LICHENS – POTENTIAL INDICATORS OF AIR POLLUTION

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Abstract. The present paper gives a research review on lichens flora in the Republic of Moldova beginning with 1934 until present time. It also presents the Lichens Toxitoleration Scale (LTS) and Air Quality Gradation Scale (AQGS), which were developed to assess the air quality both in urban and forest ecosystems. Thus, 40 lichens indicator species and a standard species (*Parmelia sulcata*) are proposed to monitor environment quality in the Republic of Moldova.

Keywords: lichens, bioindication, air pollution, standard species.

Rezumat. Lichenii – potenţiali indicatori ai poluării aerului. Lucrarea prezintă o retrospectivă a cercetărilor lichenologice în Republica Moldova, începând cu anul 1934 şi până în prezent. Totodată se prezintă Scala Toxitoleranţei Lichenilor (STL) şi Gradaţii de Evaluare a Calităţii Aerului (GECA), care au fost aplicate în testarea calităţii aerului din ecosistemele forestiere şi urbane. Sunt propuse 40 de specii indicatoare şi o specie standard (*Parmelia sulcata*) de licheni pentru monitorizarea calităţii mediului în condiţiile Republicii Moldova.

Cuvinte cheie: licheni, bioindicaţie, poluarea aerului, specie standard.

INTRODUCTION

Lichens are very often recommended for monitoring air pollution with heavy metals, even more often then mosses, due to the fact that they allow both testing air pollution with gaseous compounds and heavy metals. Lichens are widespread in nature and these contributed to the development of different environmental quality monitoring scales (i.e. SO_2 content in air) at national and international level. Furthermore, heavy metal content in lichens correlates well with their level in atmosphere, decreasing with the removal of the pollution source (BURTON, 1986). Because many of the proposed species are common for European space, they can be applied in the European Network for the Assessment of Air Quality in forest, forest-petrophyte, and urban ecosystems, equivalent to European Network Program EMEP (50x50 km), promoted by the Convention on Long-range Transboundary Air Pollution (Geneva, 1979). Out of 4 tested transplant species, *Parmelia sulcata* species, preponderant in forest ecosystems, has proved to be the most responsive to chemical air pollutants, particularly to SO_2, recording evident morphological and biochemical changes. Wildly spread, highly frequent and dominant in forest ecosystems permits *P. sulcata* species to be used as a standard indicator species in geographical mapping of air pollution with SO_2 and heavy metals environment pollution, as required by the European Commission Program on Standardization, Measurement and Testing. *P. sulcata* is already being used as a standard species for these purposes in The Netherlands and Italy.

MATERIAL AND METHODS

The research methodology was undertaken from various scholars who applied the method in their bio indication work (HAWKSWORTH & ROSE, 1970; GARTY & HAGEMEYER, 1988; VAN HALUWYN & LEROND, 2000). Lichens indicator species toxitolerance to air pollution with SO_2 was determined through scales proposed by LEBLANC & RAO, 1973; BARTOK et al., 2003; BEGU, 2008. The analysis of the scales allowed us to see that at the most authors the concentration of $SO_2 < 0.05$ mg/m^3 air is indicated for the area with clean air and the harmful effects start at 0.1 to 0.3 mg/m^3 air, some indicating the stage > 0.3 mg/m^3 as very polluted air, others indicating that the fatal concentration as being $SO_2 > 0.5$ mg/m^3 air. Thus, based on the analysis of data published by other authors and based on our tests performed in laboratory and field conditions, we propose the Lichens Toxitoleration Scale (LTS) for different SO_2 concentrations. Considering that any presence of lichens is an indication of the true criterion, as previously exposed to the higher plants, lichens appreciation will be truthful when the lichen indicator species coverage will be over 10% of substrate surface.

