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Original Research

Deciphering Factors Controlling Manganese Concentrations in the Leaves of Silver Birch (*Betula pendula* Roth) in Relation to Recent Acidification of Mountain Forest Soils

Gabriela Bílková[1,2]*, Jitka Elznicová[1], Eliška Wildová[1], Michal Hošek[1,2], Tomáš Matys Grygar[1,2]

[1]Faculty of Environment, J. E. Purkyně University in Ústí nad Labem, Czech Republic
[2]Institute of Inorganic Chemistry of Czech Academy of Sciences, Řež, Czech Republic

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Abstract

Manganese concentrations in silver birch leaves were studied in the Ore Mountains and several reference localities in Czechia, both mountainous (up to 950 m MSL) and lowland (ca. 300 m MSL). The studied mountainous areas have been seriously damaged by acid rains in the last decades and forests there have still not recovered from that crisis. The aim of this study was to identify the major controls on the foliar Mn concentration variability, which was more than one order of magnitude, from 200 mg kg^{-1} (10th percentile) to 2000 mg kg^{-1} (90th percentile) in the mountainous sites, that is, under uniform environmental conditions and for the same emission history of mountainous forests. The foliar Mn concentrations in the highest 5% samples were >3000 mg kg^{-1} which can be close to toxicity for persistent tree growth and thus indicate actual local geochemical stress in plants. Manganese uptake by plants has been enhanced by acid rain in the last decades, inferred from low foliar Mg (ca. 0.15%) and soil acidity (soil pH down to 4.0 in aqueous extracts). Certain bedrocks in the Ore Mountains, particularly local granitic porphyry and mafic lavas, have elevated Mn concentrations; however, in the mafic lavas, the soil Mn excess is compensated by elevated concentrations of soil Mg and pH. The highest foliar Mn concentrations were found in specimens growing in soils on granitic porphyry and on certain granites. The Mn concentration in birch leaves could be proposed as a proxy for the impact of soil acidification in central European mountain forests.

Keywords: acid soils, phytotoxicity, manganese, geochemical mapping

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*e-mail: gabriela.bilkova@ujep.cz

Introduction

In the 20th century, forests in west and central Europe and north-east USA were damaged by acid rain, particularly those on nutrient-poor soils developed from felsic rocks. Leaching of Mg and Ca nutrients and the increasing mobility of Al and Mn were soon recognised as a consequence of acidification that is most detrimental to trees [1-3]. Czech spruce forests declined mainly in the 1970s and the 1980s due to acidification [4, 5]. It also concerned the Ore Mountains along the Czech-Germany state boundary, where the forest decline was attributed mainly to SOx emissions from fossil fuel combustion. By the end of the 20th century, although coal mining, fossil fuel combustion, and SOx emissions had declined throughout the Ore Mountains, forests have not recovered and remained unstable [5, 6]. SOx emissions were partly replaced by NOx emissions, acidification has thus persisted [3, 5, 7] and is now combined with eutrophication. The evaluation of soil recovery after the acid rain peak is hindered by the paucity of information on the soil chemistry before the damage was done [3, 6, 8], which prompts a deeper understanding of soil element cycles in acidified areas. Mapping of acidification impacts on trees would be useful for understanding the extent of the problem.

The uptake by plants of Al and excessive amounts of Mn is not controlled exclusively by soil pH. The Mn uptake results from the interplay of Ca, K, Mg, Mn, N, and P uptake [2, 3, 9-13]. Vice versa, excess Mn in plants can suppress uptake and cellular utilisation of nutrients such as Ca, Mg, and Fe [13, 14]. Our preliminary evaluation of foliar Mn concentrations in trees from the Ore Mountains indicates the possibility that Mn uptake by birch and larch is enhanced at a higher availability of Ca^{2+} and a lower availability of Mg^{2+} [15], but this hypothesis needs verification. The Ore Mountains have a complex geology, mostly with felsic rocks, commonly with ore veins, and were severely impacted by industrial activities based on coal use on both sides of the German Czech border and, to a lesser extent, in Poland.

