LICHENS TRANSPLANT FROM AN UNPOLLUTED AREA IN TIMISOARA CITY

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Abstract
In this paper, was followed the accumulation of heavy metals (Cd, Ni, Pb, Cu, Mn, Zn and Cr) in the tissue of lichen species, transferred from an unpolluted area to an intersection with medium traffic in the city of Timisoara (Romania). The lichen species studied were *Flavoparmelia caperata* (L.) Hale and *Hypogymnia physodes* (L.) Nyl., harvested from a forest located at 70km from the city. The study also took into account the affinity of these two species for the various heavy metals in the atmosphere. Of the study, results that lichen species had accumulated metals in the range 2.16-172%, from the initial moment. Some metals have been removed from lichenic tissue by up to 30% from baseline.

Introduction
Biomonitoring can be divided into 3 categories: the transplant method, methods of laboratory testing and in situ testing. Under the transplant procedure, appropriate organisms (mainly lichens) are transplanted from unpolluted areas to the site considered polluted. Transplantation techniques are particularly useful at relatively high levels of pollutants. A distinct advantage compared to the use of indigenous species is that of well-defined exposure time. The technique does not seem to be satisfactory for the absorption rate. [1].
The epiphytic lichen, *Flavoparmelia caperata*, has been used as a heavy metal bioaccumulator (Cd, Cr, Cu, Hg, Ni, Pb and Zn) in the surrounding area of Pistoia in the north-central area of Italy. Concentrations of Cd, Cr, Ni, Hg and Pb were compared with those found in areas where there is no atmospheric pollution. Copper and especially zinc have been found in quite high concentrations. Fertilizers and pesticides have been the main source of atmospheric contamination [2].
Guttova [3], conducted a study illustrating the response of epiphytic lichens to changes in atmospheric conditions in Central Europe, where air pollutant emissions have dropped significantly since 1990 in and around Bratislava. The variation of 7 metals (Cu, Cd, Cr, Mn, Ni, Pb and Zn) was assessed in the talls of *Evernia prunastri, Hypogymnia physodes* and *Parmelia sulcata*.
Another study on the accumulation of metals by lichens was carried out by Affum [4], and it collected lichens from an unpolluted forest in November 2004, which were transplanted into 41 sites along the road Madina-Tetteh Quarshie. In 2005, February, samples were analyzed and found to contain higher concentrations of Mn, V, Pb, Cd, Cr and Ni than the initial samples. Observations showed that in all the samples studied, the Mn concentration was highest with maximum values around traffic lights, an intersection and a car workshop.
Transplants of epiphytic lichens from the natural environment to polluted sites are commonly used in bioindication studies. Individuals are transferred into new sites together with substrata on which they previously grew or attached on the new substrate, with carefully. [5].

An advantage of using lichens is that they continue to accumulate metals throughout the year [6]. Starting from the above, we realised a study on the accumulation of heavy metals in the tissue of the species *Flavoparmelia caperata* and *Hypogymnia physodes.*

Experimental

The species *Flavoparmelia caperata* and *Hypogymnia physodes*, taken from an unpolluted forest located at 70 km from Timisoara, have been transplanted into an intersection with medium traffic. Heavy metal determinations were made both at the sampling time and after a month in which samples stood around this intersection. The lichens were collected from the oak (*Quercus robur*), all with substrate, from a height of 1-1.5m and transferred to the city, being watered once a few days with tap water.

After a month, the lichens were detached from the substrate and washed well with distilled water and dried. The dry tissue was weighed 0,25-0,50 g and were calcined at 550 °C. The ash was digested with a mixture of HCl+HNO_3 3:1 (aqua regia). The residue was taken with 3ml HCl 1:1. The crucible was washed 3 times with 3ml of HCl 1:1. Solutions to take over the residue and solutions for washing were filtered through paper Sartorius filter papers 2-206 FT and quantitative moved in flasks of 25 ml. They were filled to sign with the HCl 1:1. Determination of metals was carried out with a spectrometer type Avanta AAS.

Results and discussion

In Table 1, the concentration of heavy metals in lichenic tissue is presented. It should be mentioned that for Cd and Cr there was no variation of the concentration in the conditions in which the experiments were performed for none of the lichen species being taken into work.

The initial concentration in Table 1 represents the concentration determined on 02.08.2017, and the final one is the concentration determined after a month, the period during which the lichens were placed in an intersection with medium traffic.

The data in Table 1 reveals an increase in concentration for Cu, Mn and Zn in the case of *Flavoparmelia caperata*. For *Hypogymnia physodes*, the increase in concentration was recorded for Cu, Mn, Zn and Ni.

However, due to their large surface area, relatively low growth rate, and lack of waxy cuticle and stomata, lichens can also absorb and accumulate inorganic and organic contaminants such as heavy elements directly from the air. Moreover, several authors have shown that a direct relationship exists between heavy element concentrations in thalli and those in the environment [7].

Table 1. Concentration of heavy metals in species *Flavoparmelia caperata* and *Hypogymnia physodes*

Species	Concentration of heavy metals [mg/kg D.M.]									
	Cu		***Mn***		***Zn***		***Ni***		***Pb***	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Flavoparmelia caperata	3,55	5,98	97,9	100	47,9	130	1,54	1,22	8,23	7,61
Hypogymnia physodes	5,23	7,14	269	542	76,7	132	2,46	3,33	4,92	3,45

In table 2 it is observed that within 30 days, heavy metals accrued in the range of 2,16-172%, with the exception that *Flavoparmelia caperata* eliminated Ni in a proportion of 21% and Pb

in a proportion of 7,5%, and the *Hypogymnia physodes* eliminated Pb in a proportion of 30% from baseline.

From table 2 shows that *Flavoparmelia caperata* has an affinity to accumulate Cu and Zn by up to 50% higher than *Hypogymnia physodes*, and the latter has a much higher affinity to accumulate Mn and Ni with up to 100% of *F. caperata.*

As Backor and Loppi [8] said, the ideal exposure time for accumulation of metals is 1-3 months. This study has shown that in a short period (one month), lichens have the ability to accumulate some heavy metals. After longer exposures, they become saturated with elements, lose biomass, and change their surface structures and physiological performance [8].

Table 2. Increase / decrease of heavy metal quantities, in percent (%), after a month in an intersection with medium traffic

Species	Cu	Mn	Zn	Ni	Pb
Flavoparmelia caperata	68,5%	2,16%	172%	-21%	-7,5%
Hypogymnia physodes	36,5%	102%	72%	35,5%	-30%

Conclusion

This study shows that for more efficient monitoring of air pollution with heavy metals, several species of lichens must be used, because each species has a higher or lower affinity for some metals. As can be seen above, in order to monitor the pollution with Cu, Mn, Zn and Ni, *Flavoparmelia caperata* and *Hypogymnia physodes* can be used.

After a month, this lichens have the ability to accumulate heavy metals in rather high proportions. The ideal exposure time is 1-3 months.

The loss of metals in the lichens may be due either to the insufficient exposure time or to the fact that the atmosphere in which they were kept for one month does not present such heavy metals and in conclusion they have been eliminated, because it is known that there is a direct relationship between the concentration of heavy metals in the lichen thalli and the concentration in the air.

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