# **Indicators of restoration in beech stands after air pollution: trees and macromycetes**

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## **Abstract**

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The aluminium smelter in Žiar nad Hronom has operated since 1953. As a result, the surrounding area is now one of the most polluted regions in Slovakia. Since the implementation of new production and filtration technologies in 1996, the amount of emissions has significantly decreased. Our aim was to evaluate the long-term restoration of an environment that has been damaged by fluorine-based air pollutants. We analysed the contamination of forest ecosystems in three beech stands at various distances from the emission source (2, 7, and 18 km). Signs of restoration in adult beech trees were observed through a decrease in defoliation and a reduction in the necrotic disease of the bark in tree crowns. However, the impacts of air pollution on ectomycorrhizal associations persist. In the reduced number of ectomycorrhizal fungal species (16 species in the polluted stand compared to 38 species in the control stand), the low representation of sensitive fungal orders (Cantharellales, Gomphales, and Boletales), and the indices of species richness and heterogeneity (Hill, Margalef, Simpson, and Shannon–Weaver). In some respects, the findings indicate that the beech ecosystem is capable of revitalization within 25 years after a reduction in air pollution. However, much more sensitive indicators of successful restoration, compared to the characteristics of the trees, are the communities of macromycetes.

## **Keywords**

beech bark disease, defoliation, *Fagus sylvatica* L., fluorine, fungal diversity, ecosystem recovering

## **Introduction**

Although the European beech (*Fagus sylvatica* L.) is not the most endangered tree species within its range, it is an economically significant tree (MICHEL et al., 2023; Michopoulos et al., 2023). Beech became dominant in Europe after the last glaciation (VACHER et al., 2008) and formed important types of forest ecosystems (FODOR, 2020). Long-term pollution can negatively impact entire ecosystems (MIKULENKA et al., 2020; ZÁBOJNÍKOVÁ et al., 2024). Therefore, research on the survival, damage, and regeneration of forest ecosystems in anthropogenically disturbed environments is a current and pressing issue not only in forest ecology but also in climate research in general (van der LINDE et al., 2018; CHAZDON et al., 2021).

The area surrounding the aluminium smelter in the Žiar region is one of the most contaminated regions in Slovakia (MAŇKOVSKÁ and STEINNES, 1995). The primary cause of this region's ecological and environmental problems was the launch of industrial aluminium production in 1953. Since 1957, aluminium has been produced from bauxite, involving the calcination of the anode. By the early 1990s, the resilience potential of the beech stands had declined, and the species diversity of ectomycorrhizal (ECM) fungi was very low (Štefančík and Mihál, 1993; PAVLÍK, 1997). In 1993, the amounts of emitted fluorine (in the form of fluorides and HF) and SO2 were 364 and 3.6 tons per year, respectively. The amounts of emitted pollut-

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ants have decreased, mainly due to new aluminium production technologies (Tóth et al., 2014). However, although current pollutant production is within the legislative limit values (*Annual report*, 2021), soil contamination around the aluminium smelter still exceeds the hygienic limit for water-soluble fluorine in the soil (García-Gil et al., 2013).

In the Žiarska kotlina basin, a significantly negative effect of air pollution is the epiphytotic occurrence of beech bark disease (BBD), which negatively impacts the health, vitality, and wood quality of beech trees (Cicák and Mihál, 2005). Understanding the interactions between necrotic pathogens and host trees at the tissue anatomical level can help prevent diseases and the subsequent damage that they cause (GEORGIEVA et al., 2024). Environmental changes caused by air pollution can create favourable conditions for fungi (*Nectria* spp.), which are pathogens for weakened plants (MIHÁL et al., 2019).