RESULTS AND DISCUSSIONS

The first scientific information on lichens flora from Basarabia was published by the agricultural engineer VRABIE (1934), assistant of the National Museum of Natural History from Chişinău, which at the advice of the museum director investigated the density of lichens flora within 5 habitats around Chişinău: Durleşti and Capriana oak forests, the plum orchards near Valea Dicescu and Ghidighici, and *Sophora* trees along Şcoala Cricova road, identifying about 30 species (25 species and 5 varieties were described and 3 species remained unidentified) in over 200 samples. The aim of the research was to evaluate the density and geographical orientation (16 cardinal directions) of lichens. The author concluded that lichen density is almost uniform within cardinal directions, with the exception of isolated *Sophora* tree with only 7 species and which had maximum (49.2%) and lowest (24.6%) representation in opposite directions – NE and respectively SW. The author explains this difference as the result of natural factors action (i.e. humidity, light, wind etc.). The analysis of information displayed in author's tables and charts, allowed us to additionally emphasize some ecological indication aspects, as well. Thus, out of the 5 studied habitats, the richest in lichens flora was Capriana forest (18 species), followed by Durleşti forest (15 species), then Ghidighici habitat (14 species), and the poorest habitats were Chişinău, Valea Dicescu (7 species), Şcoala Cricova Road (7 species). Moreover, air quality can be assessed by the presence of very sensitive lichens species to pollution covering > 10% of strains, i.e.: Capriana - *Evernia prunastri* (16.3%), *Lecidea enteroleuca* (11.1%), *Physcia adscendens* (12.9%), and Ghidighici – *Ph. adscendens* (15.5%). Thus, the different ecological state between Capriana and Chişinău forest habitats is obvious and even more between Valea Dicescu and Şcoala Cricova Road.

In the Republic of Moldova, scientific investigation on lichens flora extended due to research performed by the botanical garden collaborator SIMONOV (2004), which studied lichens taxonomy, chorology, morphological and anatomical features, different environmental groups, preferred and inhabited lichens habitats. Generalizing the results of his research, SIMONOV (2004) confirmed the existence of 124 lichen species on the territory of the Republic of Moldova, attributed to 50 genera, 26 families, and 7 orders. The representatives of *Lecanoriales* (97 species) order were predominant, followed by *Lecanora* (12), *Parmelia* (12), *Cladonia* (9), *Ramalina* (7) genera. The author mentions the predominance of crustose species (59) in comparison with the foliose and fruticose species (34), the xerophytes (69) in comparison with the mesophyte (8), the photophile (64) in comparison with the sciaphyles (8). Thus, Simonov, demonstrated the dominance of arid climate in the last decades and the negative impact on nature caused by humans, which is respectively reflected in the poor state of native lichens flora. At the same time, Simonov Gh. mentioned the lichens flora wellness within forest ecosystems (90 species) and petrophyte ecosystems (35 species), both being dominated by species with crust-like thallus (43 and 29 respectively), followed by those with foliose thallus (29 and 9 species).

Begu A. initiated research in the eco bio indication field by using lichens to assess the substrate particularities of the meadow forest Chitcani (BEGU, 2000) and latter on, to study the environmental features of lichens extended around the resort Potoci (Romania) located at about 900 m altitude in the Carpathian Mountains (BEGU & BOGUS, 2001) and of those in Chişinău city, the Republic of Moldova (BEGU, 2001).

Given the undeniable value of lichens as bio indicators, which is recognized worldwide, in 2001, the National Institute of Ecology (NIE) founded the Environmental Impact and Ecobioindication laboratory, with the research mainly oriented on lichen-indication. Later, BEGU A. together with SIMONOV (2002-2005) place particular emphasis on eco bio indication particularities of lichen species within forest and petrophyte ecosystems, filling the Republic of Moldova Lichens Flora Register with 22 additional species.

