EPIPHYTIC LICHENS AS QUANTITATIVE BIOMONITORS FOR ATMOSPHERIC ELEMENT DEPOSITION

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Abstract

Epiphytic lichens are being used as passive and active biomonitors of trace elements in Slovenia. The lichen *Hypogymnia physodes* (L.) Nyl. was exposed at three locations (two in the vicinity of a coal fired-power plant, and one at a reference location) for 8 months. At the same locations air particulate matter and total deposition were collected on a monthly basis. The k_0-method of neutron activation analysis, using the TRIGA Mark II reactor at the "Jozef Stefan" Institute, was employed for multielemental nondestructive analysis of all samples. The influence of the power plant on the concentration levels of some elements in the transplanted lichens, air particulates and total deposition is discussed and their correlation presented.

1. INTRODUCTION

Growing awareness of the consequences of air pollution on the environment has led to an increased interest in epiphytic lichens, which are regarded as suitable tools for monitoring levels of atmospheric pollution [1-3]. Unlike higher plants, they have neither roots, a waxy cuticle nor stomata, hence for mineral nutrition they are largely dependent on wet or dry deposition from the atmosphere. The concentration levels in lichens are usually higher than levels in precipitation or in air filters, sampling is easier and no expensive equipment is needed. Although some literature exists about quantitative relationships between elemental levels in biomonitors and those measured in atmospheric deposition [4-7], these relationships are not yet known in detail. The aim of the present study was to obtain insight into the response of lichens exposed in the vicinity of a thermal power plant to trace element air pollution by comparing the levels in lichens with atmospheric levels as measured by air particulates (fine and coarse) and total deposition.

2. EXPERIMENTAL

2.1.Sampling and sample preparation

The experiment was performed from September 1992 to May 1993 at three sampling locations; 2 locations were selected within a 10 km radius around the coal fired thermal plant (TPP) at Šoštanj and one in a rural area at Podvolovljek (35 km SW of the TPP). At each sampling location three types of samples were collected, namely air particulate matter, total deposition and epiphytic lichens.

Branches of apple trees covered with *Hypogymnia physodes* were collected at a remote location, about 20 km NE of the TPP and the same day exposed to the atmosphere at each location for 3, 6 and 8 months. They were tied with nylon thread onto a wooden holder at a height of 1.5-2 m above the ground. Some lichen material was transported to the laboratory to determine the initial concentration of elements. It was planned that the exposure period would be one year; however due to visible damage to the transplanted lichens, especially at Veliki vrh which is only 2.5 km distant from the power plant, the experiment was terminated after 8 months. In the laboratory the lichen samples were moistened with distilled water and carefully separated from the bark substratum using nylon tweezers and lyophilized.

TABLE I. TRACE ELEMENT CONCENTRATIONS (μg g^{-1} DRY WEIGHT) IN TRANSPLANTED *H. PHYSODES* EXPOSED FOR 3. 6 AND 8 MONTHS (3M. 6M. 8M) AND 3. 6 AND 8 MONTH AVERAGES IN TOTAL AIR PARTICULATES (COARSE + FINE FRACTION).

LICHENS (μg/g dry weight)

