Can saxicolous lichens be used to monitor atmospheric deposition? Disentangling the influence of bedrock and canopy cover on biodiversity and elemental content of lichens

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Abstract

Saxicolous lichens are known to be affected by air pollutants, but are often overlooked as potential biomonitors, even though they may be the only viable option for data collection in certain environments. In this study, we investigated the influence of bedrock and forest canopy on the biodiversity and the elemental content of native epiphytic and saxicolous lichens in metal rich environments (serpentinite and granite outcrops in the Slavkov Forest, Czech Republic). We compared the accumulation of potentially toxic elements in the saxicolous lichen *Parmelia saxatilis* (L.) Ach. with that in the epiphytic species *Hypogymnia physodes* (L.) Nyl. and *H. tubulosa* (Schaer.) Hav.. Generalized linear models (GLMs) models revealed similar accumulation patterns for Fe, As, Cr, and Ni, for which both substrate type (tree vs. rock) and bedrock composition (granite vs. serpentinite) were significant factors. Thalli collected from serpentinite rocks exhibited significantly higher concentrations of Fe, Cr, Ni and As, regardless of canopy cover; while Al was more abundant in saxicolous lichens on granite. Zn and Cd accumulated more in epiphytic lichens, whereas Pb was higher in saxicolous ones. Secondary metabolites as well as anatomical and morphological differences, rather than substrate, may possibly explain such divergences in Pb, Zn and Cd contents. Nevertheless, the concentrations of Cu, along with those of Zn, Cd, and Pb, were of the same order of magnitude across both lichen types and not affected by bedrock. These findings suggest that saxicolous lichens can serve as effective biomonitors in environments where epiphytic lichens are not available.

Keywords: bioaccumulation, epiphytic lichens, environmental monitoring, granite, heavy metals, secondary metabolites, serpentinite

Highlights:

Saxicolous and epiphytic lichens were compared in terms of diversity and elemental contents

Forest canopy cover, more than bedrock type, influences saxicolous lichen diversity

Bedrock composition affects Fe, As, Cr, Al and Ni accumulation in saxicolous thalli

Saxicolous and epiphytic species share similar contents of Cd, Cu, Pb and Zn

Saxicolous lichens can be used to monitor certain elements where epiphytic species are absent

Introduction

Due to their sensitivity to phytotoxic gases and their capacity to accumulate airborne toxic elements, epiphytic lichens are commonly used to monitor the effects of atmospheric pollution (Kularatne and Freitas 2013; Giordani and Brunialti 2014; EN 16413, 2014; ISPRA, 2020; Fortuna et al. 2021). Similarly, saxicolous lichens are also known to respond to atmospheric pollutants and serve as effective collectors of atmospheric deposition (Gilbert 1970; Seaward 1987; Bargagli 1998; Purvis et al. 2000). However, their role as biomonitors is often underappreciated due to the additional complexity introduced by substrate chemistry. Nevertheless, in some contexts, saxicolous lichens can provide valuable or even the only available data. For instance, elemental profiles of umbilicate foliose thalli of saxicolous *Umbilicaria* Hoffm. and *Rhizoplaca* Zopf have been found to reflect N and S deposition in high altitude habitats, where epiphytic lichens are absent (Kovasi et al. 2022). Other studies have explored the accumulation of metals in foliose thalli of *Xanthoparmelia* (Vain.) Hale species, identifying for certain elements (Sb, Pd, Cu, Cd, Sn, Pb, Zn, V) positive correlations with anthropogenic sources, while also acknowledging the contribution of the underlying rock substrate for other elements (Zschau et al. 2003; Puy-Alquiza et al. 2017). Species- (and elemental-) specific differences in metal accumulation were also reported within certain groups of foliose and crustose saxicolous species (Sawidis et al. 1995; Puy-Alquiza et al. 2017), similarly to epiphytic ones (Bennett and Wetmore 1999). Despite these findings, datasets integrating both epiphytic and saxicolous lichens are rare (e.g. Sawidis et al. 1995; StClair et al. 2002; Essilmi et al. 2019). Moreover, even when both groups are included, direct comparisons often lack a systematic evaluation of environmental variables that could influence accumulation patterns.While epiphytic lichens mostly receive their nutrients from atmospheric sources, the intimate contact of saxicolous lichens with the rock substrate determines a significant contribution to their mineral uptake (Brodo 1973). As a result, saxicolous communities tend to differ significantly depending on the chemistry of the rock, such as between carbonate and silicate rocks (Purvis et al. 2014; Armstrong 2015). In contrast, cases of a narrow substrate specificity, with distributions limited to a certain carbonate or silicate lithology, are less defined and restricted to few species. Although several species show a preference for more acidic or base-rich silicate rocks, they generally display a tendency rather than a strict exclusivity (Wirth 1972). Saxicolous communities even show a scarce specificity on ultramafic substrates as serpentinites (Favero-Longo et al. 2004). These contain potentially toxic elements (PTEs, such as, e.g., Ni, Co, Cr) and display other edaphic limitations (primarily, low Ca:Mg ratios and deficiency in essential nutrients) determining a strong selectivity for plants and thus yielding poor floras with plenty of endemisms (Harrison and Rajakaruna 2011). Instead, the lichen diversity is generally not lower than in adjacent areas with different silicate rocks, and confirmed reports of endemism are extremely limited. Although differences in the community composition can characterize serpentinites and neighbouring lithologies, the effect of the substrate seems secondary to that of other environmental factors (Favero-Longo et al. 2018; Mulroy et al. 2022). On other metalliferous rocks, several lichen species demonstrated a remarkable ability to tolerate extremely high metal concentrations, with mechanisms of exclusion, sequestration and stress tolerance being variously involved (Purvis and Pawlik-Skowrońska 2008). In this context, saxicolous lichens have been deeply characterized in terms of metal homeostasis, showing a crucial role of species- and population-specific secondary metabolites in enabling tolerance to rock chemistry and influencing lithological preferences (Hauck et al. 2009a, 2009b). Secondary metabolites, indeed, display specific affinity to different metal ions and thus variously condition their adsorption, influencing mineral nutrition, but also affecting the tolerance of excess metal concentrations (Hauck et al. 2007, 2009a). However, these findings have not been evaluated with respect to a potential scale of specific tolerance of different saxicolous taxa to airborne metal deposition. This remains an important gap in research, especially given that secondary metabolite production in epiphytic lichens has been observed to respond to metal air pollution (Hauck et al. 2013).

