more Fe against the other elements (Table 4).

In terms of Mn accumulation from the Galați Steel Works, another significant result was obtained in the positive relationship between the central parts of *X. parietina* and the tree circumferences in direction of the dominant winds ($\tau = 0.65$; $p = 0.002$). The increase of distance from the pollution sources was closely related to the decrease in the element concentrations in the lichen thalli (Rosamilia & al. 2004). The old trees offered an adequate substratum for high cover of the lichen species, which played an important role in the accumulation of atmospheric pollutants due to the long exposure time. Another pollution source that released heavy elements was the vehicular traffic responsible for the higher accumulation of Cr, Cu, Fe, and Pb in lichens (Loppi & Frati 2006).

Otherwise, such elements as Fe and Mn are essential in the metabolic activities and are found normally in the living organisms (Ite & al. 2014). Industrial pollution sources in Romania are responsible for high concentrations of pollutants in the atmosphere, especially those caused actively by the metallurgical and mining activities in the course of three

Table 2. The elements content [mg kg^{-1} dry weight] in the central and peripheral parts of the *Xanthoria parietina* thalli along the spatial gradient of each pollution source (only the samples with measured elements are given).

Pollution source

Elements	Central parts (M±SD)	Peripheral parts (M±SD)	Number of site	Number of replicates	SG (km)	DWD	CT (m) (M±SD)
Copșa Mică Works							
Al	0.85*	1.14*	3	1	30	OPDW	0.75
Fe	13.05*	5.66*	3	1	30	OPDW	0.75
Cernavodă Nuclear Power Plant (CNPP)							
Al	1.34*	1.51*	2	1	60	DDW	3.91*
	1.41*	0.25*	3	1	30	DDW	1.84*
	1.23*	1.18*	5	1	30	OPDW	0.41*
	1.15*	1.89*	6	1	60	OPDW	1.10*
Fe	20.37*	17.32*	2	1	60	DDW	3.91*
	21.37*	18.30*	3	1	30	DDW	1.84*
	19.77*	22.86*	5	1	30	OPDW	0.41*
	27.71*	27.81*	6	1	60	OPDW	1.10*
Mn	0.60*	0.43*	2	1	60	DDW	3.91*
	0.85*	0.55*	3	1	30	DDW	1.84*
	0.92*	0.84*	6	1	60	OPDW	1.10*
Cuprom Refinery (CR)							
Al	1.05±0.13	1.06±0.11	1	1	90	DDW	0.73±0.007
	1.53*	1.04*	6	1	60	DDW	2.10*
Fe	11.40±2.93	9.47±3.40	1	1	90	DDW	0.73±0.007
	10.72*	6.42*	6	1	60	DDW	2.10*
Galați Steel Works (GLSW)							
Al	0.69±0.65	1.09±0.50	1	2	90	DDW	0.67±0.22
	1.84*	2.06*	2	1	60	DDW	0.51*
	1.50*	1.09*	3	1	30	DDW	2.20*
	0.71*	0.89*	4	1	0.7	DDW	2.38*
	0.76±0.07	1.04±0.29	5	2	30	OPDW	0.71±0.08
	1.22*	0.74*	6	1	60	OPDW	1.76*
	0.66±0.10	0.83±0.21	7	2	90	OPDW	0.92±0.67
Cr	1.73*	1.47*	2	1	90	DDW	0.83*
	0.80*	1.65*	3	1	60	DDW	0.51*
	0.49*	0.87*	4	1	0.7	DDW	2.38*
Zn	1.00±0.07	0.90±0.50	1	2	90	DDW	0.67±0.22
	1.25±0.18	1.09±0.66	2	3	60	DDW	0.61±0.18
	2.34±0.63	1.74±0.01	3	2	30	DDW	1.98±0.30
	1.22*	1.18*	4	1	0.7	DDW	2.38*
	1.50±0.39	1.78±0.64	5	3	30	OPDW	0.65±0.11
	0.87*	0.84*	7	1	90	OPDW	0.45*
Fe	30.17±2.21	31.42±3.50	1	2	90	DDW	0.67±0.22
	35.03±5.05	28.87±8.34	2	3	60	DDW	0.61±0.18
	38.69±2.82	32.43±9.36	3	3	30	DDW	2.33±0.51
	38.64±7.44	36.51±6.70	4	3	0.7	DDW	2.11±0.31
	33.48±3.62	36.44±4.10	5	2	30	OPDW	0.65±0.16
	24.32±7.28	25.91±2.64	6	2	60	OPDW	2.28±0.74
	33.93±3.86	27.28±3.28	7	3	90	OPDW	0.84±0.49
Mn	0.67*	0.65*	1	1	90	DDW	0.51*
	0.56±0.39	0.59±0.22	2	3	60	DDW	0.61±0.18
	1.44±0.13	1.18±0.24	3	3	30	DDW	2.33±0.51
	1.15*	1.35*	4	1	0.7	DDW	2.20*
	0.97*	1.18*	5	1	30	OPDW	0.65*

Table 2. Continuation.

