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Localisation of Manganese, Sulphur, Potassium and Magnesium in Needle Tissues of Spruce (*Picea abies* (L.) KARST.) in Eastern Parts of Erzgebirge Mountains on Sites with different Manganese and Magnesium Supply

By

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With 10 figures

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Summary

WIENHAUS O., ZIMMERMANN F. & BÄUCKER E. 2001. Localisation of manganese, sulphur, potassium and magnesium in needle tissues of spruce (*Picea abies* (L.) KARST.) in eastern parts of Erzgebirge Mountains on sites with different manganese and magnesium supply. – Phyton (Horn, Austria) 41 (2): 179–202, with 10 figures. – English with German summary.

These investigations focus on the determination of nutrient element contents, water-soluble proportions of sulphate, phosphate, nitrate and chloride, as well as the contents of various ions in the vacuoles of spruce needle tissues. Subject matter of the examinations are all needle age classes available on four spruce (*Picea abies* (L.) KARST.) stocking on two sites characterised by abating SO_2 immission, but with different nutrient element supply, in particular manganese and magnesium. Accumulation of sulphurous compounds in mesophyll vacuoles with increasing needle age were verified by X-ray microanalysis combined with cryo-scanning electron microscopy

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(SEM). The counterions involved are primarily potassium, and also magnesium on the magnesium rich site, and manganese on the manganese rich site.

On the manganese rich site, manganese is stored at high concentrations in the mesophyll vacuoles. Except for endodermis vacuoles and mesophyll intercellular spaces manganese is detected here at measurable concentrations in transfusion parenchyma, epidermis and cell walls.

Zusammenfassung

WIENHAUS O., ZIMMERMANN F. & BÄUCKER E. 2001. Lokalisierung von Mangan, Schwefel, Kalium und Magnesium in den Nadelgeweben von Fichten (*Picea abies* (L.) KARST.) im Osterzgebirge auf Standorten mit unterschiedlicher Mangan- und Magnesiumverfügbarkeit. – Phyton (Horn, Austria) 41 (2): 179–202, 10 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Nährelementgehalte, wasserlösliche Anteile von Sulfat, Phosphat, Nitrat und Chlorid sowie die Gehalte an verschiedenen Ionen in Vakuolen von Fichtennadelgeweben wurden untersucht. Untersuchungsobjekte waren alle vorhandenen Nadeljahrgänge von insgesamt vier Fichten (*Picea abies* (L.) Karst.) auf zwei Standorten mit abklingender SO_2-Immission, aber unterschiedlicher Nährelementverfügbarkeit besonders an Mangan und Magnesium. Durch Röntgenmikroanalyse in Verbindung mit Kryo-Rasterelektronenmikroskopie (REM) konnte die Anreicherung von Schwefelverbindungen in Mesophyllvakuolen mit zunehmendem Nadelalter nachgewiesen werden. Als Gegenkation fungierten hauptsächlich Kalium, auf dem magnesiumreichen Standort auch Magnesium, auf dem manganreichen Standort auch Mangan.

Auf dem manganreichen Standort wurde Mangan in hohen Konzentrationen in Mesophyllvakuolen eingelagert. Mangan wurde hier im Transfusionsparenchym, in der Epidermis, in Zellwänden, aber nicht in Endodermisvakuolen und auch nicht in Mesophyllinterzellularen in messbaren Konzentrationen nachgewiesen.

Introduction

Over several decades Erzgebirge Mountains were subject to extreme atmospheric pollution with annual mean values of 75–85 $\mu g/m^3$ SO_2 (WIENHAUS & al. 1994). Owing to the installation of flue gas desulphurisation equipment in the large power generating stations, the conversion from solid fuels to oil and gas in domestic heating, and the shutdown of industrial plants, the threshold values for noxious impact of SO_2 on the vegetation are no longer reached. Nevertheless, sulphur concentrations in older compartments of the plants and in A-horizons of the forest soils are still excessive. Moreover, considerable quantities of noxious substances still accumulate in fog waters (LANGE & STERZIK 2000).

On a global scale, SO_2 immission is becoming critical in developing countries that depend on coal combustion and where air pollution approaches extreme values. Hence, to continue the investigations concerned with the action pathway of SO_2 immissions in woody forest plants is of outmost importance.

According to SLOVIK & al. 1995, SO_2 detoxification in spruce needles takes place chiefly via oxidative processes.

In this connection, the excess sulphur compounds jointly with cations are deposited in the mesophyll vacuoles in the form of sulphate. In this regard, great importance is given to potassium and a lesser one to magnesium (HÜVE & al. 1995, SLOVIK & al. 1996).

The importance of potassium as counterion to the enriched sulphate is emphasised by the determination of the water-soluble components of spruce needle extractives as well as by Cryo-SEM investigations on needles of young spruce (ZIMMERMANN & al. 1999).

In contrast little is known about the role of manganese in sulphur detoxification on sites with increasing soil acidification, where manganese is taken up at large rates (ZIMMERMANN & al. 2000).

Manganese is an essential micro-nutrient (SCHEFFER & SCHACHTSCHABEL 1992), required by plants only in minute quantities. Numerous enzymes are activated by it and it is physiologically important for the regulation of oxidation and reduction as well as the carboxylation processes in carbohydrate and protein metabolism. If plant-available, manganese is taken up by spruce (*Picea abies* (L.) KARST.) with no limitation in concentration (KAZDA & ZVACEK 1989).

While needle manganese contents of 80–500 µg/g dry weight confirm a sufficient manganese supply (ZÖTTL 1990), actual concentrations in needles of more than 4000 µg/g dry weight were measured in spruce stocking on sites with high manganese supply (NEBE & ROßBACH 1990, KREUTZER & BITTERSOHL 1986).

Manganese is taken up by the plants primarily in bivalent form. Under increasing soil acidification the percentage of plant available manganese increases (FÜRST 1993), because at pH values between 5 and 4, soils are becoming incapable of fixing manganese(II) ions in the solid phase (ULRICH 1981).

Based on a correspondingly high supply, manganese is subject to an intensive internal turnover in the forest ecosystems (ULRICH & al. 1979, ZÖTTL 1985). Considerable quantities are leached from the crown and enter the soil via throughfall deposition (SCHMIDT 1987).

In a chamber experiment, BURKHARDT & DRECHSEL 1997 found a connection between manganese leaching from spruce needles and the rate of sulphate deposition. In the experimental set treated with manganese, the SO_2-flux to the trees was twice as much as in the set with a low manganese supply. BURKHARDT & DRECHSEL 1997 attributed the increased SO_2 flux to the capacity of manganese to act as a catalyst in oxidative detoxification of SO_2.

BUSSLER 1958 showed that manganese taken up in excessive quantity is detoxified in the form of MnO_2 and deposited on the periphery of the plants. In contrast LANGHEINRICH & al. 1992 concluded that the oxidation level of manganese in spruce seedlings treated with manganese was low, because precipitated MnO_2 was not observed. Large portions of manganese were found by LANGHEINRICH & al. 1992 in cell walls of the xylem and in the epidermis cells of the needles of manganese-treated spruce seedlings.

In field studies GONSER 1960 showed that manganese follows the same distribution patterns as calcium and SiO_2, which appear to be mainly stored in organs or tissue compartments liable to abscise later on.

WYTTENBACH & al. 1995 suggested for high manganese needle contents a detoxification in terms of oxalate, similar to that of calcium.

The internal tolerance of high manganese concentrations can be enhanced by compartmentalisation within the tissues and cells, a more homogenous distribution in the tissues, by oxidation and complexing in the plant and by high internal magnesium concentrations (HORST 1988).