Thus, it is not easy to distinguish between natural and anthropogenic factors in this area, known by anomalously high concentration of risk elements [16]. Deciphering the anthropogenic impacts in such regions is a challenging task that requires particular attention.

Manganese concentration variability in birch leaves is a major topic of this study. Birch is one of the most common tree species in the study area [17] and in other temperate and boreal European forests. Previous studies in the Ore Mountains attributed the high Mn concentrations in the foliage of forest plants exclusively to industrial emissions [18, 19] . This conclusion needs critical evaluation, because interelement relationships in soils and plants and the impact of bedrock geology have not been studied yet, although bedrock geology is known to affect the response of soils and surface waters to acid rain [4, 20]. Reimann et al. [21, 22] also found that Mn in birch leaves in the Oslo transect varied by one order of magnitude, between 500 and 5000 mg kg^{-1}, probably as a consequence of variable bedrock geology [23], but they did not attempt to investigate the controlling mechanisms. Our study sheds light on the factors driving Mn uptake by birch. We assume high birch foliar Mn concentration observed in some sites in Ore Mountains could be suitable for monitoring impacts of the past acidification of soils on felsic rocks. The topic seems urgent, because poor health state of forests in central Europe is obvious from recent bark beetle outbreak. For this, we need to characterise whether bedrock geology, Mg content, or other possible factors can control Mn uptake by birch. The aim of this work is to decipher major factors behind foliar Mn concentrations in the target tree species as a consequence of soils geochemistry, likely considerably impacted by emissions.

Material and Methods

Study Area

The study area covered mainly the mountains of western Czechia (Fig. 1). For comparison, lowland localities were also included to test the variability in Czech environmental conditions.

The eastern part of the Ore Mountains and the Děčínský Sněžník area were the main focus of this study. The Ore Mountain ridge has an elevation of 700-1000 m MSL. Gneisses, metamorphic rocks derived from both intrusive volcanic rocks and sandy sediments are dominant rocks in the Ore Mountain-Sněžník ridge, with minor areas covered by granite, metagranite, mafic effusive rocks (basalts), ignimbrite and granitic porphyry. The high geological variability of the Ore Mountains is documented in the Litvínov-Fláje belt (Fig. 2) with almost all types of rocks present in the entire mountain range outcropping here. Two types of reference localities were also sampled: mountainous and lowland. Reference mountain terrains included sites in the Slavkov Les Mountains, Český Les Mountains, and the NW edge of the České Středohoří Mountains. The second type of reference area included lowlands between the foothills of the Ore Mountains and the NW edge of the České Středohoří Mountains in the area of Teplice. Major sources of acid emissions near the study areas were related to coal mining and processing in the Ohře Rift, just downslope of the Ore Mountains [5, 16].

Sampling and Sample Processing

The sampling sites are shown in Fig. 1. Initial sampling of soils started in 2018 in the Ore Mountains-Děčínský Sněžník area [15] within preceding monitoring network [24]. Most sites in that network were situated in colluvia and boundaries of geological units. Novel sampling was performed in August 2021, both to adjust