El.	Initial value		Podvolovljek			Veliki vrh			Zavodnje		
	0 M	std	3M	6M	8M	3M	6M	8M	3M	6M	8M
As	0.72	0.07	0.88	1.00	1.08	1.21	2.01	1.70	1.01	1.32	1.15
Fe	835	115	1038	1207	1453	2223	2429	3531	1169	1443	1477
K	4187	1145	4452	3482	3334	3619	1840	1229	4146	3630	2081
Mo	0.51	0.27	0.56	0.58	0.66	0.99	1.42	1.55	0.71	0.50	0.90
Hg	0.05	0.01	0.06	0.07	0.09	0.08	0.16	0.14	0.06	0.08	0.09
Sb	0.20	0.02	0.29	0.23	0.31	0.29	0.46	0.58	0.22	0.27	0.30
Sc	0.25	0.04	0.30	0.35	0.42	0.39	0.54	0.67	0.35	0.42	0.42
Th	0.20	0.04	0.24	0.30	0.30	0.34	0.43	0.51	0.28	0.36	0.33
U	0.09	0.01	0.10	0.12	0.13	0.24	0.40	0.35	0.14	0.17	0.19
W	0.12	0.01	0.75	0.77	0.76	0.18	0.30	0.38	0.18	0.23	0.28
Zn	59.3	6.0	78.0	71.3	70.0	68.1	75.9	52.0	67.6	74.4	66.9
					AIR PARTICULATES (ng m^{-3})						
As			0.57	0.93	0.97	0.88	1.38	1.41	0.72	0.89	0.89
Fe			56.2	95.4	84.2	174.3	163.0	158.6	206.2	139.7	126.8
K			104	137	131	217	200	191	158	131	127
Mo			0.26	0.27	0.28	0.44	0.79	0.70	0.32	0.29	0.28
Hg			0.02	0.02	0.02	0.02	0.03	0.03	0.04	0.03	0.03
Sb			0.30	0.37	0.39	0.76	1.04	1.00	0.60	0.57	0.54
Sc			0.01	0.02	0.02	0.04	0.03	0.03	0.05	0.04	0.03
Th			0.01	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02
U			0.01	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02
W			0.16	0.13	0.14	0.25	0.20	0.19	0.39	0.25	0.21
Zn			27.7	26.6	26.5	117.5	75.5	65.3	65.5	42.9	38.8

The samples were not washed, as our aim was to measure the elements that were physically trapped on the surface of the thallus as well as chemically bound to the cell wall. The samples were made brittle by immersion in liquid nitrogen and were then crushed and ground in a zirconium mortar with Zr ball in a Fritch vibration micro-pulverizer. About 100-200 mg of dry lichen powder was then used to make tablets for neutron activation analysis.

Air particulate matter (APM) was collected at each station using an in-house constructed single jet facility, with a cut-off point of ~2.5 μm [8]. The fraction ~2.5 μm to 15 μm was collected on a Nuclepore polycarbonate membrane filter, 37 mm diameter, pore size 0.45 μm. The particulate fraction finer than ~2.5 μm was collected on a Nuclepore polycarbonate membrane filter, 47 mm diameter and the same pore size. Both filters were held in the original Nuclepore holders. The arithmetic median value of the flow rate was about 400 L h^{-1}. The flow rate was measured by a rotameter at the beginning of sampling, checked every second day and finally at the end of the sampling period. Sampling times varied, depending on the minimal flow rate necessary for successful operation of the separator and the content of air particulates, being from 2 to 7 days. Filters were weighed before and after exposure and kept in petri dishes. The APM loaded filters collected during each month were pressed in a pellet die of 5 mm using a manual press, for neutron activation analysis.

Total deposition was collected monthly in a 5 L high density polyethylene sampler with a funnel of 20 cm diameter and vertical collar of 10 cm. The funnel-bottle system was mounted inside a stainless steel cylinder placed on a concrete base plate, keeping the upper edge of the funnel 1.5 m above ground level. The opening of the funnel was protected by a coarse filter mesh made of polyethylene and a nylon net (pore size of 250 μm) in order to prevent inlet of coarse particles and insects. Before each sampling

TABLE II. TOTAL EXPLAINED VARIANCE (R^2) AND ITS SIGNIFICANCE (P VALUE) USING A MULTIPLE LINEAR REGRESSION MODEL TO DESCRIBE THE RELATIONSHIP BETWEEN ELEMENT CONCENTRATIONS IN LICHENS AND 3 INDEPENDENT VARIABLES: AVERAGE TOTAL AIR PARTICULATE MATTER (NG M^{-3}), TOTAL DEPOSITION AND TOTAL AMOUNT OF PRECIPITATION DURING THE EXPOSURE PERIOD. ($P \leq 0.001$ HIGHLY STATISTICALLY SIGNIFICANT ; $P \leq 0.01$, $P \leq 0.05$ STAT.SIGNIFICANT; $P > 0.05$ NOT STAT. SIGNIFICANT)