In this study, we conducted a comparative investigation of saxicolous and epiphytic lichens in the Slavkov Forest (Czech Republic), a region of Central Europe historically affected by severe air pollution (Král et al. 1992; Petrash et al. 2023). The area has been monitored for air, water, and soil chemistry since the late 1980s and is part of the national Long-Term Ecosystem Research network (LTER-CZ; www.lter.cz/en/lysina-pluhuv-bor-catchments). We surveyed lichen diversity in areas with serpentinite and granite bedrock, considering sites both within and outside forest stands. In addition, we quantified the accumulation of elements (Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Zn) in thalli of saxicolous (*Parmelia saxatilis* (L.) Ach.) and epiphytic (*Hypogymnia physodes* (L.) Nyl. and *H. tubulosa* (Schaer.) Hav.) foliose species, occurring in both serpentinite and granite areas. The primary hypothesis of our study was that saxicolous and epiphytic lichens can serve interchangeably as biomonitors of metal pollution, thus extending bioindication potential to habitats lacking epiphytic communities. Specifically, we aimed to (1) assess how bedrock chemistry and forest canopy influence the diversity of saxicolous and epiphytic lichens, and (2) determine whether bioaccumulation patterns (a) differ between saxicolous and epiphytic species, and (b) are affected by bedrock type and/or forest canopy cover.

Materials and Methods

Study area

Lichen communities and element accumulation in selected saxicolous and epiphytic species were studied in the Slavkov Forest Protected Landscape Area, located in the Karlovy Vary Region of Czech Republic. The study area has a temperate climate with warm summer without a dry season (Cfb, according to the Köppen-Geiger Climate Classification; Kottek et al. 2006). Investigations were carried out in areas characterized by serpentinite and granite bedrock (Fig. 1). Partly or completely serpentinized peridotites (approx. 9 km^2) are part of the Mariánské Lázně ophiolitic complex, reaching a maximum elevation of 883 m (Vlčí Kámen) (Krám et al. 2009). Leucogranites and subordinate quartz monzonite and alkali-feldspar granites (approx. 70 km^2) are associated to granitic intrusions of the Younger Intrusive Complex (Lesný Lysina Massif), reaching a maximum elevation of 983 m (Mt. Lesný) (Hayes et al. 2020). Eutrophic and podzolic brown earth soils prevail in the serpentinite and granite areas, respectively (Krám et al. 1997). Rock outcrops of both lithologies are limited in extension (approx. 50-1000 m^2) and mostly distributed at the top and on the sides of the main forested peaks of the peneplain landscape, but few isolated outcrops also occur in open fields close to the forest margin.

Atmospheric depositions of sulphur and metal pollutants, related to lignite-based power and heat generation plants, were prominent over the 20th century, reaching a peak in 1982, and then drastically declining in the 1990s (Král et al. 1992; Hruška and Krám 2003; Petrash et al. 2023).

Slavkov Forest is a large forest of planted *Picea abies* (L.) H.Karst., with oldest stands of more than 140 years. In the ultramafic areas, stands of old and stunted *Pinus sylvestris* L. also occur (Krám et al. 2009), while in the granite areas the coniferous forest consists almost exclusively of *Picea abies* (Hayes et al. 2020). A rich lichen diversity in the Slavkov Forest was documented by several floristic studies, started around the mid of 19th century (1837-1862), deepened between the end of 1920s and early 1930s, with a particular focus on the serpentinite areas (Suza 1928, 1934), and renewed by recent surveys around 2010 (Peksa 2011; Steinová 2013).

Biodiversity

Saxicolous lichen diversity was surveyed on serpentinite (S) and granite (G) outcrops, located in forested (F) and open field (O) areas. In particular, 42 plots of 50 cm × 50 cm were distributed in 14 localities (3 plots for each locality) representative of the above-mentioned conditions: FS (n=9 plots), FG (n=12), OS (n=12) and OG (n=9) (Table S1; Fig. 1). A square grid divided into 25 quadrats (10 × 10 cm) was used to calculate the frequency of each species, as the sum of its occurrences within the quadrats, and to visually estimate its cover through the whole plot. For each plot, the diversity and abundance of saxicolous lichens were thus evaluated in terms of (a) alpha diversity, (b) total specific frequencies in the grid, and (c) total coverage (in %).

At each locality, the diversity of epiphytic lichens was examined by surveying the three trees (each tree treated as an individual plot) closest to the studied outcrop, not more than 50 m far away. On each tree, the total specific frequency in terms of Lichen Diversity Values (LDVs, according to standard protocols used in lichen monitoring studies; e.g. EN 16413, 2014) was calculated as the sum of occurrences of epiphytic lichens in a sampling grid consisting of four 50 cm × 10 cm ladders, each divided into five 10 cm × 10 cm units. The grid was placed on the N, E, S and W cardinal sides of the boles of each tree (mostly *Picea abies*, alternatively *Quercus petraea*), 1 m above the ground. Tree boles were considered as suitable if with girth >60 cm, trunk almost straight (inclination <20°), not damaged and with a coverage of bryophytes <25%.

For each species, indicator values informative of the specific ecological ranges with respect to pH of substratum (pH), solar irradiation (SI), aridity (AR), eutrophication (EU) and poleotolerance (PO) were considered (Nimis 2024). Moreover, a dataset of specific functional traits was compiled, including information on thallus growth form (GRO), prevailing reproductive strategy (REP), main photobiont (PHO), and the putative (literature-based) content in secondary metabolites (see Favero-Longo et al. 2018). In particular, functional attributes were assigned with reference to information available for each species on ITALIC 7.0 (Nimis 2024).

Bioaccumulation

At each locality, thalli on rock (*Parmelia saxatilis*; forming up to 10-15 cm wide rosettes) and on bark (*Hypogymnia physodes* or alternatively, *H. tubulosa*; forming up to 4-5 cm wide rosettes) were collected for chemical analyses. Lichen samples were manually cleaned to remove extraneous material such as bark pieces, soil particles, moss fragments and milled with liquid nitrogen using ceramic mortar and pestle. Only the peripheral parts of the thalli (up to 1 cm from the lobe tips) were selected for the analyses. About 200 mg of powdered lichens were then mineralized with a mixture of 3 mL of 70% HNO_3, 0.2 mL of 60% HF and 0.5 mL of 30% H_2O_2 in a microwave digestion system (Milestone Ethos 900). Elements (Al, As, Cd, Cr, Cu, Fe, Ni, Pb, Zn) were quantified by inductively coupled plasma mass spectrometry (ICP-MS, Perkin- Elmer NexION 350) and the concentrations were expressed on dry weight basis. The analytical quality was checked with the Standard Reference Material IAEA-336 (lichen). Recoveries were in the range 89-109%. The precision of analysis was estimated by the variation coefficient of three replicates (range of variation within 6%).

