Patterns in content of phenolic compounds in leaves of mountain birches along a strong pollution gradient

J. Loponen a,*, K. Lempa b, V. Ossipov a,b, M.V. Kozlov b, A. Girs a, K. Hangasmaa a, E. Haukioja b, K. Pihlaja a

a Laboratory of Environmental and Physical Chemistry, Department of Chemistry, University of Turku, FIN-20014 Turku, Finland
b Section of Ecology, Department of Biology; University of Turku, FIN-20014 Turku, Finland

Received 1 September 2000; accepted 13 October 2000

Abstract

The contents of individual low-molecular weight phenolic compounds (LMWPs) in mountain birch, Betula pubescens ssp. czerepanovii, leaves collected during 1996–1998 in six plots 7–65 km south of the nickel–copper smelter at Monchegorsk, Kola Peninsula, NW Russia, were reported. A high-performance liquid chromatography–electrospray ionisation–mass spectrometry (HPLC–ESI–MS) was used for the rapid identification of low-molecular weight phenolics. Quantification was performed by the analytical high-performance liquid chromatography with UV-detection. Contents of (+)-catechin and some gallic acid derivatives decreased significantly, and contents of flavonol glycosides slightly increased with the distance from the smelter. Hydroxycinnamic acid derivatives remained unaltered. These changes in birch leaf phenolics are probably related to the effect of environmental contamination on the biosynthetic reactions both in the shikimate and phenylpropanoid pathways. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Betula pubescens; Foliar; Heavy metals; Polyphenols

1. Introduction

Plants in heavily polluted areas exhibit changes in physiological and biochemical traits (Malhotra and Khan, 1984; Darrall, 1989; Dixon and Paiva, 1995). Effects on plant phenolic compounds were found under abiotic and biotic stresses (Zobel and Nighswander, 1990, 1991; Bernards and Lewis, 1992; Masuch et al., 1992; Christie et al., 1994; Feucht et al., 1994; Lois, 1994; Yalpani et al., 1994; Karolewski and Giertych, 1994, 1995; Dixon and Paiva, 1995; Giertych et al., 1999). In our former works it was resulted that contents of several low-molecular weight phenolic compounds (LMWPs) in leaves of white birch, Betula pubescens Ehrh., were higher in birches from the polluted sites than in birches from the control area (Loponen et al., 1997, 1998). These studies indicated that the biosyntheses of gallic acid derivatives and p-coumaroylquinic acid derivatives in the birch leaves were activated by pollution, possibly due to an intensified competition for the common precursor, dehydroquinate, between the gallotannin pathway and the pathway leading to p-coumaroylquinic acid (Loponen et al., 1998). However, some of the detected patterns were not significant, and it was supposed that this was due to the relatively low pollution pressure (Derome and Lindroos, 1998) at the site in which our earlier data have been collected.

The content of individual phenolic compounds in mountain birch leaves in the most severely polluted area of Northern Europe was investigated. The special emphasis was placed on changes in the contents of the three

* Corresponding author. Tel.: +358-2-3336-828; fax: +358-2-3336-700.
E-mail address: jyrki.loponen@utu.fi (J. Loponen).
groups of phenolics: gallic acid derivatives (GAs); hydroxycinnamic acid derivatives (HCAs); flavonoids (FLAs). The following questions were aimed to answer:
1. Are the contents of phenolic compounds in mountain birch leaves higher close to the pollution source than in control area?
2. Does the content of LMWP change gradually with an increase in pollution, or is there a threshold, distancing at which the content is higher than in control site?
3. Do the major classes of phenolic compounds or individual phenolics change consistently along the strong pollution gradient?
4. Do the contents of the main groups of phenolic compounds in birch leaves vary with the sampling year and sampling date?
5. Is this variation similar in heavily polluted and clean (control) sites?

2. Materials and methods

2.1. Study area

The area surrounding the city of Monchegorsk (68°N, 33°E) on the Kola Peninsula is one of the most extreme examples of terrestrial pollution on the boreal zone (Rigina and Kozlov, 1999). Vast quantities of sulphur and heavy metals emitted since late 40s have caused widespread destruction of soils and vegetation and imposed additional environmental gradients in the region around the smelter (Kozlov and Haukioja, 1995, 1997). The total amount of pollutants emitted by the smelter was in 1990 2.64 × 10^8 kg, which included 2.33 × 10^8 kg of SO2, and 1.58 × 10^5 kg of dusts containing heavy metals (2.7 × 10^6 kg of nickel, 1.8 × 10^6 kg of copper) (Berlyand, 1991). The area influenced by aerial pollution is estimated to exceed 10,000 km². Complete destruction of forest ecosystems occurs within 6–10 km from the smelter (Kryuchkov, 1993). Visible effects of the pollution on vegetation have been recorded up to 60–80 km, and measurable traces of pollutants up to 200 km from the smelter (Tikkanen and Niemelä, 1995).