The presence of fungi can be used as an indicator of natural and healthy forests (EGLI, 2011). Macromycetes, a significant group of fungi, are good indicators of species diversity loss in forest ecosystems, and they indicate the degree to which these ecosystems are disturbed by human activity (Luptáková et al., 2018; Salerni et al., 2020). In this context, fungi forming ectomycorrhizal symbioses are significant, as these symbioses are essential for the optimal growth and development of all forest tree species. There are two main effects of emissions on mycorrhizae. First, the impact of emissions on the above-ground parts of trees can reduce photosynthesis and increase respiration, thereby reducing the allocation of carbon to the roots, which is needed for their growth and support of mycorrhizae. Second, changes in soil pH and chemical composition can increase the mobility of toxic elements and, together with the direct input of nitrogen, increase nutrient requirements (DIGHTON and JANSEN, 1990; Frey et al. 2004). Ectomycorrhizal fungi play a major role in early primary and secondary succession even after large anthropogenic disturbances, for example, on mine tailings (Kałucka and Jagodziński, 2016) as well as in air-polluted forest stands (Mihál and Barna, 2022; Kowal et al., 2022).

Our goal was to analyse the ecological indicators of the recovery process in beech ecosystems damaged by air pollution. We addressed this by evaluating two main features: i) the health status of adult beech trees, characterized by the presence of necrotic diseases in stems and crowns and the defoliation of crowns, and ii) the diversity of ectomycorrhizal macromycetes in beech stands at three research sites established at different distances from the aluminium smelter.

## **Materials and methods**

## **Study area**

This study was conducted in three mature beech forests in the Western Carpathians (Central Slovakia, Fig. 1). The forests were selected at varying distances from an aluminium smelter near Žiar nad Hronom, with different levels of pollution. Two sites orographically belong to the Štiavnické vrchy mountains (S1: 2 km from the aluminium smelter; S2: 7 km), while the third and most distant location is in the Kremnické vrchy mountains (K: 18 km).

Site S1, the closest to the aluminium smelter, was directly affected by fluoride pollution from 1953 to 1996 (Kontrišová and Holub, 1991; Jamnická et al., 2007). The electrolysis process originally used Söderberg technology, which was environmentally unsuitable. The Söderberg technology negatively impacted the entire surrounding area and led to habitat degradation. The flora and fauna in the vicinity of the polluting sources began to show adverse effects from the emissions shortly after the plant began operations (Maňkovská and Steinnes, 1995).

Although new aluminium production technology was introduced in 1996, the fluoride concentrations did not fall below the permissible pollution limits until after 1998 (Schwarz et al., 2009, Table 1).

The dominant species in the forest stands at sites S1 and S2 are mature beech trees with natural development, without long-term silvicultural intervention (SHARMA et al., 2019). The forest stand at site K exhibits similar characteristics (Horemans et al., 2016). The basic characteristics of all three sites are presented in Table 2.

#### **Experimental design**

## **Trees**

Mature beech trees were randomly selected in a 50 m wide strip at each research site. From each stand, 24 undamaged trees were selected for analysis; the trees were not direct neighbours, and the distance between them was at least 30



Fig. 1. Location of research sites (S1: 2 km from the smelter; S2: 7 km; and K: 18 km), Slovakia, Central Europe.

Table 1. Mean pollutant emission estimates from the aluminium smelter in  $\check{Z}$ iar nad Hronom (t yrs<sup>-1</sup>) for the following periods: 1995 (old smelter technology), 1996 (transition to new smelter technology), since 1997 (new smelter technology). Source: https://www.slovalco.sk/ [accessed 07. 07. 2019]

Pollutant	1995	1996	1997	1998	1999	2000
$F^*$	0.5	22.0	7.8	'7.5	2.0	$\sim$ 1
HF	319.5	51.5	41.7	40.3	31.7	28.1
SO.	1.943.2	1,543.0	1,108.8	1,108.8	1,511.0	1,179.0

\*Fluorine in dust





\*Dubová and Bublinec (1994); \*\*Bučinová (2008).

m. Only trees from the first three of Kraft's classes were considered (1. predominant, 2. dominant, and 3. codominant), since it was assumed that they mostly influence the plot reflectance (ICP Forests, EICHHORN et al., 2020). Tree defoliation was the main parameter measured in the survey and was classified from 0% (no defoliation) to 100% defoliated (dead tree) in 5% increments (EICKENSCHEIDT et al., 2019; Michel et al., 2023). The defoliation value for a plot was calculated as the mean defoliation value of the 24 trees from the dominant stand which received some light from above (Kraft's classification, Hawryło et al., 2018).

