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THE SIGNIFICANCE OF EPIPHYTIC LICHENS AS BIOINDICATORS OF AIR POLLUTION FOR HUMAN HEALTH

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Urban dwellers are subjected to an extensive range of air contaminants that pose a risk to their well-being. It is difficult to measure most of these substances because their concentrations vary greatly over time and space and typically occur at very low levels. A high spatial and temporal density of sampling sites is made possible by biological monitors in this situation, and they also provide a useful "time integral" of potential biological effects of airborne pollutants. We provide a case study as an illustration of how lichens are used as bioindicators of air quality in Cluj-Napoca and the surrounding areas. The techniques included counting the species of macrolichens in the research region and analyzing the levels of heavy metal deposition (Pb and Zn) in both native and transplanted lichens. By employing lichens as bioindicators, scientists can monitor changes in environmental conditions over time, pinpoint pollution sources, and evaluate the quality of the air.

Keywords: lichens, bioindicators, pollution, heavy metals, Cluj-Napoca.

INTRODUCTION

Currently, over half of all people live in cities, and this percentage is rising. With detrimental effects on ecosystem function and human health, this massive and frequently uncontrolled occurrence has drastically altered the environmental conditions of earlier rural and natural places[17].

The most intense interaction between humans and their surroundings is found in urban settings. Negative effects such as air pollution and associated health effects may arise from this interaction. Serious health effects of air pollution have been documented, some of which include cardiovascular and pulmonary issues, cancer, compromised host defenses, low birth weight, and infant death. A concentrated effort should be made to monitor air pollution, particularly in urban areas, given the detrimental effects that it has on human health[13].

Changes in the spatial distribution of environmental contamination are brought about by the intensive expansion of industry, transportation, and urbanization, which links local to global phenomena. One of the most prominent signs of anthropogenic pressure in metropolitan environments is pollution from many sources, including heavy metals. The quantity, kind, and size of industrial zones, as well as the volume of traffic, population density, and waste disposal infrastructure, all affect how polluted a city is. Utilizing analytical bioindicators with varying degrees of sensitivity is vital since it is exceedingly challenging to track the detrimental effects of contaminants on human health[19].

Lichens can be particularly helpful as biomonitors of air quality in urban and industrialized regions[12,14], where the high density of various emitting sources makes it exceedingly difficult to monitor air pollution using traditional physico-chemical methods due to the range of pollutants. Furthermore, data suggests that lichen biomonitoring could be utilized as a stand-in for the impacts of air pollution on human health[15]. Lichens possess several characteristics[6,7,9,10,11], that make them excellent bioindicators, particularly for assessing air quality and environmental health.

Many lichen species are highly sensitive to sulfur dioxide (SO_2) levels in the air, their response to SO_2 makes them valuable indicators of industrial emissions and air pollution. Lichens can absorb and accumulate heavy metals from the atmosphere, such as lead and cadmium, providing a record of metal pollution. Lichens lack true roots and a protective cuticle, so they absorb water and nutrients directly from the air and precipitation. This characteristic makes them vulnerable to atmospheric pollutants, allowing them to reflect changes in air quality[20,21]. Lichens grow slowly, often at rates measured in millimeters per year. This slow growth allows them to provide a historical record of environmental conditions, making them useful for assessing long-term trends in air quality. There are numerous lichen species, and different species exhibit varying sensitivities to environmentalconditions. This diversity allows for a nuanced assessment of air quality and pollution levels based on the presence or absence of specific lichen species. Lichens can be found in a wide range of environments, from urban areas to remote wilderness. This ubiquity makes them versatile indicators that can be used in various geographical and ecological contexts. Many lichen species have unique and easily identifiable morphologies, facilitating their recognition in the field. This characteristic enhances the accessibility of lichens for citizen science projects and community-based monitoring efforts. Collecting lichen samples is non-destructive and has minimal impact on the environment, fact that allows for repeated sampling over time without causing harm to ecosystems. At the end of 20th Century lichens were recognized as valuable tools for monitoring air quality, and their inclusion in environmental impact assessments became more common. In recent years, the use of lichens as bioindicators has expanded globally. Researchers have applied lichen biomonitoring techniques to assess air quality in diverse environments, including urban areas, industrial zones, and remote regions[2].

