ACTIVE BIOMONITORING OF ATMOSPHERIC ELEMENT DEPOSITION USING *EVERNIA MESOMORPHA* IN TANGSHAN, CHINA

LIN, D.[1] – MENG, J. W.[2] – LI, M. N.[1] – WU, Q. F.[1] – WANG, L. P.[2] – LI, X. J.[2] – SONG, J. J.[2] – ZHAO, L. C.[2] – XU, P.[1] – XIA, Y.[1] – LIU, H. J.[1*]

[1]School of Life Sciences, Institute of Life Science and Green Development, Hebei University, Baoding 071002, China
(e-mails: 2754546286@ qq.com – D. Lin; Limeina139@163.com – M. N. Li; wqf45@126.com – Q. F. Wu; 2334970784@qq.com – P. Xu; ayaxiayu@foxmail.com – Y. Xia)

[2]Hebei Key Laboratory of Mineral Resources and Ecological Environment Monitoring, Hebei Research Center for Geoanalysis, Baoding 071051, China
(e-mails: mjw678@sina.com – J. W. Meng; wlp0907@126.com – L. P. Wang; 1056816294@qq.com – X. J. Li; 1733235207@qq.com – J. J. Song; zhao.l.c@126.com – L. C. Zhao)

**Corresponding author*
e-mail: liuhuajie@foxmail.com

(Received 27th Sep 2023; accepted 27th Nov 2023)

Abstract. Tangshan is a typical heavy industrial city in North China. *Evernia mesomorpha* (*EV*) from a remote site was exposed at the non-industrial and industrial sites of Tangshan in the winter heating period of 2016-2017 for 4 months. Concentration of 50 elements in the samples before and after exposure was measured using ICP (Inductively Coupled Plasma). The results show that exposure concentrations of the 49 elements are significantly higher than the background concentrations, coinciding with the fact that the exposure area had higher pollutant contents than the background area. The results show that exposure concentrations are significantly higher than the background concentrations which had a low variability, confirming the applicability of this lichen in active biomonitoring of atmospheric deposition of 49 elements in China. The elements are classified into crustal (Ag, Al, As, Cs, K, P, Rb, Si, Th, U, Sc, and 15 Lanthanides), atmospheric-crustal (Be, Bi, Cd, Co, Cr, Cu, Fe, Ge, Li, Mg, Mn, Mo, Nb, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, and V), and atmospheric groups (S and Zn). PLI_{site} and PLI_{zone} often reveal one class of bioaccumulation higher at industrial sites than at non-industrial sites, regardless of element sources. After taking all groups together, the bioaccumulation is evaluated as heavy and moderate at the industrial and non-industrial sites, respectively. The bioaccumulation is the highest for the atmospheric elements (heavy to severe) and the lowest for the crustal elements (low to moderate). These results indicate that *EV* is a good biomonitor for all elements except P. Air quality of Tangshan in winter heating period is a concern due to industrial, transport and coal combustion emissions.
Keywords: *lichen, heavy metal, element concentration, lichen bioaccumulation, air pollution*

Introduction

Lichens are a symbiont of fungi and algae/cyanobacteria. These organisms grow on trees, rocks, and bare ground in crustaceous, foliose and fruticose forms. Lichens are often used as biomonitors for assessing the level of atmospheric element deposition. This is because they 1) have no real roots and depend on atmospheric deposition for nutrients. 2) have no waxy cortex or stomata and can easily obtain atmospheric elements. and 3) have strong permeability of, high bioaccumulation capacity of, and high toxic tolerance to atmospheric deposits (Garty, 2001). Lichens have become a reliable and efficient tool for the biomonitoring of atmospheric element deposition at a

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

low cost (Abas, 2021). This method can be a good solution to overcome the shortcomings of the traditional monitoring methods, such as high cost, cumbersome operation, high technical requirement, limited number of elements and monitoring sites especially in the remote areas (Abas, 2021).

There are two methods in lichen biomonitoring: passive biomonitoring and active lichen biomonitoring. In the active biomonitoring method, lichens are collected in a background area ("clean" sites or areas with very low pollution) and are exposed to exposure sites. After a period of exposure, the level and spatial/temporal pattern of atmospheric element deposition in the exposure sites are qualitatively/quantitatively studied according to changes in lichen element composition (Brunialti and Frati, 2014; Jia et al., 2020; Zhao et al., 2019; Liu et al., 2016a, b, 2017). The passive biomonitoring mainly collects local lichen samples, which can get the information of total deposition of accumulated atmospheric elements in a certain time. The active biomonitoring method is mostly used in the areas without lichens due to severe air pollution or other factors and *EV* is lack in the exposure area Tangshan, so this method is used. This is especially true in the cities and industrial areas, whereas of this method has been validated at non-industrial and industrial sites (Abas, 2021; Boonpeng et al., 2020).

In recent decades, atmospheric pollution in Eastern China has been quite serious and caused an adverse impact on human health and ecosystem security (Peng et al., 2019). Tangshan is the world's largest city in terms of iron and steel output and has diverse traditional industries, such as electric power, coal, building materials, chemical industry and coking. Over the past decade, the city has kept decreasing the iron and steel output and energy consumption. However, it is still faced with considerable industrial emissions and low air quality, especially in winter heating period (November to March next year). In this period, severe haze events lasting for several days occurred frequently in a large area of North China (Wang et al., 2013). According to the air quality rankings of 74 cities released by the Ministry of Ecology and Environment of the People's Republic of China, Tangshan City came in fourth to last in 2016 and fifth to last in 2017. Therefore, biomonitoring of atmospheric element deposition in winter heating period of Tangshan City is typical for air quality monitoring of heavy industrial cities.