We surveyed the forests for defoliation (leaf loss) on 24 July 2019, when the trees were in full leaf. The main objective of crown condition monitoring by ICP Forests (Eichhorn et al., 2020) is the acquisition of periodic information on the temporal variation in tree vitality in relation to biotic and abiotic stress factors. Therefore, in our results, we also include our previous measurements from 2014 and 2016 from the same sample plots (unpublished). We assessed BBD (beech bark disease) on both the stem and the crown of the trees separately. These evaluations were conducted outside the growing season, when the trees were leafless (November 2021), according to the rating scale defined by Cicák and Mihál (1997). We quantified BBD from grade 0 (no necroses) to grade 4 (presence of large and "breakthrough" necroses). The necrosis of branches in the beech crowns was rated on a 4-point scale (0–3). The degree of stem necrosis was visually assessed around the entire stem circumference, from the root flares to the crown base, and the health status of the branches in the crowns was evaluated using binoculars (Mihál et al., 2019).

#### **Macromycetes**

In each stand at each site, we established three research plots measuring  $15 \times 50$  m, i.e., 750 m<sup>2</sup>. For submontane beech forests, this area is sufficient for mycocenological observations (ARNOLDS, 1981; Bučinová, 2008). The species spectrum and abundance of ectomycorrhizal (ECM) macromycete fruiting bodies were recorded using the transect walk method along the contour lines within each research plot area. We conducted these surveys in the first week of October in 2019 and 2020, when the conditions for macromycete growth were optimal. Within the

research plots, we documented the species spectrum of ECM fungi along with the abundance of produced fruiting bodies. Unidentified or ambiguous species in the field were characterised under laboratory conditions. The systematic classification of macromycete species was based on macroscopic and, if necessary, microscopic identifying features using available mycological atlases and keys (see Mihál and Barna, 2022). The taxonomic classification and scientific nomenclature of the determined macromycete species largely follow the CABI Bioscience Index Fungorum database (Kirk, 2024).

#### **Quantification of macromycetes species diversity**

Species diversity is based on species composition, which refers to the identity and variety of elements in a group (Hlôška et al., 2022). Macromycete species diversity was quantified using the following diversity indices: i) indices based on the number of species, and (ii) indices of species heterogeneity, which combine species richness and evenness. From the available indices, those that were most suitable for the numerical assessment of diversity in the examined populations (Merganič et al., 2016) were selected as follows:

i) Two indices were used to evaluate species richness:

Hill (1973): *N*0 = S

MARGALEF (1958):  $R1 = (S - 1) / \ln(N)$ .

The calculation of the Margalef index is based on the number of species in relation to the size of the population; the higher the value, the greater the species richness.

ii) Two indices were used to evaluate species heterogeneity. SIMPSON (1949):  $D = 1 - \sum p_i^2$ 

Simpson's index measures the probability that two randomly selected organisms are of the same species. It is defined as  $D = \lambda$ ; however, to ensure that the values logically grow from 0 to 1 in accordance with increasing heterogeneity,  $D = 1 - \lambda$ .



Fig. 2. Defoliation at the studied sites (S1, S2, K) by year (2014, 2016, 2019). The sites were situated at different distances from the aluminium smelter (S1: 2 km; S2: 7 km; K: 18 km). Error bars indicate the 95% confidence intervals.

$$
SHANNON and WEAVER (1949): H'=-\sum_{i=1}^{8} (p_i \ln(p_i))
$$

The Shannon–Weaver (*H'*) index of overall diversity combines both species richness and evenness aspects. It shows how often the species are encountered. When the number of species is the same in two sample sets, the index has a higher value in the set where individual species are more evenly represented. The value of the Shannon index generally ranges from 0 to ln S.

