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The Efficiency of Lichens in Air Biomonitoring in Teleorman County

Maria Magdalena Cernat Popa and Carmen Otilia Rusănescu *

Faculty of Biotechnical Systems Engineering, National University of Science and Technology Politehnica Bucharest, 313 Spl. Independentei, 060042 Bucharest, Romania; maria.cernat@stud.isb.upb.ro
* Correspondence: carmen.rusanescu@upb.ro

Abstract: In this work, we collected samples of lichens from the oaks of Pădurea Troianul, in the area of Teleorman county, to analyze the air quality, using the lichen biodiversity index and its determining factor. We transplanted them to the points to be monitored and analyzed them to detect and quantify the concentration of heavy metals or other toxic substances accumulated in the lichen biomass. This research was conducted at transplant sites, where five sample sites were chosen. We investigated the concentrations of the heavy metals Cd, Pb and Hg, making a comparison between the concentrations indicated by lichens and conventional measurements. The sampling, observation and analysis of lichens, we carried out in the months of September, October and November 2021, because in September the temperatures are higher and the degree of precipitation is lower than in November, so we followed a development of lichens covering different periods in terms of temperature and humidity. The comparison was made starting from the contamination factors obtained and measured in the sampling station. The application of the biomonitoring method allows obtaining an exact index of the purity of the atmosphere based on the tolerance of the transplanted lichens, the results being obtained quickly.

Keywords: bioindicators; lichens; heavy metals; transplantation; pollution sources

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1. Introduction

The quality of the air we breathe is a major concern in our modern society. Air pollution can have negative effects on human health and ecosystems in general. To monitor and assess the level of pollution, researchers have developed different methods of analysis [1–12]. One of these involves the use of lichens as indicators of air quality. Lichens are symbiotic organisms that consist of an association between a fungus and an alga or cyanobacterium. These organisms are sensitive to environmental changes and can provide valuable information about air quality in a given region.

Lichens are known for their sensitivity to air pollutants such as sulfur dioxide, nitrogen dioxide, ozone and heavy metals. These chemicals can be absorbed by lichens through their pores and affect the body's physiological processes. Due to this sensitivity, lichens can be used as indicators of the level of pollution with harmful substances in a specific geographical area [13–20].

The lichen air analysis approach involves collecting these organisms and evaluating them in the laboratory. Researchers study the diversity, density and general condition of lichens in a given area. By comparing these characteristics with past data and a set of references, the level of air pollution at that location can be determined.

Lichens are also used in long-term biomonitoring studies to assess the evolution of air quality in a region [20–27].

The lichen-based approach to air analysis has several advantages. First, lichens are non-invasive bioindicator organisms, which means that their collection does not require the destruction of the environment. Lichens can also be found on different surfaces, such as trees, rocks and buildings, which allows monitoring of air quality in diverse areas. In addition, lichens are very hardy and can survive in extreme conditions, which makes them useful in monitoring pollution in industrial areas or near pollution sources [28–30].

The biomonitoring of air pollution with lichens has the advantage that they can be found everywhere in terrestrial environments, lichens react to the toxic effect of pollutants.

The analysis of the degree of pollution in an area using lichens is carried out by comparing the concentrations of heavy metals detected in lichens with the reference values. The identification of pollution sources is done by analyzing the lichens collected from different areas. Biomonitoring of heavy metals in Teleorman County is important for evaluating the degree of environmental pollution and its impact on human health and ecosystems. Passive biomonitoring with lichens provides information regarding the impact of pollution on ecosystems and human communities.

The analysis may include the determination of the content of heavy metals, organic chemicals or other pollutant compounds. Biomonitoring with lichens provides a long-term assessment of air quality, as lichens can accumulate in the environment over a longer period of time [31]. Lichens can also be used to assess the effects of pollution reduction measures and air quality at different points. The history of research into the use of lichens as bioindicators reflects the progress of our knowledge of the sensitivity of these organisms to atmospheric pollution [32]. From the first observations and discoveries to the development of monitoring methods and ecological implications, research in this field has made significant contributions to the understanding of the relationship between lichens and air quality. The use of lichens as bioindicators in air quality monitoring has had and will continue to have an important impact on the protection of the environment and human health. The use of lichens as bioindicators in the study of air quality has been explored and documented by several authors over time [33]. These authors have made significant contributions to the field of research on lichens as bioindicators, providing valuable insights [34–47]. Lichen transplanting is a practice used to transfer and install lichens on different surfaces or substrates [2,7,25,34,36,40–47].

