TESTING THE QUALITY OF THE ENVIRONMENT THROUGH THE LICHENOINDICATION METHOD

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Abstract
The monitoring of the state of the environment through the method of biological monitoring based on the principle of eco-bioindication, i.e. non-instrumental method - direction promoted at the European level and by art. 7 "b" of the Convention *on Long-Range Transboundary Air Pollution (Geneva, 1979). The most well-known bioindicators of air quality are lichens, successfully used in active and passive ecological monitoring in many countries of the world. In the Chisinau area, laws were established regarding the modification of the chlorophyll content or the total degradation of the lichen thallus under the action of SO2 and the capacity to accumulate heavy metals, depending on the source of pollution, geographical conditions, the species sensitivity, the exposure duration, etc. We propose the Parmelia sulcata species as a reference object (standard) in the air quality monitoring at the national level.*

Keywords: lichen indication, monitoring, pollution, standard species.

1. INTRODUCTION

The eco-bioindication is based on the study of some species and communities of organisms, sensitive to the change in the conditions of the external environment, or with cumulative particularities of chemical pollutants. The eco-bioindication method makes it possible to obtain information about fluctuations in time and space, the accumulation or synergistic effect between certain abiotic factors and the response of living organisms to environmental changes (Blandin, 1986). Traditionally, the strict monitoring of negative phenomena is carried out through the instrumental method, installing a complex ecological network (Андреев, 2002), which signals, non-stop, the dynamics of the phenomena. Making a retrospective of the phyto- and zoo-indication, the biomonitoring method is proposed in the supervision of the forest ecosystems state as an alternative to the instrumental method (Măciucă, 2003) and was applied by us, both for the whole territory of Moldova and in the Chişinău area (Begu, 2011).

Toxicity of SO2 and heavy metals. SO2 pollution affects chlorophyll, transforming it into pheophytin by replacing one Mg atom with one H, thus reducing the capacity for photosynthesis (Bercea, Bathory, Rusu, 1991-1992); (Garrou e Castrogiovanni, 1991). The problem of the organisms' tolerance to heavy metal pollution becomes quite important and current. An increased toxicity manifests Hg, Pb and Cd, the others having more moderate effects on plants and animals (Израэль, Филиппова, Ровинский, 1976); (Munn, 1973); (WMO operations manual...,1971). The

excess of heavy metals, in association with SO2, inhibits the photochemical processes of photosynthesis (Bessonova, Kozyukina, Lyzhenko, 1990).

Indicator lichens and standard species. Very frequently in the monitoring of atmospheric pollution with SO2 and heavy metals, lichens are recommended, even more often than mosses, because they very well allow testing air pollution with gaseous compounds and heavy metals. The heavy metals content in lichens correlates quite well with their level in the atmosphere, decreasing with distance from the pollution source (Burton, 1986). Lichens are more widespread and environmental quality monitoring scales have already been developed for them at national and international level.

The authors (Sloof, Wolterbeek, 1993) discuss the possibility of calibrating (using) some common species, as a precision tool to check pollution parameters. (Kovács, 1992). They propose 70 indicator species of heavy metals, of which 15 species are found on the Republic of Moldova territory. It is necessary to develop a standardization method regarding the use of lichens, which would ensure the accuracy of the results and the minimization of errors, because the Program of the European Commission for Standardization, Measurements and Testing proposes to use lichens as a reference material for all of Europe (Quevauviller, Herzig, Muntau, 1996). For example, for the Netherlands, in the geographical mapping of pollution, the species *Parmelia sulcata* is proposed as a bioaccumulator of heavy metals (Sloof and Wolterbeek, 1991), and the italians (Bargagli, 1990) and (Loppi, Chiti, Corsini, Bernardi, 1994) explain the priorities of using this species. The authors (Thompson, Ramelow, Beck, et al. 1987); (Walther, Ramelow, Beck et al., 1990) propose the local species *Parmelia praesorediosa* and *Ramalina stenospora*, and studies from Yugoslavia (Jovanovic, Morkov, Deschamps, Vukotic, 1995) advocate the use of the species *Hypogymnia physodes, Diploicia canescens* and *Lecanora expallensis.*

Thus, a dense network of test organisms can ensure the monitoring of the state of the environment through bioindicators with permanent activity in the monitored environment, which incorporate heavy metal concentrations in their tissues, simultaneously reacting to other harmful factors (SO2, NOx, HF etc.). Chemical analysis allows the assessment of concentrations of harmful substances, which highlights synergistic effects, ecological valence, the law of the minimum and other forms of correlation between the studied elements and external factors.