In the above equations, S is the number of species, N is the number of fruiting bodies,  $p_i$  is the relative frequency of fungal species ( $p_i = n_i / N$ ), and  $n_i$  is the frequency of species *i*.

The statistical analyses were performed using STA-TISTICA software (Stat Soft Inc., Tulsa, OK, USA). The variability in the measured characteristics between the monitored beech ecosystems was tested using a Kruskal– Wallis ANOVA model. Multiple comparisons of p-values (two-tailed) were conducted using the K-W test for the three analysed sites. Spearman's correlation was used to analyse the linear relationship between variables (bark disease of stems versus bark disease of crowns of the same trees).

## **Results**

#### **Trees: defoliation**

On all the plots, beech trees were classified as class 1 for defoliation, indicating slight defoliation (10–25% defoliation). In all the studied sites for the measured years (2014, 2016, 2019), we did not find significant differences in crown defoliation. The negative impact of the aluminium smelter was not confirmed (Kruskal–Wallis ANOVA 2019:  $H = 4.273$ :  $P = 0.118$ ). The differences between groups were not significant (Fig. 2).



Fig. 3. Degrees of necrotic diseases of beech at sites at different distances from the aluminium smelter (S1: 2 km; S2: 7 km; K: 18 km) in November 2021. Different letters indicate significant differences at  $P < 0.05$ ; N = 24; error bars show 95% confidence intervals.



Fig. 4. Abundance of ECM fungi at research sites (S1: 2 km from the smelter; S2: 7 km; K: 18 km) per are  $(100 \text{ m}^2)$  in 2019 and 2020. Different letters indicate significant differences at *P* < 0.05; error bars show 95% confidence intervals.

## **Trees: beech bark disease**

The impact of the aluminium smelter on BBD manifested only on the tree stems, with both sites S1 and S2 (2 km and 7 km from the pollution source) having significantly worse health status compared to the control site K (18 km away). For the beech crowns, we observed a decrease in disease severity with increasing distance from the source, but this trend was not significant (Fig. 3). Interestingly, the relationship between stem and crown diseases on the same trees was statistically significant according to Spearman's correlation (R =  $0.528, P \le 0.001$ ).

## **Macromycetes: abundance**

The dynamics of ECM macromycete fruiting body abundance showed a similar trend in both years of the study (Fig. 4). In the beech forest closest to the aluminium smelter (S1: 2 km from the pollution source), the abundance of ECM species was the lowest. A significant difference in ECM density compared to other sites was observed at site K. Table 3 shows that proximity to the pollution source significantly affected the abundance of macromycetes in both study years.

## **Macromycetes: species richness**

In the environment with the highest level of air pollution (S1), we found the lowest total number of ECM species

among the studied sites. Species richness (*N*0) significantly increased with distance from the emission source, and Margalef's species richness index results also indicated a significant impact of the aluminium smelter (Fig. 5, Table 3).

#### **Macromycetes: orders and species**

A total of 54 ECM macromycete species were recorded. Agaricales was the most numerous order across all sites (Table S1, Fig. 7). The fruiting bodies of the species *Hygrophorus eburneus* were found every year at all the research plots in individual locations. Other species from Agaricales found at all locations were *Laccaria amethystina* and *Inocybe fastigiata*. From the order Russulales, species such as *Lactarius blennius*, *L. subdulcis, Russula cyanoxantha*, and *R. foetens* were present. Fungi from the orders Cantharellales and Gomphales were not found at all at location S1, which was the most affected by air pollution. Furthermore, the order Boletales was very rare at location S1 (we found only one fruiting body each of the species *Xerocomus subtomentosus* and *Xerocomellus chrysenteron* in 2019). Unique species distributions were observed at each site: *Lactarius quietus, Tricholoma album, Russula pseudoaeruginea and R. virescens* were only found at S1; five species were only present at S2; and eight species were only observed at K. These findings indicate greater species diversity at site K.