The vacuoles of adult plant cells are characterised by a great buffering and storage capacity. In addition to water, primary metabolites and inorganic nutrients, water-soluble secondary metabolites can be accumulated, with the possibility of a significant concentration gradient towards the cytoplasm emerging (MATILE 1978).

Thus, the investigation of the cell saps may be regarded as an indirect approach to gain insight into the conditions existing in cytoplasm where relative constant conditions are maintained (MATILE 1987).

The objective of this investigation was to localise excess manganese in spruce needles. Hints to a possible physiological importance were expected on the basis of manganese distribution within the needle and the distribution of the other nutrient elements in the case of high manganese contents. In particular, one objective of the investigations was to assess the role of manganese in its capacity as counterion in the case of sulphur accumulation in needle vacuoles subsequent to SO_2 immission.

The role of Mn in neutralising sulfate accumulation as a result of SO_2 immission will also be examined (ZIMMERMANN & al. 2000).

Materials and Methods

Sites

For the investigation into the localisation of sulphate-sulphur and the cations of manganese, magnesium and potassium in spruce needles, two sites were selected in the eastern parts of Erzgebirge Mountains – a region that was exposed to intensive atmospheric pollution.

The site in Forest District Tharandt (T) is typical of eastern Erzgebirge rhyolite sites (porphyry, poor in quartz), (FIEDLER & al. 1985), characterised by both low pH values and low trophic level (lower mountainous belts, 370 m a.s.l., precipitation 850 mm/a, annual mean temperature 7.6 °C).

According to their nutrient content the rhyolites with 3–4 g/kg MgO + CaO are poor in alkaline earths, and with >50 g/kg K_2O they are rich in potassium, and with a content of < 0,3 g/kg P_2O_5 they are extremely poor in phosphorus (NEBE & al. 1998).

The occupation of the exchangers with alkaline cations (BS) accounts for 4–7% in middle debris (Hauptfolge), and for 6–9% in basal debris (Basisfolge). The portion of exchangeable alkaline earths ions, decisive for soil functions, accounting for 2–5%, appears to be extremely low. Increased percentages of exchangeable manganese are indicated for Tharandt site (NEBE & al. 1998).

The experimental plot Geising (G) is representative of the basaltic sites occurring only sparsely in the eastern parts of Erzgebirge Mountains, which are characterised by a relatively high nutrient supply, proper base saturation and magnesium supply (upper mountainous belts, 740 m a.s.l, 900 mm/a rainfall, mean annual temperature 4.5-5.5 °C).

The basaltic volcanic rocks of the eastern parts of Erzgebirge Mountains are rich in alkaline earths (>210 g/kg MgO + CaO), moderate in potassium (14 g/kg K_2O) (NEBE & HOFMANN 1982), and rich in phosphorus (7.5 g/kg P_2O_5).

Nutrient-rich brown earths have formed on the basaltic sites, containing well above 5 g/kg of calcium in fine soil. The occupation of the exchangers with alkaline cations in Ah-horizon with ~30% and in B- horizon with ~ 85% is much more favourable than in rhyolite soil types. In this context, the percentage of exchangeable Calcium and Magnesium ions, being crucial for soil functions, dominates.

Immission situation and deposition over the study period

The SO_2 immissions were similar at Tharandt site (lower elevations) and Geising site (upper ridges of Erzgebirge Mountains), both in the average concentration of noxious substances and the trend over time (Tab. 1). Over the years 1994 to 1996 relatively high SO_2 concentrations were still recorded. Starting from 1997, and more

Table 1.

Immission (annual mean of SO_2 [µg m^{-3}]) , bulk and throughfall deposition (SO_4-S and Mn [kg ha^{-1} a^{-1}]), and bulk and throughfall precipitation [mm a^{-1}] over the period of formation of the needles investigated. (The monitoring station Oberbärenburg is located in the vicinity of Geising experimental plot.)

	Tharandt Forest						Geising (measuring station Oberbärenburg)					
	immission	deposition					immission	deposition				
		bulk		throughfall				bulk		throughfall		
year	SO_2	SO_4-S	precipitation		SO_4-S	Mn	SO_2	SO_4-S	precipitation		SO_4-S	Mn
1994	36	9.6	879	500	48.7	1.83	38	11.2	989	708	56.5	0.52
1995	26	9.4	1032	669	45.7	2.02	35	13.9	1307	1064	44.3	0.54
1996	37	7.4	769	378	35.0	1.96	30	8.2	975	756	42.3	0.47
1997	24	5.3	715	334	27.3	1.55	25	7.2	962	704	33.7	0.39
1998	10	6.5	914	548	18.1	1.20	15	7.9	1174	877	28.6	0.39
1999	8	*	826	467	*	*	7	6.6	959	718	23.3	0.40
2000	8	4.4	803	456	11.5	1.07	8	5.9	996	803	20.0	0.48

*data only available for the second half of the year pronouncedly in 1998, the measures taken for flue gas desulphurisation in the large power generating stations, as well as the reduction of solid fuel combustion in domestic heating, had the effect of contributing substantially to the reduction of SO_2 emissions (ZIMMERMANN & WIENHAUS 2000).

Starting from 1998, the threshold value of the noxious substance SO_2, beyond which damage to vegetation may occur (20 µg m^{-3} according to UN ECE and EU Guideline 96/62/EG as of 27. 09. 1996) has no longer been exceeded in the study area of the eastern parts of Erzgebirge Mountains.

The values of the sulphate deposition, both in terms of bulk and throughfall deposition, show a downward trend, although not as steeply sloping as in the immission of SO_2.

Manganese throughfall depositions result mainly from needle leaching. The values being about the fourfold in Tharandt Forest reflect the different manganese supply at the experimental sites. Starting from 1997, reduced manganese depositions were measured here as compared to the preceding years.

Trees

Two medium-aged spruces (*Picea abies* (L.) KARST.) which were healthy by outer appearance were selected at Tharandt (age 40, trees T4 and T5) as well as at the Geising site (age 65, trees G1 and G2). Referring to a group of 20 trees (previously analysed by RINGEL 1999, personal communication), manganese supply was maximal in spruce T4, whilst in tree T5 it was average. The sulphur contents of the needles of trees T4 and T5 were in the range of the area average. As for the Geising site, no analyses were available from previous investigations.

Sampling

In late October 1999 a branch facing south was removed from each sample tree at the top crown (7^{th}–9^{th} whorl). SEM preparations were obtained from all available needle classes (T4: 6, T5: 5, G1: 8, G2: 6 needle age classes). The youngest four needle age classes (tree G1: 3 needle age classes) were sampled for the purpose of conducting total element analyses. Water-soluble contents of cations and anions were determined in the youngest four to six needle age classes.

X-ray microanalysis

Measurements of the relevant ions contained in the vacuoles were performed by X-ray microanalysis (EDX) in conjunction with cryo-scanning electron microscopy (STELZER & al. 1988, VAN STEVENINCK & VAN STEVENINCK 1991, ZIMMERMANN & al. 2000, BRUNNER & FREY 2000). This method allows the preservation of samples by rapid-freezing, which largely avoids a displacement of ions. This examination combines electron-optical visualisation with analysis in high spatial resolution.

Original specimen stubs made of copper (EMITECH K1250 preparation system) were reconstructed into an adapter by milling out an opening for insertion of two types of cylindrical (10 mm) specimen stubs (Fig. 1-1).