Statistics

Generalized linear models (GLMs) were used to analyse the influence of bedrock and the presence/absence of forest canopy on saxicolous and epiphytic lichen diversity. In particular, factorial ANOVAs were carried out with SYSTAT 10.2 (Systat Software, San Jose, CA, USA) to evaluate significant differences in alpha-diversity, total specific frequency and total cover values, according to the different predictors. Significant differences between the studied conditions (forest with granites, FG; forest with serpentinites, FS; open field with granites, OG; open field with serpentinites, OS) were evaluated by ANOVA with a post-hoc Tukey test ($P<0.05$ as significant). Moreover, the relative importance of components of gamma-diversity (i.e. similarity (S), relativized richness difference (D), relativized species replacement (R)) was analysed for plots in the different environments, considering the different substrates and forest canopy conditions altogether and separately. The analysis was carried out on presence/absence matrices following the Simplex method and using the SDR Simplex software (Podani and Schmera 2011).

Ordinations of plots were performed on the basis of specific frequency data (and also cover data for saxicolous communities) by Principal Co-ordinate Analysis (PCoA: symmetric scaling, centring samples by samples, centring species by species; ter Braak and Šmilauer 2002). Moreover, a Canonical Correspondence Analysis (CCA) was carried out with the matrix of specific frequencies of saxicolous and epiphytic taxa and those of autoecological indices available in Nimis (2024), choosing the highest value for each index (CCA1), and of the functional trait dataset, also including secondary metabolites reported in at least three species, in order to partition variation explained by each variable and construct a model of significant variables (biplot scaling for interspecies distances, Hill's scaling for inter-sample distances; forward selection of variables option; Monte Carlo permutation test on the first and all ordination axes) (ter Braak and Verdonschot 1995). The ordinations were performed using CANOCO v. 4.5 (ter Braak and Šmilauer 2002).

GLMs were also used to evaluate the influence of bedrock type, the presence/absence of forest canopy, and the type of substrate (rock vs. bark) on the concentrations of PTEs within the lichen thalli, following the above-mentioned approach.

Results

Saxicolous and epiphytic lichen diversity in serpentinite and granite areas

A total of 113 lichen taxa was recorded through the surveyed outcrops and trees, consisting of 82 saxicolous taxa, 27 epiphytes and 4 taxa (*Cladonia pyxidata* (L.) Hoffm., *Parmelia saxatilis* (L.) Ach., *Parmelia sulcata* Taylor, *Physcia adscendens* H. Olivier) which colonized both substrates (Table S2).

GLM models (Table 1) calculated for saxicolous lichens showed that both variables, i.e. bedrock and the presence/absence of the forest canopy, significantly affected lichen cover, while only the latter factor was significant for the alpha-diversity and the total specific frequency. In particular, a higher number of saxicolous species characterized plots in open field areas with respect to forest ones, with similar values for granite and serpentinite (Fig. 2A). Consistently, total specific frequencies (Fig. 2B) per plot were lower in forest stands, on both granite and serpentinite outcrops, while significantly higher cover values characterized granite outcrops in open fields (Fig. 2C). In the case of epiphytic communities, species richness (Fig. 2D) and total specific frequency (Fig. 2E) per plot were similar in granite and serpentinite areas as well as in the forest stands and open fields, in agreement with an insignificant effect of bedrock and canopy presence displayed in GLM models (Table 1). In open fields, epiphytic diversity per plot was significantly lower than saxicolous diversity; instead, there were no differences in forest stands, although median values calculated for the epiphytes were the lowest.

SDR analysis (Table S3), defining the importance of gamma-diversity components, showed higher similarities in the case of epiphytic communities (22.0<S<38.0%) than for saxicolous ones (13.7<S<24.5%). Saxicolous communities in the forest and on granite displayed the highest beta-diversity (R+D=86%), and those in open fields showed the highest replacement (R=58.0%). Accordingly, in the PCoAs of specific cover (PCoA1; Fig. S1A) and frequency data (PCoA2; Fig. 3A) in saxicolous communities, plots in the different conditions were distinctly separated along the first (OG) and the second (FG, FS, OS) axes, while a weaker separation characterized epiphytic communities in the different conditions of bedrock and forest canopy occurrence (PCoA2; Fig. 3B).

In Canonical Correspondence Analysis 1 (CCA1), crossing specific frequency data and autoecological indices (Fig. 3), the first axis mostly displayed a positive correlation with aridity (AR, weighted correlation, w.c. 0.71) and eutrophication (EU, w.c. 0.70), generally separating saxicolous and epiphytic plots in the right and left sides of the diagram. The second axis mostly showed a negative correlation with poleotolerance (PO, w.c. -0.70), pH (w.c. -0.66), eutrophication (w.c. -0.60) and solar irradiation (SI, w.c. -0.39), separating forest plots in the upper quadrants and most plots in open field areas in the lower quadrants. Epiphytic and saxicolous plots mostly showed a positive and negative correlation with poleotolerance, respectively. According to forward selection, eutrophication (F=3.37, P=0.002) and poleotolerance (F=3.19, P=0.002) showed the highest conditional effects, followed by pH (F=2.30, P=0.006) and solar irradiation (F=1.57, P=0.040).

CCA2, crossing specific frequency data and functional traits, including secondary metabolites (Fig. S2), extracted four axes which only accounted for 42.2% of their relationships. Nevertheless, the first and all canonical axes were significant (Monte Carlo test; P<0.05), as well as some conditional factors, including the following metabolites and morphophysiological traits: rhodocladonic acid (F=4.03, P=0.002), physodic acid (3.87, 0.0012), fumarprotocetraric acid (3.25, 0.010), physodalic acid (2.74, 0.044), thamnolic acid (2.78, 0.010), barbatic acid (2.64, 0.014), atranorin (1.96, 0.018), norstictic acid (1.74, 0.030), reproductive mode (1.99, 0.016), and growth form (1.69, 0.042).