2. MATERIALS AND METHODS

In order to carry out the active biological monitoring, 60 samples of four lichen test species were transplanted in the 15 locations in five sectors of Chisinau, depending on the geographical position of the sector and the stations, the altitude, the source of pollution, etc. (Fig. 1).

During 9 months of exposure of the samples (24.12.06-23.09.07), 16 photographs of four lichen-transplant species were taken, approximately, every 10 days.

3. RESULTS AND DISCUSSION

Changes of the thallus. In general, the first symptoms of SO2 damage to transplants appeared approximately after one month of exposure, constituting 3-5% of the thallus surface, by 21.01.2007 as of 04/03/2007. Only in 17 variants from 60 totally, the transplants were not affected, the rest, about 73%, showed obvious symptoms of the negative impact of atmospheric pollutants, sometimes reaching 20-27% of the thallus surface, in particular, 3 variants from the Botany sector.

The damage degree intensifies in the summer period, especially on Albişoara Street (70-90% *Evernia prunastri*), Dacia Avenue (70-80% *Parmelia sulcata* and 55-60% *Parmelia caperata*), Paris Street (70% *Evernia prunastri*, 80 % *Hypogymnia physodes*, 90% *Parmelia sulcata*),

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Academy Street (60% *Evernia prunastri*, 50-80% *Hypogymnia physodes*). At the experiment end, complete or almost complete destruction of the thallus was, sometimes, reported: Albişoara Street– 100% *Hypogymnia physodes*, 90% *Evernia prunastri*; Academy Street – 90% *Evernia prunastri*, 85% *Hypogymnia physodes*.
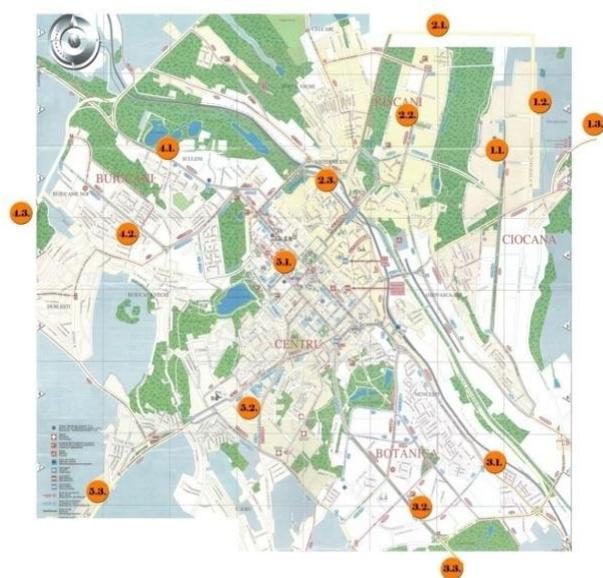
Sector	Number	Research stations
1. Ciocana	1.1	Zadnipru street
	1.2	Milescu Spătaru street
	1.3	Poiana Nucului zone
2. Râşcani	2.1	Bucovinei street
	2.2	Miron Costin street
	2.3	Albişoara street
3. Botanica	3.1	Munceşti street
	3.2	Dacia Avenue (Zimbru Stad.)
	3.3	Dacia Avenue (Instit. Prot. Plant)
4. Buiucani	4.1	Calea Ieşilor street
	4.2	Paris street
	4.3	Balcani street
5. Centru	5.1	Maria Cebotari street
	5.2	Academiei street
	5.3	Hânceşti street

Figure 1. Location scheme and legend of research stations in Chisinau urban space

Overall, the most polluted with SO_2, from the point of view of air quality, are: the residential area adjacent to Paris Street (Buiucani sector) - all the transplanted samples were affected from 50 to 90% of the surface and Albişoara Street- 10-100%, followed by the area of Academy Street - 15-90% and Dacia - 15-80%.

Among the cleanest areas, the outskirt areas, around Bucovina Street (Râşcani sector) stands out, where, practically, the transplanted samples remained unaffected. Next, come the other outskirt areas, where the air quality does not bear the obvious impact of pollutants - str. Hâncesti (affection 7-15%), Balcani Street (3-20%) and Poiana Nucului zone (10-20%) from the surface of the valley.