Pollution source
Galați Steel Works (GLSW)

Elements	Central parts (M±SD)	Peripheral parts (M±SD)	Number of site	Number of replicates	SG (km)	DWD	CT (m) (M±SD)
	1.15*	1.42*	6	1	60	OPDW	1.76*
	1.49±0.84	1.38±0.49	7	2	90	OPDW	1.03±0.51
Ag	2.37*	4.48*	1	1	90	DDW	0.83*
	9.54*	7.28*	2	1	60	DDW	0.51*
	12.98*	8.73*	3	1	30	DDW	2.85*
	7.64*	4.12*	4	1	0.7	DDW	2.20*
	12.83±6.71	9.36±1.51	5	2	30	OPDW	0.59±0.07
	5.99*	12.84*	7	1	90	OPDW	0.67*
Ișalnița Power Station (IPS)							
Al	1.46±0.14	1.14±0.28	3	2	30	DDW	1.33±0.12
	1.39*	0.29*	4	1	0.3	DDW	2.86*
	1.81*	2.34*	5	1	30	OPDW	2.25*
Fe	21.92±12.34	18.83±6.46	3	3	30	DDW	1.35±0.08
	32.37*	19.72*	4	1	0.3	DDW	2.86*
	13.94*	10.74*	5	1	30	OPDW	2.25*
Mn	1.51±0.94	1.28±1.29	3	2	30	DDW	1.40±0.02
	0.69*	0.49*	5	1	30	OPDW	2.25*
Ag	10.94±4.85	7.48±0.47	3	2	30	DDW	1.31±0.09
	5.15*	7.93*	4	1	0.3	DDW	2.86&

Legend: *data are insufficient for calculation of univariate statistics (mean and standard deviation); M-mean; SD-standard deviation; SG-spatial gradient; DWD-dominant wind direction; CT- circumference of trees; OPDW-in the opposite direction from dominant wind; DDW-in the direction of dominant wind.

Table 3. Elements content [mg kg^{-1} dry weight] in the central and peripheral parts of the *Flavoparmelia caperata* thalli along the spatial gradient of each pollution source (only samples with measured elements are given).

Pollution sources
Oltchim Works (OW)

Elements	Central parts (M±SD)	Peripheral parts (M±SD)	Site number	Number of replicates	GS (km)	WD	CT (m) (M±SD)
Al	0.47±0.28	0.41±0.02	3	2	30	DDW	1.18±0.16
	1.24*	0.99*	6	1	60	DDW	0.60*
	0.33*	0.69*	7	1	90	DDW	1.50*
Fe	4.07±1.45	4.69±0.76	3	2	30	DDW	1.18±0.16
	15.6*	12.37*	6	1	60	DDW	0.60*
	2.80*	4.21*	7	1	90	DDW	1.50*

Legend: see Table 1 and Table 2.

Table 4. Elements content [mg kg^{-1} dry weight] in *Physcia adscendens* thalli unsplit into central and peripheral parts (only samples with measured elements are given).

Pollution sources
Galați Steel Works (GLSW)

Elements	Contents (M±SD)	Site number	Number of replicates	GS (km)	DWD	CA (m) (M±SD)
Al	1.25*	1	1	90	DDW	0.83*
	1.41±1.69	2	2	60	DDW	0.67±0.22
Zn	1.08±0.31	1	2	90	DDW	0.67±0.22
	1.12*	2	1	60	DDW	0.83*
	2.19*	5	1	30	OPDW	0.54*
Cr	1.51*	1	1	90	DDW	0.83*

Table 2. Continuation.

	Pollution sources Galați Steel Works (GLSW)					
Elements	**Contents (M±SD)**	**Site number**	**Number of replicates**	**GS (km)**	**WD**	**CA (m) (M±SD)**
	1.06*	3	1	60	DDW	0.51*
Fe	23.69±4.41	1	2	90	DDW	0.67±0.22
	26.07±3.29	3	2	60	DDW	0.67±0.22
	26.61*	7	1	90	OPDW	0.54*
Mn	1.06±0.19	1	1	90	DDW	0.67±0.22
	0.76*	2	1	60	DDW	0.83*
Ag	3.23±0.76	3	2	60	DDW	0.67±0.22

Legend: see Table 1 and Table 2.

centuries (Bartók & Rusu 2004). Biomonitoring by lichens is necessary for prediction of environmental quality closely related to human health (Frati & Brunialti 2006).

A particular case within this study was the Copșa Mica Works, where terricolous lichens, such as *C. pyxidata* and *C. glauca,* accumulated more Pb, Fe and Zn as compared to the other elements (Table 5).

No significant results have been obtained for any other relationship between the elements and selected variables.

Table 5. Elements content [mg kg^{-1} dry weight] in the terricolous lichen species collected near Copșa Mică Works (CMW)

Elements	*Cladonia glauca*	*Cladonia pyxidata*
Pb	38.58*	22.60*
Ni	NA	0.46*
Al	3.14*	3.78*
Zn	16.59*	15.23*
Cu	1.28*	0.78*
Fe	32.46*	31.06*
Mn	0.65*	NA

Legend: *only raw data were presented; NA – not available data

Conclusions

The major conclusion was that old trees with large circumferences (0.51–2.85 m) play an important role in the colonization and longevity of lichens. Since a significant positive correlation was found between the Mn content in the central parts of *X. parietina* and tree circumferences in direction of the dominant winds, it could be maintained that lichens on the old trees serve as good indicators of a high degree of Mn contamination in the case of Galați Steel Works. Furthermore, lichens are small in size and, therefore, could not be used to mitigate atmospheric pollution, but they can be used successfully as indicators of that pollution.

Since a lower content of Fe was measured at a greater distance from the Galați Steel Works, especially in lowland areas (Romanian Plain, South Romania) in direction of the prevailing wind, it could be concluded that the winds had a mitigating effect on atmospheric pollution.

Xanthoria parietina is a valuable indicator of elements and thus could be used in biomonitoring of the investigated sites.

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