Content of potentially toxic elements

Both the saxicolous *Parmelia saxatilis* and the epiphytic *Hypogymnia* species showed accumulation of potentially toxic elements within their thalli (Fig. 5). The highest content was recorded for Fe (average values recorded in the whole study area ± SD: 891±867 mg kg^{-1}), followed by Al (345±116 mg kg^{-1}), Zn (61 ± 21 mg kg^{-1}), Cr (18±33 mg kg^{-1}), Ni (19±31 mg kg^{-1}), Cu (7.6 ± 1.0 mg kg^{-1}), Pb (5.3 ± 2.0 mg kg^{-1}), As (0.7 ± 0.6 mg kg^{-1}) and Cd (0.3 ± 0.2 mg kg^{-1}). GLM models (Table 3) showed similar patterns for Fe, As, Cr, and Ni on the one side, and Cd, Pb and Zn on the other. For the former group, both the substrate (tree vs. rock) and the bedrock (granite vs. serpentinite) were significant factors, while for the second only the substrate influenced the elemental contents detected in the thalli. In particular, significantly higher contents of Fe, Cr, Ni and As were detected in thalli from serpentinite rocks, independently from the presence or absence of forest canopy cover. In the case of Zn, Pb and Cd, different elemental contents characterized saxicolous and epiphytic lichens, independently from the bedrocks and the forest canopy occurrence, and metal-specific trends were recognized. For Zn and Cd, metal accumulation was higher in the epiphytic lichens, while it was higher in saxicolous ones for Pb. In the case of Cu and Al, no significant differences were detected according to the experimental condition, with the exception of a higher Al content in saxicolous lichens on granite with respect to all epiphytes and the thalli on serpentinites in open fields.

Discussion

The guidelines for biomonitoring airborne element bioaccumulation using epiphytic lichens recommend the use of a single species to ensure comparable data across studies (e.g. ISPRA 2020). Indeed, an interspecific variability in the bioaccumulation performance of epiphytic lichens was early recognized and, although initially related to several factors, including different environmental inputs, it has recently (unequivocally) been associated to species-specific morpho-chemical features (Fortuna et al. 2021). To improve dataset consistency, it is therefore advisable to limit the number of species used as biomonitors, focusing research efforts on a few well-characterized taxa (as carried out, for instance, with *Pseudevernia furfuracea* (L.) Zopf; Cecconi et al. 2018, 2019). Concerns over the influence of substrate on the elemental composition of lichen thalli (including in epiphytic species) (Bargagli and Mikhailova 2002) have further discouraged the use of saxicolous lichens in biomonitoring. However, in one of the few studies comparing metal accumulation in both saxicolous and epiphytic lichens, morphological traits such as growth form (e.g., foliose vs. fruticose) were found to influence concentrations of elements like Cu and Zn more than the nature of the substrate (rock vs. tree) (St Clair et al. 2002). In this regard, our work verified the hypothesis that, at least for a group of predominantly anthropogenic elements including chalcophiles such as Cd, Pb, Zn and Cu, foliose saxicolous lichens (*Parmelia saxatilis*) growing on different bedrock types and under different environmental conditions (forest canopy) may exhibit similar bioaccumulation patterns. Our results further demonstrated that the concentrations of these elements in saxicolous lichens were comparable to those found in nearby epiphytic species (*Hypogymnia physodes* and *H. tubulosa*), with only minor differences. These differences are likely attributable to species-specific functional traits, in line with patterns previously observed among epiphytic lichens (Fortuna et al. 2021). Accordingly, the use of foliose saxicolous lichens may be considered a viable option for biomonitoring certain elements, especially where epiphytic species are absent. The investigated lithologies (chemically distinct ultramafic serpentinite and acid granite), significantly influenced the composition of corresponding lichen communities in the Slavkov Forest (Fig. 3). However, their impact appeared subordinate to other environmental factors, particularly the presence or absence of a forest canopy. Previous studies have shown that climatic conditions strongly affect lichen diversity on different silicate rocks, from global to regional scales, prevailing on the rock chemistry even in the case of metal-rich serpentinites (Favero-Longo et al. 2018; Mulroy et al. 2022). In our study, the significant influence of forest canopy was also evident at the local scale, with higher saxicolous lichen diversity observed in open areas compared to forested plots (Fig. 2A), likely due to the crucial role of light availability. This aligns with other local-scale surveys that identified solar radiation as the primary factor influencing the functional traits of saxicolous lichens (Giordani et al. 2014).

Epiphytic communities across different bedrock and canopy conditions showed lower variability in alpha diversity and total specific frequency – reflected in the Lichen Diversity Value (LDV; Fig. 2D-E). It is important to note that the epiphytic survey has been limited to the 1 m band of tree trunks, as prescribed by the EN 16413 (2014) biomonitoring protocol, which likely led to an underestimation of the overall diversity. In contrast, rock environments tend to offer greater microhabitat heterogeneity and support higher functional redundancy, contributing to the observed higher saxicolous lichen richness (Giordani et al. 2019). Despite this, beta-diversity (R+D) was also lower in epiphytic than in saxicolous communities (approx. 70% vs. 80%; Table S3). This suggests that saxicolous and epiphytic communities correspond to different successional stages. Saxicolous communities, being more mature, appear to diverge due to deterministic factors - such as adaptation to different rocks and light regimes (Armstrong 2015), while epiphytic communities likely reflect early successional stages, influenced more by stochastic processes related to recent (re-)colonization dynamics (Giordani et al. 2018). The presence of poleotolerant species (mean PO index ± SE = 2.35±0.13), often represented by young thalli, characterized the epiphytic communities on the approx. 150 years old coniferous trees (Fig. 4). This pattern suggests a recent (re-)appearance of epiphytes, likely following a prolonged period of lichen absence – commonly referred to as a “lichen desert”, observed in Central Europe during the period of strong industrial activity during the last century (Paoli et al. 2020). The effects of historical industrial pollution likely interacted with lichen sensitivity to forest management practices (Nascimbene et al. 2013), as reflected in the land-use history of the Slavkov Forest (Krám et al. 2009, Hayes et al. 2020). In this context, epiphytes were forced to recolonize new substrates, which were periodically renewed in some areas, whereas rock surfaces offered greater ecological continuity over time.