The first type of stubs (Fig. 1-2) with vertical drill holes (1.4 mm) was provided for the preparation of the transverse needle fracture. The second type with three bore holes into the surface at an obtuse angle was provided for the preparation of longitudinal needle fracture (Fig. 1-3).

Spruce needles were glued into the apertures of the specimen stubs, using TISSUE TEK-cryo-adhesive (MILES) and rapidly frozen under subcooled ("slush")

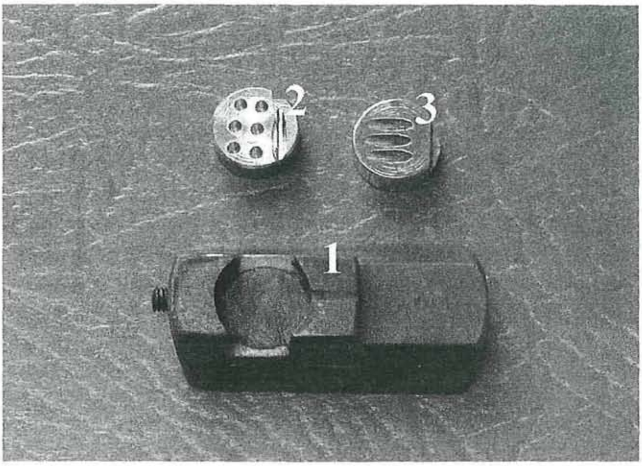

Fig. 1. Specimen stub (adapter) (1) of the cryo-transfer system, stub for preparation of transverse (2) and longitudinal needle fracture (3).

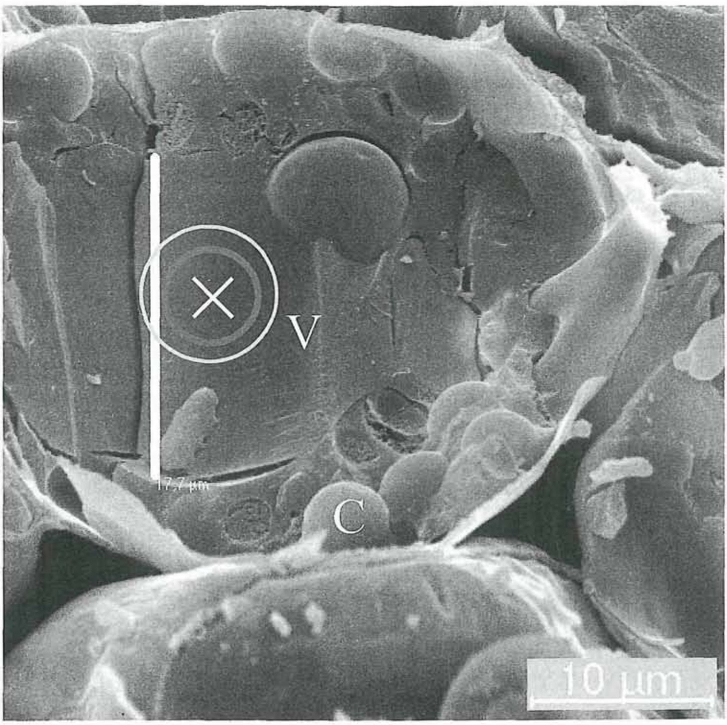

Fig. 2. Cryofracture through a mesophyll cell after needle longitudinal fracture. Vacuole measuring 17.7 µm (vertical bar), (X) zone of X-ray excitement in the centre of the (V) vacuole, (C) chloroplasts.

nitrogen (approx. –210°C). Subsequently, the prepared needle samples were placed into the storage of a nitrogen Dewar (KB 32, FUNKE MEDINGEN).

For examining the samples in SEM, the specimen stubs were assembled in a liquid nitrogen bath onto precooled adapters. The freezing fracture was performed in the preparation system, using a knife tool. Then the newly formed surface with exposed needle tissue and the cells broken up was vapour-coated with carbon and transferred into the SEM JEOL T330 A.

Excitation zones for X-ray analysis of slightly more than 5 µm can be calculated based on the parameters of influence: sample density, line energies of the nutrient elements and excitation energy (RÖNTEC Wintools). In general, this suffices for the order of magnitude of the spruce needle vacuoles in mesophyll and endodermis cells (Fig. 2).

Measurements were conducted constantly on the exposed cell vacuoles with an accelerating voltage of 15 KeV and a measuring time of 200 seconds at 1200 cps (counts per second) and an excited area of ca. 10 μm^2. At least five X-ray spectra were recorded of each sample per compartment.

The measured X-ray spectra were evaluated qualitatively and quantitatively ("without standards", using the Peak-to-Background ratio), applying the system software (RÖNTEC WINTOOLS). Usable results were obtained by this method even in the case of rough fracture surfaces. By means of the COAT correction (RÖNTEC) the proportion of carbon that was introduced by the coating was eliminated from the quantitative analyses.

Elements starting from atomic number 5 (B) are detectable by this analysis system. The nitrogen concentration could not be measured in the needle samples, because the X-ray peak of nitrogen was superimposed by the peak of oxygen (>95 %) which dominated due to the water content.

Thus, problems of quantification caused by the the frozen-hydrated bulk samples could not be completely overcome (STELZER & LEHMANN 1993, BRUNNER & FREY 2000). Hence, amongst others, the quantifications in the range of light elements (atomic number < 11) are connected with a greater error than in elements of an atomic number > 11.

Errors resulting from the quantitative analysis are, among others, attributable to the examination of only a small number of needles (2–5) per variant, to the analysis of only 5–10 cells per needle and to the fact that the surface properties of the preparations vary within certain limits.

Thus, the results obtained by quantitative X-ray micro-analysis become available for considerations from the viewpoint of plant nutrition, without necessitating expert knowledge in physics.

Chemical analyses

The total contents of sulphur and nitrogen were determined in terms of total sulphur and nitrogen, using VARIO EL (Elementaranalytik Hanau GmbH). Subsequent to wet ashing by means of nitric acid the remaining nutrient elements (P, K, Ca, Mg, Mn, Zn, Al, Fe, Cu) were measured using pressure digesters and analysis on ICP, Perkin Elmer.

The water-soluble contents of cations (K, Mg, Ca, Mn und Zn), as well as of the anions sulphate, phosphate, chloride and nitrate were determined according to HOESS 1986 on DIONEX DX 300 (ZIMMERMANN & al. 2000).

Results

Assessment of the nutrient status of the sample trees based on total element contents of the needles

Depending on the site and the situation of immission, the nutrient status of the sample trees differed. The comparison of the limit values designating that growth of spruce was devoid of deficiency (NEBE & ROßBACH 1990, ZÖTTL 1990) with the results of the total needle analyses revealed that the two sample trees on Tharandt experimental site were subject to sufficient nitrogen supply. Low relative nitrogen contents, distinctly below the limit value for a nutrient status devoid of deficiency, were apparent for both sample trees growing on the basalt site of Geising (G). The water soluble nitrate nitrogen contents were low in all needles examined, accounting for as much as approx. 0.1% in total nitrogen and all sample trees were free of any phosphorus deficiency. With a proportion of 20% to 30%, the water-soluble phosphate closely correlated with total phosphate.

Trees T4, T5 and G1 were subject to good, and tree G2 to sufficient potassium supply. The limit value for growth devoid of deficiencies was exceeded also in the three-year-old needles in all sample trees. In tree G2 the potassium contents increased with needle age starting from second needle age class. On average, 71% of the total potassium contents were water-soluble.