2.2. Study sites and the description of study years

Six sampling plots were located in lowlands (altitude 160–240 m) within the following damage zones (Kozlov and Haukioja, 1995): industrial barrens (7 and 9 km south of the smelter), birch transitional community (15 km S), severely (20 km S) and slightly (29 km S) damaged spruce-dominated forests. The most distant plot (65 km SE of the smelter) was situated in primary spruce forest. The foliar contents of copper and nickel in mountain birch showed significant negative correlation with the distance from the pollution source, being increased by the factor 5–120 near the smelter compared to the regional background level (Kozlov et al., 1995). It is also necessary to mention that in 1996, in the Kola area, there was a late spring, leaf growth was slow, and the size of the leaves remained small. In 1997: early spring, fast leaf growth, normal size leaves. In 1998: normal spring, normal leaf growth, normal size leaves.

2.3. Trees and sampling protocol

Twenty young mountain birch [B. pubescens ssp. czerpanovii (Orlova) Hämet–Ahti] ramets (trunk diameter 12–25 mm) were sampled in each plot during 1996–1998. Leaf samples were collected twice during the summer: the first one – just after the termination of leaf growth; the second one – 20 days after the first sampling date. Additionally, in 1997 and 1998, leaves from ten birch trees (different sets were sampled in different years) were sampled in each site to control the effect of previous-year sampling on the phenolic content. Fifteen to twenty undamaged short-shoot leaves (one leaf from each shoot) were randomly sampled from the crown of each tree and, after clipping the petioles, divided in two sets: the leaves of the first set were put into the vial with acetone, whereas the leaves of the second set were put into the dry vial. Before the sampling, vials for storing of the first set of leaves (10 ml dark brown glass vials with a plastic cap) were numbered, filled with acetone, tightly closed and weighed. Dry vials for the second set were also numbered and weighed. All vials were transported to the experimental plots in cool boxes over wet ice, which allowed leaf fixation with acetone within 15 min after sampling. Samples were transported back to the laboratory in cool boxes. Vials with leaves fixed in acetone were weighed to estimate fresh weight of leaves, and the caps were then wrapped by parafilm. These samples were transported to the Department of Chemistry in Turku University in cool boxes and stored at −20°C until the analysis. The second set of leaves (vials without acetone) was used to determine the tree-specific water content in the leaves. The vials with fresh leaves were weighed immediately, dried at 80°C for 48 h and then weighed again. The results were used to calculate the dry weight of leaves fixed in acetone. Total of 960 samples have been collected and analysed.

2.4. Extraction of plant material

The acetone fraction from leaf containing vials was separated, and the leaf material left to dry in an air hood. Dried leaves were ground with mortar and pestle. The milled material was transferred to 60 ml brown glass bottle equipped with a cap. For the extraction, 70% aqueous acetone was added (the required volume of extraction solvent was 1 ml per 20 mg of dry leaf
of water was adjusted in the sample so that the ratio between the dry weight of the leaves and the volume of water was 0.022 g ml⁻¹.

2.5. HPLC analysis: separation and quantification

Qualitative and quantitative determinations of LMWPs were performed with a high-performance liquid chromatography system consisting of a Merck-Hitachi L-7100 HPLC pump (Hitachi, Tokyo, Japan) connected to a Merck-Hitachi L-7200 Autosampler (Hitachi, Tokyo, Japan) and Merck-Hitachi L-7400 UV-detector (Hitachi, Tokyo, Japan). Linking between the HPLC-apparatus and a computer including a HSM Manager-program was done through a Merck-Hitachi D-7000 Interface (Hitachi, Tokyo, Japan). Additional analyses were done with LC-235 diode array detector (Perkin–Elmer, Norwalk, CT, USA) connected to a Graphic Printer GP-100 (Perkin–Elmer, Beaconsfield, England). The column used was 5 μm Spherisorb ODS2 (250 × 4.6 mm I.D., Waters, UK). The injection volume was 20 μl. the constant flow rate was 1 ml min⁻¹, and a column back pressure varied from 70 to 146 bar during the gradient. The detection wavelength was 280 nm. Analyses were made at room temperature (20°C).