Evernia mesomorpha (EV) is a common corticolous lichen in mountain forests of Northern China (*Fig. 1a)*. In the previous studies, the species was used as a biomonitor for atmospheric element deposition of only 16 elements outside of China (Bennett and Wetmore, 1999). The element concentrations are specific to the element and lichen species (Zhao et al., 2019) and may be related to the geographical area of monitoring. Therefore, it is feasible to conduct monitoring studies for more elements in China. In this study, *EV* was collected in a remote background area (Saihanba Forest Farm, Hebei; *Fig. 1b*) and exposed to industrial and non-industrial sites of Tangshan for 4 months (*Fig. 1c*). Changes in concentration of 50 elements in lichens are studied before and after exposure to verify the following hypotheses. (1) *EV* is suitable as a biomonitor for the 50 elements with exposure concentrations higher than the background concentration. and (2) *EV* has a higher element concentration and PLI at industrial sites than that at non-industrial sites. This is the first active biomonitoring study comparing lichen bioaccumulation levels at industrial and non-industrial sites in the cities of Eastern China and also one of the studies involving the most elements.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

Materials and methods

Study area and lichen

Saihanba Forest Farm, Chengde, Hebei, China (116°53′-117°31′E, 42°22′-42°31′N) is selected as the background area (*Fig. 1b*). It is a remote area, located in the forest-steppe ecotone at the junction between Inner Mongolia Plateau and Greater Khingan-Yinshan Mountains, and belongs to typical semi-arid and semi-humid continental monsoon climate. It has a high forest coverage (up to 80%) with abundant thalli of *EV*. The air pollution is low due to few human activities. During the experiment, the concentration of suspended atmospheric pollutants (SO_2, $PM_{2.5}$ and PM_{10}) in this area was lower than that in Tangshan City (*Fig. 1d*).

The exposure area (118.23425~117.99625°E, 39.53423~39.72491°N; *Fig. 1b, c*) is located in Tangshan City, Hebei, covering an area of 343 km^2. The exposure area consists of different environments, such as farmland, parks, residential areas, commercial areas and industrial sites (*Table 1; Fig. 2c-e*). Most of the industrial sites are distributed in the east and cover several large-sized factories and many scattered small factories. 20 sites are selected in consideration of the spatial distribution of industrial facilities, including 10 industrial sites (with a distance of less than 1 km to industrial facilities) and 10 non-industrial sites (with a distance of more than 2 km to industrial facilities). It is worth mentioning that the city has been intensifying pollution control, emission reduction and overall environmental renovation in an effort to improve the environmental quality as declared by the Municipal Bureau of Ecology and Environment during the experiment. With the above effort, some iron and steel works were shut down or relocated.

Table 1. *Location and type of sample sites in background and exposure areas*

Exposure site No.	Site type	Location	
		Latitude north	Longitude east
Background	Remote; forest	42.43528°	117.15810°
1	Farmland; non-industrial	39.72491°	117.99625°
2	Farmland; non-industrial	39.69036°	118.05951°
3	Farmland; non-industrial	39.65806°	118.04721°
4	Commercial areas; non-industrial	39.67434°	118.16684°
5	Residential areas; non-industrial	39.65059°	118.14167°
6	Parks; non-industrial	39.62154°	118.17544°
7	Commercial areas; non-industrial	39.57700°	118.15194°
8	Commercial areas; non-industrial	39.66686°	118.11128°
9	parks; non-industrial	39.56431°	118.07168°
10	farmland; non-industrial	39.53423°	118.04815°
1	Factory; industrial	39.64245°	118.20338°
2	Factory; industrial	39.69956°	118.20488°
3	Factory; industrial	39.56950°	118.23425°
4	Factory; industrial	39.54981°	118.15585°
5	Factory; industrial	39.71471°	118.09051°
6	Factory; industrial	39.58894°	118.12149°
7	Factory; industrial	39.54389°	118.22259°
8	Factory; industrial	39.69757°	118.13554°
9	Factory; industrial	39.61481°	118.22193°
10	Factory; industrial	39.62849°	118.05354°

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

EV (*Evernia mesomorpha; EV; Fig. 1a*) was selected as a biomonitor for the following reasons. (1) It has been verified as a biomonitor for atmospheric element deposition (Bennett and Wetmore, 1999). (2) It is common and widely distributed in China (Wei, 2020). (3) It is a corticolous fruticose lichen and therefore can be easily and cleanly collected. (4) It has abundant soredia on the surface, making it easy to intercept and retain atmospheric deposits (Di Lella et al., 2003; Wu et al., 2020).

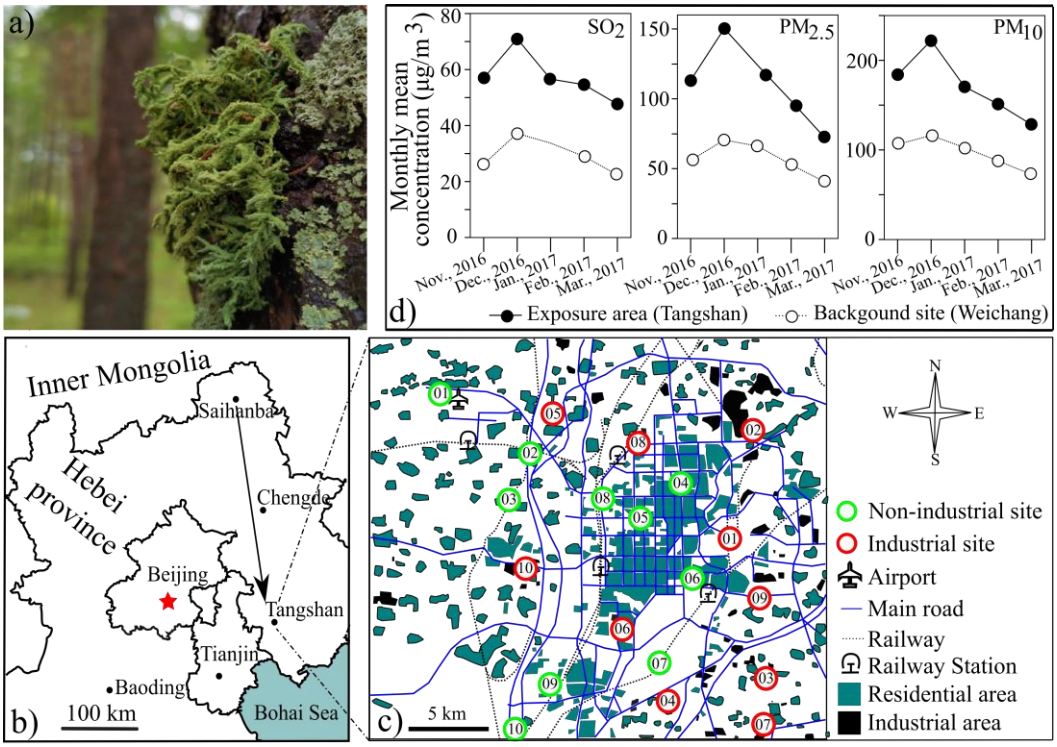

Figure 1. *Evernia mesomorpha (EV), exposure area and monthly mean pollutant concentration of SO_2, $PM_{2.5}$ and PM_{10}. (a) EV. (b) Location of the experimental sites. (c) Exposure sites. (d) Monthly mean concentration of SO_2, $PM_{2.5}$ and PM_{10} in the background (data were obtained from an air quality monitoring station located in Weichang County) and exposure areas (data were obtained from 6 air quality monitoring stations in Tangshan)*