Element	R^2	P value
As	88.1	0.0095
- APM (ng m^{-3})		0.002
- deposition (μg m^{-2})		0.91
- precipitation (mm m^{-2})		0.21
Mo	88.7	0.0084
- APM (ng m^{-3})		0.003
- deposition (μg m^{-2})		0.529
- precipitation (mm m^{-2})		0.599
Hg	84.3	0.0187
- APM (ng m^{-3})		0.04
- deposition (μg m^{-2})		0.0036
- precipitation (mm m^{-2})		0.017
Sb	91.7	0.0039
- APM (ng m^{-3})		0.0009
- deposition (μg m^{-2})		0.465
- precipitation (mm m^{-2})		0.013
Se	74.2	0.06
- APM (ng m^{-3})		0.073
- deposition (μg m^{-2})		0.648
- precipitation (mm m^{-2})		0.881
W	94.8	0.0012
- APM (ng m^{-3})		0.809
- deposition (μg m^{-2})		0.03
- precipitation (mm m^{-2})		0.0038

period, 10 ml conc. suprapur HNO_3 was added to the bottle to preserve the precipitation collected. In the laboratory the samples were filtered through Nuclepore polycarbonate 0.45 μm membrane filters, and the water and filtrate analysed separately.

2.2. Sample analysis

The k_0-standardisation method was used for analysis of all samples. All irradiations were performed in the carousel facility of the TRIGA Mark II reactor of the Jozef Stefan Institute (IJS) at a thermal fluence rate of 1.1×10^{16} $m^{-2}s^{-1}$. The irradiation time for each sample was 18 hours. All samples were irradiated together with an Al-0.1 % Au alloy disc and Zr foil, serving as comparator and fluence rate monitor, respectively. After irradiation samples were transferred to clean 5 ml polypropylene mini scintillation vials for measurement. The radionuclides used in the determination of 50 elements in each sample , their half lives and gamma energies measured are given elsewhere [9]. The samples were measured on an absolutely calibrated Ortec HP Ge detectors, connected to a Canberra Series 90 multichannel analyser. Each sample was measured twice; for 1 hour after 2 days of cooling time and for 20 hours 8 days after the end of irradiation. The details of the measurement procedures and quality assessment of the method applied are given elsewhere [9-10].

3. RESULTS AND DISCUSSION

More than 40 elements were determined in each sample but only the most indicative results for those elements in transplanted lichens which showed a significant increase or decrease (twice the standard

deviation of the initial value) after 3 months of exposure are presented in Table I. The results are not corrected for the initial concentration, which is presented as the mean value of three subsamples collected from the reference location, and its standard deviation, in the first column of Table I. In the second part of Table I the results for mean total air particulate matter for the exposure period of the lichens are presented. The mean values were calculated from the monthly results for coarse and fine fractions. The results for the levels of trace elements in precipitation were presented elsewhere [11].

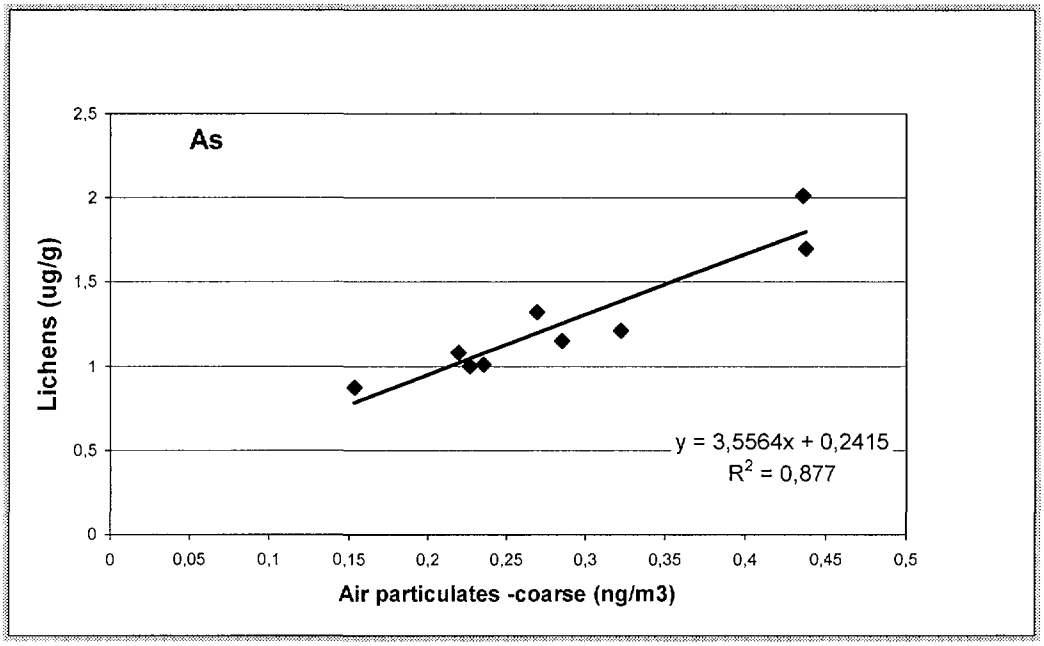
As shown in Table I, except for K, the elemental levels in lichens at all locations slightly increase with increasing exposure time. As expected the highest elemental levels were found at Veliki vrh, the sampling location only 2.5 km distant from the TPP. The elements that increase most significantly are

