Assessment of Lichens as Biomonitors of Heavy Metal Pollution in Selected Mining Area, Slovakia

Amer H. Tarawneh¹, Ivan Salamon^{2*}, Rakan M. Altarawneh³, Jozef Mitra¹
and Anastassiya Gadetskaya⁴

¹Tafila Technical University, Department of Chemistry and Chemical Technology, P.O.Box 179,
Tafila 66110, Jordan.

²University of Presov, Faculty of Humanities and Natural Science, Department of Ecology, 01, 17th November St.,
081 16, Presov, Slovakia.

³Chemistry Department, Faculty of Science, Mutah University, Karak 61710, Jordan.

⁴School of Chemistry and Chemical Technology, Al-Farabi Kazakh National University,
Almaty 050040, Kazakhstan.

*Corresponding Author Email: ivan.salamon@unipo.sk

Received 07 September 2020, Revised 23 April 2021, Accepted 26 April 2021

Abstract

Lichens have widely been used as bioindicators to reflect the quality of the environment. The present study was conducted to investigate the lichens diversity that grows on the surface of waste heaps from an abandoned old copper mine in Mlynky, Slovakia. In spite of the heavy metal-contaminated environment, we documented twenty species of lichens in the selected site. Taxonomically the most numerous group were represented by *Cladonia* with seven species, as well other species; namely, *Acarospora fuscata*, *Cetraria islandica*, *Dermatocarpon miniatum*, *Hypogymnia physodes*, *Hypogymnia tubulosa*, *Lecanora subaurea*, *Lepraria incana*, *Physcia aipolia*, *Porpidia macrocarpa*, *Pseudevernia furfuracea*, *Rhizocarpon geographicum* and *Xanthoria parietina*. The content of selected heavy metals (Cu, Fe, and Zn) in the predominant lichens *Cetraria islandica*, *Cladonia digitata*, *Cladonia pyxidata*, *Hypogymnia physodes* and *Pseudevernia furfuracea* were analyzed. The highest content of Cu, Fe, and Zn was found in *Cladonia pyxidata* collected from mine-spoil heaps with concentration 46 ± 4.4 , 82.5 ± 22.6 , 4.8 ± 1.6 mg/kg, respectively. Interestingly, *Cladonia pyxidata* collected from the forest surrounding the location showed 15 times lower concentration for Cu. Additionally, similar results were found for Fe and Zn.

Keywords: Mineral mining, Accumulation, Metal contamination, Lichens, Tolerance, Toxicity.

Introduction

Lichens were first described in Greek literature about 300 BC by Greek botanist Theophrastus in his work "De Historia Plantarum" which he characterized as nodules on the bark of olive trees. Lichens represent the dominant life form for about 8% of terrestrial ecosystems [1]. Recent studies have shown that Lichens species are widely used as biomonitoring for atmospheric heavy metals contamination [2-12].

Since the lichens have no root, they depend mainly on atmospheric inputs for their mineral nutrition [13, 14]. Lichens lack a protective outer cuticle and stomata, and thalli can absorb mineral elements over their surface, including heavy metals [3, 15]. These properties of lichens make them very important bioindicators of air pollution [2, 16-18]. The rate of accumulation of heavy metals by lichens and their ability to tolerate them is

specific to the genus of lichen [19, 20]. The ability of lichens to accept metals depends on many factors, such as the chemical form in which the metals are bound and their solubility [21, 22].

Through surveys of changes in lichen communities and the setting of selected ion concentrations (cations such as heavy metals), we are able to obtain valuable information about the quality of the environment, particularly in industrial and densely populated areas [21]. Lichens respond to air pollutants with changes on all biotic organizational levels, from the cellular to the community level. Their growth in environmentally extreme sites confirms their highly significant role in the long-term transformation of nature. According to observations, along with some bryophytes, these are the first organisms to develop in an inhospitable environment, and they pave the way for the attachment of vascular plants [23].