Sample collection, cleaning, exposing and recovery

Sample collection. A line transects with the size of 50 × 1,000 m was set in the larch forest on a south-facing slope at 1,500 m in Saihanba Forest Farm. Slight altitude fluctuations were selected because attitudes would affect element concentration in lichens (Bačkor and Loppi, 2009; Bajpai et al., 2014). *EV* was collected 1 to 2 m (Fernández et al., 2015) away from trunk bases at a site (117°9’29”E, 42°26’7”N) with abundant lichens on the line transect on October 25, 2016 and March 20, 2017. Lichen thalli were cut away together with barks by a knife and put into a kraft bag. Then they were air-dried in a shady and ventilated place. The sampling method has been published through other articles (Zheng et al., 2023).

Cleaning. Lichen thalli were washed twice with deionized water and each washing cycle lasted 5 s in order to wash away all impurities on the lichen surface and reduce the heavy metal concentration in thallus and its inter-sample variation (Boonpeng et al., 2020; Demiray et al., 2012). The samples were put into sealed kraft bags and air-dried

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

in a shady place at room temperature. The sample cleaning method has been published through other articles (Zheng et al., 2023).

Five samples collected in Oct 2016 were selected as a "before exposure" control and sealed into kraft bags for storage till the experiment ended. 60 samples were exposed to 20 sites (n = 3 for each site). At the end of experiment 6 samples were collected in the background area in March 2017 as an additional control to evaluate seasonal effects on lichen element composition. Each sample was a composite sample consisting of 10 to 15 thalli with the size of 3 to 5 cm so as to enhance the representativeness of samples (Zheng et al., 2023). Exposed sample was put into nylon bags (10×15 cm), each containing 2 to 4 thalli.

Sample exposure and recovery. The nylon bags containing lichen thalli were hung in the tree 2.5-3 m above ground at each exposure site in mid-November 2016. These samples were recovered in mid-March 2017 after four months of exposure, put into sealed kraft bags and sent to the laboratory immediately for analysis.

Figure 2. *Photographs of sample site habitats in background and exposure areas. (a-b) background area, (c) residential areas, (d) farmland, (e) industrial sites*

Sample processing and element determination

Optimized ICP (Inductively Coupled Plasma) method was used for determining elements in lichen thalli (Zhao et al., 2017). Impurities were removed from lichen surfaces under stereomicroscope. After that, these samples were oven-dried for 72 h at

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

70°C and crushed with a ball grinder (Retsch MM400; Retsch GmbH, Haan, Germany). Then they were filtered with a 10-mesh sieve. 200-300 mg of homogenized samples were digested using a HNO_3-H_2O_2 microwave digestion system. Concentrations of 50 elements (Ag, Al, As, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Dy, Er, Eu, Fe, Gd, Ge, Ho, K, La, Li, Lu, Mg, Mn, Mo, Nb, Nd, Ni, P, Pb, Pr, Rb, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Tb, Th, Ti, Tl, Tm, U, V, Y, Yb and Zn) were tested by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700X, Agilent Technologies, Tokyo, Japan) in Hebei Research Center for Geoanalysis. Three reference materials were used for quality control: GBW10014 (cabbage), GBW10015 (spinach) and GBW10052 (green tea). These reference materials were provided by the Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences.

EC, PLI and bioaccumulation scale

EC (Exposed-to-Control ratio) was calculated using the following formula:

$$EC_x = C_{[x]exposed} / C_{[x]control} \quad \text{(Eq.1)}$$

where "C" represents the concentration, "x" represents the studied element, "exposed" represents "exposed" and "control" represents the background value.

PLI (pollution load index, PLI)

PLI was calculated on the basis of EC (Tomlinson et al., 1980). The average bioaccumulation level for a single element x in a study area with k sites was expressed with $PLI_{zone\text{-}x}$:

$$PLI_{zone\text{-}x} = (EC_{site1\text{-}x} \times EC_{site2\text{-}x} \ldots \times EC_{site\text{-}k\text{-}x})^{1/k} \quad \text{(Eq.2)}$$

The average bioaccumulation level for an element group containing m elements at a single site was expressed with $PLI_{site\text{-}group}$:

$$PLI_{site\text{-}group} = (EC_1 \times EC_2 \ldots \times EC_m)^{1/m} \quad \text{(Eq.3)}$$

The average bioaccumulation level for an element group in a study area containing k sites was expressed with $PLI_{zone\text{-}group}$:

$$PLI_{zone\text{-}group} = (PLI_{site\text{-}group\text{-}1} \times PLI_{site\text{-}group\text{-}2} \ldots \times PLI_{site\text{-}group\text{-}k})^{1/k} \quad \text{(Eq.4)}$$

among which subscript numbers "1, 2...k" indicate element types.

Interpretive bioaccumulation scale

The 12-week interpretive bioaccumulation scale is used to evaluate the bioaccumulation level (Cecconi et al., 2019) of elements or element groups in *EV*. Five classes and the corresponding thresholds and descriptions are listed briefly as follows: Class 1, EC or PLI $\leq$ 1, absence of bioaccumulation; class 2, EC or PLI = 1-1.8, low bioaccumulation; class 3, EC or PLI 1.8-3.1, moderate bioaccumulation; class 4, EC or PLI = 3.1-3.7, heavy bioaccumulation; class 5, EC or PLI > 3.7, severe bioaccumulation.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

Statistical analysis

Type-R cluster analysis was performed on EC of 50 elements at 20 sites. In the cluster analysis, Euclidean distance was used for distance measurement and Ward's method for clustering. Elements were grouped according to the clustering results and $PLI_{site\text{-}group}$ (*Eq. 3*) and $PLI_{zone\text{-}group}$ (*Eq. 4*) were calculated for each group.