The main purpose of the work is to monitor the quality of the urban air in Teleorman County by means of bioindicators. Bioindicators can replace or complement instrumental monitoring, and lichens can provide indications about the variation over time, the accumulation or the effects of certain polluting substances. To carry out this research, we studied and inventoried the species of lichens and the sampling points, and we transplanted them to different points with the aim of obtaining measurements related to the concentrations of heavy metals in the five cities of the county. We found from the samples analyzed in the laboratory that atmospheric pollution induces certain morphological changes in lichens, such as discolored thallus due to the accumulation of heavy metals, a feature that is the basis of air quality evaluation. Following the research, we found that the diversity of lichens is low, which indicates moderately polluted air. The scientific originality of the results is due to the conduction of a complex study by applying the ecological methods of transplantation and chemical determination of heavy metals which led to the obtaining of new information regarding toxic tolerance to atmospheric pollutants.

2. Materials and Methods

The location of the studied area is Teleorman County, and from an ecological point of view, it is in the plain region of southern Romania, being located in the southeastern part of the Muntenia region. There are a number of ecological characteristics and natural resources specific to this geographical area, such as extensive agricultural lands known as one of the most important agricultural regions in Romania. Agriculture has a significant impact on the environment, and agricultural practices can affect soil and water quality, as well as local biodiversity and urban anthropogenic impact.

The process of transplanting lichens can be carried out by different methods, depending on the type of substrate and the lichens involved. Some common methods include the following.

Fragmentation and manual transfer: involves dividing a healthy lichen into smaller pieces, called fragments, and transferring them to a new substrate. This process can be carried out manually, and it is conducted carefully to preserve the structure of the lichen. The method works better for lichens with a more robust and adherent structure, such as crusts and folio lichens.

Sometimes lichen fragments can be sampled using borders, such as pieces of wood or paper. Lichens can attach to the supports, and then the supports can be placed in the new desired location for transplantation as seen in Figure 1.

Figure 1. Preparation of the sample for transplantation.

For certain species of lichens, a special paste containing mycelium or lichen fragments, mixed with a binder and other substances, can be used. This paste can be applied directly to the target substrate to initiate lichen growth.

This process involves the collection of lichen fragments from a healthy donor surface, in this case the Protected Natural Area of the Troianul Teleorman Forest, and their transfer to a target surface. The lichen fragments are attached to the target substrate with the help of natural resin and are allowed to grow and develop there. It is important to take into account the specific requirements of lichens and the environment in which they are transplanted. Some lichens can be sensitive to pollution or environmental changes, and selection of the right species and suitable environmental conditions is essential for transplant success. In the biomonitoring process, lichens transplanted to certain points in the studied area were used. The fixing points in Teleorman County are formatted as follows: station number, zone of transplanting, town:

TR-01 SUD Alexandria Teleorman urban, TR-02 SUD Turnu-Măgurele Teleorman urban, TR-09 SUD Turnu Măgurele Teleorman urban traffic, TR-10 SUD Turnu Măgurele Teleorman urban industrial and TR-11 SUD Zimnicea Teleorman urban. The establishment of target areas for passive biomonitoring of heavy metals at the level of Teleorman County was achieved by fixing 5 monitoring points. The locations of the biomonitoring points were chosen considering two categories of areas:

- Areas that, although they are not in the vicinity of heavy metal pollution sources, show signs of contamination identified with the help of bioindicators;
- Areas referred to as "control areas" where no heavy metal contamination has been reported.

In this way, the monitoring of the presence and accumulation of heavy metals is ensured, as well as the estimation of their dynamics in time and space. The monitoring took place between 11 September 2021 and 11 November 2021, during three months, choosing to present the obtained results in the present paper. The transplanted lichen species is the following: biomonitor species identification sheet, scientific name: *Parmelia saxatilis* (L.) Ach. The details about the species chosen for monitoring are present in Figure 2a.