In the middle ages, Slovakia was part of the Austria-Hungarian Kingdom, which is considered the biggest producer of precious metals in Europe; Slovakia was exported copper to all of Europe during the 16th and 17th centuries [24]. At the beginning of the 19th century, the Austria-Hungarian Kingdom produced half the European production of gold, 1/3 of its silver, and 1/6 of its copper. Whereas, Mlynky was considered one of the most historic mining areas for Cu, Fe, and Zn in Slovakia [24].

Since lichens are a valuable tool to determine the quality of the environment and can be considered an inexpensive tool for evaluating air pollution. The present study aims to evaluate the content of Cu, Fe, and Zn present in highly dominant lichens found in mine spoil heaps and in the surrounding forest at Mlynky area.

Materials and Methods

Geographical Characteristics of the Surveyed Sites

The samples were collected from Mlynky, in the middle east of Slovakia (geographic coordinates: 48 ° 51'11 "N 20 ° 25'48" E). The mining area lies in the upper part of the Hnilecká Valley on the southern edge of the Slovak Paradise National Park. It is located in the so-Spišský Orel region, which is the most important subsoil unit of the Slovak Rudohorie.

The territory has a complex geological structure. Paleozoic rocks predominate, such as sandstones, cremations, slate, phyllites, diabase, diabase tuffs. The northern part consists of Mesozoic limestones.

Collection of Lichens and Their Identification

The samples collected in April and May 2016 at an altitude of 760 m up to 1100 m above sea level. We collected lichens in woodland and rocks. Herbarium items of lichens were stored in the private herbarium of J. Mitra. In order to identify lichen to species, Purvis *et al.*, determination key was used to identify the lichens [25]. Thin layer chromatography (TLC) was also used to identify lichens based on their secondary metabolites compositions as previously described [26].

Analysis of Cu content in Soils

Determination of Cu content was carried out in the Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov, Slovakia. Flame Atomic Absorption Spectrometry (FAAS) was used to determine the Cu metal content in the soil and lichens growing on the top of the spoil-mine heap.

Soil samples were collected from sites where our lichens have assembled. Three replicates were taken from each place. After the removal of visible organic material and stones, the samples were sieved through a sieve (0.8 mm) and dried to a constant weight at 80°C for 24 h. Total Cu was measured after digestion 0.5 g of sample in 50 mL Aqua Regia for 24 h; solutions were then evaporated to dryness in a water bath and dissolved in 5% HNO₃ prior to measurement by FAAS. Detection wavelength for Cu assigned at λ_{max} = 324.8 nm.

Analysis of Heavy Metals Content in Lichen Thalli

Determination of Cu, Fe, and Zn contents was carried out in the Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov, Slovakia. FAAS was used to determine the heavy metal contents (Cu, Fe, Zn) in the thalli of selected lichens. Macroscopic foreign material adhering to lichen surfaces (e.g., soil particles) was removed with forceps and the lichens were rinsed with deionized water. The collected lichens were dried at 80°C for 24 h and 100 mg of dry material was weighted. Prepared samples were located into DAP-60K pressure vessels regard to microwave mineralization, 9 mL of HNO₃ (65%) and 2 mL of H₂O₂ (30%) (supplied by Sigma-Aldrich) were added to each flask. The temperature program consisted of four steps. The digestion process was performed with the Speedwave Two mineraliser (Berghof, Germany). The other experimental conditions were as follows: supply voltage ~ 230 V, frequency 50/60 Hz, power 1610 W, magnetron frequency 2450 MHz. DAP-60K pressure vessels, volume 60 mL, maximum pressure 40 bar, maximum temperature 230°C, maximum load < 300 mg, minimum acid volume > 5 mL. The mineralized samples were allowed to cool to room temperature.

From each sample, the mineralized material was quantitatively removed into a 50 mL volumetric flask and diluted to the mark with 2% HNO₃ and deionized water with a conductivity of <0.1 μ S. Subsequently, the samples were measured on the Fe, Cd, Zn, Cu and Pb content against the standard (supplied by ULTRA Scientific) using an atomic absorption spectrophotometer (AAS) at 248.3 nm (Fe), 213.9 nm (Zn), 324.8 nm (Cu). The analysis was performed using a Shimadzu AAS 7000. It is a fully automatic double-wired device with a 3D-optical system, automatic 6-lamp holder, D-lamp background correction with SR-correction of spectral interference.