The normal distribution of data was tested using Shapiro-Wilk test. Independent samples T test was used to test concentration difference between the control samples and the exposed samples for each element at each exposure site. Difference in element concentration and $PLI_{site\text{-}group}$ between industrial and non-industrial sites was tested using independent sample T test or Mann-Whitney U test according to data normality. The difference of $PLI_{site\text{-}group}$ for a specific type of sites between different element groups was tested using Wilcoxon test for paired samples with a Bonferroni correction for multiple comparisons. All the analyses were conducted using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA).

Results and analysis

Element concentration

The background and exposure concentrations of 50 elements in *EV* were listed in *Table 2*. The background concentrations were not significantly different between the two collection times (Independent Samples t-test, $P > 0.05$) for all elements but Tm, which had a slightly lower concentration in March 2017 than in Oct 2016. Therefore, the background concentrations in Oct 2016 were taken hereafter as a control. The background concentrations are Si > K > Al > Fe > S > P > Mg > Ti > Mn > Zn > Sr > Rb > V > Cu > Pb > Ce > Cr > La > Ni > As > Nd > Li > Y > Co > Sc > Th > Cs > Pr > Se > Sm > Gd > Sn > Ge > Dy > Cd > Mo > Nb > U > Sb > Er > Yb > Be > Bi > Tl > Eu > Ag > Ho > Tb > Tm > Lu. This rank is roughly followed after exposure, with the exception of 11 elements (Bi, Cd, Cr, Li, Mo, Nb, Pb, Sb, Se, Sn, Yb) which move forward 2-4 positions in the exposure area and 10 of them are Group II (atmospheric-crustal origin) elements.

The background concentration of P had a CV of 38.67% and was not significantly different with the exposure concentrations (Independent Samples t-test, $P > 0.05$). The background concentration of the other 49 elements had a CV of less than 25% (6.03-24.03%) and was significantly lower than the exposure concentrations, regardless of industrial or non-industrial sites (Independent Samples t-test, $P \leq 0.05$).

The average element concentration tends to be higher at industrial sites than at non-industrial sites. However, this trend is not significant (Independent Samples t-test, $P > 0.10$) for 6 elements (Ag, As, Co, Sn, P and Tl), marginally significant ($P = 0.05$~0.07) for 3 elements (Cu, Nb and Sb) and significant (Independent Samples t-test, $P \leq 0.05$) for the remaining 41 elements.

At local (site) scale, the exposure concentrations are not significantly different from the background concentrations at some sites for several elements. This pattern is observed at 90% and 20% of the sites for P and As respectively regardless of site types, and at 40%, 10% and 10% of the non-industrial sites for Al, A and K, respectively. The exposure concentrations are significantly higher than the background concentrations for the other 45 elements at all the exposure sites ($P \leq 0.05$; Independent Samples t-test or Mann-Whitney U test).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

Table 2. *Mean and CV for background and exposure concentrations of 50 elements in EV*

Element	Background concentration				Exposure concentration			
	Oct 2016 (*n* = 5)		Mar 2017 (*n* = 6)		Non-industrial (*n* = 10)		Industrial (*n* = 10)	
	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)
Ag	0.059*	21.94	0.053	11.08	**0.110A**	14.66	**0.123A**	29.73
Al	3576*	16.3	3803	19.84	4956B	18.09	6419A	19.88
As	2.011*	21.43	2.068	10.1	**2.969A**	13.65	**3.350A**	18.85
Be	0.089*	15.77	0.087	12.9	0.184B	12.59	0.230A	22.34
Bi	0.084*	6.09	0.087	5.98	0.217B	15.05	0.290A	21.1
Cd	0.202*	9.41	0.185	3.86	0.526B	23.64	0.865A	55.27
Ce	4.062*	14.22	4.456	9.82	7.228B	15.24	9.357A	19.4
Co	0.728*	13.35	0.754	10.45	**2.071A**	18.26	**2.290A**	34.86
Cr	4.049*	14.55	4.013	10.14	10.85B	17.03	14.66A	37.27
Cs	0.464*	11.12	0.432	11.00	0.780B	14.92	0.955A	21.03
Cu	4.453*	13.47	4.222	7.66	**9.649b**	20.59	**12.30a**	30.37
Dy	0.212*	11.14	0.199	9.62	0.388B	14.11	0.499A	19.72
Er	0.107*	11.75	0.099	9.20	0.209B	14.29	0.265A	20.44
Eu	0.068*	13.01	0.061	9.11	0.127B	14.14	0.170A	23.54
Fe	1828*	16.75	1912	14.97	4310B	13.74	5919A	27.31
Gd	0.277*	11.78	0.294	8.5	0.499B	14.26	0.638A	19.94
Ge	0.226*	12.41	0.261	17.31	0.492B	14.55	0.591A	23.01
Ho	0.040*	11.9	0.037	9.05	0.075B	13.79	0.095A	20.83
K	4300*	14.72	4005	5.69	6491B	14.19	7531A	13.45
La	2.078*	14.35	2.093	9.57	3.663B	14.53	4.701A	18.89
Li	1.642*	15.09	1.791	15.77	4.486B	13.22	5.578A	28.34
Lu	0.014*	14.22	0.014	7.93	0.027B	13.69	0.033A	21
Mg	895.3*	16.85	839.4	10.82	2472B	13.16	3454A	36.3
Mn	61.03*	11.65	64.08	8.4	153.5B	17.27	222.1A	35.85
Mo	0.194*	7.85	0.206	11.62	0.449B	19.4	0.625A	31.86
Nb	0.190*	10.42	0.182	6.07	**0.499b**	19.87	**0.610a**	24.28
Nd	1.734*	11.98	1.844	9.54	3.158B	15.34	4.035A	18.57
Ni	2.074*	14.88	2.171	11.6	5.092B	11.35	6.690A	26.86
P	1056	**38.67**	986.3	5.52	**958.6A**	31.59	**1172A**	36.01
Pb	4.388*	7.99	4.013	9.79	12.65B	17.58	16.17A	21.91
Pr	0.449*	12.58	0.497	9.59	0.810B	15.62	1.049A	19.41
Rb	8.214*	7.92	7.932	4.35	13.31B	15.8	15.72A	19.83
S	1246*	10.73	1278	2.76	4748B	20.82	7095A	44.01
Sb	0.120*	8.64	0.127	14.9	**0.394b**	22.78	**0.505a**	34.87
Sc	0.665*	17.57	0.630	14.79	1.181B	16.92	1.507A	21.25
Se	0.365*	7.05	0.368	7.76	0.819B	9.87	1.100A	29.06
Si	10648*	16.38	10932	16.44	18054B	19.77	22456A	18.43
Sm	0.320*	12.08	0.337	10.23	0.569B	14.81	0.725A	19.73
Sn	0.260*	9.79	0.273	15.7	**0.649A**	16.31	**0.917A**	50.46
Sr	11.84*	24.03	11.95	13.54	29.11B	18.83	38.93A	23.87
Tb	0.040*	13.41	0.038	8.84	0.069B	13.75	0.088A	19.41
Th	0.497*	12.69	0.479	8.57	0.873B	16.13	1.136A	23.67
Ti	120.0*	10.24	126.6	11.23	274.1B	18.92	339.6A	23.53
Tl	0.073*	9.89	0.066	16.15	**0.184A**	15.75	**0.213A**	30.46
Tm	0.015*!	12.12	0.013!	9.18	0.029B	15.18	0.037A	20.61