The concerns about the impacts of air pollution on human health have driven extensive research into bioindicators. Bioindicators are organisms or biological systems that provide information about the health of an ecosystem and can indicate the presence and intensity of environmental pollution[18].

Lichens are excellent bioindicators and bioaccumulators of elements and trace elements, since the concentrations found in their thalli can be directly correlated with those in the environment[8].

Lichens serve as passive indicators, reflecting the quality of the environment in which they grow. By studying the changes in lichen populations, researchers can gain insights into the levels and types of pollutants present in the air. Certain lichen species are sensitive to specific environmental conditions, especially air quality. Therefore, their presence, absence, or changes in abundance can be correlated with the levels of pollutants in the air and other environmental factors. This information can then be used to assess the impact of pollution on the ecosystem and, by extension, on human health[16].

CASE STUDY – CHARACTERIZATION OF THE LEVEL OF POLLUTION IN THE CITY OF CLUJ-NAPOCA AND THE PERI-URBAN AREA USING LICHENS

Regarding the corticolous macrolichen flora, the Cluj-Napoca urban environment was largely unexplored; Codoreanu et al.'s 1960[3] study on corticolous lichen flora was restricted to the Botanical Garden.

In Cluj-Napoca and the surrounding peri-urban area, two studies were conducted between 1998 and 2000[4,5], to assess the level of development of the epiphytic macrolichen flora in green spaces that are found in both densely populated areas and on the outskirts. The sampling points (Tabel 1) were chosen based on factors such as exposure, road traffic, human population density, and the existence of lakes or streams with flowing or stagnant water.

The inventory proved that foliose and fruticose lichens were present in the study area, with a focus on specific areas that provided favorable conditions for the establishment of corticolous macrolichen, while also accounting for areas with less potential. 31 species of foliose and fructicose lichens were identified as a result of the investigation of sampling sites. However, the author of the study considered that the situation at the time could not be categorically considered as a dynamic phenomenon of tree repopulation with macrolichens, due to the depollution of the urban environment, in the absence of a previous study covering a significant area that could confirm a definite decrease in pollution.

Table 1

The investigated sampling points in Cluj-Napoca municipality and peri-urban area in 1998-2000

Sampling points	Subsystem	Wet environment	No. of tree species surveyed
Botanical Garden	Urban dense	Ţiganilor stream	15
Cetăţuia Park	Urban dense	–	4
Ethnographic Museum – open-air section	Peri-urban	–	6
Central Park	Urban dense	River Someş	3
Sports park „I. Haţeganu”	Urban dense	River Someş	1
Tineretului Park	Urban dense	Lake	3
Calea Turzii	Urban dense, busy car traffic axis	–	1
Zărneşti Street	Open urban, low traffic car axis	–	1
Hoia Forest	Peri-urban	–	3
St. Ion Pine Plantation	Peri-urban	–	3
M. Kogălniceanu Street	Urban dense	–	1
Gr. Alexandrescu Street	Urban dense	–	4
Popii Valley	Peri-urban	Stream	5
Snagov Alley	Urban	–	7
Teleorman Street	Urban dense	–	4
Someşeni Baths	Peri-urban	Lakes	11

A concentration of macrolichen was observed in the peri-urban area of the city (Table 2), a special situation being represented by the Botanical Garden, located very close to the city centre, where the highest number of lichen species was identified (20 sp.). The situation of this sampling site is particular, the Botanical Garden with a considerable area (about 13 ha), having also special microrelief conditions, a wet environment ensured by the presence of a stream and benefiting from a special protection status, due to the scientific interest it presents. In addition, lichens have been collected from a large number of supporting tree species, which due to their high age, rhytidome characteristics (roughness, pH), provide particularly favourable conditions for macrolichen settlement. Of the species of foliose and fruticose lichens collected here, 10 sp. are common to those appearing in the 1962 study; the presence of a further 10 sp. of macrolichen seems to indicate an improvement in conditions at this stationary site, which is gratifying considering that the site is located near the central area of the town.

The low number of species recorded in the dense urban environment, in the sampling points located in the immediate vicinity of busy roads, was correlated with road traffic, probably the main source of pollution in the study area.