Quality Assurance

Quality control methods were used to evaluate the reliability of the proposed procedures and the efficiency of the FAAS method. The linearity, range, precision, and recovery were investigated in this study. A recovery in the range of 94–98% was obtained in this work, providing good method accuracy. The recovery study was performed as follows: eight analysed samples were spiked with diluted standard solutions of Cu, Fe, Pb, Cd and Zn [27, 28]. The average recovery percentages obtained were 95.2 ($\pm$ 1.3), 94.1 ($\pm$ 2.7), 95.8 ($\pm$ 0.8), 97.3 ($\pm$ 2.1), and 96.2 ($\pm$ 1.9) % for Cu, Fe, Pb, Cd, and Cr, respectively.

Statistical Analysis

To investigate the correlation between different types of lichens and heavy metals accumulation among all investigated sites, a Principle component analysis was carried out using SPSS Statistics version 22.0.

Results and Discussion

As part of this study, lichen surveys were carried out in Mlkyne area at forest and

mine sites. The preliminary observations on the flora of targeted sites indicate that interesting lichens occur (Table 1). Most of the identified species, up to seven, belonged to the genus *Cladonia*. In the selected place, we also confirmed the extension of two representatives of the genus *Hypogymnia* and eleven different species were documented for the first time at Mlynky area.

Out of twenty species, five were found to be predominant in the selected sites, namely; *Cetraria islandica*, *Cladonia digitata*, *Cladonia pyxidata*, *Hypogymnia physodes* and *Pseudevernia furfuracea*. In addition to those taxa mentioned in Table 1, several unidentified lichens have been seen.

Table 1. Location of collected lichens at Mlynky.

Lichens	Mine heaps	Forest
<i>Acarospora fuscata</i>	-	+
<i>Candelariella aurella</i>	-	+
<i>Cetraria islandica</i>	-	+
<i>Cladonia coccifera</i>	-	+
<i>Cladonia digitata</i>	-	+
<i>Cladonia fimbriata</i>	+	+
<i>Cladonia furcata</i>	-	+
<i>Cladonia macilenta</i> subsp. <i>floerkeana</i>	-	+
<i>Cladonia pleurota</i>	+	+
<i>Cladonia pyxidata</i>	+	+
<i>Dermatocarpon minutum</i>	-	+
<i>Hypogymnia physodes</i>	-	+
<i>Hypogymnia tubulosa</i>	-	+
<i>Lecanora subaurea</i>	-	+
<i>Lepraria incana</i>	-	+
<i>Physcia aipolia</i>	-	+
<i>Porpidia macrocarpa</i>	-	+
<i>Pseudevernia furfuracea</i>	-	+
<i>Rhizocarpon geographicum</i>	-	+
<i>Xanthoria parietina</i>	-	+

+ detected

- not detected

Interestingly, *Cladonia pyxidata* was found in abundance at both sites. Furthermore,

Cladonia pyxidata shows a higher ability to accumulate Cu in comparison with other predominant lichens in the same region, Table 2. The total concentration of Cu in the *Cladonia pyxidata* was found to be 3.10 ± 0.92 and 46 ± 14.4 for a sample collected from the forest and spoil mine heap, respectively (Table 2). These results are in good agreement with previous studies explaining the effect of the surrounding environment on the heavy metals accumulation in lichens [7-14]. On the other hand, the lowest concentration of Cu was found in *Pseudevernia furfuracea* collected from the forest site.

In 2010 Bačkor investigated the lichens diversity in spoil-mines area and their Cu content; the concentration of Cu content in the thalli of *Cladonia pyxidata* was 242 ± 46.3 mg/kg [29].

Table 2. Cu content (mg/Kg) in the selected lichens species and soil collected at Mlynky.

Lichens	<i>Cetraria islandica</i> ²	<i>Cladonia digitata</i> ²	<i>Cladonia pyxidata</i> ²	<i>Cladonia pyxidata</i> [*]	<i>Hypogymnia physodes</i> ²	<i>Pseudevernia furfuracea</i> ²	Spoil heaps
Cu	2.65	2.45	3.10	46	2.80	2.30	384
Conc.	± 0.71	± 0.88	± 0.92	± 14.4	± 1.06	± 1.01	± 67.4

² - lichens collected from forest, * - collected from spoil mine heap.