This scenario is further supported by the low average concentrations of Cd, Cr, Cu and Zn in our epiphytic lichens - likely reflecting the decline in S and metal depositions from the 1990s. These concentrations are fully comparable to those previously reported for *H. physodes* in pine and spruce forests in Czechia and Poland (Kłos et al. 2011) as well as in natural parks in Poland (Kozłowsky and Strzyż 2020) (Table S4). Notably, Pb levels in the Slavkov Forest have decreased compared to past decades. For instance, average Pb concentrations in snow were 13.9–20.3 µg L^{-1} during 1981–1983 (Král et al. 1992), whereas bulk Pb deposition declined to 0.63 mg m^{-2} yr^{-1} during 1996–2011 (Bringmark et al. 2013). Moreover, concentrations of several elements (including Cr, Ni and

Pb) were lower in the Slavkov Forest than in spruce forests used as reference sites for trace element monitoring in Slovakia (Guttová et al. 2011). Cd, Pb and Zn were also significantly lower than those recorded in remote spruce forests of the Western Carpathians in Poland (Klimek et al. 2015) (Table S4). Most elements in *Hypogymnia* from the Slavkov Forest (with the exceptions of As, Cd and Zn) aligned with background concentrations found for *P. furfuracea* (Cecconi et al. 2018), with Cr level being even lower. For saxicolous communities (av. PO maximum indices ± SE = 1.69±0.09), high cover values and thallus dimensions suggest a persistence of the observed populations through the industrial era. Higher presence of saxicolous species tolerant to eutrophication in the case of open areas aligns with adjacency of surveyed outcrops with cultivated and intensively grazed fields (Zarabska-Bożejewicz 2020). This also contributes to a higher species richness with respect to forested areas (Pinho et al. 2012). In several cases, saxicolous lichens have been found to be more resilient to air pollution than epiphytic species, a phenomenon attributed to the buffering capacity of rock substrates, particularly in calcicolous communities that better tolerate SO_2 pollution (Gilbert 2000). With this regard, no differences in lichen abundance (species richness, cover, total specific frequency) were detected on the investigated acid (granites) and ultramafic (serpentinites) rocks (Fig. 2). Adaptation to metal stress, mediated by certain secondary metabolites that regulate uptake and exclusion processes - some of which were significant conditional factors in CCA2 (Fig. S2) - may also contribute to explain long term saxicolous tolerance and population persistence (Hauck et al. 2007, 2009a,b; Pawlik-Skowrońska and Bačkor, 2011; Favero-Longo 2014; Paukov et al. 2022). Norstictic acid, for instance, supports metal acquisition at slightly alkaline conditions, but also extracellularly binds metals, preventing the toxic effects (Purvis 2014). Thamnolic acid, produced by several *Cladonia* P. Browne species, was associated with the tolerance to acidic air pollution (Hauck et al. 2009c). Among epiphytic communities, higher correlation of open-field plots with physodalic acid, a major compound in *H. physodes* and *H. tubulosa*, suggests its role in increasing the adsorption of Fe^{3+} and decreasing that of other heavy metals, including Cu^{2+} (Hauck and Huneck 2007; Hauck et al. 2013).

The content of potentially toxic elements in *P. saxatilis* was quantified in the peripheral parts of lobes, up to 1 cm from the tips. As a radial growth of 2-3 mm yr^{-1} is documented for the species in favourable growth conditions (e.g., in Wales; Armstrong, 1981), the detected contents may be confidently related to the recent period of low atmospheric deposition. This likely justifies the overall congruence, in terms of order of magnitude, with results on epiphytes (Fig. 5). Higher contents may be instead expected in the inner parts of the saxicolous thalli, related to longer times and more intense patterns of deposition (Bargagli et al. 1987; Bargagli 1998).

Differences in elemental concentrations between *P. saxatilis* and *Hypogymnia* species may be attributed to their distinct secondary metabolite profiles and anatomical traits. While both share atranorin and its derivatives in the cortex, *P. saxatilis* contains salazinic acid in the medullary layer, whereas *Hypogymnia* species primarily produce physodalic and physodic acids (St Clair et al. 2002; Fortuna et al. 2021). These differences, rather than the nature of the substrate, may account for the slight divergences in elemental concentrations observed between saxicolous and epiphytic collections, particularly for Pb, Zn and Cd. Such species-specific differences were also observed in a study where thalli of various epiphytic lichens (including *H. physodes* and *Parmelia sulcata*, which share the same secondary metabolites as *P. saxatilis*) were exposed for three months in the city of Bratislava (Slovakia) (Guttova et al. 2011). In that study, Cd accumulated more in *H. physodes* than in *P. sulcata*, consistent with our findings showing higher Cd concentrations in *H. physodes* compared to *P. saxatilis* in the Slavkov Forest (Fig. 5I). Conversely, Pb displayed the highest accumulation in *P. sulcata*, and in our study showed significantly higher contents in *P. saxatilis* than in *H. physodes* (Fig. 5G). However, this pattern does not hold for Zn: while Zn accumulated most in *P. sulcata* in the Bratislava study, *P. saxatilis* showed lower Zn levels than *H. physodes* in our data (Fig. 5E). Similarly, Cu concentrations were comparable between epiphytic and saxicolous lichens in our study, despite higher Cu accumulation reported in *P. sulcata* elsewhere. The elevated Cu levels in *P. saxatilis* on serpentinites may partly reflect uptake from the substrate (Fig. 5F). The fact that many potentially toxic elements can be accumulated in particulate form (Loppi et al. 1998) may explain some of these inconsistencies and suggests that the contribution of secondary metabolites, while relevant, might be limited. A proper assessment of the role of morpho-chemical and anatomical traits would require dedicated experiments, which are beyond the scope of this study. Additional extrinsic factors may also help explain the differing accumulation pattern of Pb compared to Zn, Cd and Cu. For instance, with reference to trace metal budgets for forested catchments in Europe, Cd, Cu and Zn are characterized by higher internal circulation than Pb. In epiphytes lichens, this could imply a repeated, plant-mediated metal supply (Bringmark et al. 2013), potentially leading to higher accumulation than in saxicolous species.

Conclusions

Saxicolous and epiphytic lichens were compared in terms of diversity and elemental content across forested and open stands on serpentinite and granite areas. Forest canopy presence had a stronger influence than bedrock on saxicolous lichen diversity. In contrast, epiphytic lichen communities showed similar composition regardless of bedrock type or canopy cover. Accumulation in saxicolous lichens, specifically Fe, As, Cr, Al and Ni, was influenced by the underlying bedrock. For chalcophile elements (Cd, Zn, Cu, Pb), differences in concentrations between saxicolous and epiphytic foliose lichens may be attributed to intrinsic species traits, such as secondary metabolite profiles, rather than direct substrate uptake. The detected concentrations of Cd, Zn, Cu, and Pb (regardless of lichen, canopy presence, or bedrock) were consistently within the same order of magnitude. Moreover, the differences in accumulation between *Parmelia saxatilis* and *Hypogymnia physodes* did not exceed 250% (with the exception of Cd in open granite areas, around 320%), a range comparable to differences observed among epiphytic species alone (Fortuna et al. 2021). These findings support the use of saxicolous lichens as effective biomonitors of metal pollution in areas lacking epiphytic species. With appropriate considerations (in particular for those elements displaying negligible uptake from the bedrock), they may also complement epiphytic lichens in integrated biomonitoring studies.