We note the fact that, contrary to our assumptions and the laws of the accumulation of noxes, the variants arranged in depressed places were not the most affected, but on the contrary, those on high ground, but located in the area of residential spaces. With certainty, the emissions, mainly those of automotive origin, are retained in the spaces between residential and administrative blocks (mostly with 9-16 floors). Those produced by the chimneys of companies, start to be deposited from top to bottom, i.e. from the height of the chimneys of emission, on high ground (with increased concentrations of noxes), towards depressions (with more diluted concentrations of noxes). Thus, in the sedentary sectors, the dominant direction of the winds and the altitude cannot ensure the air purification effects, specific to the open sectors of the plains. Pollution decreases from "high ground, intra-village" to "depression, intra-village" and "high ground, extra-village". In the municipality of Chisinau, the dominant direction of the winds moved, as a matter of priority, the air masses with the pollutants accumulated in it, from the NW to the SE, increasing the air pollution in

the Center and Botanica sectors. The Botanica sector (without industrial macro-enterprises) is a victim of the frontal displacement of pollutants from the mobile sources in the Center sector (transport with intense car and rail traffic) and from the fixed sources in the area of the Ciocana de Jos sector.

Changes in chlorophyll content. A direct expression of the negative impact, caused to lichens transplanted in the municipality of Chisinau, is represented by the content of photosynthesis pigments - chlorophyll "a" and "b" and carotenoids. Photosynthesis is very sensitive to environmental pollution with SO_2, As_2O_3, H_2S, herbicides, insecticides and chloroform (Duca, 2006). Thus, in the control variety (samples from the Codrii Scientific Reserve) the content of chlorophyll "a" and "b" ranged from 0.800 mg/g d.m., in *Hypogymnia physodes*, to 1.564 mg/g d.m., in *Parmelia sulcata* (Fig. 2). The most intensively degraded the chlorophyll content of the species *Parmelia sulcata,* decreasing in all the variants analyzed from about 2.5 times (Tohatin) to about 40 times (Paris Street), the difference between the variants being statistically significant, and compared to *Parmelia caperata* – 1.5 times (Fig 2).
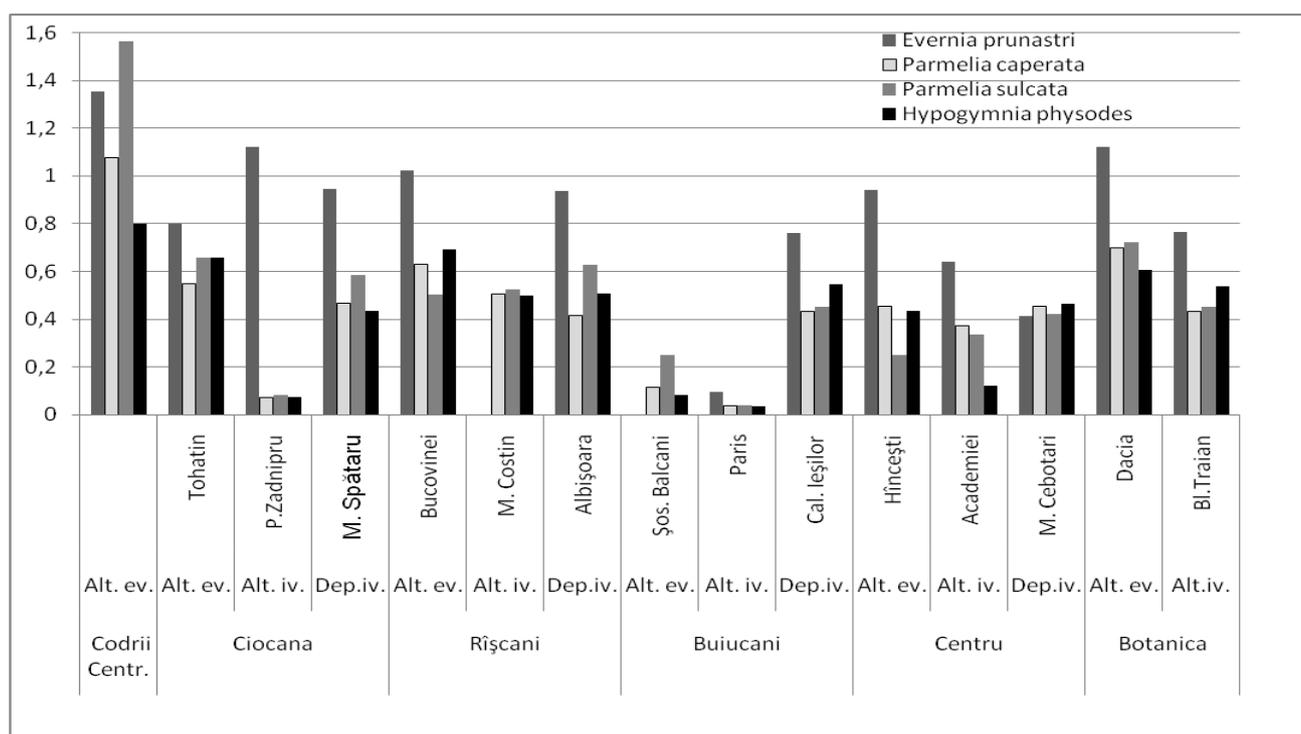