Its taxonomic classification is as follows: class *Ascomycotina*, order *Lecanorales* and family *Parmeliaceae.*

Its taxonomic description is as follows: It has a foliose thallus in the shape of a rosette, weakly attached to the substrate; the lobes are clothed and 3–5 mm wide, with arched lobulate margins. The upper part is gray, whitish gray, brown–gray or greenish gray, with numerous isidia; the lower part is black and covered with resins. The apothecaries have a brown disc, with a diameter that reaches up to 10 mm. Ascospores are 14–18 μm × 8–12 μm in size. By applying KOH, the upper part of the thallus acquires a yellow color, and by applying the same chemical reagent, the medulla acquires a yellow color.

Habitat, cenology existing in the Central European Mediterranean area is: *Parmelietum omphalodis*, *Umbilicarietalia cylindricae* (upper montane), *Chrysotrichetalia chlorinae*, *Rhizocarpetalia obscurati* (moister habitats, shaded or near the soil), *Parmelietalia saxatilis* (rocks with influence of soil), *Physcietalia caesiae*, *Pseudevernietum furfuraceae* [48].

Importance for biomonitoring (characteristics that recommend it for biomonitoring): the species shows high toxic tolerance to atmospheric pollution.

The species is among the most efficient bioaccumulators of pollutants. The species was transplanted in the immediate vicinity of the monitoring stations for a good assessment of the quality of the environment. The samples did not require any preparation before transplanting into the monitored areas. They were fixed at a height of 1.50 m from the ground, the transplant areas were marked, and then natural resin was applied to them.

The biomonitoring points were established in the immediate vicinity of the air biomonitoring stations. These are: TR-01-Alexandtia urban; TR-02-Măgurele urban tower;

TR-09-Turnu Măgurele urban heavy road traffic; TR-10-Turnu Măgurele urban industrial. TR-11-Zimnicea urban.

When establishing the biomonitoring points, the direction of circulation of the air currents and the frequency of the emissions were taken into account, with the samples being positioned 1.50 m from the ground on the bark of trees. For the collection of biological material, the aim was to quantify the emissions for a certain episode in a time interval, 11 September–11 November 2022, during the autumn period when the species is able to accumulate emissions. The average temperature recorded was between 6 °C and 10 °C, registering a negative deviation from the median of the standard reference interval (1991–2020) of approximately 2.4 °C. Humidity is an essential factor influencing lichens in several ways, including the following categories.

Lichens require a certain amount of moisture to grow and develop optimally. When moisture is sufficient, lichens can absorb water from the environment to support their metabolism and achieve photosynthesis. The wetter the environment, the more chances they will have to grow and develop. On the other hand, lichens are sensitive to desiccation, that is, excessive drying of the environment. When the humidity level drops significantly, lichens enter a resting stage and become inactive in order to conserve their resources and protect themselves from dehydration. This may limit their ability to survive and reproduce in arid environments or prolonged periods of drought.

The degree of moisture affects the distribution and abundance of lichens in different ecosystems. Lichens are more common in areas with moderate or high humidity, such as wetlands in forests, swamps or regions with humid climates. On the other hand, in arid or semi-arid areas, lichens may be less widespread or have less development.

Another important aspect of humidity is that lichens can be sensitive to air pollution. In humid environments, lichens can more easily retain and accumulate toxic substances from the air and suffer from the effects of pollution. Therefore, the absence of lichens or their low presence in certain regions can be an indicator of air quality. In general, lichens have a wide range of adaptability to different humidity conditions, and this is just one of many variables that can influence their distribution and development. Other important factors include light levels, the type of substrate they grow on, temperature, pollutants and the presence of other organisms in the ecosystem.

In the September–November period, the degree of humidity increases due to more abundant rainfall. At an interval of one month, the samples were collected after the exposure period in plastic bags, brought to the laboratory, dried and homogenized. They were subjected to chemical analysis.

During chemical analysis, the heavy metal microelements were analyzed: Cd, Hg and Pb. Bioindicator samples:

- They were subjected to drying at ambient temperature.
- Foreign biological debris (tree bark) was removed.
- They were dried in an oven at approx. 100 °C.
- The determination of the concentration of heavy metals was carried out using an atomic absorption spectrophotometer with a GBC Avanta PM type graphite furnace, with a spectral source, and lamps with a cavitary cathode, manufacturer: GBC Scientific Equipment PTY. Ltd.—Keysborough, Australia; Model: Avanta ULTRA Z 933 PLUS, year of production: 2003 a method which has a much higher sensitivity than flame absorption spectrophotometry.