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Ethical Approval and Consent to Participate
Not applicable.

Consent to Publish
Not applicable.

Authors contribution
SEF-L, LP and SL conceived the study. SEF-L, LP, EB, PK performed the field investigation; ZF, LP, EB, SEF-L revised the lichen material; SL provided data on toxic elements in lichens. All authors (SEF-L, LP, EB, ZF, PK, SL) contributed to data analysis and interpretation, prepared the first draft of the manuscript and read and approved the final version. SEF-L coordinated the project DiESCALiDi.

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Competing Interests
The authors have no relevant financial or non-financial interests to disclose.

Data Availability statement
All data analysed during this study are included in the article and the Supplementary Material

Tables

Table 1. Summary of the generalized linear models carried out on lichen diversity data.

	FOREST CANOPY (F/O)			**BEDROCK (G/S)**			**ERROR**	
	Mean-Square	**F-ratio**	**P**	**Mean-Square**	**F-ratio**	**P**	**Sum of-Squares**	**Mean square**
Saxicolous								
Diversity	291.050	41.000	**0.000**	21.050	2.965	0.093	276.855	7.099
Cover	4771.172	16.272	**0.000**	3506.029	11.957	**0.001**	11435.554	293.219

Frequency	24528.286	43.925	**0.000**	178.571	0.320	0.575	21777.905	558.408
Epiphytes								
Diversity	10.028	0.441	0.511	0.694	0.031	0.862	772.278	22.714
Cover	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Frequency	498.778	0.317	0.577	441	0.28	0.6	53468.222	1572.595

Table 2. Summary of the generalized linear models carried out on elemental content in lichen thalli.

	Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Al	Bedrock	10438.006	1	10438.006	0.072	0.789
	Canopy	5738.550	1	5738.550	0.040	0.843
	Substrate	351760.053	1	351760.053	2.423	0.125
	Error	9002353.684	62	145199.253		
Fe	Bedrock	1.73050E+07	1	1.73050E+07	9.821	**0.003**
	Canopy	44733.607	1	44733.607	0.025	0.874
	Substrate	1.95777E+07	1	1.95777E+07	11.111	**0.001**
	Error	1.09247E+08	62	1762048.827		
As	Bedrock	7.259	1	7.259	6.721	**0.012**
	Canopy	0.109	1	0.109	0.101	0.752
	Substrate	10.315	1	10.315	9.552	**0.003**
	Error	66.956	62	1.080		
Cd	Bedrock	0.396	1	0.396	2.517	0.118
	Canopy	0.489	1	0.489	3.108	0.083
	Substrate	0.664	1	0.664	4.220	**0.044**
	Error	9.758	62	0.157		
Cr	Bedrock	20196.037	1	20196.037	6.675	**0.012**
	Canopy	3281.901	1	3281.901	1.085	0.302
	Substrate	20670.837	1	20670.837	6.832	**0.011**
	Error	187595.800	62	3025.739		
Cu	Bedrock	113.810	1	113.810	1.720	0.194
	Canopy	13.286	1	13.286	0.201	0.656
	Substrate	3.343	1	3.343	0.051	0.823
	Error	4101.441	62	66.152		
Ni	Bedrock	22635.678	1	22635.678	18.836	**0.000**
	Canopy	589.585	1	589.585	0.491	0.486
	Substrate	19779.022	1	19779.022	16.459	**0.000**
	Error	74507.016	62	1201.726		
Pb	Bedrock	34.720	1	34.720	0.909	0.344
	Canopy	0.135	1	0.135	0.004	0.953
	Substrate	203.218	1	203.218	5.321	**0.024**
	Error	2367.716	62	38.189		
Zn	Bedrock	264.749	1	264.749	0.064	0.801
	Canopy	652.669	1	652.669	0.158	0.693
	Substrate	23216.003	1	23216.003	5.607	**0.021**
	Error	256731.448	62	4140.830		

Figures

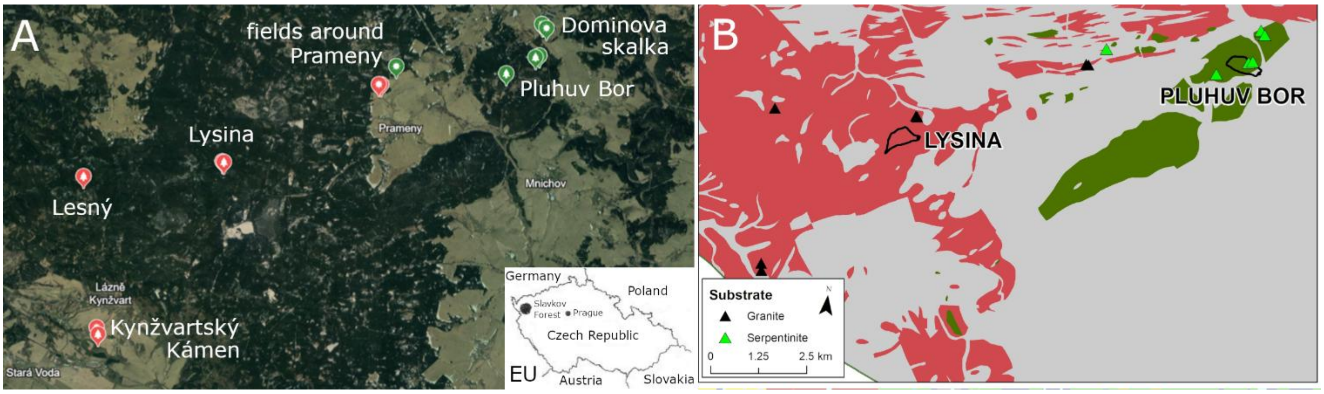

Fig. 1. Slavkov Forest Protected Landscape Area: (A) map of the surveyed localities in serpentinite (green marks) and granite (red marks) areas; (B) geological map (green: serpentinite areas; red: granite areas; triangles, surveyed localities).