Along with other Environmental Impact and Ecobioindication laboratory collaborators (later on Ecobioindication and Radioecology) there were studied over 80 forest ecosystems, forest-petrophyte, meadow and hill, registering epiphleoid lichen flora (particularly eco bio indication species). For each ecosystem, there was assessed the air quality in accordance with the indicator species abundance, specific diversity and toxitolerance (BEGU, 2004, 2006) as well as the capacity to accumulate certain heavy metals. At the same time, studies concerning principles and methodology of biologically active monitoring applications in evaluation of Chişinău city green spaces environmental state (BEGU & CREȚU, 2005) were continued.

The accumulated knowledge and experience allowed us to propose the concept of environmental standard based on lichen indication (BEGU et al., 2003) and develop certain criteria to assess the environment quality (BEGU & BREGA, 2009).

The results of biologically active monitoring concerning air quality performed in 15 Chişinău stations has allowed us to establish the degree of chlorophyll degradation and accumulation capacity of heavy metals by different lichens transplant species and to propose a country standard species (BEGU, 2008). In addition to purely environmental aspect, the research has contributed to expanding our database and the Republic of Moldova Lichens Flora Register (BEGU, 2009).

Preceding from the fact that ample information on the concentration of the most toxic pollutants in the atmosphere that cause disruptions to vital activity of lichens has accumulated in recent times, some authors specify gradations scale towards SO_2 concentrations in the atmosphere (HAWKSWORTH & ROSE, 1970). Indicated concentrations differ greatly from one author to another, assumable due to the fact that certain data are obtained in laboratory whereas others in the field, and emission structure, climatic conditions, research methodology differ etc.

The analysis of gradations enable us to ascertain that at most of the authors, SO_2 concentration < 0.005 mg/m^3 is indicated for zones with clean air and harmful effects start at 0.1 to 0.3 mg/m^3, some authors indicating the level > 0.3 mg/m^3 as a zone with very polluted air, whereas others indicate a SO_2 concentration > 0.5 mg/m^3 air as fatal for lichens.

Thus, based on the results of our tests performed in laboratory conditions (by gassing), natural conditions (biologically active monitoring) and on the analysis of the data published by other authors, the toxitolerance scale was developed. The scale is composed of 6 levels, out of which 5 are characterized by particular lichens indicator species and the sixth level is completely devoid of lichens indicator species (Table 1).

Table 1. Scales concerning SO_2 concentrations in the air and air quality expressed through lichens toxitolerance (BEGU, 2009).
Tabel 1. Gradaţii ale concentraţiilor de SO_2 în aer şi calitatea aerului exprimată prin toxitoleranţa lichenilor (BEGU, 2009).

SO_2 concentration in the air, mg/m^3 air	**Air quality**	**Lichens toxitolerance level within the studied area, presence of:**	**Toxitolerance level**
< 0.05	Clean air	Highly sensible species	I
0.05 – 0.1	Slightly polluted	Sensible species	II
0.1 – 0.2	Moderately polluted	Species with moderate resistance	III
0.2 - 0.3	Highly polluted	With increased resistance	IV
0.3 – 0.5	Heavily polluted	With high resistance	V
> 0.5	Critically polluted	Complete absence of lichens	VI

Considering that not any presence of lichens is a true indication criterion, fact previously presented for higher plants, in case of lichen species the assessment will be accurate only when the indicator species substrate coverage is over 10% of the total surface area. The threshold is important, especially for Toxitolerance level I and II, due to the fact that the air can not be affirmed as clean if only one (or even 2-3) exemplar has been identified in the studied area and particularly when the exemplar has pale appearance and covers an insignificant surface.

Given the abundance and indicator species toxitolerance, the Air Quality Graduations Scale was developed to assess the air quality (Table 2).

Table 2. Air Quality Graduations Scale (AQGS), based on abundance of lichen species with different toxitolerance level (BEGU, 2009).
Tabel 2. Gradaţii de Evaluare a Calităţii Aerului (GECA) în baza abundenţei speciilor de licheni cu diferit grad al toxitoleranţei (BEGU, 2009).