Owing to a proper calcium supply at the two sites, a distinct calcium accumulation became apparent with increasing needle age in all four trees investigated. The soluble calcium percentages were on average only 14% relative to total contents, with a clear age trend being missing.

The four spruces investigated were free of a magnesium deficiency, and tree G2 was characterised by an above average magnesium supply. With an average proportion of 86%, a high percentage of the magnesium was water-soluble.

The manganese nutrient status of all 4 sample trees was at least sufficient (Fig. 3). The trees of Tharandt experimental site were virtually luxuriously manganese supplied with manganese contents 20times (T4) to 15times (T5) the limit value.

The analysed manganese was water-soluble on average to 79%.

The analyses of total sulphur contents revealed differences between the individual trees (Fig. 4). In trees T5 and G2, an upward trend with increasing needle age was dominating. In contrast to this, the sulphur contents of the analysed three youngest needle classes of tree G1 were comparatively low, not revealing a noticeable trend.

The sulphur contents of current-year needles stated by HÜTTL 1992 are given as reference values for insufficient (800 µg/g dry weight corresponding to 0.08%) sulphur nutrition and for anthropogenic excessive nutrition

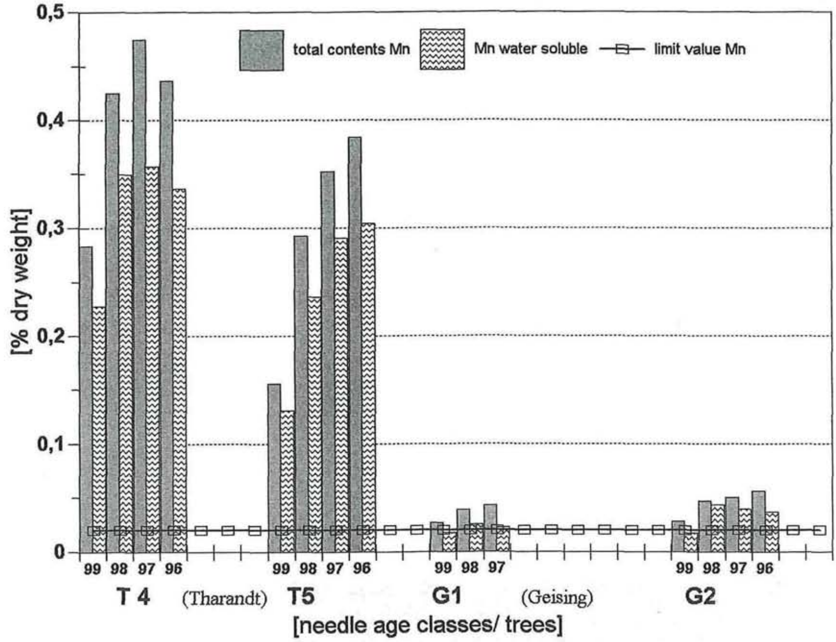

Fig. 3. Manganese total contents, soluble manganese contents and limit value of spruce needle manganese contents for nutrient deficiency.

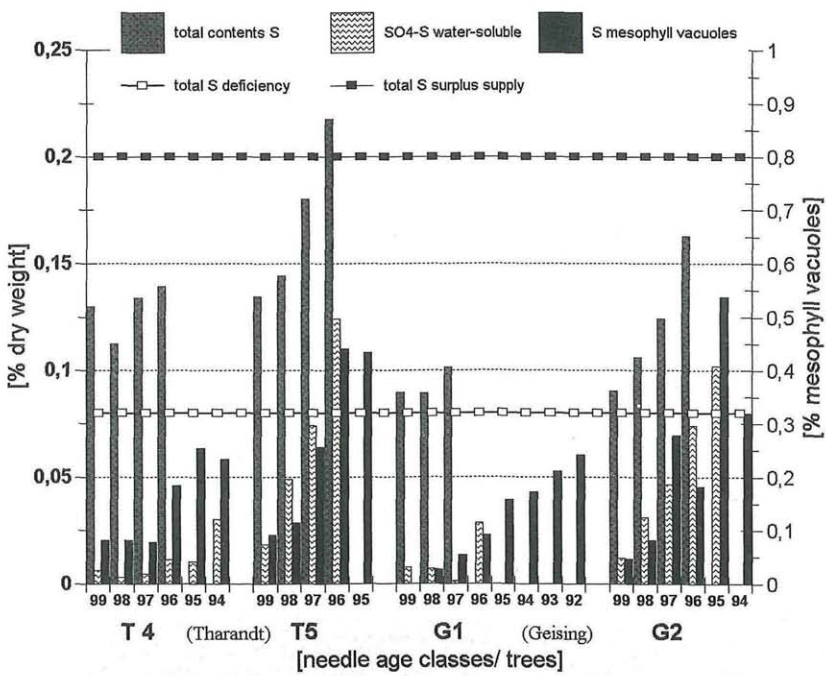

Fig. 4. Total sulphur (maximally four needle classes), water-soluble SO4-S, limit values for problematic nutrient status for sulphur according to HÜTTL 1992 – (left axis) and sulphur in the mesophyll vacuoles (right axis) of the examined needle classes of spruces T4 and T5 (site Tharandt) and spruces G1 and G2 (site Geising).

(>2000 µg/g dry weight, corresponding to 0.2%). Sulphur contents between 800 µg/g dry weight and 1100 µg/g dry weight designate, according to HÜTTL 1992, sufficient sulphur nutrition, while 1100 µg/g dry weight up to 2000 µg/g dry weight designate optimal sulphur nutrition.

According to the limit values for one-year-old needles, the spruces investigated are characterised by a sufficient to optimal sulphur nutrient status.

The sulphur nutrient status is, even if taking the N:S quotient into account, generally unproblematic, regarding the needle age classes involved in total analyses.

Total sulphur contents, water-soluble proportions and sulphur in the mesophyll vacuoles

In general the storage of sulphur was lower in younger needle age classes than in older ones. Total sulphur contents were low in the needles formed during periods of already drastically reduced immission (T4, G1).

By means of X-ray microanalysis it was possible to investigate a few of the older needles, which were not involved in the total chemical analyses, (Fig. 4). Total sulphur contents, water-soluble sulphate and mesophyll vacuole contents determined by X-ray microanalysis (Fig. 4), showed very similar trends of sulphate and mesophyll sulphur, when comparing trees and needle age classes.

Thus, the hypothesis of sulphate accumulation in the mesophyll vacuoles of the older needle age classes was verified based on our measured values.

Another result of our investigations is the fact, that the individual trees growing on the same site accumulate sulphur at very different concentrations. These individual differences in sulphur accumulation behaviour were already obvious in the young needle age classes.

Contents of magnesium, phosphorus, sulphur, potassium and manganese in mesophyll and endodermis vacuoles

Basic differences of element contents were found in the vacuoles of mesophyll and endodermis cells (Tab. 2).

Magnesium in mesophyll of Tharandt site needle samples was identifiable only in a few X-ray spectra (Tab. 2).

In contrast, magnesium was detectable in the mesophyll of all needles of the sample trees growing on the basaltic Geising site, although no connection was observed between the contents and needle age.

Magnesium was significantly high in the endodermis vacuoles in all represented variants and the concentrations were at least ten times higher than those measured in the mesophyll. In all four trees investigated the highest magnesium concentrations were measured in the endodermis of the three younger needle age classes, whereas the lowest concentrations were ascertained in older needles. On Geising site (basaltic bedrock), the mag-

Table 2.