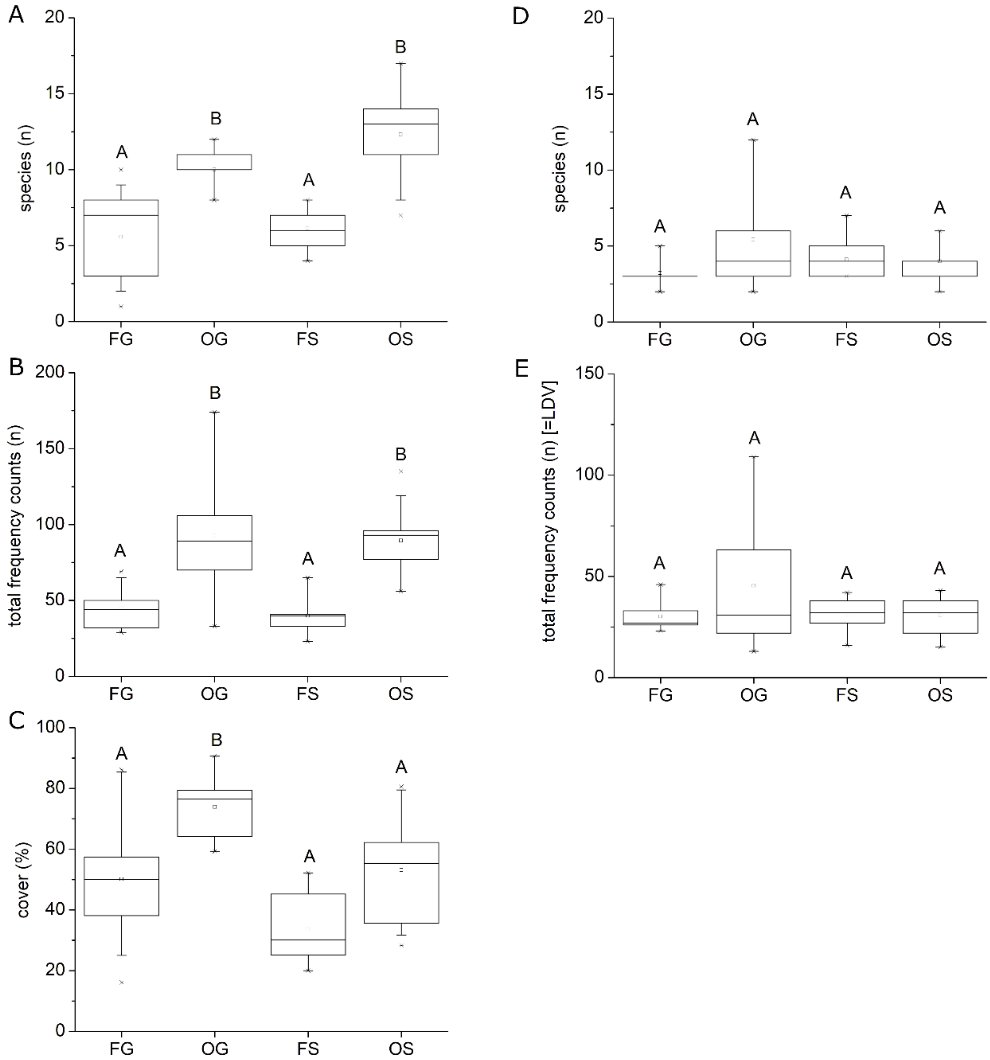

Fig. 2. Alpha diversity (A, D), total frequency counts (B, E) and cover (%, C) of saxicolous (A-C) and epiphytic (D-E) lichen communities in forested granite (FG) and serpentinite (FS) areas and in open field granite (OG) and serpentinite (OS) areas. Box-plots not sharing at least one letter significantly differ (ANOVA, Tukey's test, $p < 0.05$).

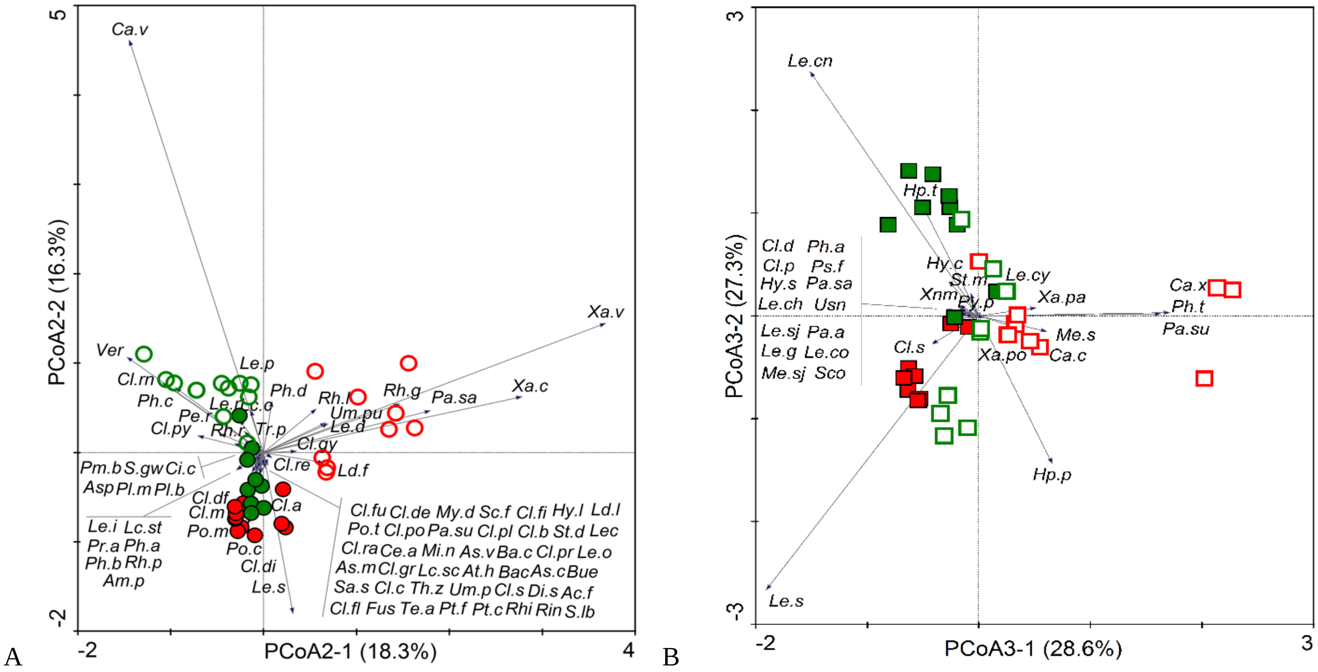

Fig. 3. Ordination of plots on the basis of specific frequencies of saxicolous (A; PCoA2) and epiphytic (B; PCoA3) lichens. Species abbreviations are listed in Table S2. Filled symbols, forest plots; empty symbols, open field plots; red, granite bedrock; green, serpentinite bedrock. Additional comment in the note to Fig. S1 (PCoA1).