Samples were filtered before analysis by a Titan Syringe filter (0.45 μm, 13 mm I.D., Scientific Resources, Eatontown, NJ, USA). HPLC gradient program for individual LMWPs was developed earlier (Ossipov et al., 1995, 1996). The eluent composition was: A – acetonitrile; B – formic acid/water (5/95, v/v). The elution profile was: 0–5 min, 100% B (isocratic); 5–60 min, 0–30% A in B (linear gradient); 60–70 min, 30–60% A in B (linear gradient); 70–85 min, 60% A in B (isocratic); 85–90 min, 60–0% A in B, (linear gradient); 90–110 min, 100% B (isocratic).

Quantification of GAs, HCAs and FLAs was performed with standard agents: gallic, p-coumaric and chlorogenic acids, (+)-catechin, quercetin (obtained from Sigma), myricetin and kaempferol (purchased from Fluka) (Ossipov et al., 1996). Similar molar extinction coefficients were assumed for aglycones and glycosides (Winter and Herrmann, 1986). Mean values of contents of phenolic compounds are expressed in milligrams per gram (mg g⁻¹) of dry leaf weight (DW).

2.6. HPLC–ESI–MS determination

The high-performance liquid chromatographic–mass spectral analyses of individual phenolic compounds were carried out with a Perkin–Elmer SCIEX API 365 triple–quadrupole mass spectrometer (PE Sciex, Toronto, Canada) incorporated into a Perkin–Elmer Series 200 HPLC system with UV/VIS-detector and Apple Macintosh 8.1 data system. The ionisation technique was an electrospray (pneumatically assisted electrospray; Turbo IonSpray Ionisation Chamber, PE Sciex, Toronto, Canada). The HPLC system was comprised of two Perkin–Elmer Series 200 micro pumps (Perkin–Elmer, Norwalk, CT, USA) and 785A UV/VIS-detector (Perkin–Elmer, Norwalk, CT, USA). Samples were introduced into the system by a Perkin–Elmer Series 200 Autosampler (Perkin–Elmer, Norwalk, CT, USA). The separation of individual compounds was achieved on a 5 μm Spherisorb ODS2 (250 × 4.6 mm I.D., Waters, UK) column. The chromatographic conditions were generally similar to those used in quantitative analysis. The HPLC solvent system consisted of acetonitrile and formic acid/water (0.4/99.6, v/v). The flow rate was 1.0 ml min⁻¹. Before the ESI, main part of the flow was split and only minor part (20% of the flow) was introduced into the ion source.

The mass spectrometer was operated in the negative ion mode. Mass data were acquired by the scan mode, which consisted of scanning over a mass range of m/z 100–1100 using a 0.30 atomic mass unit step size. The needle voltage for the negative ion experiments was −4000 V. Orifice plate voltage was set at −35 V; ring voltage was set at −220 V. Nebulizer gas (purified air) flow was set to value 8, and curtain gas (N₂) flow was set to value 10. Heated nitrogen gas temperature was 300°C.

2.7. Statistical analysis

The contents of phenolic compounds were constrained by both ecological and temporal factors. Therefore, effects of distance to the smelter, year and date on the content of phenolic compounds were analysed by using repeated measures analysis of variance (Potvin et al., 1990; von Ende, 1993). Sampling year and date were within subject effects and the gradient (six study sites) was between subject effect. The contents of individual compounds were pooled. Consequently, total content of phenolic compounds and contents of three major classes of phenolic compounds, GAs, HCAs and FLAs were analysed. The pooling reduced considerably inflatory effects of high number of response variables on critical significance values (P values) in statistical analyses (see Rice, 1989). To fulfil the assumptions of analysis of variance, contents of three major phenolic groups were log transformed to normalise the distribution of model residuals.
3. Results