Air quality	**SO_2 content in the air, mg/m^3**	**Abundance of species with different toxitolerance degree, % of substrate surface**	**Conventional colour**
1. Clean	<0.05	I > 10 or I < 10 and II > 75	blue
2. Slightly polluted	0.05-0.1	I – 0 -10 or II – 50-75	green
3. Moderately polluted	0.1-0.2	II - 10-50 or III > 50	violet
4. Polluted	0.2-0.3	III - 10-50 or IV > 50	yellow
5. Heavily polluted	0.3-0.5	IV - 10-50 or V - 1-100	red
6. Critically polluted	>0.5	Complete absence of lichens	black

Standard species. Several authors consider important to take into account the biological features of lichens species and their sensitivity to certain pollutants, the effects of which can be noticed through visible signs immediately or in time, but with no apparent thallus damage (BURTON, 1986). In this context, fruticose species are recommended, because they can be easily detached from the substrate and further investigated. SLOOF & WOLTERBEEK (1993) discuss the possibility of calibration (use) of certain common species as an instrument to assess the pollution parameters. Due to this and the fact that the European Committee for Standardization, Measurement and Testing Programme proposes to use lichens as reference material within all Europe (QUEVAUVILLER et al., 1996), it is important to develop a standardized and well acknowledged method, which will provide accurate results and minimize errors.

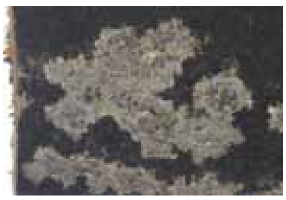
In this context, *Parmelia sulcata* is proposed for the Netherlands, as an indicator lichen species for heavy metals accumulation and is noted in the geographical mapping of pollution (SLOOF & WOLTERBEEK, 1991). The Italian scholar BARGAGLI (1990) explains the priorities concerning the use of this species. The Swiss HERZIG (1989) performed passive monitoring applying *Hypogymnia physodes* and established a good correlation between the Pb and Cu site deposition and Pb and Cu accumulated in lichens. Taking into consideration Switzerland conditions, it is proposed to combine the Calibration of Indicator Lichens Method with the Biologically Passive Monitoring Method into a biologically integrated system for monitoring of air pollution.

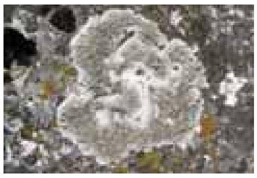
Monitoring of heavy metal content in the environment using bioindicators can be ensured though a dense network of test organisms and ongoing monitoring activities in the studied environment (soil, water, biota, air etc.), which incorporates within their tissues summary concentration of heavy metals, reacting simultaneously to other harmful factors (i.e. SO_2, NO_x, HF etc.). In this case, we offer 40 lichen indicator species to asses the air pollution with SO_2, commonly found in the habitats of the Republic of Moldova (Table 3). Chemical analysis allowed us to assess the concentrations of harmful substances in the recommended species, either spontaneously growing in different habitats or transplanted into habitats with a strong anthropogenic impact and almost devoid of lichens. It is important to highlight that this kind of networks do not require any financial expenditure, both for organising and maintaining them available at any time. There will be costs only related to the study activities performed in the filed: i.e. collecting of samples, laboratory analysis and interpretation of partial results and development of recommendations.

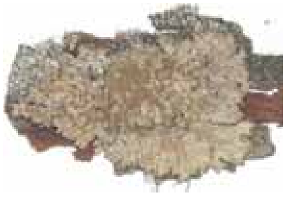
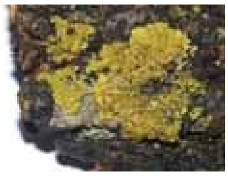
Among the 4 transplant species tested in conditions of Chişinău city, *Parmelia sulcata* was found to be the most responsive to the chemical air pollutants, particularly SO_2, recording obvious morphological and biochemical changes (colour, thallus, and photosynthetic pigments degradation). These findings allowed us to transfer the species from toxitolerance level 3 to toxitolerance level 2.

