Cd ACCUMULATION IN *PARMELIA SPP.* SPECIES

Masu Smaranda[1], Amalia Raluca Dumbrava[2]

[1]National R & D Institute for Industrial Ecology, ECOIND,
Branch of Timisoara, 300004, 1 Regina Maria Square, Timisoara, Romania
[2]Administration of Natural Park Portile de Fier, 90 Banat street, Orsova, Romania
e-mail: andamasu@yahoo.com

Abstract
Lichens are a group of organisms with a large geographical spread. They live permanently with their surrounding environment. They show a great sensitivity to the composition and concentrations of the substances found in the environment.The concentration of metals in the environment can dramatically affect the lichens' health. The aim of this study was to determine the degree of bioaccumulation of Cd in the tissue of the *Parmelia spp.* species of lichens from synthetic aqueous environments with different concentrations of Cd between 20-50 $mg \cdot L^{-1}$.The study was conducted in the laboratory on samples of lichen the acacia support. The lichens originatefrom a Natural Reservation in the South-West of Romania. The bio-accumulation of Cd was tracked over a period of 16 weeks of exposing the lichens to pollution with synthetic solutionsat pH =3.5, simulating acid rain. The lichens have bio-accumulated amounts of Cd between 4,0-4.9$mg \cdot kg^{-1}$D.M. The studies on the lichens metal bioavailability could further enhance their utility as environmental bio-prospections and for air quality biomonitoring tools.

Introduction
In 1867 Schwendener announced to the scientific world his hypothesis that lichen was formed by two separate organisms, a fungus and an algae. It was later reported that lichens are symbiotic association between the algae *Cianobacteriae* or *Chlorophyceae* and *Ascomiycetes* fungus, or in rare occasions with *Basidiomycetes or Pycomycetes* fungus.Algae provides nutrients and the fungus provide water and minerals. Lichens are spread out all over the globe: trees, bushes, rocks, walls, rots, etc. Lichens were designated as species with high potential for environmental monitoring characteristics modifications 1-4]. The basis of this consideration are the following aspects: long life, slow growth and ability to accumulate certain pollutants in ambient air, in wet and dry deposition [5]. Lichens differ in their sensitivity to various substances in the environment. Was noted that lichens are relatively resistant to metal pollution [6].Lichens in areas with low rainfall, which obtain most of their water from fog and dew, are particularly vulnerable to air quality and weather pattern changes[7].Fog, which often increases with increasing elevation, contains higher levels of dissolved ions, including H^+, than precipitation rain or snow. Although the effects of occult deposition from fog on lichen communities have not yet been critically examined, acid fog has been implicated in changes to cryptogamic plants [8].According to recent studies, lichens are the best indicators for low and moderate bioaccumulation of metals in industrial and urban areas. Metal content in lichens were correlated with the level of annual air pollution.Considering the previous reports in the literature [1, 6-7], the current study evaluates the bioaccumulation of cadmiumin a artificial polluted environment.Cd is a component of fly-ash and presumably travels long distances in the atmosphere; its presence can be correlated with power plants, industrial centers, etc. [7].Higher metal concentrations were recorded in the higher rainfall autumn season compared with spring for most metals. An inter-species comparison of several hundred measurements confirmed the

higher affinity of *Parmelia spp*. for Cd.In order to improve data quality inbio-prospectionsandbiomonitoring environmental, it is suggested to analyses lichens tissue and the introduction species like *Parmelia spp.* in monitoring the presence or absence of Cd in air [9-10].

Experimental

Experimental study aim to evaluate the ability of lichens to incorporate metals in case of exposure to acidic synthetic solutions. The studied lichens were *Parmelia spp.*Lichens have been taken with acacia wood support from a Natural Reservation in the South-West of Romania. Lichens were placed in three beakers and have been sprayed periodically with nutrient solutions and polluting synthetic solutions. Nutritional solutions contain sodium acetate, urea and dipotassium hydrogen phosphate. Polluting synthetic solutions having pH=3.5 contain cadmium in concentrations of 2.0-5.0mg $\cdot L^{-1}$. The experiment lasted 16 weeks. The structure of the experiment is shown in Table 1.