What is worth mentioning is that Cu content in the substrate at the Mlynky site was found to be 384 ± 67.4 , which is lower than what was reported by Bačkor *et al.*, [29]. This difference in Cu concentrations can be attributed to the removal of the spoil-mine heap in the Mlynky mining area. The amount of Cd and Pb accumulated by the selected lichens were found to be lower than the detection limit of the AAS (0.009 mg/Kg). The lowest content of Fe was found in *Cetraria islandica* with a value of 33.5 mg/kg (Table 3).

The highest Fe accumulation was recorded in *Cladonia digitata* and *Hypogymnia physodes* with values of 82.5 and 81.4 mg/kg, respectively. On the other hand, the content of Fe was not significantly lower in both *Cladonia pyxidata* and *Pseudevernia furfuracea* species. When Zn content was determined, remarkable differences were observed in the content between predominated species of lichens (Table 3). Higher concentrations have been demonstrated in *Cladonia digitata* and *Pseudevernia furfuracea*, which have been collected from the forest site with concentrations 4.80 ± 1.58 and 4.10 ± 1.78 mg/Kg, respectively. The lower concentration for Zn metal was measured in *Cladonia islandica* with a value of 0.75 mg/kg, (Table 3).

Table 3. The amount of iron and zinc accumulated by the selected lichens during growth.

Lichens	Metal (mg/kg)	
	Fe	Zn
<i>Cetraria islandica</i>	33.50±3.94	0.75±0.22
<i>Cladonia digitata</i>	82.50±22.57	4.80±1.58
<i>Cladonia pyxidata</i>	67.80±16.20	1.55±0.72
<i>Hypogymnia physodes</i>	81.40±19.67	3.85±1.05
<i>Pseudevernia furfuracea</i>	56.55±17.60	4.10±1.78

The present study documented twenty species of lichens, where the *Cladonia* genus is the most present in such contaminated regions. Also, we have confirmed the presence of three species of *Cladonia* lichens growing on spoil-mine heaps, Table 1. These species were found on both non-contaminated and metal-contaminated soil, as previously reported [29].

The results of the principle component analysis have been graphically displayed as loading plots. Fig. 1 illustrates the analyzed heavy metal concentrations (Cu, Fe, and Zn) in four types of lichens family (*Pseudevernia furfuracea*, *Cetraria islandica*, *Cladonia*, and

Hypogymnia physodes) in two different sites (forest and mine slag heaps).

Figure 1. Principle component analysis of lichens based on heavy metal Levels. components 1, 2 and 3 represent correlation matrix (correlations among several variables, site, heavy metal concentration, and lichens)

Firstly, different symbols F1, F2, F3, and F4 were used to represent the concentrations of analyzed heavy metals in forest samples, namely *Pseudevernia furfuracea*, *Cetraria islandica*, *Cladonia*, and *Hypogymnia physodes*, respectively. Group 1 is consisting of lichens (F1, F3, and F4), which were characterized by similar Cu and Zn concentration profiles. Whereas lichen F4 was classified as a separate group 2 as it shows a high concentration of Fe. Whereas the level of Cu and Zn are similar to F1, F2, and F3.

Whereas symbols M1, M3, and M4 were used to represent the Fe contents in *Pseudevernia furfuracea*, *Cladonia*, and *Hypogymnia physodes*, respectively. The *Cetraria islandica* lichen was not collected in those industrial sites. Lichens (M3 and M4) within group 3 were characterized by a high concentration of Fe. Finally, lichen (M1) was classified as a separate group 4 because it was characterized by a low level of Fe. In this regard, the *Cladonia*, and *Hypogymnia physodes* were found to have a high tendency to accumulate Fe rather than *Pseudevernia furfuracea*.