Figure 2. Change in the content of chlorophyll a+b in active monitoring transplants, mg/g s.u., Chisinau.
Note: *Evernia, Hypogymnia - Cystococcus* **alga;** *Parmelia - Trebouxia* **seaweed**

The degradation of chlorophyll in the thallus of *Parmelia sulcata* was assumed by the increased degree of damage to the thallus, described previously. Thus, *Parmelia sulcata* is a very sensitive indicator of air pollution, the symptoms of the damage being the most obvious, both visually and analytically. In second place, *Parmelia caperata* is placed - the reduction of the chlorophyll content from about 1.5 times (the location of the Plant Protection Institute), to about 25 times (Paris Street), compared to the control, the last case confirmed above and through the degradation of the thallus.

The content of pigments in the thallus of the species *Evernia prunastri* decreased more weakly, highlighting, in particular, the variants str. Paris - by about 15 times and M. Cebotari Street- by about 6.5 times. The content of pigments in *Hypogymnia physodes* decreased from about 1.1 times (Bucovinei Street), to about 10 times (Balcani Street).

Thus, we can confirm that there is a good correlation between the pigment destruction degree and the morphological damage degree to the transplanted lichens thallus. The chlorophyll "a" and "b" content decreased considerably in the samples of the most polluted variant – Paris Street, recording extremely low values in all studied species; the stations follow – Zadnipru Street, low values for 3 species; Balcani Street and Academiei Street. Likewise, as in the case of the evaluation based on photo images, the cleanest air is characteristic for the Râşcani sector - here the chlorophyll content remained quite high, followed by the Tohatin zone and Spătaru Street, from the Ciocana sector.

Accumulation of heavy metals. According to the prescriptions of the Program of the European Commission for Standardization, Measurements and Testing (CESMT), lichens are approved as a reference material in air quality testing, certified for all Europe (Quevauviller, Herzig, Muntau, 1996). Among the four species used by us in the active biological monitoring, better and more obvious results were recorded with the species *Parmelia sulcata*. For a more convincing evaluation, we also analyzed the content of heavy metals in the species *Parmelia caperata*. We note that the values of the indices recorded for the 2 species, in the vast majority of cases, are similar. As a rule, *Parmelia sulcata* accumulates Pb and Cr better, and *Parmelia caperata* – Ni, partially Cd (Fig. 3).
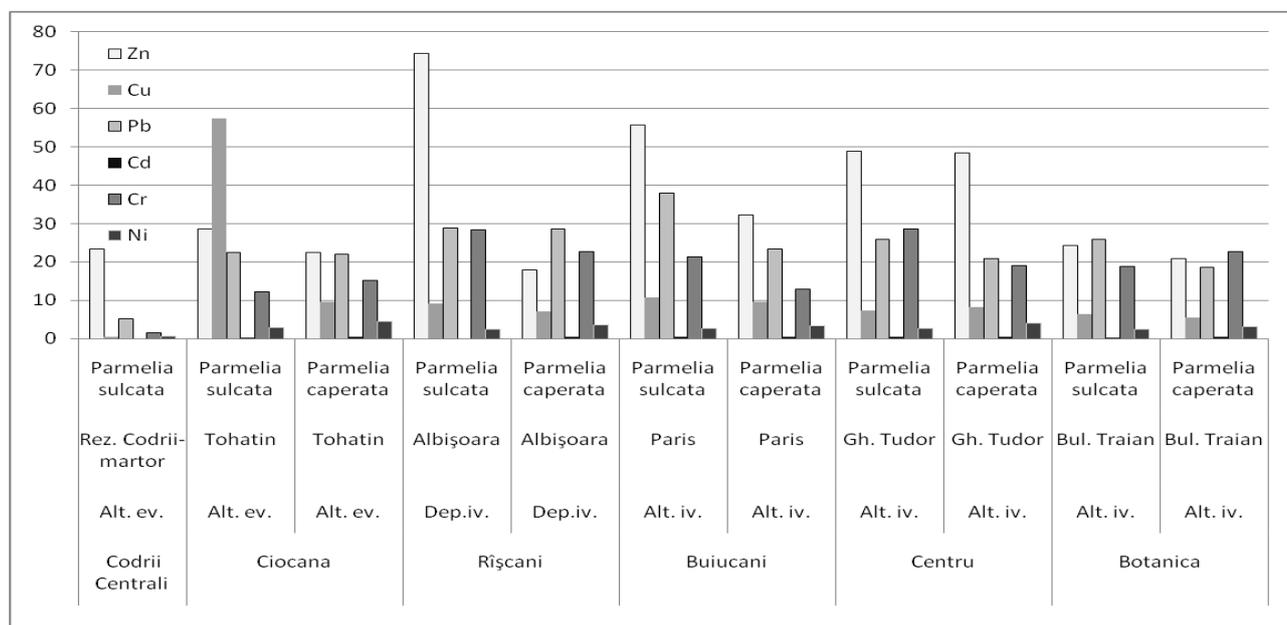