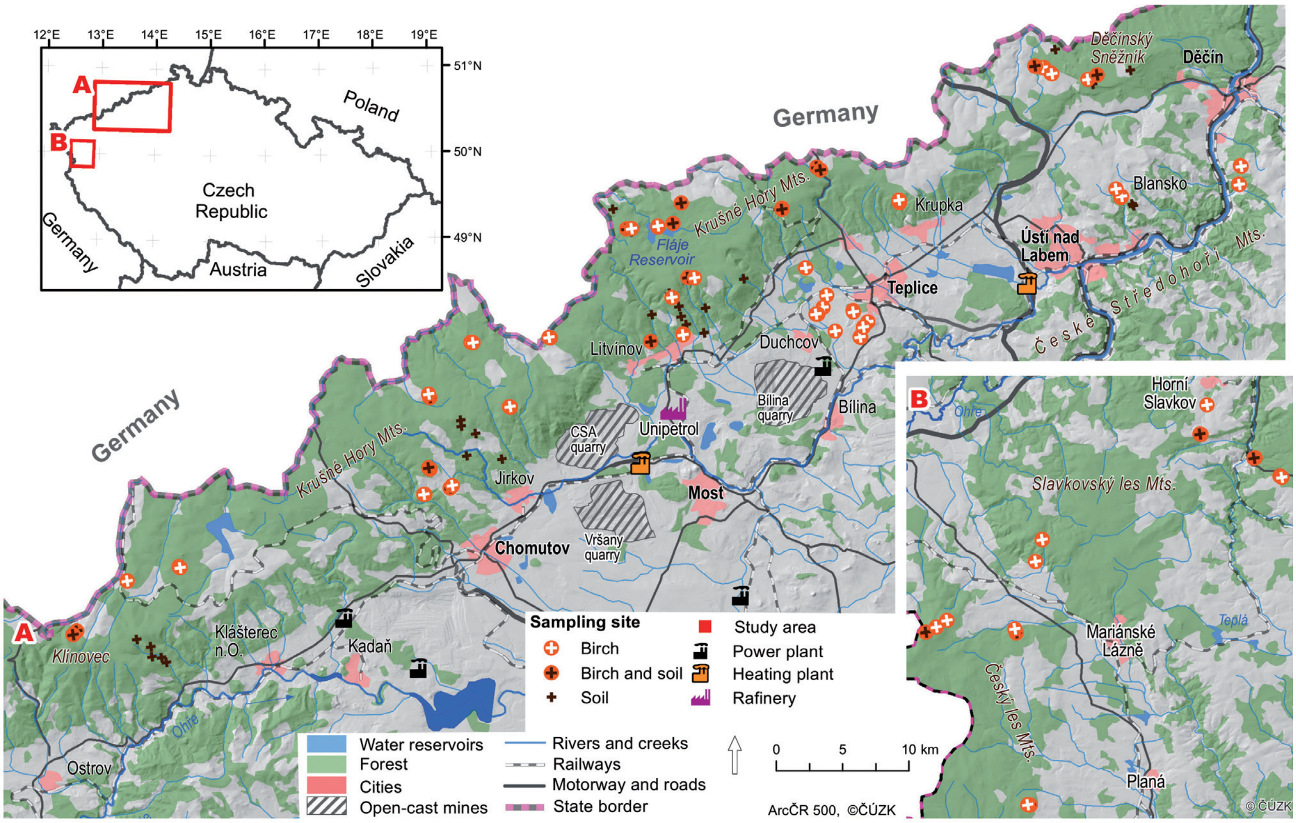

Fig. 1. Map of the Czech Republic with positions of soil and plant samples.

the sampling sites to areas with clearly defined bedrock geology (geological map of Czech Republic 1:50 000, GeoČR50) and to extend the sampling geographically. In 2021, soils and birch leaves were sampled. For evaluation, the samples were divided into three subsets. Subset 1 covered the main study area: a continuous mountain ridge of the Ore Mountains and the Děčínský Sněžník with very similar environmental conditions; this subset included soils (2018), soils with birch leaves (2021), and birch leaves (2021) (Fig. 1). Reference mountain areas (subset 2) included samples from the Slavkov and Český Les mountains as well as the edge of the České Středohoří Mountains, with more diverse bedrock geologies. Lowland samples of subset 3 were taken from the area near Teplice downslope of the Ore Mountains, with different climate than the mountainous areas (subsets 1 and 2) and mostly sedimentary rocks as soil forming substrate.