Physcia adscendens, *P. caesia*, and *Xanthoria parietina*, three nitrophilic species, were the most frequently found species. This suggests a relatively high atmospheric concentration of nitrogen oxides, which is supported by data from the Cluj Environmental Protection Inspectorate, which shows that nitrogen oxide emissions during that period appeared to be slightly but continuously above the allowable thresholds. It has to be acknowledged that following 1989, Cluj-Napoca saw a record number of cars, the majority of which were not ecologically friendly, as road traffic values rose dramatically.

The identification of numerous species with low toxitolerance (*Flavoparmelia caperata, Ramalina farinaceea*, *Usnea subfloridana*, *Physcia stellaris*, etc.) and the presence of several lichen species, such as *Cladonia fimbriata*, *Melanelia exasperata* and *Phlyctis agelaea*, which are thought to be highly sensitive to SO_2 pollution, seem to indicate a stagnation or even reduction in the level of this pollutant.

Table 2

Correspondence between the investigated sampling points and lichen species

Sampling point	Botanical Garden	Cetățuia Park	Ethnographic Museum	Central Park	„I. Hațeganu" Park	Tineretului Park	Calea Turzii	Zărnești Street	Hoia Forest	St.Ion Pine Plantation	M. Kogălniceanu Street	Gr. Alexandrescu Street	Popii Valley	Snagov Alley	Teleorman Alley	Someșeni Baths	**Attendance**
Lichen species																	
Candelaria concolor	X																1
Candelariella vitellina	X																1
Cladonia fimbriata									X								1
Evernia prunastri	X		X						X							X	4
Flavoparmelia caperata	X																1
Hypogymnia physodes										X							1
H. tubulosa										X							1
Melanelia exasperata	X		X						X								3
M. glabratula									X				X				2
M. subaurifera										X							1
Parmelia saxatilis	X												X				2
P. sulcata	X	X	X			X			X	X			X				7
Parmelina tiliacea	X																1
Pseudevernia furfuracea	X									X						X	3
Punctelia subrudecta	X		X														2
Usnea subfloridana										X							1
Phaeophyscia orbicularis	X	X	X			X											4
Physcia adscendens	X	X	X		X		X	X	X	X		X	X	X	X	X	**13**
Ph. aipolia		X	X						X				X	X	X		**6**
Ph. caesia	X								X		X	X		X	X		**6**
Ph. dubia																X	1
Ph. Stellaris	X		X						X								3
Ph. tenella	X	X	X			X							X			X	6
Physconia distorta													X				1
Physconia. grisea	X			X	X												3
Pertusaria albescens	X																1
Ramalina farinacea	X																1
X. candelaria	X			X	X	X											4
X. fallax									X								1
X. parientina	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	**15**
Phlyctis agelaea			X														1
No. of lichen species	**20**	**6**	**11**	2	4	5	2	2	**11**	8	2	3	**8**	4	4	**6**	
No. of tree species	15	4	6	3	1	3	1	1	3	3	1	4	5	7	4	11	

The investigation made on the composition of the corticolous macrolicheni flora and its distribution in Cluj-Napoca municipality was continued and completed, in 2000–2001[1], lichens were used as bioindicators to evaluate Cluj-Napoca's level of pollution, a number of eighteen sampling points beeing selected (Table 3). These included the busiest thoroughfares, the less busy streets in the city's center and other neighborhoods, parks, and green areas. From these points, the toxitolerant corticolous lichens Physcia stellaris and Xanthoria parietina were collected. These lichens naturally grow on the bark of lime and maple trees.

Apart from the passive monitoring approach, an active strategy was also employed, which involved relocating *Pseudevernia furfuracea* – a common, acidophilic species that is sensitive to pollution – into nine of the selected sampling points, considered as heavily polluted. This species was transplanted over a five-month period, from November 17, 2000, to May 5, 2001, from Mount Băișoara, an area free of pollution.