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

Element	Background concentration				Exposure concentration			
	Oct 2016 (n = 5)		Mar 2017 (n = 6)		Non-industrial (n = 10)		Industrial (n = 10)	
	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)	Mean (μg/g)	CV (%)
U	0.152*	13.21	0.162	10.19	0.275B	16.1	0.335A	17.92
V	4.463*	14.93	4.630	15.14	9.227B	12.62	12.05A	24.17
Y	1.123*	13.5	1.032	7.83	2.107B	13.86	2.686A	18.69
Yb	0.096*	11.84	0.091	8.8	0.184B	13.9	0.233A	21.25
Zn	32.79*	7.17	31.33	7.69	119.9B	21.23	207.3A	57.79

Background concentrations were measured in Oct 2016 and March 2017. Unless otherwise indicated by a "!", the background concentrations are not significantly different between the measured times (Independent Samples t-test, $P \leq 0.05$). "*" means the exposure concentration is significantly higher than the background concentration (Oct 2016) regardless of site type. In column EC, different capital letters indicate that the average element concentration is significantly (Independent Samples t-test, $P \leq 0.05$) different between industrial and non-industrial sites, and different lowercase letters indicate that the average element concentration is marginally significantly (Independent Samples t-test, P = 0.05-0.07) different between industrial and non-industrial sites

Element concentration

Figure 3a shows the results of type-R cluster analysis (Ward's method, Euclidean distance) performed on EC of 50 elements at 20 sites. *Figure 3b* and *c* show PLI_{zone}, PLI_{site} and bioaccumulation class for each element and element groups. According to the results, 50 elements are divided into 3 groups at the distance of 20 (*Fig. 3a*).

The Group I consist of 26 elements (Ag, Al, As, Cs, K, P, Rb, Si, Th, U, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y, and Sc; *Fig. 3a*). Group II consists of 22 elements (Tl, Ti, Nb, Be, Ge, V, Cu, Co, Li, Se, Ni, Fe, Sr, Mo, Cr, Bi, Pb, Mn, Mg, Sn, Cd, and Sb; *Fig. 3a*). Group III consists of Zn and S (*Fig. 3a*).

In each group, PLI_{zone} is higher at industrial sites than at non-industrial sites for all elements but P. The number of elements with bioaccumulation class at industrial sites one class higher than at non-industrial sites is 12 in Group I, 13 in Group II and 2 in Group III. In terms of PLI_{zone}, bioaccumulation class is 2-3 for most of the Group I elements, 3-4 for most of the Group II elements with an exception of class 5 for Cd and Sb, and 4-5 for most of the Group III elements, regardless of site types (*Fig. 3b*).

$PLI_{site\text{-}group}$ is significantly higher at industrial sites than at non-industrial sites for each of the three groups (*Fig. 3c*; $P \leq 0.05$, Independent Samples t-test). $PLI_{site\text{-}group}$ is also significantly different between groups in the decreasing sequence of Group III > Group II > Group I, regardless of site types (*Fig. 3c*; $P \leq 0.05$, Independent Samples t-test, Bonferroni correction).

Discussion

Elements suitable for biomonitoring using EV

EV is suitable for monitoring atmospheric deposition of all elements but P, which has verified the hypothesis 1. Background concentrations were similar between before and after exposures for most elements (*Table 2*), indicating that the exposure concentration changes should be attributable to atmospheric element deposition in Tangshan rather than climate changes. No difference was detected in the concentration of P before and after exposure, regardless of site types. Statistically, this is due to the large CV of the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

background P concentration (>30%, *Table 2*). The relevant studies recommended a local variation of ≤ 25% (Frati et al., 2005; Malaspina et al., 2014) that would otherwise result in an excessive inflation of false negative rate in comparing element concentrations before and after exposure. Such a higher background variation of P relative to other elements may be the result of many complex factors such as bio-regulation or particulate matter accumulation (Gao et al., 2021; Yemets et al., 2014). Although a lot of effort has been made to control the background variability, such as narrow ranges of altitude and collection height on trees, and water washing before exposure, we failed to reduce background variation for P. Therefore, we do not recommend this type of lichen to monitor the atmospheric deposition of P.

Instead, we recommend *EV* as a good active biomonitor for the other 49 elements. For these elements, the background concentrations are rather low in variability (CV < 25%; *Table 2)*, and are significantly lower than the exposure concentrations, as observed at regional scale (*Table 2*) and at most sites. These results are consistent with the fact that the exposure area is higher in atmospheric pollutant concentration than the background area (*Fig. 1d*). In addition, a higher concentration at industrial sites than at non-industrial sites for 45/49 elements (*Table 2*) and a 1-2 higher bioaccumulation class at industrial sites than at non-industrial sites for 27/49 elements (*Fig. 3b*) are also consistent with the fact that there are more intense emissions at the industrial sites.

Element sources

The Group I elements are of crustal origin. In this group, the 16 rare earth elements are often regarded as good geochemical tracers with few atmospheric sources. Al and Si are the main crustal elements in atmospheric deposits. Accumulation of most Group I elements in lichens has been regarded as of crustal origin in Taihang Mountains of North China (Liu et al., 2016b) and Inner Mongolia deserts (Liu et al., 2016a, b). These crustal elements are affected by frequent anthropogenic activities enhancing immobilization and redeposition of soil and rock materials in exposure areas and surrounding areas (Agnan et al., 2014).