Conclusion

The present study documented twenty species of lichens, where the *Cladonia* genus is the most present in such contaminated regions. Also, we have confirmed the presence of three species of *Cladonia* lichens growing on spoil-mine heaps. These species were found on both non-contaminated and metal-contaminated soil. The Fe content in this site is high. Therefore, it appears that iron content is not a limiting factor in the expansion of lichens in our study. Rather, we are inclined to the hypothesis that the spread of lichens at this site is influenced by human activity, as the waste material accumulated here in the 1970s.

Acknowledgement

The taxonomical identity of lichens was consulted with M. Backor (University of P. J. Safarik, Kosice, Slovakia). The characteristics of the rock substrate and geological substrate were discussed with Z. Krempaska (Spis Museum, Spisska Nova Ves, Slovakia). The R&D was supported by the VEGA-project No. 1/0582/2017.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. J. Asplund and D. A. Wardle, *Biol. Rev.*, 92 (2017) 1720.
[doi: 10.1111/brv.12305](https://doi.org/10.1111/brv.12305)
2. J. Garty, *Crit. Rev. Plant Sci.*, 20 (2001) 309.
[doi: org/10.1080/20013591099254](https://doi.org/10.1080/20013591099254)
3. R. Bargagli and I. Mikhailova, *NATO Sci. Ser. (Series IV: Earth Environ. Sci.)*, 7 (2002) 65.
[doi: org/10.1007/978-94-010-0423-7_6](https://doi.org/10.1007/978-94-010-0423-7_6)
4. S. Nayaka, D. K. Upreti, M. Gadgil and V. Pandey, *Curr. Sci.*, 84 (2003) 674.
[doi: jstor.org/stable/24108503](https://doi.org/jstor.org/stable/24108503)
5. V. Pandey, D. K. Upreti, R. Pathak and A. Pal, *Environ. Monit. Assess.*, 73 (2002) 221.
[doi: 10.1023/a:1013173104533](https://doi.org/10.1023/a:1013173104533)
6. Z. Jeran, R. Jaćimović, F. Batič and R. Mavsar, *Environ. Pollut.*, 120 (2002) 107.
[doi:org/10.1016/S0269-7491\(02\)00133-1](https://doi.org/10.1016/S0269-7491(02)00133-1)
7. K. Rola, *J. Trace Elem. Med. Biol.*, 61 (2020) 126512.
[doi: org/10.1016/j.jtemb.2020.126512](https://doi.org/10.1016/j.jtemb.2020.126512)
8. G. Kosior, E. Steinnes, A. Samecka-Cymerman, S. Lierhagen, K. Kolon, A. Dołhańczuk-Śródka and Z. Ziemcik, *Chemosphere*, 171 (2017) 735.
[doi: 10.1016/j.chemosphere.2016.10.131](https://doi.org/10.1016/j.chemosphere.2016.10.131)
9. A. R. H. De La Cruz, J. K. H. De La Cruz, D. A. Tolentino and A. Gioda, *Chemosphere*, 210 (2018) 849.
[doi:org/10.1016/j.chemosphere.2018.07.013](https://doi.org/10.1016/j.chemosphere.2018.07.013)
10. A. Vannini, L. Paoli, A. Russo and S. Loppi, *Chemosphere*, 231 (2019) 121.
[doi: 10.1016/j.chemosphere.2019.05.085](https://doi.org/10.1016/j.chemosphere.2019.05.085)
11. A. Kłos, Z. Ziemcik, M. Rajfur, A. Dołhańczuk-Śródka, Z. Bochenek, J. W. Bjerke, H. Tømmervik, B. Zagajewski, D. Ziolkowski, D. Jerz, M. Zielińska, P. Krems, P. Godyń, M. Marciniak and P. Świsłowski, *Sci. Total Environ.*, 627 (2018) 438.
[doi:org/10.1016/j.scitotenv.2018.01.211](https://doi.org/10.1016/j.scitotenv.2018.01.211)
12. A. Stojanowska, J. Rybak, M. Bożym, T. Olszowski and J. S. Białowicz, *Sustainability*, 12 (2020) 8066.
[doi:org/10.3390/su12198066](https://doi.org/10.3390/su12198066)
13. O. W. Purvis, *Bot. Stud.*, 55 (2014) 23.
[doi:10.1186/1999-3110-55-23](https://doi.org/10.1186/1999-3110-55-23)
14. J. Garty, In: I. C. Kranner, R. P. Beckett and A. K. Varma (eds) *Protocols in Lichenology*. Springer Lab Manuals. Springer, Berlin, Heidelberg, 458, (2002).
[doi :org/10.1007/978-3-642-56359-1_27](https://doi.org/10.1007/978-3-642-56359-1_27)