TABLE III. CONCENTRATIONS OF ELEMENTS (μg/g) IN BAUXITE, RED MUD AND SOIL SAMPLES

	Bauxite	**Red mud**	**soil (site 7)**
Al	134000	9010	5250
As	74.7	127	9.5
Au	<0.06	<0.04	<0.016
Br	2.4	7.2	11.1
Ca	21700	<2000	-
Ce	403	662	59.1
Cl	<50	<50	<100
Co	45.4	67.8	13.5
Cr	409	715	740
Cs	3.5	6.3	2.6
Dy	23.2	0.15	3.35
Eu	2.3	0.057	0.68
Fe	149000	258000	27700
Ga	59.0	87	8.52
Hf	17.2	88.1	5.45
K	1230	304	9560
La	134	227	25
Mg	<2000	<1400	1100
Mn	1170	59	33
Na	430	4240	5380
Nd	115.5	49.9	12.1
Rb	27.3	41.5	47
Sb	4.9	9.1	1.23
Sc	70.1	121	8.7
Se	0.31	<1.3	15.8
Sm	2.04	-	4.14
Tb	2.8	4.62	2.38
Th	57.1	104	12.3
Ti	6570	348	256
U	<4	-	<4
V	123	<18	9.2
W	7.3	12.7	1.4
Zn	370	556	161

TABLE IV. CONCENTRATIONS OF ELEMENTS (μg/g) IN THREE DIFFERENT BIOINDICATORS FROM THE SAME SITE

	Lichen	**Grass**	**Pine needles**
Al	11000	253	506
As	2.25	<1.0	<0.8
Au	<0.005	0.013	<0.003
Br	34	2.8	1.55
Ca	22600	14300	26000
Ce	11.2	<1.5	0.45
Cl	415	4720	200
Co	2.43	0.13	0.16
Cr	32	2.2	2.6
Cs	0.85	<0.4	<0.5
Dy	0.62	<0.18	<0.01
Eu	0.165	<0.09	<0.10
Fe	6200	229	410
Ga	3.9	<0.4	0.27
Hf	0.62	<0.05	0.04
K	5000	23000	3100
La	5.6	<0.7	0.25
Mg	2000	1800	920
Mn	77	28	15.6
Na	585	2100	91
Nd	4.3	<1.1	<0.75
Rb	<15	18.9	<12
Sb	0.55	<0.035	0.06
Sc	2.05	0.06	0.09
Se	<2.0	<0.5	<1
Sm	0.68	<0.08	0.04
Tb	<0.15	<0.15	<0.1
Th	1.33	0.07	0.07
Ti	530	<120	<45
U	<0.7	<0.8	<1
V	20.5	<1	0.95
W	<0.4	<0.2	<0.2
Zn	76	43	15.6