## **Discussion**

## **Defoliation**

Defoliation is a critical parameter for assessing tree health in forest monitoring, indicating the loss of leaves in the crown compared to a fully foliated reference tree. The average defoliation of trees in the studied beech stands has been below 20% since 2014, with no statistically significant differences between sites ( $P = 0.4605$ ). All the studied beech stands fell into defoliation class 1, which corresponds to a defoliation range of 10–25%. According to the transnational crown condition survey from 27 countries in 2019, 44.9% of plots belonged to this defoliation class (Michel et al., 2020). The same source, the ICP Forests Technical Report on Forest Condition in Europe in 2019, reported that common beech had a mean defoliation of 21%, the lowest among the main tree species in Europe.

In the years 1988–1993, the defoliation values at site S1 were more than double the values at site K (Cicák

Table 3. Kruskal–Wallis ANOVA model results showing the significance (*P*-value) of the relationship between the aluminium smelter's distance from beech ecosystems and the species diversity of macromycetes during the investigated period (2019 and 2020)

Effect	Abundance			Species richness		Margalef		<b>Shannon</b>		Simpson	
2019	5.970	0.050	6.771	0.034	7.200	0.027	6.489	0.039	7.200	0.027	
2020	5.980	0.050	5.980	0.050	6.489	0.039	3.822	0.148	.689	0.429	



Fig. 5. Species richness of ECM mycobiota (mean number of taxa per are) at research sites (S1: 2 km from the smelter; S2: 7 km; K: 18 km) in 2019 and 2020: a) *N*0 index (HILL, 1973); b) species richness index *R*1 (MARGALEF, 1958). Different letters indicate significant differences at *P* < 0.05; error bars show 95% confidence intervals.



Fig. 6. Species heterogeneity of ECM mycobiota (per are) at research sites (S1: 2 km from the smelter; S2: 7 km; K: 18 km) in 2019–2020: a) Simpson's index (*D*); b) Shannon–Weaver index (*H'*). Different letters indicate significant differences at P < 0.05; error bars show 95% confidence intervals.



Fig. 7. Relative representation of macromycete species in beech stands (S1: 2 km from the smelter; S2: 7 km; K: 18 km) by order; a) all found fungal species; b) excluding species with only one fruiting body found during the entire study.

et al., 2011). The poor health condition of beech trees in the Žiarska kotlina basin was evident not only in the high defoliation values but also in the tree mortality, which reached 21% between 1991 and 2004 (Cicák and Mihál, 2005). A significant decline in defoliation was recorded in 1997, one year after the change in aluminium production technology. High defoliation values at site S1 have also been influenced by periodic outbreaks of folivorous insects (Šušlík and Kulfan, 1993).

## **Beech bark disease**

Air pollution negatively affects adult beech trees in forest stands through BBD (beech bark disease). Cicák et al. (2011) described several negative impacts in the stand at the S1 site. CALE et al. (2017) evaluated the impact of nitrogen pollutants on necrotic disease prevalence in *Fagus grandifolia* Ehrh. in North America, and AUGUSTAITIS et al. (2015) evaluated the impact of ozone on beech stands in Lithuania. We found a significant impact of the smelter on the stem part of beech trees (Fig. 3). The necrotization of crowns also decreases with distance from the emission source, but not significantly. The state of necrotization has improved over the last 20 years and showed similar trends at all sites (Mihál et al., 2019). In stand S1, situated closest to the smelter, the necrotization values were significantly worse than those in the other stands. This trend persisted at the S1, S2, and K sites from 1995 to 2004. However, since 2004, significant changes have occurred, and the necrotization index value at S2 is now close to the value at site K. Cicák and Mihál (2005) attribute the cause of BBD improvement at site S2 to the higher vitality of trees compared to site S1, which has experienced greater impacts of air pollution.