Table 1 Polluting solution characteristics and the experiment structure

Polluting synthetic solution characteristics	Experiment's variants		
	P 1	P 2	P 3
Type	s 1	s 2	s 3
pH simulating acid rain	3.5	3.5	3.5
Cd content [mg·L^{-1}]	2.0	4.0	5.0

The metal content of lichens samples was analyzed before and after experiment. Lichens were separated by wooden cuticle and were washed twice with double distilled water. Then, the lichens were dried for 3 days at room temperature. It was weighed an amount of 1.2 g lichens and was kept at a constant temperature (60°C) to constant weight (3-4 hours).Then the tissue was hand-milled to homogenize. It was weighed amount 0.25-0.50g of powder, then acclaimed at 550°C for 90 min. The resulting ash was dissolved on the sand bath using a mixture of 5:1 hydrochloric acid (37% Merck, Germany) and HNO_3 (69% Merck Germany) The solid residue was taken up in 3ml HCl 1: 1 and filtered through Sartorius filter papers 2-206 FT.The crucible was washed 3 times with 3 ml of HCl 1:1. The washing solutions were filtered. All the resulting solutions were transferred into flasks of 25 ml and bring to volume with HCl 1: 1. Metals analyses were performed with an AAS spectrometer type Avanta.The certified reference material BCR 482 lichen (IRMM) Geel Belgium was used to validate the analytical determinations.Determination of metals in wood support of lichens was similarly analyzed.

Results and discussion

Table 2 presents the initially amount of metals in the studied lichens.Initially lichens showed no detectable Cd and Cr in tissues. Nickel may occur in some tissue samples, such as in sample P 1. Initially Fe content in lichen samples was included in the range 1925.4-3845.0mg·kg^{-1} D.M. The amount of Pb was in the range 30.6-45.2 mg·kg^{-1} D.M.The amount of Zn was 7.5-31.1 mg·kg^{-1} D.M., of the copper and of 7.6-12.7 mg·kg^{-1} D.M.It is observed from the Table 2 that the quantities of metals in analyzed lichens, determined before starting the experiment, are correlated with the values reported by The certified reference material BCR 482 lichen[11].

Table 2 The initially amount of metals in the lichens, *Parmelia spp.* [mg·kg^{-1} D.M.]

No	Experiment's variants	**Metals [mg·kg^{-1} D.M.]						
		Cd	Cr	Cu	Fe	Ni	Pb	Zn
1	P 1	*	*	117 ±2.0	3845.0 ±43.5	7,8 ±2.3	30,6 ±3.1	16,7 ±2.7
2	P 2	*	*	7,6 ±1.7	1925,4 ±33.8	*	37,7 ±4.6	22,7 ±3.7
3	P 3	*	*	12,7 ±1.9	2864,0 ±42.8	*	45,2 ±4.6	31,1 ±3.2
4	The certified reference material BCR 482 lichen [11]	0.56	4.12	7.03	-	2.47	40.9	100.6

*Below detection limit ,** 3 replicates for each metal anal;yzed

In Table 3 is indicates the concentrations of metals initially determined on woody acacia support for *Parmelia spp.* The acacia support does not contain Cd and Ni ions, but contain Cr which was not adsorbed into the studied lichens. In the case of other metals, respectively Cu, Fe, Pb and Zn, the found amounts on acacia support are correlated with initial quantities of these metals in the tissue of lichens.

Table 3 Initially range of metals ccontent on woody acacia support for *Parmelia spp.*

No.	Experiment's variants	Metals content min.-max. [mg·kg^{-1} D.M.]							
		Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
1	acacia wood support	*	5.9-9.5	5.1-9.3	863.5-1775	22.3-29.0	*	22.9-55.7	9.8-18,6

*Below detection limit

In Table 4 are presented the metal concentrations determined in the tissue *of lichens Parmelia spp*. after 16 weeks exposure in the laboratory to artificial pollution with a single pollutant - cadmium.Initially lichen samples do not contain Cd. This metal was bio-accumulated in time the tissue of lichens.

Table 4 *Parmelia spp.*metal content after 16 weeks exposure in the laboratory to artificial pollution with a single pollutant - cadmium

No.	Experiment's variants	**Metals content [mg·kg^{-1} D.M.]					
		Cd	Cr	Cu	Fe	Pb	Zn
1	P 1	4.37 ±0.3	*	12.67 ±2.4	2698 ±46.4	30.6 ±6.2	22.1 ±4.3
2	P 2	4.90 ±0.4	*	9.74 ±1.9	2750 ±34.7	37.7 ±28	20.3 ±5.1
3	P 3	4.0 ±0.2	*	14.5 ±4.1	3021 ±52.6	45.2 ±5.7	37.3 ±3.9

*Below detection limit, **3 replicates for each metal analyzed.