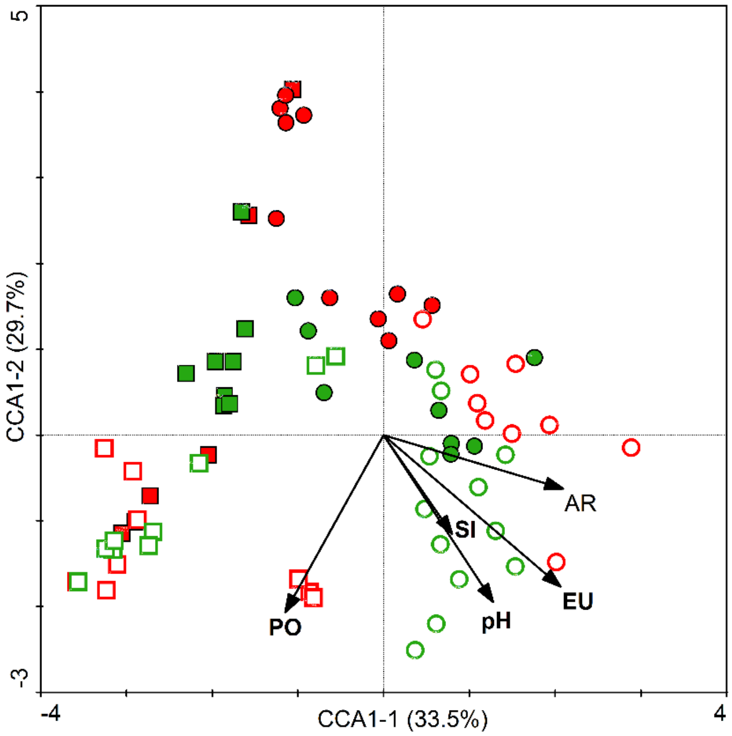

Fig. 4. Factorial maps in the canonical correspondence analysis showing the position of plots and the contribution of autoecological features of saxicolous and epiphytic species (indices of aridity, AR; pH of substratum, pH; solar irradiation, SI; eutrophication, EU; poleotolerance, PO). First and all canonical axes significant (Monte Carlo test; $P<0.005$). Squares, epiphytic; circles, saxicolous; filled symbols, forest plots; empty symbols, open field plots; red, granite bedrock; green, serpentinite bedrock. Significant conditional factors in bold.

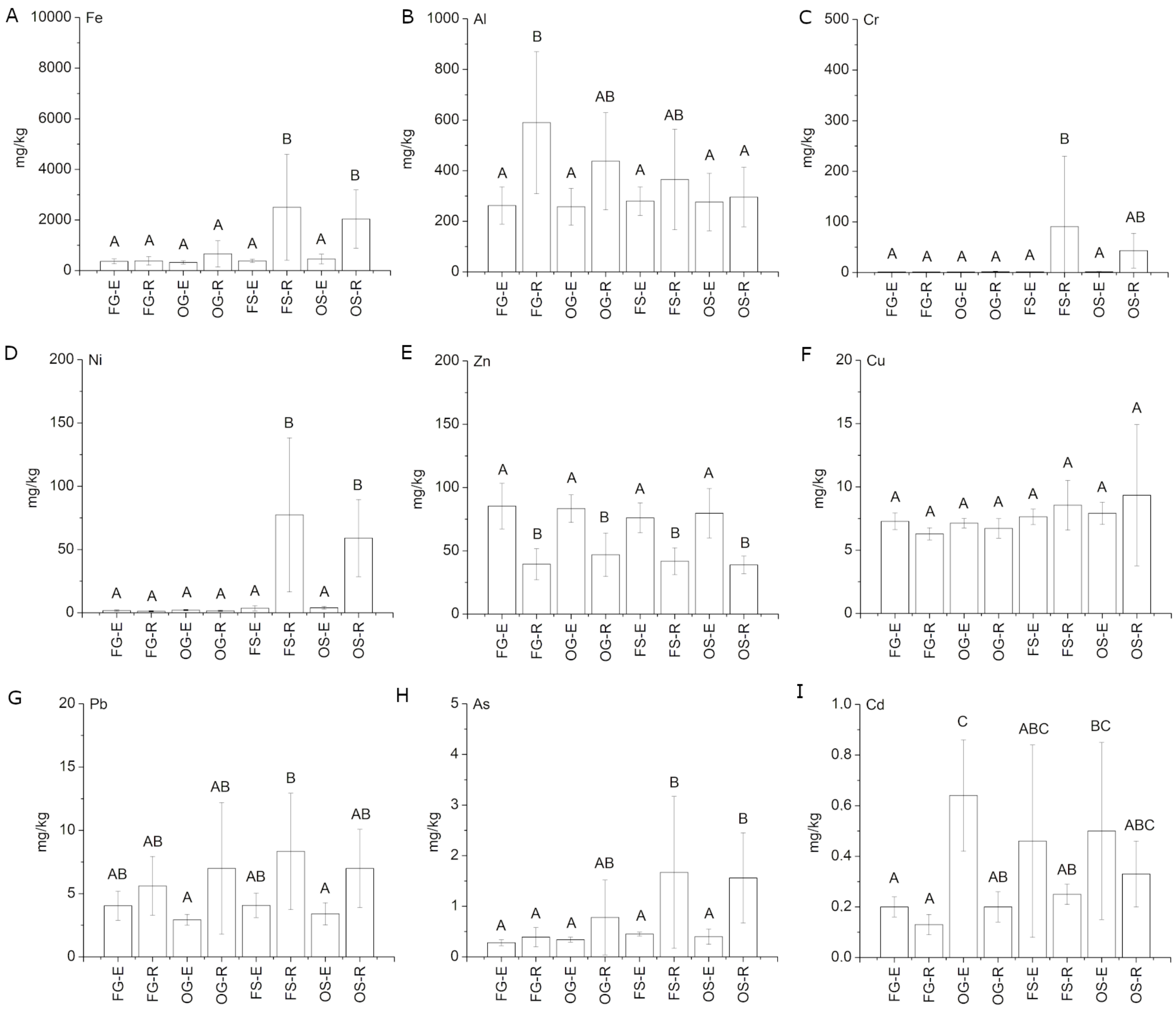

Fig. 5. Contents of Fe (A), Al (B), Cr (C), Ni (D), Zn (E), Cu (F), Pb (G), As (H), and Cd (I) in lichens (mg kg^{-1} dw). Codes marking each column indicate bedrock (G = granite; S= serpentinite), presence/absence of forest canopy (F=forest; O= open fields), and the monitored lichen (E, the epiphytic *Hypogymnia* ssp.; R, the saxicolous *Parmelia saxatilis*). Data are expressed as average values ± SD. Columns not sharing at least one letter are significantly different (ANOVA with Tukey test; P<0.05).