Element contents of the mesophyll and endodermis vacuoles measured by X-ray mircroanalysis of frozen hydrated bulk specimens (Cryo- SEM, EDX [mean values weight % in cell sap]) (n. dt.: non detectable)

tree	needle	mesophyll vacuole contents						endodermis vacuole contents					
	age class	n	**Mg**	**P**	**S**	**K**	**Mn**	n	**Mg**	**P**	**S**	**K**	**Mn**
T4	99	12	0.076	0.012	0.046	0.802	0.213	5	0.388	0.066	0.158	0.388	n. dt.
	98	5	0.032	n. dt.	0.082	0.362	0.948	3	0.167	0.230	0.087	0.790	n. dt.
	97	9	n. dt.	n. dt.	0.087	0.264	0.987	10	0.237	0.062	0.260	0.985	n. dt.
	96	6	n. dt.	0.017	0.183	0.342	1.095	4	0.298	n. dt.	0.305	0.700	n. dt.
	95	6	n. dt.	n. dt.	0.253	0.380	1.128	1	0.290	n. dt.	0.280	0.970	n. dt.
	94	8	n. dt.	n. dt.	0.234	0.219	1.105	3	0.457	n. dt.	0.413	0.457	n. dt.
T5	99	15	n. dt.	n. dt.	0.091	0.451	0.073	14	0.654	0.256	0.205	0.099	n. dt.
	98	10	0.031	0.005	0.114	0.351	0.512	5	0.524	0.234	0.278	0.274	n. dt.
	97	14	n. dt.	0.002	0.256	0.441	0.426	9	0.319	0.168	0.220	0.318	n. dt.
	96	5	n. dt.	n. dt.	0.440	0.546	0.620	5	0.482	0.338	0.286	0.618	n. dt.
	95	6	n. dt.	0.023	0.433	0.828	0.447	6	0.378	0.163	0.192	1.067	n. dt.
mean site Tharandt			**0.013**	**0.005**	**0.202**	**0.453**	**0.687**		**0.381**	**0.138**	**0.244**	**0.606**	**n. dt.**
G1	99	5	0.032	0.006	n. dt.	0.640	n. dt.	4	0.710	0.003	0.068	0.645	n. dt.
	98	6	0.012	0.038	0.027	0.610	n. dt.	3	0.773	0.283	0.087	0.550	n. dt.
	97	6	0.045	n. dt.	0.053	0.663	n. dt.	3	0.847	0.180	0.103	0.283	n. dt.
	96	17	0.027	n. dt.	0.091	0.428	n. dt.	5	0.292	0.474	0.146	1.242	n. dt.
	95	10	0.102	n. dt.	0.157	0.508	n. dt.	11	0.400	0.173	0.106	0.766	n. dt.
	94	14	0.041	n. dt.	0.171	0.550	n. dt.	18	0.531	0.278	0.168	0.656	n. dt.
G2	99	15	0.013	0.022	0.047	0.332	n. dt.	10	0.902	0.432	0.333	0.063	n. dt.
	98	17	0.049	0.008	0.081	0.323	n. dt.	10	0.688	0.172	0.240	0.227	n. dt.
	97	14	0.096	n. dt.	0.277	0.441	0.044	12	0.342	0.051	0.167	0.236	n. dt.
	96	5	0.022	n. dt.	0.182	0.390	n. dt.	4	0.423	0.178	0.085	0.358	n. dt.
	95	7	0.060	n. dt.	0.537	1.233	n. dt.	3	0.263	n. dt.	0.200	0.813	n. dt.
	94	10	0.064	n. dt.	0.320	0.783	n. dt.	3	0.513	n. dt.	0.300	0.573	n. dt.
mean site Geising			**0.047**	**0.006**	**0.162**	**0.575**	**0.004**		**0.557**	**0.185**	**0.167**	**0.534**	**n. dt.**

nesium contents of endodermis vacuoles were higher, on average, than those on the Tharandt (rhyolite bedrock) site.

Phosphorus could not be identified in most of the X-ray spectra measured at the mesophyll vacuoles. (The low mean concentrations identified for one-year needle age classes might possibly stem from cytoplasm which in the marginal zone of the ray was likewise excited in the analysis.)

In contrast, phosphorus concentrations were significantly high in the endodermis vacuoles with the exception of the older needles of trees T4 and G2. The phosphorus concentrations measured at Geising site were higher than those at the Tharandt site (Tab. 2).

Mean sulphur concentrations were similar in mesophyll and endodermis at Tharandt and Geising. While no regular changes in sulphur concentrations with increasing needle age were discernible in endodermis, a clear increase was obvious in mesophyll in older needles. Differences were apparent within the sites in the sulphur concentrations in the mesophyll vacuoles. At Geising, significantly higher sulphur concentrations were measured in needles of tree G2 than in the needles of tree G1. At Tharandt, the higher concentrations were detected in mesophyll in needles of the tree T5 (Tab. 2).

Potassium concentrations were relatively high in the vacuoles both of mesophyll and endodermis of all needles examined.

Manganese was almost exclusively found in the mesophyll vacuoles of the needles of Tharandt sample trees. Manganese in the endodermis was neither measurable in spruce needles at Tharandt nor at Geising Tab. 2). The concentrations of manganese in the mesophyll of the older needle age classes of spruce T4 was approximately twice as high as that of spruce T5. In contrast to potassium, current-year needles were characterised by significantly lower manganese concentrations than older needles.

Ion balance of the measured mesophyll vacuole contents

To assess the importance of cations for equalisation of charges of the sulphate anions deposited in the mesophyll vacuoles, equivalent quantities were compared (Fig. 5).

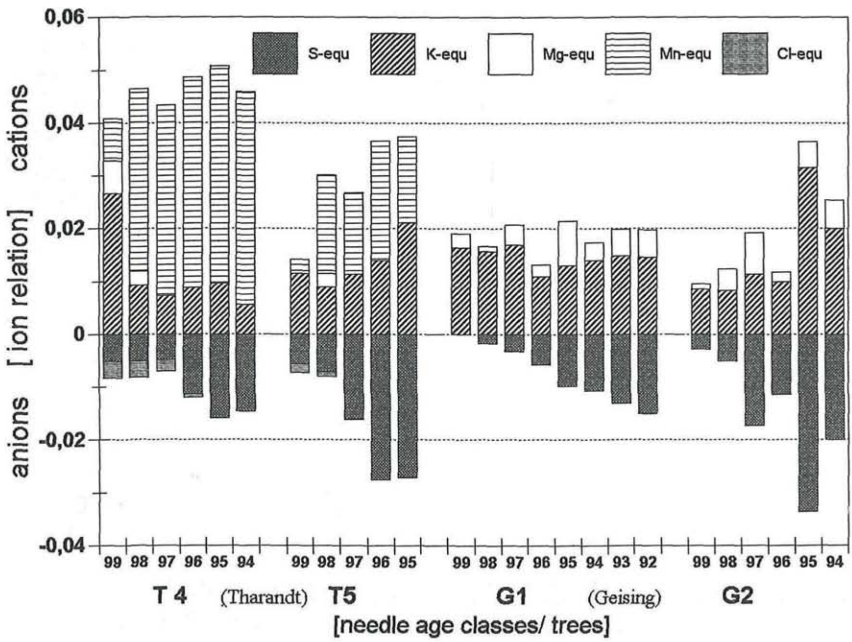

Fig. 5. Comparison of the ion balances in the mesophyll vacuoles on equivalent basis ((weight %/atomic weight) * effective valence (K, Cl = 1; S, Mg, Mn = 2)), cations: positive range of the axis; anions: negative range of the axis.