The Group II elements are of atmospheric-crustal origin. They appear to come from atmospheric sources (Cr, Ni, Mo, V, Ti, Nb, Co, Mn and Cu) superimposed on crustal input. Because heavy metal concentrations in the lichen are often correlated to those in the air (Sulaiman et al., 2018), the increased element ranks of 10 elements (Bi, Cd, Cr, Li, Mo, Nb, Pb, Sb, Se, and Sn) in the exposed lichens also highlight the importance of atmospheric sources in air quality. For example, Fe and Pb are often released in iron and steel production in which the typical alloy elements are used (Tasiþa et al., 2008). On the other hand, Fe and Mn are often regarded as crustal elements, which are easily affected by soil input. A series of measures have been taken in the exposed area for air pollution control. However, industrial emissions continue. Pollutants from industrial emission will deposit in local soil and are accumulated in lichens along with soil particulates.

The Group III elements are of atmospheric origin. Among these elements, Zn is closely related to industrial (Adjiri and Ramdani, 2022; Brunialti and Frati, 2014; Garty, 2001) and transport activities as a typical industrial metal and a reliable tracer for lead-free gasoline emissions (Monaci, 2000). S is deposited in lichens in both particulate and gaseous (SO_2) forms. Both S and Zn have a higher bioaccumulation class at industrial sites than at non-industrial sites (*Fig. 3b*), suggesting an anthropogenic origin from industrial emissions for these elements.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

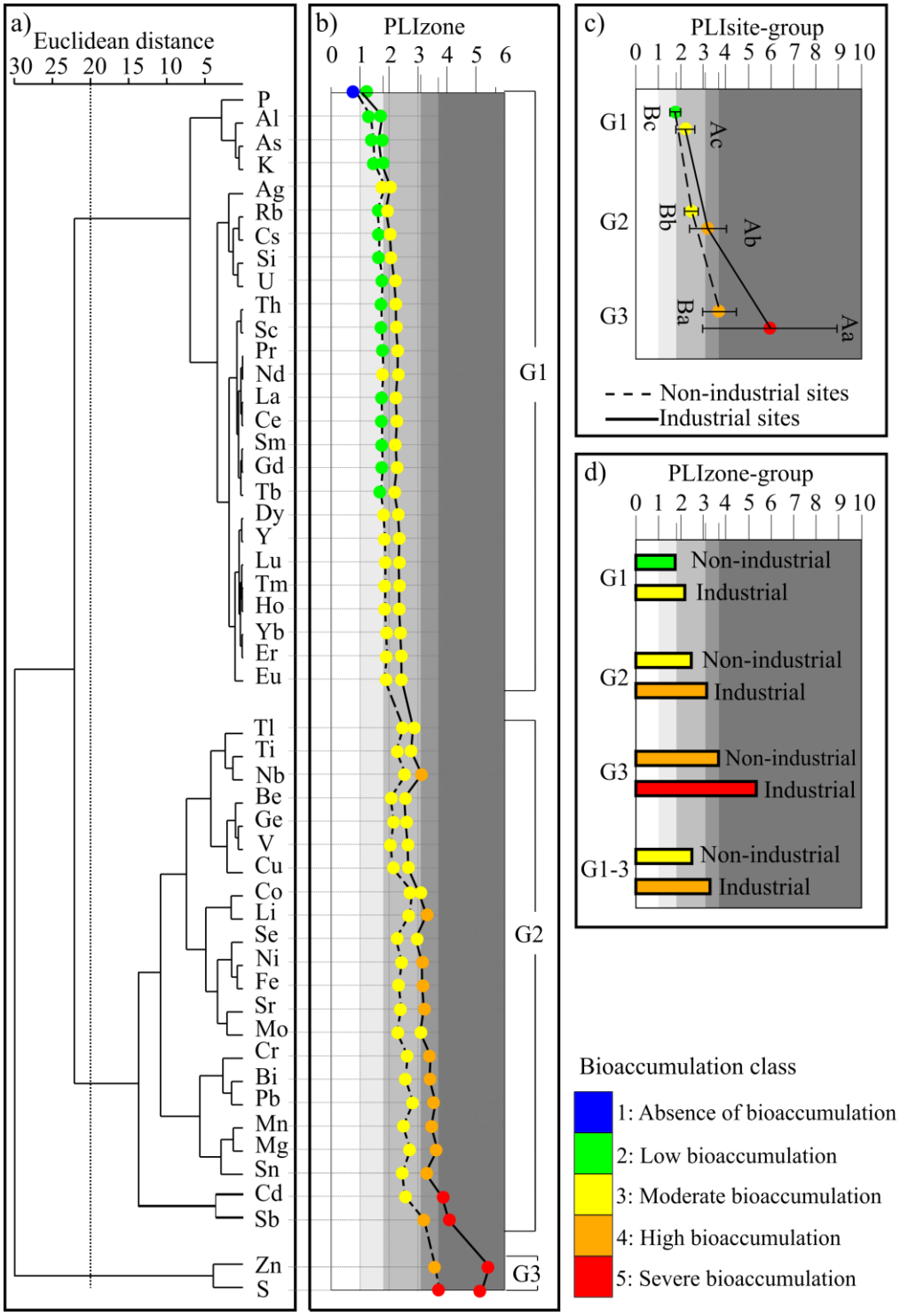

Figure 3. *Cluster dendrogram and bioaccumulation of 50 elements in EV. a), Type-R cluster analysis on EC using Euclidean distance with Ward's method. b), PLI_{zone} and bioaccumulation class of 50 elements at non-industrial and industrial sites. c), PLI_{site} of three element groups at non-industrial and industrial sites (n = 10 for each type of sites); different capital letters indicate a significant (Independent Samples t-test, $P \leq 0.05$) difference in PLI_{site} between industrial and non-industrial sites for a specific group; different lowercase letters indicate a significant difference in PLI_{site} between different element groups for a specific site type (Wilcoxon test for paired samples with Bonferroni correction: $\alpha = 0.05/3 = 0.0167$). d), PLI_{zone} of the three groups and the group combined at non-industrial and industrial sites*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

Bioaccumulation level

Our results are consistent with the hypothesis 2 suggesting a higher element concentration and PLI at industrial sites than at non-industrial sites. Indeed, the exposure concentrations are higher at industrial sites than at non-industrial sites for most elements (41/50; *Table 2*). The bioaccumulation class at industrial sites is often one class higher than that at non-industrial sites (*Fig. 3c*), as observed from the group combined, each element group (*Fig. 3c, d*) and most elements (*Fig. 3b*). PLI_{zone} reveals that the industrial and non-industrial sites belong to "heavy bioaccumulation" and "moderate bioaccumulations", respectively (*Fig. 3b*). These results indicate that air quality is a concern and industrial emissions are also a great concern in the study area, because the non-industrial and industrial sites are often spatially interconnected in the city (*Fig. 1c*).