Several hundred measurements confirmed the affinity of *Parmelia spp.* for higher Cd content [9-10]. The amount of Cd bio-accumulate in the tissue of lichens was between 4.0-4.9mg·kg^{-1} D.M.In 16 weeks of exposure to laboratory environmental pollution using aqueous solutions with different concentrations of Cdand pH=3.5 simulating acid rain, bioaccumulation of Cd levels reached a plateau at 4.45 ± 0,45mg·kg^{-1} D.M. The cadmiumbioaccumulation in lichen tissues pollution exposed has reached a limit value, relatively constant. In Table 5 it is shown

comparatively the appearance of lichens *Parmelia spp*. after 16 weeks of exposure to laboratory pollution with synthetic solutions having a single pollutant - Cd.It can be observed that after 16 weeks exposure to pollution with synthetic solution of Cd at concentrations of 2-4$mg \cdot L^{-1}$ the color of lichens was changed from greenish-gray, to greenish with brown spots (see table 5 samples P1 and P2). Exposure to environments more polluted with cadmium produced dramatic alteration of lichens with their surface restriction and from greenish-gray at brown color modification (see table 5 sample P 3).

Table 5 Lichens *Parmelia spp.* appearance of the experimental variant after 16 weeks

Experiment's variants	The appearance of the lichens (*Parmelia spp.*)	
	Initial	After 16 weeks.
P1	Greenish-gray	Greenish with brown spots
P 2	Greenish-gray	Greenish with brown spots
P 3	Greenish-gray	Brown and surface restriction

Conclusions

Lichens of the *Parmelia spp.* species exposed 16 weeks to artificial polluting environments with Cd acid aqueous solutions at pH= 3.5, have confirmed the high affinity for Cd. Levels of cadmium bioaccumulation after 16 weeks of exposure, in laboratory experiment, to polluted environments with aqueous solutions with different concentrations of Cd, reached a plateau at 4.45 ± 0.45$mg \cdot kg^{-1}$ D.M.The cadmiumbioaccumulation in lichen tissues pollution exposed has reached a limit value, relatively constant.After 16 weeks of exposure to pollution with synthetic cadmium solution at concentrations of 2-4$mg \cdot L^{-1}$ and pH=3.5 simulating acid rain, the color of lichens was changed from greenish-gray to greenish with brown spots. Exposure to more polluted environments as 5$mgCd \cdot L^{-1}$ had dramatic effects on lichenswith their surface restriction and color modification.The lichens studies of metal bioavailability could further enhance utility as environmental bio-prospections and air quality bio-monitoring tool.

References

[1] M.E.Conti, G.Cecchetti, Environ. Pollut. 14(2001) 471.
[2] H.A. Carreras, E.D.Wannaz, C.A. Perez, M.I. Pigmata, Environ, Res. 97 (2005) 50.
[3] A.M.Rusu, G.C.Jones., P.D.J. Chimodes, O.W. Purvis,Environ. Pollut. 143 (2008) 81.
[4] M.O. Obiakor, C.D. Ezeonyejiaku,Am. J. Life. Sci. Res. 1(2013) 59.
[5] T.H. Nash,Lichen Biology Cambridge University Press, Cambridge UK (1996).
[6] J. Garty, Crit. Rev. Plant Sci.20 (2001) 309.
[7] M.E. Hale, Lichens as bioindicators and Monitors of Air Pollution in the Flat Tops Wilderness Area. Colorado. Final Report USFS Contract No. ON RFP R2-81-SP35 (1981).
[8] P.A.Wolseley, P.W. James, Acidification and the Lobarion: a case for biological monitoring. *In*: P. A. Wolselely, and P.W. James. (eds.). The Effects of Acidification on Lichens 1986-1990. CSD Report 1247. The Nature Conservancy Council, Peterborough, UK.(1992).
[9] R. Bargagli, F.Monaci, F Borghini, F. Bravi,C. Agnorelli, Environ Pollut. 116 (2002) 279.
[10] P.L. Nimis, S. Andreussi,E.Pittao, Sci. Tot. Environ. 275(2001) 43.
[11] EC-IRMM, (Revised: 2007), The certified reference material BCR 482 lichen, European Comission- Joint Research Centre,Institute for Reference Materials and Measurements,Brussels, Belgium (1995).