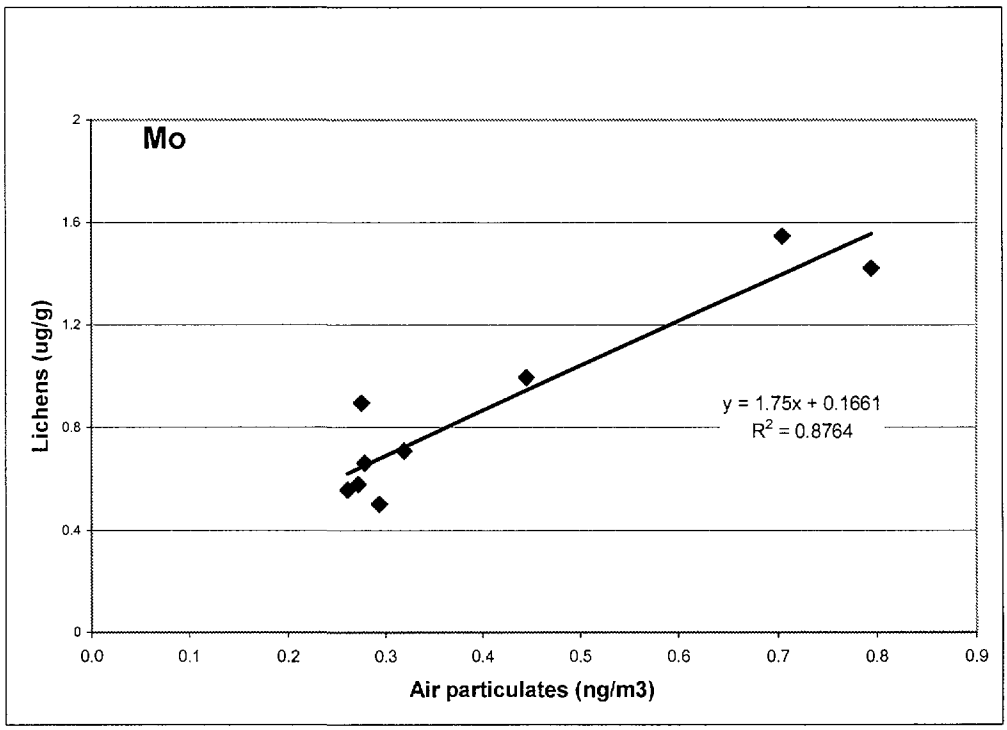


Fig.1. Plots of As and Mo concentrations in lichens exposed for 3, 6 and 8 months and mean air particulate matter (ng m^{-3} per exposure period, coarse fraction for As and total (fine + coarse) for Mo).

U, Fe, Sb, Mo, Hg and As. These elements are typical constituents of coal and are released into the atmosphere by coal combustion. However, at the reference location of Podvolovljek an extremely high value of W was obtained in all 3 exposure periods. As can be seen from the results, the concentrations of K decreased from 4187 μg g^{-1} at the reference location to 1229 μg g^{-1} after 8 months of exposure at Veliki vrh, most probably due to damage to the cell membranes and thus leaching of intracellular

K ions. In April 1993 there were elevated emissions of SO_2 from the TPP caused by full operation of all 5 units due to demand for electricity. Since the exposed lichens became completely white at the Veliki vrh sampling location but were slightly less injured at Zavodnje, it can be concluded that even for one of the most tolerant epiphytic lichens (*Hypogymnia physodes*) the levels of SO_2 in the gaseous emissions were too high.