Road traffic was – and probably still is – the primary cause of pollution in Cluj-Napoca, releasing $S0_2$, $C0_2$, NOx, and metal particles – Pb, Cu, and Zn being the most common – into the air. These elements were extracted from native and transplanted lichens using flame atomic absorption spectrophotometry using a Perkin Elmer 3030B instrument. The samples were first digested using an HNO_3 and H_2O_2 mixture. The results were expressed in mg/kg dry material. After 1990, Cluj has seen a rise in the number of cars, which has led to an increase in road traffic pollution.

The Botanical Garden, which occupies an area of roughly 14 hectares has been designated as a protected area for more than 80 years, and was selected as a reference point (the least polluted area) among the 18 sampling points selected. This was verified by analyses for the three metals examined in native lichen species, with the lowest concentrations found here.

The measured concentrations of Cu, Pb, and Zn in *Xanthoria parietina* were found to be 22–257 mg/kg, 16–147 mg/kg, and 125–416 mg/kg, respectively. Pb and Cu concentrations in the most polluted area are nine times and eleven times higher, respectively, than in the Botanical Garden control sample. Cu, Pb, and Zn were found in *Physcia stellaris* at minimum and maximum concentrations of 21–109 mg/kg, 15–166 mg/kg, and 116–347 mg/kg, respectively.

Xanthoria parietina and *Physcia stellaris* do not accumulate the metals under study in a manner similar to one another, despite growing on the same substrate and sharing morphological and thallus size similarities. Zn and Cu are accumulated more by *Xanthoria*, while Pb is more favored by *Physcia.*

Table 3

Cu, Pb and Zn concentrations (mg/kg) determined in native lichens from the 18 sampling points in Cluj-Napoca in 2000–2001

Number	**Sampling point**	**Xanthoria parietina (native)**			**Physcia stellaris (native)**		
		Cu	Pb	Zn	Cu	Pb	Zn
1	Eroilor Boulevard	159	147	401	63	166	364
2	Unirii Square	257	121	416	94	155	314
3	Calea Turzii	100	73	209	27	80	190
4	Clinicilor Street	135	119	310	a*	a	a
5	Aurel Vlaicu Street	177	65	338	a	a	a
6	Someşeni Baths	107	26	200	28	41	172
7	Gr. Alexandrescu Street	121	24	222	29	21	183
8	14 Iulie Square	253	82	236	37	50	205
9	Tineretului Park	104	67	249	121	47	347
10	Mărăști Park	162	78	323	a	a	a
11	Feroviarilor Park	130	53	255	59	101	271
12	Hoia Forest	135	42	249	36	46	167
13	Central Park	119	11	257	109	69	267
14	Ethnographic Museum	124	62	258	47	72	169
15	Sports park „I. Haţeganu”	30	39	115	79	54	153
16	Snagov Alley	120	48	265	34	87	229
17	Cetăţuia Park	123	60	281	101	85	263
18	Botanical Garden	22	16	125	21	15	116

Note: a – Stationary where samples were absent

After five months of exposure to road traffic, all examined elements were found in higher concentrations in the transplanted lichens than in the control sample (Table 4). *Pseudevernia furfuracea* had Pb concentrations ranging from 20 to 81 mg/kg in the various stationary sites, as opposed to the control sample's 18 mg/kg. As a result, in the most contaminated area, the Pb concentration increased by almost four times. The increase in lead content in lichens exposed for five months is a proportionate reflection of the amount of traffic in various parts of the city. During the study period, Republicii Street (sampling point 9) had the highest concentration of lead (Pb). Due to heavy traffic, the parallel street was closed to vehicles. Zn concentrations ranged from 137–197 mg/kg, while a concentration of only 64 mg/kg was measured in the marker sample. Cu concentrations in the transplanted lichens ranged from 28–152 mg/kg, with the marker sample containing only 15 mg/kg. Calea Turzii and Clinicilor Street's (sampling points 1 and 2), were the most polluted, based on the total concentrations of the elements under study (Zn + Cu + Pb). It is evident that Pseudevernia furfuracea quickly and extensively accumulates metals. When the concentration of the elements under study was added up for each species of lichen, it was found that *Pseudevernia furfuracea* accumulated roughly 60% of what *Xanthoria parietina* accumulated in just 5 months, and that *Physcia stellaris* accumulated roughly 80% of what it accumulated in decades of vegetation. A plausible rationale could be that *Pseudevernia furfuracea* differs physiologically from *Xanthoria parietina* and *Physcia stellaris*, has a thallus with a significantly larger contact surface area, and has numerous isidia that further increase this surface area.