From the comparison of the anions plotted in the negative range and the sum of cations, ionic imbalances resulted frequently, which might be equalised by organic anions (e.g. malate, citrate, oxalate) which can not be measured by EDX. The equivalents of potassium reveal two different trends: On the one hand, a decrease from current-year to one-year old needles, and an increase with needle age and with the sulphur content in the older needles, on the other hand.

Fig. 5 shows that in older needle age classes at Tharandt site potassium alone did not suffice as counter cation to sulphate. At the same time, manganese caused an excess of the examined cations as compared to sulphate anions. At Tharandt site magnesium hardly bears any importance for ionic equalisation in the mesophyll vacuoles. At Geising site, magnesium obviously acted partly as a counterion to the sulphate ions.

Correlation analyses – mesophyll vacuole contents

Partial correlation coefficients were computed, to deduce a possible relationship between the accumulation of sulphate and the increase of cations in the mesophyll vacuoles. The data of all or of a particular selection of the needle age classes investigated were used in the calculation of the correlations (Tab. 3).

Table 3.
Partial correlation coefficients of the relationship between sulphur contents in the mesophyll vacuoles and the contents of potassium, manganese and magnesium cations, inclusive of all investigated or selected needle age classes, sampling as of autumn 1999, Tharandt (T) and Geising (G) sites (*: $p<0.01$; +: $p<0.05$)

	S to K	S to Mn	S to Mg
tree G1 all needle age classes	0.0379	–	0.2866+
tree G1 from 2nd needle age class	0.1036	–	0.2730+
tree G1 from 4th needle age class	0.4182*	–	0.2278
tree G2 all needle age classes	0.8670*	–	0.2761
tree G2 from 2nd needle age class	0.8848*	–	0.1252
tree T4 all needle age classes	0.1811	0.2464	-0.2303
tree T4 from 2nd needle age class	0.3114	0.1624	-0.3141
tree T5 all needle age classes	0.5415*	0.2920	–
tree T5 from 2nd needle age class	0.6564*	-0.2185	–

The correlation analysis of the data of the single trees gave different results (Tab 3). In the needles of trees T4 and G1, with the inclusion of all needle age classes investigated, a significant relationship between the contents of sulphur and potassium was not found.

The needles that formed at Tharandt site in the sampling year (1999) were characterised by clearly higher potassium contents as compared to the older needle age classes. As shown in Fig. 5 the increase in potassium contents was a function of sulphur enrichment and is discernible only starting from the second needle age class. The omission of the youngest needle age classes from the correlation analysis seemed to be justified, as apparently the youngest needles were characterised by other relationships in contrast to the older needles with higher sulphur contents (Fig. 5). The validity of this was corroborated by an increase in the correlation coefficient between sulphur and potassium with respect to the older needles (Tab. 3).

A significant relationship between potassium and sulphur contents in tree G1 could be observed only after the youngest three needle age classes (1999, 1998, 1997) were excluded from the calculation (Tab. 3). While sulphur increased with needle age the cations measured in tree G1 were relatively constant. This was reflected by the smaller correlation coefficient, as a significant relationship could only be observed starting from the 4^{th} needle age class. Contrary to this, this relationship (with a correlation coefficient of 0.8543*) was highly significant for tree G2, taking into consideration all needle age classes.

Sulphur and potassium appeared to correlate most significantly in the trees with the highest sulphur contents in older needles (T5 and G2) (Fig. 5).

For tree G1 of Geising a significant relationship between sulphur and magnesium could be calculated (Tab. 3). The negative correlation coefficient for magnesium at Tharandt is due to the fact that magnesium is contained only in the two youngest needle age classes of tree T4. This correlation is of no relevance to the issues discussed in this article.

In summary, a significant relationship between sulphur and manganese could not be detected for the trees examined.

Manganese in other needle compartments

In addition to the investigations of the vacuoles of the mesophyll and endodermis, measurements were conducted in other needle compartments for orientation.

In the spectrographs the Y-axis refers to the number of recorded X-ray impulses, while the pertinent energy relates to the X-axis (Fig. 6). When observing the spectra an estimate of element concentrations may be performed based on the relation of peak height to height of the background in the respective energy band.

The X-ray spectra referring to **mesophyll plasm** of trees T4 and T5 revealed the element peaks for phosphorus, potassium and sulphur (Fig. 6) as expected. The Kα-line of magnesium (at 1.25 keV) was hardly visible in the noise of background radiation. The X-ray impulses for silicon were attributable to excited cell wall ranges. The spectrographs revealed that manganese (Kα-lines at ~5,9 keV) was undetectable in cytoplasm by X-ray microanalysis.

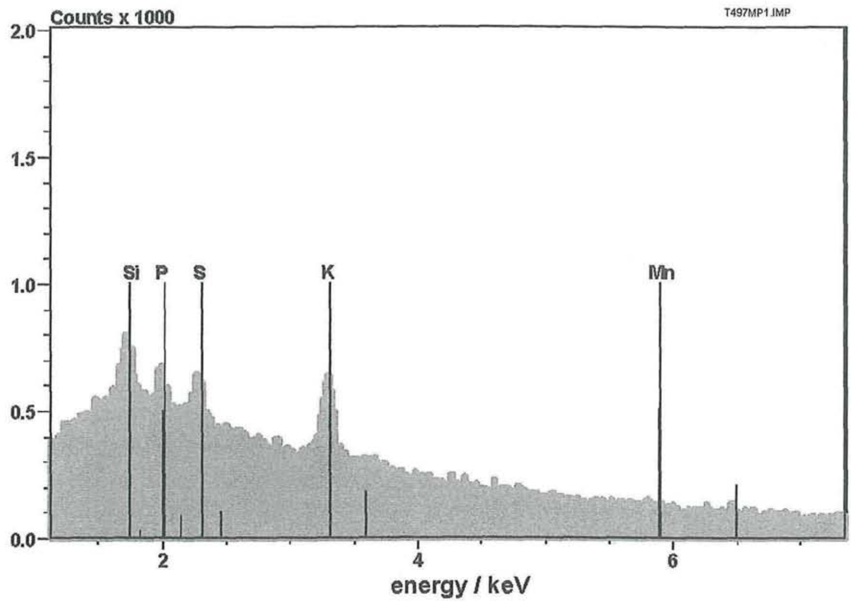

Fig. 6. EDX spectrum of the area of cytoplasm of a mesophyll cell. Tree T4, needle age class 1997.

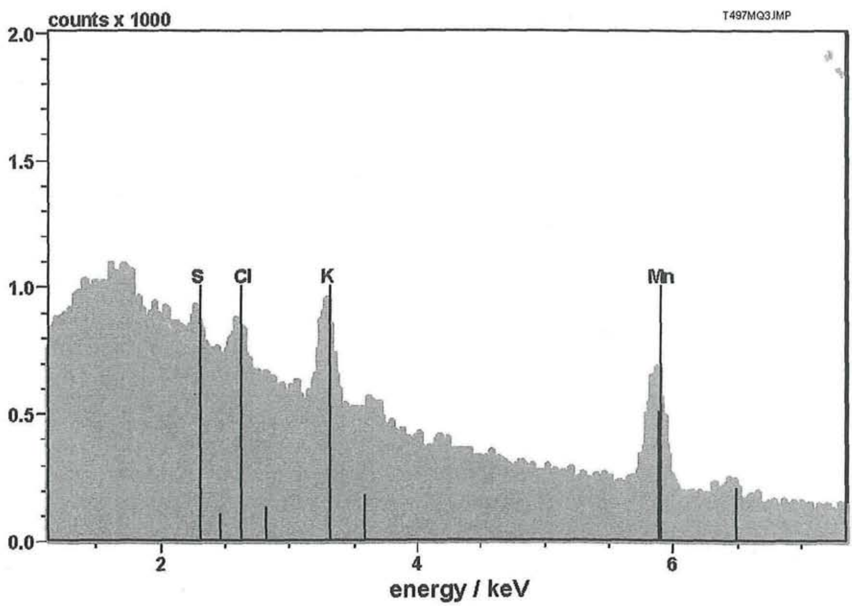

Fig. 7. EDX spectrum of a mesophyll vacuole, spruce T4, needle age class 1997.