Table 3. Republic of Moldova list of lichens indicator species recommended in assessing the atmosphere air quality (original).
Tabel 3. Conspectul lichenilor indicatori din Republica Moldova, recomandați în evaluarea calității aerului atmosferic (original).

No.	**Species/Synonym/ Protection State**	**Image**	**Toxitolerance**	**No. of habitats and country preferable zone**	**No.**	**Species/Synonym/ Protection State**	**Image**	**Toxitolerance**	**No. of habitats and country preferable zone**
1	*Anaptychia ciliaris* (L.) KOERB. Ex A. MASSAL – Rare		2	17 habitats within the entire country	21	*Pertusaria discoidea* (PERS.) MALME. (*P. albescens* (HUDS.) CHOISY & WERNER, *P. globulifera)* – Red Book of Moldova, suggested		2	5 habitats within the northern and central parts of the country
2	*Bacidia rubella* (HOFFM.) MASSAL. syn. *B. luteola* (SCHRAD.) MUDD.		2	19 habitats within the entire country	22	*Phaeophyscia nigricans* (FLORKE) MOBERG syn. *Physcia nigricans* (FLK.) STIZB.		4	6 habitats within the northern and central parts of the country
3	*Candelariella aurella* (HOFFM.) ZAHLBR.		3	14 habitats within the entire country	23	*Phaeophyscia orbicularis* (NESK.) MOBERG syn. *Physcia orbicularis* POTSCH emend. DU RIETZ.), *Ph. virella* (ACH.)		4	18 habitats within the northern and central parts of the country
4	*Candelariella vitelina* (HOFFM.) MULL. ARG.		4	14 habitats within the entire country	24	*Phlyctis argena* (SPRENG.) FLOT. - Red Book of Moldova, suggested		3	7 habitats within the northern and central parts of the country
5	*Cladonia fimbriata* f. *fimbriata* (L.) FR.		2	7 habitats from the northern zone of the country	25	*Physcia adscendens* (FR.) H. OLIVER.		3	40 habitats within the northern and central parts of the country

6	*Cladonia pyxidata* (L.) HOFFM. – Rare 	2	13 habitats within the northern and central parts of the country	26	*Physcia aipolia* (EHRH. ex HUMB.) FURNR. HAMPE emend. NYL. var. *aipolia* 	2	28 habitats within the entire country
7	*Evernia prunastri* (L.) ACH. 	3	34 habitats within the entire country	27	*Physcia caesia* (HOFFM.) FURNR. 	3	24 habitats within the northern and central parts of the country
8	*Graphis scripta* (L.) ACH. – Red Book of Moldova 	2	15 habitats within the northern and central parts of the country	28	*Physcia stellaris* (L.) NYL. 	3	24 habitats within the entire country
9	*Hypogymnia physoides* (L.) NYl. 	3	22 habitats within the entire country	29	*Physcia tenella* (SCOP.) D. C. (*Ph. hispida*) 	IV	35 habitats within the entire country
10	*Lecanora carpinea* (L.) VAINIO 	3	17 habitats within the northern and central parts of the country	30	*Physconia distorta* (WITH.) J. R. LAUNDON (*Ph. pulverulenta* f. *izidiigera* A. ZAHL.) 	3	30 habitats within the northern and central parts of the country
11	*Lecidella elaeochroma* (ACH.) HERTEL. syn. *Lecidea glomerulosa* (D. C.) STEUD. *L. enteroleuca* ACH. *L. olivacea* (HOFFM.) MASSAL 	3	35 habitats within the entire country	31	*Physconia grisea* (LAM.) POELT syn. *Physcia grisea* (LAM.) A. Z. 	3	45 habitats within the entire country