Two samples of birch leaves per a place with consistent geology were obtained at heights up to 3 m. The leaves were collected in paper bags and dried under laboratory conditions. Visually clean leaves were sampled. The leaves were not washed, as they typically contain comparable or higher content of Mn than soils. The atmospheric dust deposition cannot have a significant effect in such a high concentration as in studied leaves. Washing of the leaves could leach out manganese. One soil sample per a site with consistent geology was taken from the mineral soil horizon at

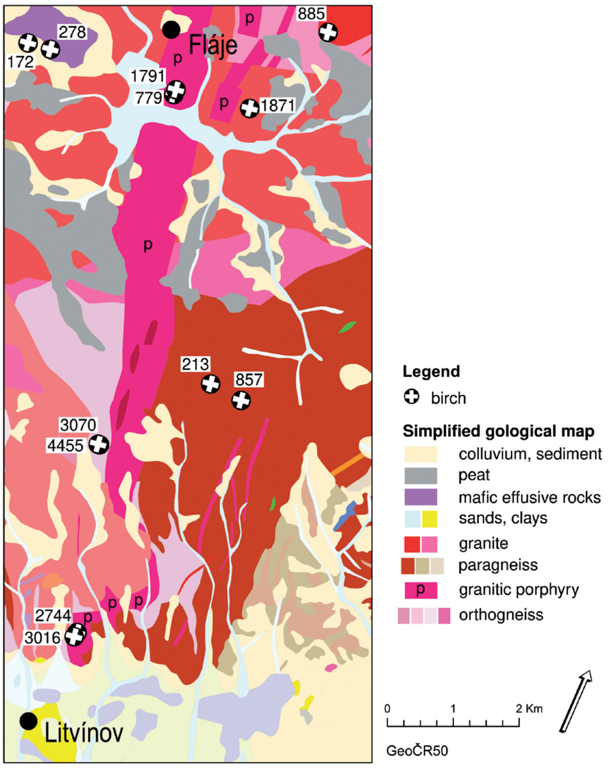

Fig. 2. Variable geology in the Litvínov-Fláje belt. Numbers show foliar Mn concentrations in mg kg 1 and document association of high foliar Mn and granitic porphyry bedrock and low foliar Mn and basalt rocks.

a depth of 10-30 cm. Stones were removed in the field, and the <2 mm size fraction was separated in the laboratory for analyses, dried, and pulverised. Pulverisation was performed using a planetary micromill (Pulverisette, Fritsch, Germany) with 80 ml agate bowl and five agate balls (2 cm).

Elemental analyses were performed using X-ray fluorescence spectroscopy (XRF). An Epsilon 3^X XRF spectrometer (PANalytical, The Netherlands) with an X-ray tube (Ag cathode, up to 50 kV) and a Peltier-cooled large-area Si-drift detector was used. Single-element calibration curves were constructed for soil analysis with a set of certified reference materials, as described in a previous paper [25]. Because of the lack of plant reference materials with sufficient concentrations of Mn, calibration was performed using ICP-OES analyses of a collection of plant materials described in a previous paper [26]. The analyses were performed after biomass digestion in an ultrapure nitric acid/hydrofluoric acid mixture (6 mL/30 µL) in a microwave dissolution system (Topex+, rotor type GT240) at 180ºC for 20 min. The excess acid was evaporated on a hot plate after digestion, and the samples were diluted to 25 mL using ultrapure water. The elemental concentrations were quantified using an ICP OES (Agilent 5100).

The acidity of soil leachates was obtained after treating 10 g of sieved soil (<2 mm) and 25 ml deionised water (boiled and cooled to laboratory temperature in a closed vessel). The suspension was shaken for 5 min using a glass stick and left to remain for 2 to 4 h before pH measurement.