15. M. E. Conti and G. Cecchetti, *Environ. Pollut.*, 114 (2001) 471.
[doi:org/10.1016/S0269-7491\(00\)00224-4](https://doi.org/10.1016/S0269-7491(00)00224-4)
16. P. Adamo, S. Giordano, S. Vingiani, R. C. Cobianchi, and P. Violante, *Environ. Pollut.*, 122 (2003) 91.
[doi.org/10.1016/S0269-7491\(02\)00277-4](https://doi.org/10.1016/S0269-7491(02)00277-4)
17. M. Bačkor, and S. Loppi, *Biologia Plantarum*, 53 (2009) 214.
[doi: 10.1007/s10535-009-0042-y](https://doi.org/10.1007/s10535-009-0042-y)
18. V. Banášová, O. Horak, M. Čiamporová, M. Nadubinská and I. Lichtscheidl, *Biologia*, 61 (2006) 433.
doi.org/10.2478/s11756-006-0073-1
19. R. Bargagli, F. Monaci, F. Borghini, F. Bravi and C. Agnorelli, *Environ. Pollut.*, 116 (2002) 279.
[doi: 10.1016/s0269-7491\(01\)00125-7](https://doi.org/10.1016/s0269-7491(01)00125-7)
20. J. E. Sloof, *Atmos. Environ.*, 29 (1995) 11.
[doi.org/10.1016/1352-2310\(94\)00221-6](https://doi.org/10.1016/1352-2310(94)00221-6)
21. A. Beeby, *Environ. Pollut.*, 112 (2001) 285.
[doi: 10.1016/s0269-7491\(00\)00038-5](https://doi.org/10.1016/s0269-7491(00)00038-5)
22. G. Sarret, A. Manceau, D. CUNY, C. V. Haluwyn, S. Déruelle, J. L. Hazemann, Y. Soldo, L. Eybert-Bérard and J. J. Menthonnex, *Environ. Sci. Technol.*, 32 (1998) 3325.
[doi:org/10.1021/es970718n](https://doi.org/10.1021/es970718n)
23. Y. Sueoka, M. Sakakibara and K. Sera, *Metals*, 5 (2015) 1591.
[doi:org/10.3390/met5031591](https://doi.org/10.3390/met5031591)
24. G. W. Buck and D. H. Brown, *Annals Botany*, 44 (1979) 265.
[doi:org/10.1093/oxfordjournals.aob.a085730](https://doi.org/10.1093/oxfordjournals.aob.a085730)
25. O. W. Purvis, B. J. Coppins, D. L. Hawksworth, P. W. James and D. M. Moore, London: Natural History Museum Publications in association with the British Lichen Society, (1992) 710.
26. A. Orange, P. W. James and F. J. White, *British Lichen Soc.*, 34 (2001) 181.
[doi:org/10.1006/lich.2002.0376](https://doi.org/10.1006/lich.2002.0376)
27. R. M. Altarawneh, *Int. J. Environ. Anal. Chem.*, 2019.
[doi:org/10.1080/03067319.2019.1675653](https://doi.org/10.1080/03067319.2019.1675653)
28. Q. M. Jaradat and A. Tarawneh, *Jordan J. Chem.*, 146 (2014) 1.
[doi:jjc.yu.edu.jo/index.php/jjc/article/view/151](https://doi.org/jjc.yu.edu.jo/index.php/jjc/article/view/151)
29. M. Bačkor, O. Peksa, P. Škaloud and M. Bačkorová, *Ecotoxicol. Environ. Saf.*, 73 (2010) 603.
[doi:org/10.1016/j.ecoenv.2009.11.002](https://doi.org/10.1016/j.ecoenv.2009.11.002)