12	*Lepraria incana* (L.) ACH. (*L. aeruginosa* SM.) 	5	19 habitats within the entire country	32	*Protoparmeliopsis muralis* (SCHREB.) M. CHOISY *Placolecanora muralis* (SCHREB.) RAS. 	2	5 habitats within the northern and central parts of the country
13	*Parmelia acetabulum* (NECK.) DUBY. 	3	23 habitats within the entire country	33	*Pseudevernia furfuracea* (L.) ZOPF. (*Evernia furfuracea* MANN.) - Red Book of Moldova (Vulnerable) 	2	14 habitats within the northern and central parts of the country
14	*Parmelia caperata* (L.) ACH. 	2	14 habitats within the northern and central parts of the country	34	*Ramalina farinacea* (L.) ACH. - Red Book of Moldova (Vulnerable) 	2	13 habitats within the northern and central parts of the country
15	*Parmelia olivacea* (L.) ACH. 	3	11 habitats within the northern and central parts of the country	35	*Ramalina fraxinea* (L.) ACH. var. *fraxinea* - Red Book of Moldova (Vulnerable) 	1	18 habitats within the entire country
16	*Parmelia quercina* (WILLD.) VAIN. – Red Book of Moldova, suggested 	2	6 habitats within the northern and central parts of the country	36	*Ramalina polynaria* (WESTR.) ACH. var. *polynaria* - Red Book of Moldova (Vulnerable) 	2	9 habitats within the northern and central parts of the country
17	*Parmelia scortea* f. *scortea* ACH. 	3	16 habitats within the northern and central parts of the country	37	*Ramalina roesleri* (HOCHST. ex SCHAER. HUE 	2	10 habitats within the northern and central parts of the country

18	*Parmelia sulcata* TAYLOR 	2	54 habitats within the entire country	38	*Usnea hirta* (L.) F.C.WEBER ex F. H. WIGG. – Red Book of Moldova (Endangered) 	1	6 habitats within the northern and central parts of the country
19	*Parmelia verruculifera* NYL. 	3	9 habitats within the northern and central parts of the country	39	*Xanthoria candelaria* (L.) TH. FR. (*X. ucrainica*) 	3	6 habitats within the northern and central parts of the country
20	*Peltigera polydactyla* f. *polydactila* (NECK.) HOFFM – Red Book of Moldova (Endangered) 	1	4 habitats from North zone of the country	40	*Xanthoria parietina* (L) TH. FR.var. *parietina* 	5	77 habitats within all zones of the country

CONCLUSIONS

Among approximately 200 lichen species and varieties known within the Republic of Moldova, based on literature sources, field research, laboratory testing and monitoring (through photographing), transplants responsiveness, there were identified 115 species sensitive to pollution with SO_2, 34 species - NO_x, 3 species - the Cl, 2 species - F, 27 species to environment pH, 37 species were calciphyles and 14 – siliciphyle.

Preceding the indicator species frequency within forest, forest - petrophyte and urban ecosystems as genuine indicators to be applied in the monitoring of forest sector, there are 40 species, which form certain associations sensitive to environmental air pollution with SO_2, NO_x etc., and accumulate in their body large concentrations of heavy metals. Of these, 3 species register a toxitolerance level 1, 15 - level 2, 16 - level 3, 4 - level 4, and 2 species - toxitolerance level 5.

Furthermore, preceding the Republic of Moldova conditions, in which the lichens flora is represented by only approximately 200 species and varieties, and the fact that major sources of intense pollution are lacking, etc., the Lichens Toxitolerance Scale was proposed, which includes six conventional levels/classes of air pollution with SO_2. Subsequently, the Air Quality Graduations Scale based on the indicator abundance/coverage, level of toxitolerance, co-relation between different eco bioindications species was developed to be applied in forest and urban ecosystems.

Widespread, high frequency and predominance of *Parmelia sulcata* species in forest ecosystems, allows its use as a standard species and to be applied in geographical mapping of air pollution with SO_2 and heavy metals, requirement imposed by the European Committee for Standardization, Measurement and Testing Programme (ECSMTP). *P. sulcata* already serves as a standard species in the Netherlands and Italy.

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