Results and Discussion

Overview of Element Concentrations in Soils and Leaves: Impact of Geology

The influence of bedrock on Mn concentration in soils is shown in the boxplots in Fig. 3a). The two most Mn-rich parent geologies are basalts and granitic porphyry. At the opposite side there were Mn-poor soils developed on the sandy sediments in the Děčínský Sněžník area. The foliar Mn concentrations (Fig. 3b) do not simply mirror soils Mn concentrations, however, soil-forming substrates play an obvious role. In particular, birch leaves show maximal concentrations in sites close to granitic porphyry in the bedrock, similar to the elevated Mn concentration in the corresponding soils. On the other hand, Mn was not elevated in birch leaves from mafic lavas despite elevated soil Mn concentrations. The geological control is obvious from Fig. 2: here birch leaves were sampled in very nearby sites, at the same elevation, terrain, and emission history, but with contrasting geologies and shows quite contrasting foliar Mn concentrations.

Fig. 4 compares soils and foliar concentration Mn and nutrients we supposed can interact with Mn based on literature survey. Only samples from mountain subsets 1 and 2 are shown in Fig. 4. Besides Mn, Mg also shows considerable scatter within the soils in this subset. Magnesium content in leaves is, however, much less variable due to cellular homeostasis mechanisms, however, its content is generally low relative to the sufficiency ranges for plants, also indicated in Fig. 4b). Oppositely, foliar Mn is by two orders of magnitude above those ranges. The second highest foliar Mn concentration was found in areas with granitic bedrock (Fig. 3). Areas with granite bedrock are indeed known to be prone to acidification [4].

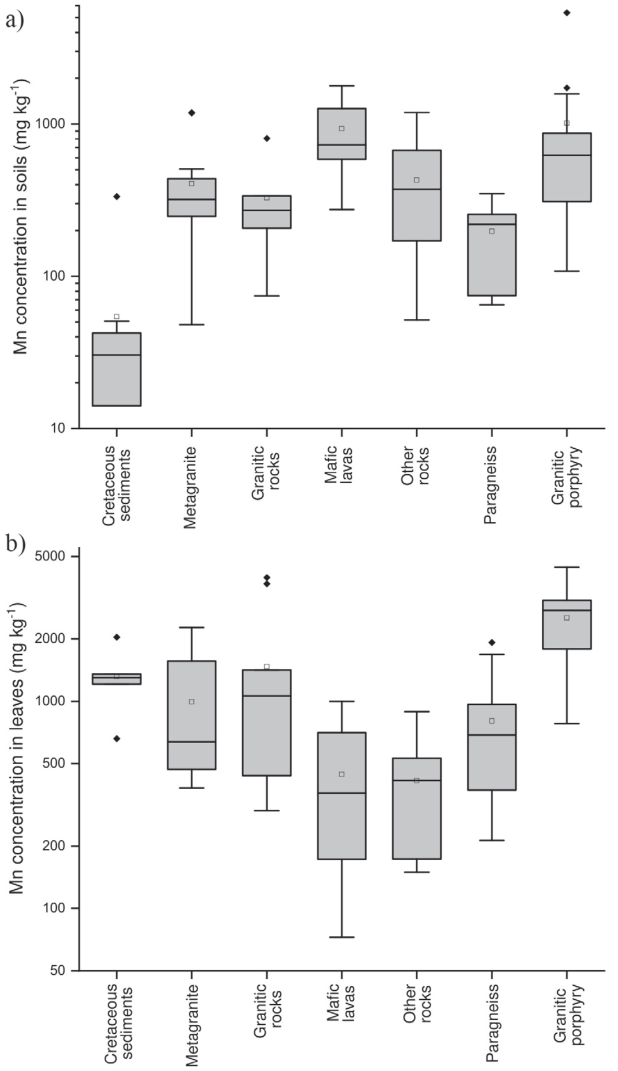

Fig. 3. Soil a) and foliar b) concentrations of Mn according to the major bedrock geologies in subsets 1 and 2. Soil collection included samples taken in 2018 and 2021. The label "other rocks" covers metamorphic rocks (paragneiss, gneiss undistinguished and phyllites). Noteworthy is rock-specific dissimilarity of soil and foliar concentration distributions.