Finally, the metal content of native lichens was used to rank the Cluj-Napoca sites that were investigated in order of degree of pollution (Table 5). Weakly and strongly contaminated areas can be identified for each of the three metals; Cu and Zn show less variation. Since lead accumulation is the most distinct and highly toxic element, we will solely discuss it in detail. Thus, five distinct pollution-prone zones were identified in Cluj-Napoca, taking into consideration the lead contamination:

– 119–147 mg/kg Pb on the busiest roads (sampling points 1, 2, 4) indicate excessive pollution.

– extremely contaminated; 73–82 mg/kg (sampling points 3, 8, 10).

– 60–67 mg/kg of medium-level pollution (sampling points 9, 5, 14)

– 39–53 mg/kg in parks (sampling points 11, 12, 15) – slightly polluted

– 11–26 mg/kg of extremely mild pollution (sampling points 6, 7, 18, 13)

According to data gathered in the early 2000^{S}, Cluj-Napoca might be categorized as a moderately polluted urban region.

CONCLUSION

In conclusion, we can state that the main polluting factor is, in the absence of pollution of industrial origin, car traffic, in Cluj-Napoca being mainly represented by non-ecological cars. A consequence of this pollution is the presence of numerous nitrophilic species (indicated in bold in the table), as car engines eliminate important quantities of nitrogen oxides.

In Cluj-Napoca, the study carried out by Codoreanu, V. in 1960, although limited to the area of the Botanical Garden, allows us to affirm a continuity of colonisation with epiphytic lichens, the disappearance of the major sources of industrial pollution - mainly due to the cessation of activity of these economic units - creating the premises for the extension of the range of rare species, very sensitive to pollution.

Efforts to reduce urban air pollution are crucial for safeguarding public health.. Monitoring air quality and taking steps to mitigate pollution can significantly improve the well-being of urban populations. The use of bioindicators is a powerful tool in environmental monitoring, offering a dynamic and responsive approach to studying the health and changes in ecosystems. Their ability to provide early warnings, integrate responses to multiple stressors, and involve communities in monitoring efforts makes them valuable components of environmental research and management. Lichens possess several characteristics that make them excellent bioindicators, particularly for assessing air quality and environmental health. Their sensitivity to environmental conditions, particularly air quality, allows them to indicate the overall health of ecosystems and provide information about the presence and impact of pollutants. Studying lichens helps researchers assess the quality of the air, identify sources of pollution, and monitor changes in environmental conditions over time.

Table 4

Cu, Pb, and Zn accumulation in transplanted *Pseudevernia furfuracea* (November 17, 2000 – April 5, 2001) compared to blank sample

No.	Sampling point	Sum of elements	Zn	Cu	Pb
1.	Unirii Square	228	153	29	45
2.	Calea Turzii	332	187	95	49
3.	Clinicilor Street	331	136	152	43
4.	Aurel Vlaicu Street	298	181	68	49
5.	Gr. Alexandrescu Street	225	177	28	20
6.	Feroviarilor Park	291	197	53	41
7.	Ethnographic Museum	198	137	23	38
8.	Botanical Garden	256	157	76	43
9.	Republicii Street	288	176	30	81
10.	Blank sample Băişoara	110	64	15	18

Table 5

Cu, Pb, and Zn concentrations (mg/kg) in native and transplanted lichens are compared

Sampling point	Element	*Xanthoria parietina* (native)	*Physcia stellaris* (native)	*Pseudevernia furfuracea* (transplanted)
Unirii Square	Cu Pb Zn	257 121 416	94 155 314	29 45 153
Calea Turzii	Cu Pb Zn	100 73 209	27 80 190	95 49 187
Aurel Vlaicu Street	Cu Pb Zn	177 65 338	86 69 215	68 49 181
Grigore Alexandrescu Street	Cu Pb Zn	121 24 222	29 21 183	28 20 177
Feroviarilor park	Cu Pb Zn	130 53 255	59 101 271	53 41 197
Ethnographic Museum	Cu Pb	124 62	47 72	23 38

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