Spectra of the cell wall of mesophyll cells, which were X-rayed from the outside (direction of the intercellular space) did also not include any manganese peaks but comparatively high silicon concentrations ($\bar{x}$ = 1.64%, SD:0,352).

Contrary to this, manganese was significantly detectable in the **mesophyll vacuole** in the needles of Tharandt sample trees (Fig. 7).

In the vacuoles of **endodermis cells** of the examined spruce needles high manganese concentrations were measurable in addition to sulphur, potassium and phosphorus, as compared to mesophyll. Manganese, however, could not be detected at methodologically measurable concentrations in the endodermis vacuole (Fig. 8). Manganese was detected in the endodermis radial cell walls and also in composite cell walls of adjacent endodermis and mesophyll cells. Likewise, manganese was contained in the epidermis cells.

In the cell vacuoles of **transfusion parenchyma**, potassium ($\bar{x}$ = 0.22%) was detected on a regular scale. At Tharandt site, manganese was likewise identifiable with on average 0.21% (SD: 0.159) in transfusion parenchyma.

The spectra from the regions of the outer **epidermis cell wall and cuticle** contained silicon ($\bar{x}$ = 0.84%) and calcium ($\bar{x}$ = 3,28%) at a regular rate. At Tharandt, manganese contents of on average $\bar{x}$ = 0.22% (SD: 0.04) were calculated from these X-ray spectra. In the intercellular spaces of mesophyll, crystalline oxalate deposited on the mesophyll cell walls was measured microanalytically on several crystals for each of the needles examined. In addition to traces of silicon ($\bar{x}$ = 0.566%, SD: 0.4963) high concentrations of calcium ($\bar{x}$ = 21.13%, SD: 8.04) were measured in crystals in each case. Manganese however, could not be detected within the range of oxalate crystals (Fig. 9, Fig. 10).

The high magnesium supply at Geising (basaltic bedrock) compared to Tharandt (rhyolitic bedrock), was reflected in the X-ray spectra of the tissue compartments of the needles investigated. Here, manganese could not be identified in any of the examined tissues by means of X-ray microanalysis.

Discussion

Valuation of the method

The activity of the cytoplasm, which uses the large extra-plasmatic compartment – the vacuole – for various purposes, becomes obvious by the different element concentrations in the vacuoles. Therefore, the investigation of the cell saps may be regarded as an indirect approach to the understanding of the conditions prevailing in the cytoplasm where relative constant conditions are maintained (Matile 1987). This indirect approach was taken as the basis for the described investigations on mesophyll vacuoles of the spruce needles.

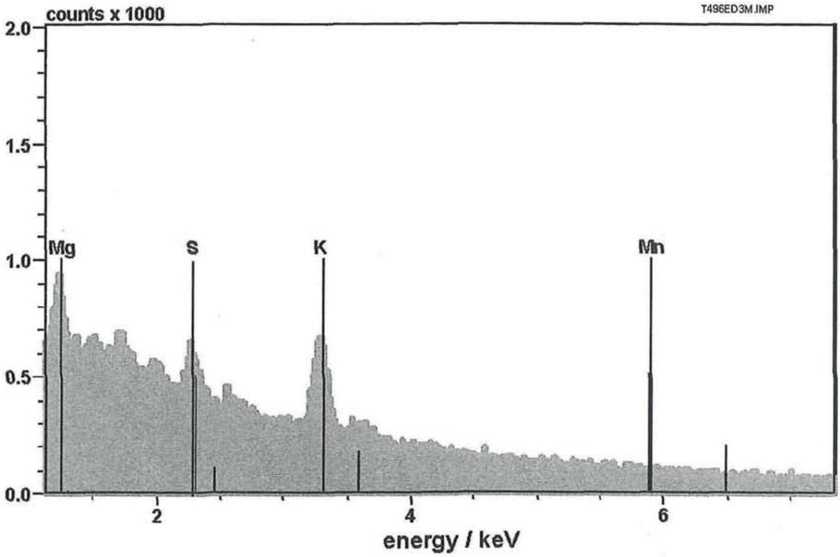

Fig. 8. EDX spectrum of an endodermis vacuole, spruce T4, needle age class 1996.

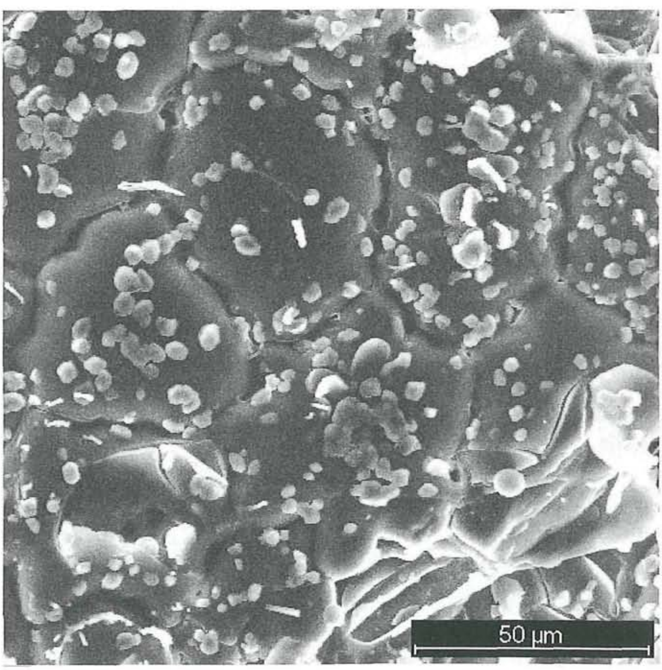

Fig. 9. Crystalline calcium oxalate in the intercellular space of mesophyll, spruce T4, needle age class 1997.

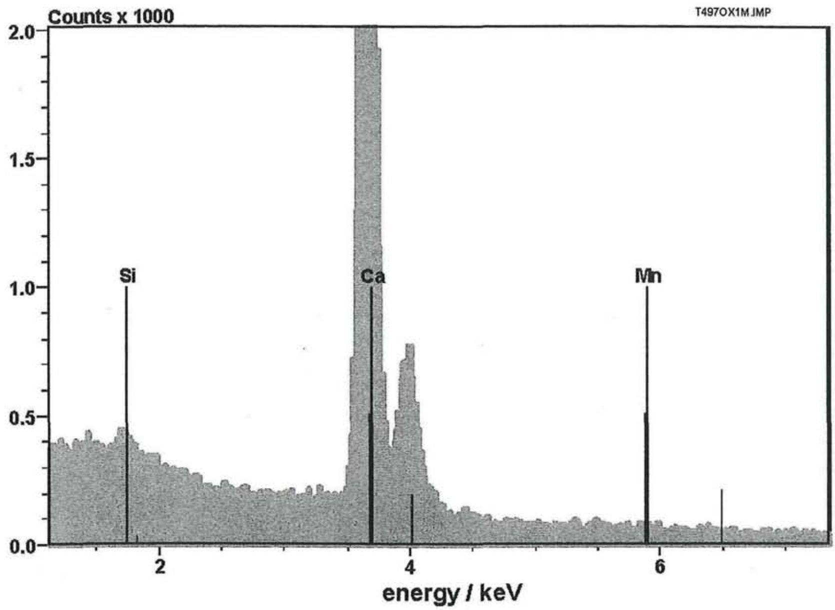

Fig. 10. EDX spectrum of an oxalate crystal the intercellular space of mesophyll, spruce T4, needle age class 1997.