Following the analysis, the presence of constant sources of pollution with heavy metals was found, but it was within acceptable limits. The high bioaccumulation capacity of lichens shows in all five locations a very small difference compared to the values indicated by the monitoring stations. Heavy metals are considered toxic pollutants and can be dangerous to human health and the environment.

3. Results

Processing and interpretation of the results: Following the analysis, the presence of constant sources of pollution with heavy metals was found, but it was within acceptable limits. The high bioaccumulation capacity of lichens shows in all five locations a very small difference compared to the values indicated by the monitoring stations. Heavy metals are considered toxic pollutants and can be dangerous to human health and the environment. Here are the values obtained following the analysis of heavy metals in the air according to the analyses carried out and determined by bioindicators:

3.1. The Concentration of Lead in the Air (Pb)

By comparing the values with those of the Environmental Protection Agency, we recorded differences of 0.003 $\mu g/m^3$ and 0.33 $\mu g/m^3$, respectively. The legal limit for lead in air in the European Union is 0.5 $\mu g/m^3$ as an annual average. The average value of the lead concentration in the TR-01-Alexandria-urban traffic station area was 0.30 $\mu g/m^3$, at the other four stations in the county there were no values above 0.1 $\mu g/m^3$.

3.2. The Concentration of Mercury in the Air (Hg)

The value of mercury concentration in the outside air must not exceed 0.5 $\mu g/m^3$. The average annual, legal concentration of mercury in the European Union, in the ambient air is 5 ng/m^3.

In all the samples we took, the values recorded for mercury were below 1 $\mu g/m^3$. A single value of 0.33 $\mu g/m^3$ was found in the vicinity of the monitoring station TR-10-Turnu Măgurele (industrial area). Comparatively, the values determined by AMP-Teleorman are approximately equal to 0.36 $\mu g/m^3$.

For outdoor air, the recommended imita value for Hg is 1 $\mu g/m^3$. In the European Union, the annual average value as the legal limit for mercury in ambient air is 1 ng/m^3.

3.3. The Concentration of Cadmium in the Air (Cd)

Compared to the value determined by the Environmental Protection Agency of 0.16 $\mu g/m^3$, we recorded a difference of 0.04 $\mu g/m^3$.

In all analyzed stations, the cadmium concentration values were below 0.2 $\mu g/m^3$. Only in the TR-11-Zimnicea urban station area, the average value of the cadmium concentration in the outdoor air was 0.2 $\mu g/m^3$.

Figure 2b show the establishment and marking of the transplant points by means of a 10 cm × 10 cm border at 1.5 m from the ground. In these points, the biomonitor species will be attached with the help of a natural resin.

(**a**) (**b**)

Figure 2. Transplant areas: (**a**) *Parmelia saxatilis* (L.) Ach.—detail of the lichen species chosen for transplantation; (**b**) marking the transplant point.

Figure 3 shows the map of Teleorman County, with the places where the air pollution monitoring stations are located also indicated.

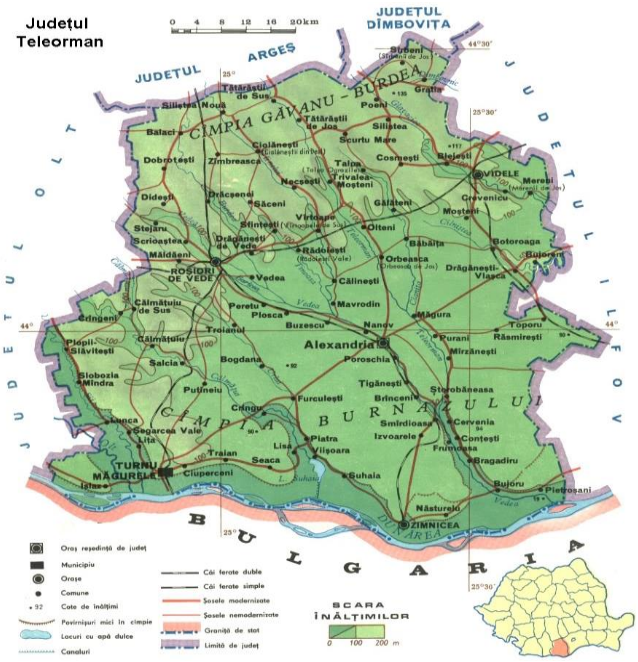

Figure 3. Location of the county on the territory of Romania.