Greatest concern should be paid to the emissions of group Group III (atmospheric group) elements. This group generally showed "severe bioaccumulation" and "heavy bioaccumulation" at industrial and non-industrial sites, separately (*Fig. 3c, d*). However, S showed "severe bioaccumulation" at all site types, obviously an element with the highest bioaccumulation rate. This might be related to the fact that Tangshan City has higher SO_2 emissions than the background area (*Fig. 1d*) and SO_2 has stronger transfer ability than particulate matters. It is reported that the bioaccumulation rate of S in lichens is often closely related to ambient SO_2 concentration (Gries et al., 1997). The high ambient SO_2 concentration in North China Plain is related to industrial activities, and also arises from coal combustion emissions during the heating period (Zhao et al., 2019; Jia et al., 2020). In addition, Cd and Sb in Group II (atmospheric-crustal group) elements also showed "severe bioaccumulation" in industrial sites (*Fig. 3b*). These elements and Zn are typical heavy metals released from industrial and transport activities (Adjiri and Ramdani, 2022; Brunialti and Frati, 2014; Garty, 2001).

Although showing lower bioaccumulation rate than Group III elements, the other Group II elements are still a concern as revealed by their "heavy bioaccumulation" at industrial sites and "moderate bioaccumulation" at non-industrial sites (*Fig. 3b-d*). Furthermore, greater concern should be given to some toxic heavy metals in Group II, such as Cr, Bi, and Pb, because they are harmful to human and ecosystem health when excessively deposited. Group I (crustal) elements have the lowest bioaccumulation rate compared with other groups (*Fig. 3c*), indicating a higher contribution of industrial, transport and coal combustion emissions than that of soil and rock substances resulting from anthropogenic disturbances in the study area.

Conclusions

EV is a good active biomonitor of atmospheric deposition for 49 elements but not for P. Concentration of the 49 elements significantly increased after exposure, coinciding with the fact that the exposed area has higher atmospheric pollutant concentrations than the background area. Lichen element composition changes show that Tangshan experienced nonnegligible air pollution arising from industrial, transport and coal combustion emissions during the study period. The industrial sites are worse in air quality than the non-industrial sites, as revealed by the higher bioaccumulation class in the former ("heavy bioaccumulation") than the latter sites ("moderate bioaccumulation").

The most concerned elements are those of atmospheric origin (Group III consisting of S and Zn) and some of atmospheric-crustal origin (Cd and Sb in Group II). S has the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

highest bioaccumulation rate ("severe bioaccumulation") compared with all other elements. Cd, Sb and Zn showed "severe bioaccumulation" at industrial sites and "heavy bioaccumulation" at non-industrial sites.

The other atmospheric-crustal elements (Group II) showed "heavy bioaccumulation" and "moderate bioaccumulation" at industrial and non-industrial sites, respectively. The crustal elements (Group I) showed lower bioaccumulation rate than the atmospheric-crustal and atmospheric element, highlighting the importance of industrial, transport and coal combustion emissions on air quality.

Acknowledgements. The authors would like to thank the funding support from Natural Science Foundation of Hebei Province (D2020201002).

REFERENCES

[1] Abas, A. (2021): A systematic review on biomonitoring using lichen as the biological indicator: a decade of practices, progress and challenges. – Ecological Indicators 121: 107197.
[2] Adjiri, F., Ramdani, M. (2022): Distribution maps of the different levels of elemental concentrations accumulated by the lichen in the Northeast of Algeria. – Journal of Environment and Earth Science 12: 14-22.
[3] Agnan, Y., Séjalondelmas, N., Probst, A. (2014): Origin and distribution of rare earth elements in various lichen and moss species over the last century in France. – Science of the Total Environment 487: 1-12.
[4] Bačkor, M., Loppi, S. (2009): Interactions of lichens with heavy metals. – Biologia Plantarum 53(2): 214-222.
[5] Bajpai, R., Shukla, V., Upreti, D. K., Semwal, M. (2014): Selection of suitable lichen bioindicator species for monitoring climatic variability in the Himalaya. – Environmental Science and Pollution Research 21(19): 11380-11394.
[6] Bennett, J. P., Wetmore, C. M. (1999): Changes in element contents of selected lichens over 11 years in northern Minnesota, USA. – Environmental and Experimental Botany 41(1): 75-82.
[7] Boonpeng, C., Sangiamdee, D., Noikrad, S., Watthana, S., Boonpragob, K. (2020): Metal accumulation in lichens as a tool for assessing atmospheric contamination in a natural park. – Environment and Natural Resources Journal 18(2): 166-176.
[8] Brunialti, G., Frati, L. (2014): Bioaccumulation with lichens: the Italian experience. – International Journal of Environmental Studies 71(1): 15-26.
[9] Cecconi, E., Fortuna, L., Benesperi, R., Bianchi, E., Brunialti, G., Contardo, T., Di Nuzzo, L., Frati, L., Monaci, F., Munzi, S. (2019): New interpretative scales for lichen bioaccumulation data: the Italian proposal. – Atmosphere 10(3): 136.
[10] Demiray, A. D., Yolcubal, I., Akyol, N. H., Çobanoğlu, G. (2012): Biomonitoring of airborne metals using the lichen *Xanthoria parietina* in Kocaeli Province, Turkey. – Ecological Indicators 18: 632-643.
[11] Di Lella, L. A., Frati, L., Loppi, S., Protano, G., Riccobono, F. (2003): Lichens as biomonitors of uranium and other trace elements in an area of Kosovo heavily shelled with depleted uranium rounds. – Atmospheric Environment 37(38): 5445-5449.
[12] Fernández, J. A., Boquete, M. T., Carballeira, A., Aboal, J. R. (2015): A critical review of protocols for moss biomonitoring of atmospheric deposition: sampling and sample preparation. – Science of the Total Environment 517: 132-150.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