Figure 3. The content of heavy metals in two species of Parmelia, mg/kg d.m.

The transplanted lichen samples confirmed the increased capacity to accumulate heavy metals during 9 months. The highest accumulations are recorded by Cu and Cr - about 10-20 and 8-17 times higher than the control (except Tohatin zone - about 100 times Cu); followed by Pb and Ni - about 3-8 times and ending with Cd - about 1.5-6.0 times more than the control sample. If Cu, Cd and Ni remain below the values of Maximum Permissible Concentrations (MPC) for plants

(respectively 100; 3 and 10 mg/kg dry matter (d.m.), then Pb and Cr concentrations exceed 1.8-3.8 and 2.2- 5.3 times the MPC (MPC Pb=10, MPC Cr=5 mg/kg d.m.).

Likewise, Pb and Cr, totally, and Cd, partially, exceed the critical levels for plants, established for Europe. Thus, the risk presented by these two elements (Pb, Cr) is quite high, both for the vegetation and for the city population. Pb registers maximum values in the variants of Zadnipru Street (38.5 mg/kg d.m.) and Paris Street (37.9 mg/kg d.m.), and Cr – Albişoara Street (28.4 mg/kg d.m.) and Academiei Street (28,7 mg/kg d.m.). These variants were nominated as quite polluted areas, also based on other indices, previously described. In general, more polluted with heavy metals proved to be the residential areas – Paris Street (Zn, Pb, Cu, Cr), Academiei Street (Zn, Pb, Cr) and Albişoara Street (Pb, Cr).

4. CONCLUSIONS

The capacity to accumulate heavy metals by lichens is quite high; the indices obtained are similar to the data from the specialized literature and confirm the possibility of their application in the air quality biological monitoring, and in our case, especially the *Parmelia sulcata* species.

Among the four transplanted test species, *Parmelia sulcata* is the most responsive to atmospheric chemical pollutants, especially SO2, registering obvious morphological and biochemical changes (color change, thallus degradation, degradation of photosynthetic pigments). This fact allows us to transfer it from the third toxic tolerance class to the second toxic tolerance class. The wide spread, high frequency and preponderant dominance of the *Parmelia sulcata* species in forest and dendrological habitat, allows its use as a standard in the geographical mapping of atmospheric pollution with SO2 and heavy metals, a requirement provided by the Program of the European Commission for Standardization, Measurements and Testing. *Parmelia sulcata* already serves as a standard in Holland and Italy.

The results obtained in the framework of biological monitoring allowed us to, theoretically, argue the possibility and effectiveness of the eco-bioindication application in the air quality monitoring in forest and urban ecosystems. The eco-bioindication method is easily achievable based on the specific diversity, abundance and degree of toxic tolerance by applying the impact assessment gradation scales, adjusted to the country's conditions.

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