The map in Figure 3 was made using the ArcGis program version 10.3. Figure 4 represents details of the lichen species Parmelia saxatilis (L.) transplanted from a spot of oak bark. Transplantation was performed in all 5 selected biomonitoring points. Lichens have a slightly dehydrated thallus and a weak color.

Figure 4. Lichens transplanted at the September 2021 transplant point.

The figures above show the adhesion of biomonitors and the development of the thallus through toxic tolerance to heavy metals. Their coloring indicates an acceptable limit of heavy metals, also specified in Table 1.

Table 1. Comparative analysis between the values obtained by the biomonitors and conventional measurements [μg/m^3] September 2021–November 2021.

Measurement Points	Cd-Measurement	Cd-Biomonitor	Hg-Measurement	Hg-Biomonitor	Pb-Measurement	Pb-Biomonitor
TR-01	0.15	0.17	0.31	0.31	0.33	0.30
TR-02	0.13	0.14	0.28	0.29	0.14	0.18
TR-09	0.17	0.16	0.27	0.25	0.19	0.21
TR-10	0.11	0.19	0.36	0.33	0.16	0.17
TR-11	0.16	0.20	0.30	0.27	0.21	0.23

The data indicated by biomonitors for heavy metals are extremely close to the data indicated by conventional measurements, with biomonitors indicating values slightly higher than conventional measurements, as seen in Table 1, which leads us to conclude that lichens as biomonitors provide a more accurate assessment than conventional measurements of pollutants.

Figure 5 shows details about the development of transplanted lichens after a period of 3 months in which the development of the thallus, their more intense color and their abundance can be observed due to the humidity of the last month.

Figure 5. Evolution of lichens after 3 months after transplantation.

4. Discussion

The incineration of municipal and industrial waste can release cadmium into the air, as it can be present in various products and materials that are burned. Cadmium dioxide resulting from incineration can be emitted into the atmosphere. Regarding transportation and vehicle emissions, vehicles that use fossil fuels such as gasoline or diesel can release cadmium and other heavy metals into the atmosphere through exhaust emissions. This includes both passenger vehicles and commercial or industrial vehicles. It is important to note that these are just a few common sources of cadmium air pollution, and there are other activities and processes that can contribute to the release of this heavy metal into the environment. Appropriate regulations and practices regarding the management and control of these sources are essential to minimize cadmium air pollution and protect human health and the environment. It is found that the values of cadmium in the atmosphere determined by bioindicators mostly result from transports, even if they do not exceed the allowed limits. The pollutants analyzed in this paper in 2021 were monitored by the air quality-monitoring network in Teleorman County.

5. Conclusions

In this paper, we analyzed the efficiency of lichen use in air monitoring in Teleorman county, Romania. We have identified the major sources of pollution in the study area. We performed conventional direct measurements of heavy metal emissions from the atmosphere, later we transplanted lichens as biomonitors that we observed during 3 months, September–November 2021, and subjected them to analyzes for the determination of heavy metals in the thallus of lichen species near pollution sources. We compared the measurements from several selected urban locations, respectively the industrial area. The concentration differences between conventional measurements and those indicated by lichens are very small, highlighting the fact that lichens represent an effective bioindicator through the accuracy of the data, providing information about the degree of pollution and the distribution of pollutants in the region. This finding is made by cross-validation comparing the results obtained from both methods. If invasive measurements and lichen analysis coherently indicate the levels of pollutants in the area, this reflects the effectiveness of lichens as biomonitors and increases confidence in the results. Comparing the results is part of a holistic interpretation of air quality. These data provide information about the health of the ecosystem, the impact on human health and offer the authorities and decision-makers support in establishing local environmental policies. Comparing the data obtained by invasive methods with the data from lichen analysis offers a complete and complementary approach for the assessment of air quality and the impact of pollution on the environment.

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