By application of X-ray microanalysis in conjunction with Cryo-SEM, ions accumulated in the vacuoles could be measured at a relatively lower cost. The results corroborated the incorporation of excess sulphur in the form of sulphate into the mesophyll vacuoles with increasing needle age. The results showed sulphur accumulation differing according to the individual trees under comparable site and immission conditions. The methodical approach is confirmed by well comparable trends both between the individual trees and between the needle age classes, referring to the water-soluble sulphate contents and the sulphur contents of the mesophyll vacuoles (mesophyll vacuoles are dominant in volume percentage of the needles).

Mesophyll and endodermis

The magnesium contents of the spruces of the two sites differ especially in the endodermis. According to STELZER & al. 1990 the endodermis vacuole in spruce needles serves as a magnesium store. Obviously, magnesium is transported into the mesophyll vacuole only, if this store is filled due to proper supply.

The function as counterion for sulphate ions, mainly in older needles containing more sulphate, is presumably not primarily fulfilled by magnesium and only refers to these properly supplied sites.

The average sulphur contents of the endodermis were comparable with those of the mesophyll. With the exception of tree T4, however, sulphur concentration did not show a clear age trend. This suggests that the incorporation of surplus sulphate in the needle is not so important in the endodermis, as compared to mesophyll. This is yet emphasised by the much smaller volume percentage as compared to mesophyll, and by the capacity of the endodermis to act as a mediator for exchange of substances between vascular bundles and assimilation parenchyma.

The results obtained from the investigations into the sulphur contents of the various tissues confirm that increased total sulphur contents in older needles are mainly localised in mesophyll vacuoles.

Ionic balances, correlation

The potassium contents in the mesophyll vacuoles of the older needles vary strongly with sulphur concentrations. Thus, the ionic balances of the mesophyll vacuoles for all four trees prove that potassium bears a great importance as a counterion to the sulphate ions stored in older needles. This is likewise confirmed by correlation analyses. As correlation analysis eventually only examines equidirectional changes of element contents- its conclusiveness is restricted, if all ions in solution are effective and, moreover, if not all ions involved are represented. Supposedly, soluble manganese is involved to certain percentages in the ionic balance at Tharandt site, although this is not indicated by the correlation coefficient between manganese and sulphur. Similarly, the magnesium located in the mesophyll vacuole of tree G2 is involved in the ionic balance as well.

Elements other than magnesium, sulphur, chlorine, potassium and manganese (and carbon and oxygen) were not detected by X-ray microanalysis. In accordance with FINK 1991, calcium was found in the tissues investigated, exclusively in the cell wall and oxalate crystals of the mesophyll intercellular spaces, but never in the vacuoles. Hence, the significance of calcium as counterion for sulphate ions is excluded.

Manganese detoxification in case of hypernutrition

Total needle analyses of Tharandt, a site characterised by a very high manganese supply, revealed very high manganese concentrations; and X-ray microanalysis on cryo-SEM showed that concentrations in the single needle compartments were different. The highest concentrations were found in the vacuoles of the mesophyll, where manganese was stored in bivalent form. Furthermore, manganese could be identified by EDX also in the transfusion parenchyma and in the epidermis cell wall, where apparently manganese is transported or enriched. In the endodermis vacuole, enrichment of manganese was not detectable, although manganese was contained in detectable quantities in the endodermis cell wall.

Likewise, manganese accumulation could not be identified in mesophyll cytosol and in mesophyll cell walls. This partly contradicts the conclusions drawn by GODDE & al. 1991, who investigated the distribution of various cations in needle tissues by Protone-Induced X-ray analysis (PIXE). GODDE & al. 1991 discovered similar potassium and manganese distribution patterns within the spruce needles. The highest concentrations were detected, in accordance with the EDX examinations, in the assimilation parenchyma. As potassium concentrations are highest in cytoplasm, GODDE & al. 1991 supposed also a concentration of manganese in cytoplasm where we however found no manganese.

The calcium oxalate crystals in the mesophyll intercellular spaces did not contain manganese measurable by EDX.

The pathway of manganese through the spruce needle can be shown by way of measurements. It is transported from the xylem to the needle. The enrichment in transfusion parenchyma points to its possible shifting to other tree components via the phloem. This might be a confirmation of an internal redistribution taking place in connection with fluctuations in manganese supply, found by TRÜBY & LINDNER 1990 in spruce (*Picea abies* (L.) Karst.).

By EDX measurements manganese is detected in the radial walls and also in some tangential walls of the endodermis, but neither in the vacuole nor in cytosol. This suggests an apoplastic traversal of the endodermis. SCHOLZ & BAUCH 1973, however, found that the water conductivity of the radial walls of endodermis of pine needles was low. Possibly, a symplastic transport through the endodermis would be imaginable (with merely passive incorporations into the cell wall like in wood). In this case EDX analysis would not show any enrichment (below detection limit) in manganese in cytosol and vacuole fluid.

After endodermis traversal, manganese is deposited in the mesophyll vacuole. Here relatively high concentrations are reached, and the sulphate anions which were accumulated under the conditions of declining immission are by far insuffient as counterions to the manganese(II) cations. For equalisation of charges organic ions such as malate or citrate are possible here. The detoxification in terms of oxalate supposed by WYTTENBACH & al. 1995, comparable to calcium, is opposed by the poor solubility of manganese(II) oxalate and because crystals containing manganese have so far not been found in needles examined. Likewise, the high proportion of soluble manganese which was ascertained would contradict the detoxification in the form of oxalate. The increase of an internal tolerance to high manganese concentrations through compartmentalisation within the tissues and cells and complexing in the plant, supposed by HORST 1988, seems to come closer to the observations made by these investigations.

Presumably, manganese is leached via the epidermis cell wall and the cuticle. According to LYR & al. 1992 leaching from leaf organs takes place via the exchange of cations located at the exchange places of the cuticle by hydrogen ions of precipitation or fog water. In this context, the rate of leaching of anions is, in general, not equivalent to the leaching of the cations. Leaching is also indicated by the low concentration increase of manganese, starting from the second needle age class. High leaching rates were identified for manganese at Tharandt site (Tab. 1) and have been found to coincide with the results of other investigators (TRÜBY & LINDNER 1990, WIENHAUS & al. 1994). Lower manganese contents in the throughfall deposition over the past few years (Tab. 1) suggest a connection between manganese leaching and immission.

The relationship between manganese leaching from spruce needles and the sulphate deposition rate found by BURKHARDT & DRECHSEL 1997, could, on the one hand, explain the still relatively high sulphur needle contents at Tharandt site, despite diminishing immission. On the other hand, a comparison of the two examined trees shows that the higher manganese contents are linked here with a lower accumulation of sulphate. The lower accumulation of sulphate in the needles of tree T4, which apparently takes up a greater amount of manganese than tree T5, may also suggest a joint leaching of manganese and sulphate from the needles. The joint leaching of manganese and sulphate could also be an explanation for the regressive manganese contents of throughfall deposition.

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