#### **Macromycetes**

The smelter in Žiar nad Hronom was mainly observed to have a negative impact on ECM (ectomycorrhizal) macromycetes, as these species never appeared among the most dominant macromycete species. Rather, the most dominant fungi were saprotrophic macromycetes (Mihál and Bučinová, 2005). A negative impact of aluminium ions on ECM macromycetes was also reported by  $\check{Z}_{EL}$ et al. (1993). They found that increased concentrations of aluminium ions in soils around an aluminium smelter evidently damage the mycelium cell membranes of symbiotic fungi, thereby hindering or completely stopping the intercellular transport of other elements, such as biogenic elements. According to RUOTSALAINEN and KOZLOV (2006), the effects of air pollution from industrial factories (including six aluminium smelters) decreased the species abundance and diversity of fungi with increasing emission loads. Pollutants with a longer history of emission exhibited more adverse effects on fungi.

We found that the impact of air pollution from the smelter on the ectomycorrhizal mycobiota still persists today, affecting both species diversity and density (Figs 4 and 5). From 1990 to 2020, the species diversity of ECM macromycetes in beech stand S1 near the smelter (6–14 species) was always lower compared to that in the control site K in the Kremnické vrchy mountains (18–33 species), which are 18 km from the smelter, with a difference of 52– 71% (Mihál and Barna, 2022). At the S2 site (7 km from the smelter), the number of ECM species was 21–44% lower than that at site K. With increasing distance from the smelter, the production of biomass and abundance of fruiting bodies significantly increase (Mihál et al., 2019).

Interestingly, according to Šmelko (2008), species richness (*N*0 index) is completely and positively dependent on the number of individuals, which is true for sites S1 and S2. At site K, however, this trend does not apply due to the much higher number of species that only grew there. In particular, the number of fruiting bodies present at site K represented up to 74% of the total number of fruiting bodies found at all three sites. At sites S1 and S2, these shares were only 8% and 18%, respectively, resulting in more similarities between sites S1 and S2 compared to site K.

Beech stands with similar growth (decomposition and ecological) conditions generally have a uniform species composition and distribution of fungi (FODOR, 2020). Using Simpson's diversity index, we found that, in 2019, sites S2 and K had significantly higher species heterogeneity than site S1, which was near the smelter (Fig. 6a). However, in 2020, there was no significant difference between S1 and S2, as the Simpson's diversity index at S1 significantly increased from 0.54 to 0.73 that year. The general increase in the abundance (Fig. 4) and diversity (Fig. 5) of ECM macromycetes in 2020 compared to the previous year was significant only in the beech stand near the smelter (S1). In terms of the Shannon index, biodiversity showed higher values at all sites in 2020 compared to 2019. This implies that the growth conditions in that year were more favourable for species heterogeneity and the overall diversity of ECM macromycetes at all three sites (Fig. 6b).

## **Conclusions**

The impact of the environment on growth conditions and forest stand damage depends on a wide range of ecological relationships that govern forest ecosystems. For these reasons, it is important to clarify the revitalization and restoration processes taking place in anthropogenically disturbed beech ecosystems, which was the aim of this study. We observed signs of restoration in adult beech trees in forest stands damaged by air pollution through a reduction in defoliation and necrotic branch bark (in the crowns). However, a significant negative impact of pollution from the smelter still persists in the necrotization of beech stems. Information on the causes of tree damage and their impact on crown conditions is essential for studying the impacts of air pollution on forests. These data can also contribute to other aspects that are relevant to forest policy, such as sustainable forest management. Another indicator of the health status of beech ecosystems damaged by air pollution is mycological conditions. In the stands around the smelter, which were heavily affected by air pollution in the past, the diversity and abundance of ectomycorrhizal fungal fruiting bodies continue to be negatively impacted.

The results confirm a capacity for the revitalization of trees in emission-affected beech ecosystems, even 25 years after a reduction in emission pollution. In the case of mycobiota, recovery from the strongly negative impact of air pollution is gradual. However, under certain climatic conditions, such as those observed in 2020, Simpson's (*D*) and Shannon–Weaver (*H'*) indices no longer measured a significant difference in the species heterogeneity of ECM fungi between air-polluted stands and the control forest.

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## **Supplementary material**

Table S1. List of recorded ECM fungal species



● occurrence of the species; ● occurrence rare, only one fruiting body found; ● abundant occurrence, every year on all plots.