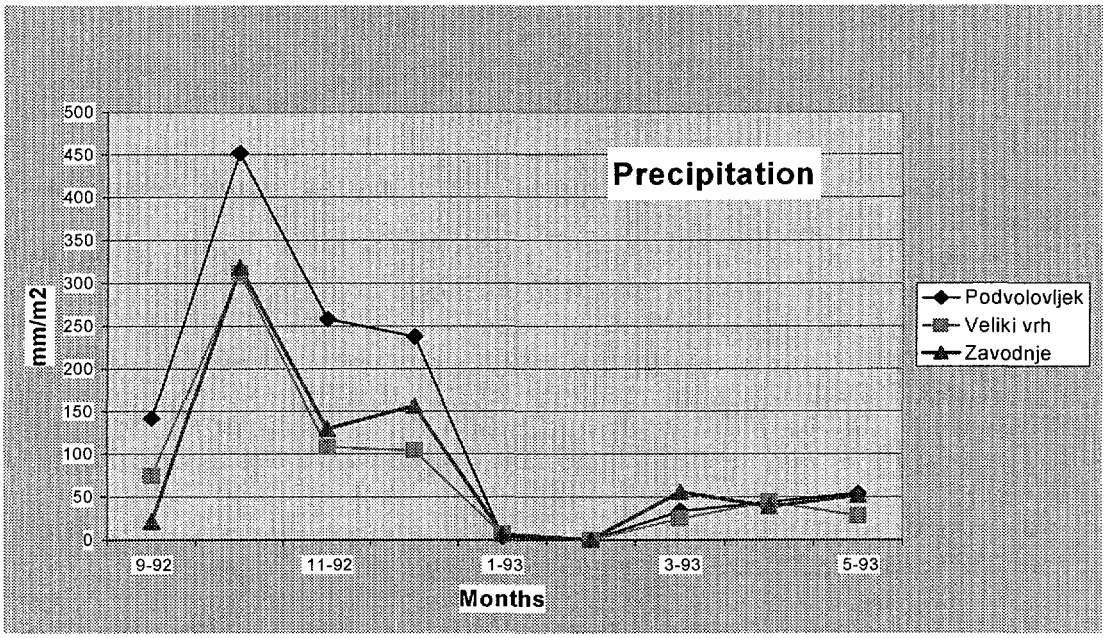


Fig.2. The amount of precipitation (mm/m^2) at the sampling stations.

Knowing that trace elements are deposited on the lichen surface either as dry particulates or as material dissolved or suspended in wet precipitation, some initial multiple linear regression analysis was performed to find out which process was the dominant one at this particular environment. Since the experiment was performed only at three locations (two of them very close to the emission source) and because of the limited data set no other statistical analysis was meaningful.

The approach in the present field study was to compare the elemental concentrations in lichens exposed for 3, 6 and 8 months with air particulate matter (mean value per exposure period), total deposition (wet and dry for the exposure period) and the total amount of precipitation. Statgraphics routines [12] were used and a summary of the results are presented inTable II. For As, Mo, Hg, Sb, Se, and W significant relationships between the variables used in the model were found (expressed as P value), and the model explained from 74 (Se) to 95 % (W) of the total variance of elemental concentration in lichens. For Ca, Ce, Cs, Fe, K, La, Na, Rb, Sc, Sm, Th, U, and Zn the total explained variance was less than 65 %. Based on the P value which was the highest for total deposition (except for Hg and W), the initial statistics showed good correlation between the concentrations in air particulates, the amount of precipitation and the concentrations in lichens; for example for Sb, Fe, Sc, and Th these relationships were highly significant ($0.01 > P > 0.0001$). However, for As and Mo it was found that a simple linear model can be applied (Fig. 1) which explained more than 82 % (depending on particle size) of the total variance in the lichen data. For example for As better correlation was obtained between lichens and the coarse fraction of APM, but in the case of Mo with total APM (fine + coarse). Our results are in a way contrary to some literature data where a good correlation between lichen values and bulk precipitation was obtained [4-7]. However, one reason for this discrepancy could be the vicinity of the emission source and entrapment of fine or coarse particles directly onto the lichen surface, and another the meteorological conditions during the experiment (Fig.2). There was heavy rain in October, but the winter period was very dry with low levels of precipitation. The first statement appears to be in agreement with Ross [6] who found no correlation between wet deposition and the

levels for Mn, Cr and Ni in mosses and suggested than some other factors are more important than adsorption of wet deposition. He also concluded that atmospheric wet deposition is of primary importance only at baseline stations, not near local sources.

Our field experiment, which as explained, was performed under the extreme environmental conditions (high SO_2 emission, heavy rain in one month) showed that even in such conditions, good correlation between physical measurements and lichen values exist.

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