Fig. 4 thus shows unfavourable Mg/Mn ratio in birch leaves in Ore Mountains-Děčínský Sněžník ridge. Plants can accommodate excess of Mn by cellular sequestration [13], but it needs synthesis of additional ligands for Mn transfer to cellular species and their

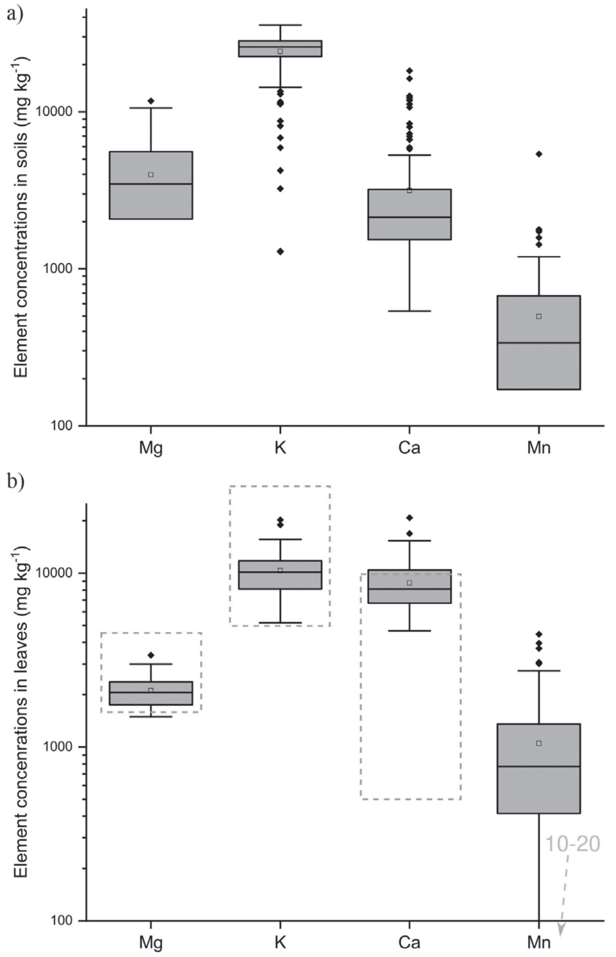

Fig. 4. Soil a) and foliar b) concentrations of Ca, Mg, K and Mn in subsets 1 and 2. Rectangles (green dashed lines) show minimal concentration sufficiency for plants, according to White and Brown (2010).

imobilisation in these spaces, that is energetically demanding for the plant.

Further Factors Controlling Foliar Mn

We searched for other possible factors controlling foliar Mn concentrations in birch, such as soil acidity and site elevation. It was not possible with the entire collection because of extreme values of foliar Mn found in sites on granitic porphyry (Fig. 3b), which were thus excluded from this analysis. Still the relation of foliar Mn to possible controlling factors did not produce strong linear regressions.

To examine the possible controlling factors, we chose graphic representation of data using boxplots. These plots demonstrated that high foliar Mn is more likely at low foliar Mg (Fig. 5). The foliar Mg content in the lowest decile and quartile is associated with elevated foliar Mn. Apparently, Mg is not limiting for majority of samples with its content in range Q2 to Q4. High scatter of foliar Mn in Mg sufficient plants shows impacts of further controlling factors, actually unknown to us.