[13] Frati, L., Brunialti, G., Loppi, S. (2005): Problems related to lichen transplants to monitor trace element deposition in repeated surveys: a case study from Central Italy. – Journal of Atmospheric Chemistry 52(3): 221-230.
[14] Gao, J., Wu, Y. Y., Liu, B. Y., Zhao, R. K., Liu, A. Q., Li, X., Chen, Q. Z., Sun, L. W., Guo, X. P., Liu., H. J. (2021): Vertical distribution patterns of element concentrations in podetia of Cladonia rangiferina from Huzhong Natural Reserve, Heilongjiang, China. – Polish Journal of Environmental Studies 30(1): 103-110.
[15] Garty, J. (2001): Biomonitoring atmospheric heavy metals with lichens: theory and application. – Critical Reviews in Plant Sciences 20(4): 309-371.
[16] Gries, C., Sanz, M. J., Romagni, J. G., Goldsmith, S., Kuhn, U., Kesselmeier, J., Nash III, T. H. (1997): The uptake of gaseous sulphur dioxide by non-gelatinous lichens. – New Phytologist 135(4): 595-602.
[17] Jia, S. J., Zhang, X., Liu, Q. X., Chen, Q. Z., Li, X., Pang, X. M., Li, J. J., Wu, Q. F., Zhao, L. C., Liu, H. J. (2020): Spatial-temporal patterns of element concentrations in *Xanthoparmelia camtschadalis* transplanted along roads. – Polish Journal of Environmental Studies 29(1): 121-129.
[18] Liu, H. J., Fang, S. B., Liu, S. W., Zhao, L. C., Guo, X. P., Jiang, Y. J., Hu, J. S., Liu, X. D., Xia, Y., Wang, Y. D., Wu, Q. F. (2016a): Lichen elemental composition distinguishes anthropogenic emissions from dust storm inputs and differs among species: evidence from Xilinhot, Inner Mongolia, China. – Scientific Reports 6: 34694.
[19] Liu, H. J., Zhao, L. C., Fang, S. B., Liu, S. W., Hu, J. S., Wang, L., Liu, X. D., Wu, Q. F. (2016b): Use of the lichen *Xanthoria mandschurica* in monitoring atmospheric elemental deposition in the Taihang Mountains, Hebei, China. – Scientific Reports 6: 23456.
[20] Liu, H. J., Wang, J. G., Xia, Y., Yang, M. J., Liu, S. W., Zhao, L. C., Guo, X. P., Jiang, Y. J., Li, X., Wu, Q. F., Fang, S. B. (2017): Elemental compositions of lichens from Duolun County, Inner Mongolia, China: origin, road effect and species difference. – Scientific Reports 7(1): 5598.
[21] Malaspina, P., Giordani, P., Modenesi, P., Abelmoschi, M. L., Magi, E., Soggia, F. (2014): Bioaccumulation capacity of two chemical varieties of the lichen *Pseudevernia furfuracea*. – Ecological Indicators 45: 605-610.
[22] Monaci, F., Moni, F., Lanciotti, E., Grechi, D., Bargagli, R. (2000): Biomonitoring of airborne metals in urban environments: new tracers of vehicle emission, in place of lead. – Environmental Pollution 107(3): 321-327.
[23] Peng, H., Chen, Y., Weng, L., Ma, J., Ma, Y., Li, Y., Islam, M. S. (2019): Comparisons of heavy metal input inventory in agricultural soils in North and South China: a review. – Science of the Total Environment 660: 776-786.
[24] Sulaiman, N., Fuzy, S. F. F. M., Muis, S. I. N. A., Sulaiman, N., Ismail, B. S. (2018): Use of lichens as bioindicators for determining atmospheric heavy metal concentration in Malaysia. – Pakistan Journal of Botany 50: 421-428.
[25] Tasiþa, M., Rajšiþa, S., Tomaševiþa, M., Mijiþa, Z., AniĀiþa, M., Novakoviþa, V., Markoviþa, D. M., Markoviþb, D. A., Laziþc, L., Radenkoviþd, M. (2008): Assessment of air quality in an urban area of Belgrade, Serbia. – Environmental Technologies.
[26] Tomlinson, D. L., Wilson, J. G., Harris, C. R., Jeffrey, D. W. (1980): Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. – Helgoländer Meeresuntersuchungen 33(1-4): 566-575.
[27] Wang, L., Yang, J., Zhang, P., Zhao, X., Wei, Z., Zhang, F., Su, J., Meng, C. (2013): A review of air pollution and control in Hebei Province, China. – Open Journal of Air Pollution 02(03): 47-55.
[28] Wei, J. C. (2020): The Enumeration of Lichenized Fungi in China. – China Forestry Publishing House, Beijing.
[29] Wu, Y. Y., Gao, J., Zhang, G. Z., Zhao, R. K., Liu, A. Q., Sun, L. W., Li, X., Tang, H. L., Zhao, L. C., Guo, X. P., Liu, H. J. (2020): Two lichens differing in element

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205

concentrations have similar spatial patterns of element concentrations responding to road traffic and soil input. – Scientific Reports 10(1): 1900.

[30] Yemets, O. A., Solhaug, K. A., Gauslaa, Y. (2014): Spatial dispersal of airborne pollutants and their effects on growth and viability of lichen transplants along a rural highway in Norway. – The Lichenologist 46(6): 809-823.

[31] Zhao, L. C., Wang, L., Jiang, Y. J., Hu, Y. Q., Xu, C. Y., Wang, L., Li, X., Guo, X. P., Liu, A. Q., Liu, H. J. (2017): Determination of elemental concentrations in lichens using ICP-AES/MS. – Bio Protocol 7(5): e2165.

[32] Zhao, L. L., Zhang, C., Jia, S. J., Liu, Q. X., Chen, Q. Z., Li, X., Liu, X. D., Wu, Q. F., Zhao, L. C., Liu, H. J. (2019): Element bioaccumulation in lichens transplanted along two roads: the source and integration time of elements. – Ecological Indicators 99: 101-107.

[33] Zheng, X., Wu, Q. F., Wang, L. P., Liu, A. Q., Xu, C. Y., Li, X. J., Zhao, L. C., Xu, D., Meng, J. W., Liu, H. J. (2023): Composite samples with a small sample size reflect mean element concentrations in three lichens differing in element-specific concentrations. – Applied Ecology and Environmental Research 21(1): 609-622.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2202_11911205