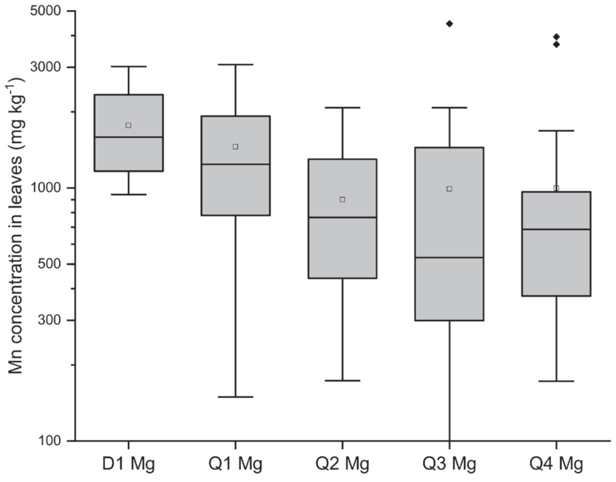

Fig. 5. Distribution of foliar Mn concentrations in classes of selected percentiles of Mg: from left, the lowest decile D1 and quartiles from lowest to highest. Samples from D1 are also included in Q1.

To test the possible impact of elevation of sampling sites as attempted by Hrdlička and Kula [24], we separated sampling sites into five categories: lowland sites and quartiles of elevation for sites in that massif. The results showed generally lower median values in both lowlands and high-elevation sites (Fig. 6). The scatter in the plot is large but we consider Fig. 6 worth discussing, in particular when seeing clear trend of medians in the five groups. The highest median Mn concentrations were found in the foothills of the Ore Mountains. We sampled only in Czech slopes of the Ore Mountains, that were exposed to maximal emission impacts on Czech mountain forest caused by atmospheric inversion typical for winters. Additionally, slopes of the Ore mountains received washouts of leaf

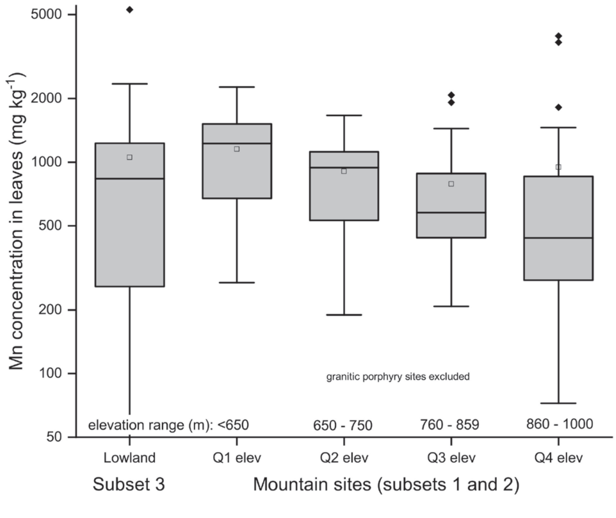

Fig. 6. Comparison of samples from lowland and mountainous sites divided into quartiles according to the site elevation. Sites affected by granitic porphyry were excluded; sites from certain granitic localities are above the Tukey upper fences in the plot.

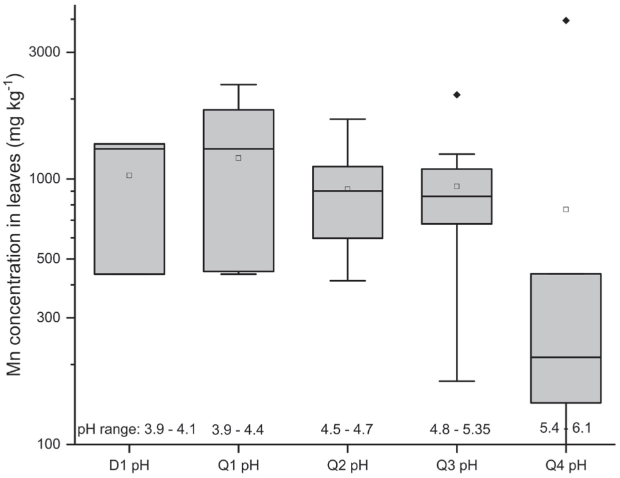

Fig. 7. Distribution of foliar Mn concentrations in classes of selected percentiles of pH (30 samples). From left, the lowest decile D1 and quartiles from lowest to highest. Samples from D1 are also included in Q1. Only three samples in D1 are shown without boxes.

litter and surface water from higher elevations, which could bring Mn leached from litter.

Association of foliar Mn concentrations and pH of soil extracts is shown graphically in Fig. 7. The pH control was much weaker than we expected. Kogelmann and Sharpe [3] found much more significant correlation of maple foliar Mn with soil leachate pH. We attribute much weaker relation in our dataset to the fact our study area was geologically and geographically much more variable. Still, low pH enhanced larger Mn uptake by birch in our dataset, while the lowest foliar Mn was found for the 4th quartile of leachate pH values.

Could Birch Foliar Mn be Suitable for Monitoring Purposes?

Acidification harms plants, among other mechanisms, through enhanced mobility of Al and Mn, but the foliar concentration of the latter element is more suitable for monitoring purposes [1, 3]. The Al concentration in biomass is too low, which would result in high instrumental demands and high contamination risk in sampling. Additionally, Al^{3+} ions in acidic forest soils are complexed by organic matter which decreases their uptake by plants [20]. Contrarily, Mn is accumulated by leaves because common mountain forest trees can detoxify excessive cellular Mn by sequestration in the parenchyma [13, 14]. This adaptability is documented by extreme concentrations found in granitic porphyry (Fig. 3b). However, the accumulation of Mn is not beneficial for plants, as they must produce the extra metabolites needed for this sequestration. Enhanced Mn uptake at higher soil acidity (Fig. 7) and low Mg uptake (Fig. 5) would favour monitoring of birch leaves composition for biomonitoring of soil acidification impacts. The fact of geological control (Fig. 2 and 3) is not contradictory: low-Mg and high-Mn soils on granites and granitic porphyry can simply be more prone to damages by acid rains. In any case, trees growing on these are endangered by acidification more than on other soils.

Kogelmann and Sharpe [3] proposed Mg/Mn and Ca/Mn element ratios in exchangeable cations and in the foliage of red maple to judge the state of health of maple stands in Pennsylvania, USA. Because Ca is not limiting in the study area (Fig. 4), Mn and Mg ratio should be a more appropriate here.

Manganese uptake by plants is not passive and results from the interplay between bioavailable Mn, biogeochemical cycling of soluble Mn^{2+} and insoluble Mn III,IV oxides, and plant regulation mechanisms, probably mainly rhizome and soil bacteria [12, 27]. Kruse et al. [27] hypothesised that Mn uptake is affected by the state of the soil microbial community, similar to the findings of Lambers et al. [12] for rhizomes. In such cases, the foliar Mn concentration could be a complex function of the overall soil condition. Thus, certain rhizome damage could be revealed by too high Mn, which would be desirable for monitoring purposes. Growing eutrophication by NOx deposition could also contribute to the changes in rhizomes, and there is a question how it could affect the Mn uptake, already rather high in some sites in the study area. Another question, to which monitoring of the Mn and Mg uptake by plants could be relevant, is how forest soil liming can affect general nutrient balance and the state of soils, which is still not fully understood [28, 29].

Conclusions

The uptake of Mn by silver birch in central European mountain forests exceed actual plant needs by two orders of magnitude. We showed the largest excess of Mn was found in Mn rich and Mg poor soils, that are expected to be particularly prone to acidification, i.e. soils on granites and granitic porphyry. Actually granitic porphyry represents major geochemical anomaly in the study area regarding foliar Mn uptake, yet not observed by preceding researchers. Contrarily, birch growing on Mn rich but also Mg rich soils on basalts show low foliar Mn concentration, supporting the geological control of the Mn uptake. After exclusion of the impact of granitic porphyry, high Mn uptake was found to be favoured by Mg deficiency and low pH of soils, although those factors by far have not represented all sources of Mn variability, that still needs further research. The state of rhizomes and impact of eutrophication of mountain soils in central Europe due to NOx emissions could be one of such possible yet unknown factors. The topic of variability in Mn foliar uptake by silver birch in central European mountain forests definitely needs further study.

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Conflict of Interest

The authors declare no conflict of interest.

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