3.1. Phenolic composition of mountain birch leaves

In leaves of mountain birches from Kola, the found individual LMWPs (Fig. 1) were divided into three groups according to chromatographic (see Ossipov et al., 1995, 1996) and mass spectral analyses. Here m/z values of the deprotonated molecules ([M−H]− ions) were shown as results. The first group, HCAs: 5-p-coumaroylquinic acid, cis- and trans-3-p-coumaroylquinic acids, m/z 337 for all (molecular weight, M.W. 338); chlorogenic acid and neocholesteric acid, m/z 353 for both (M.W. 354). The second group, FLAs: (+)-catechin, m/z 289 (M.W. 290); kaempferol-3-O-rhamnopyranoside, m/z 431 (M.W. 432); quercetin-3-O-arabinofuranoside, m/z 433 (M.W. 434); kaempferol-3-O-galactopyranoside, m/z 447 (M.W. 448); quercetin-3-O-galactopyranoside or quercetin-3-O-galactopyranoside, m/z 463 for both (M.W. 464); myricetin-3-O-rhamnopyranoside, m/z 463 (M.W. 464); quercetin-3-O-glucuronopyranoside, m/z 477 (M.W. 478); myricetin-3-O-galactopyranoside, m/z 479 (M.W. 480); quercetin-3-O-(4'-O-acetyl)-galactopyranoside, m/z 489 (M.W. 490); myricetin-3-O-glucuronopyranoside, m/z 493 (M.W. 494). Phenolics of the third group, GAs, were recognised on the basis of m/z values from 169 to 787 in mass spectra (see Salminen et al., 1999) as well as the shapes and maxima of UV-spectra and retention times in HPLC chromatograms (Ossipov et al., 1997).

3.2. Total contents of phenolic compounds

There was a considerable amount of among-year variation in the total contents of foliar phenolics in 1996–1998 (Fig. 2, Table 1). On the basis of pooled data, the total content declined during the study period from 40.4 mg g−1 DW in 1996, in the early sampling date, to 17.2 mg g−1 DW in 1998, in the late sampling date. The declining trend was not, however, induced by leaf samplings in the previous seasons, since the additional trees (not sampled in the previous season) revealed a similar trend and did not differ from the main sets of trees either in 1997 or 1998 (Fig. 2). The early leaf samples, collected at the termination of leaf growth, contained, in general, higher amounts of phenolic compounds than the late leaf samples (mature leaves) (Fig. 2, Table 1). The sampled leaves from the sites close to the smelter had

![Figure 1](https://example.com/figure1.png)

Fig. 1. Example structures of some individual phenolic compounds exist in leaves of mountain birch.
Fig. 2. Total phenolic contents in mountain birch leaves growing along the pollution gradient; mg g⁻¹ DW (dry weight of the leaves), mean ± SE (standard error of the mean). “Basic trees” were sampled every year 1996–1998, n = 20. “Additional trees” were different trees in each site, sampled in 1997–1998 to control the effect of the previous year sampling on phenolics, n = 10.

generally higher phenolic contents than in leaves from the control site (Fig. 2), albeit the decline was not linear as indicated by contrast analyses (independent contrasts for areas 7, 9 and 15 km from the smelter $F_{1,114} = 2.94; P = 0.09$, $F_{1,114} = 4.71; P = 0.03$ and $F_{1,114} = 5.11; P = 0.02$, respectively).

The sum contents of individual phenolics declined with an increase in distance from the smelter, except in 1998, in the late sampling date (Fig. 2), resulting in a significant year*gradient interaction (Table 1). Moreover, changes in the sum contents of individual leaf phenolic compounds along the pollution gradient were consistently larger in early than in late sampling date as indicated by a significant date*gradient interaction (Table 1). Contrast analyses revealed that the area 15 km from the smelter was mainly responsible for the
significant interaction (an independent contrast for the area $F_{1.114} = 9.22; P = 0.003$).

3.3. Different groups of phenolics

GAs, HCAs and FLAs had different patterns of temporal and spatial variations (Fig. 3). In main effects, GAs determined the pattern of sum content of LMWPs. The contents of GAs declined during the study period (1996–1998). Contents of GAs were higher in young leaves than in mature leaves, and increased with an increase in pollution (independent contrasts for the areas 7 and 9 km $F_{1.114} = 10.63; P = 0.001$ and $F_{1.114} = 8.72; P = 0.003$, respectively). However, any statistically significant interaction among the main effects was not detected (Table 1).

The content of HCAs showed no variation among the study sites. Their annual and seasonal patterns corresponded to those found in sum LMWPs as well as GAs (Fig. 3, Table 1): the content of HCAs declined during the study period (1996–1998), and young leaves had higher content of HCAs than mature leaves. Significant among-year and seasonal variations were found in the contents of HCAs (Table 1). In 1996, content of HCAs differed from the control in the area of 9 km (independent contrasts for early and late samples $F_{1.118} = 5.12; P = 0.026$ and $F_{1.118} = 3.92; P = 0.05$, respectively), but not in 1997 and 1998. There was among-site variation in respect to seasonal variation indicated by the independent contrasts for date * gradient (7 and 15 km; $F_{1.114} = 4.30; P = 0.04$ and $F_{1.114} = 6.05; P = 0.015$, respectively). Evidently, responses of HCAs to the environmental stressors vary in time and are spatially non-linear. The seasonal decline in contents of HCAs was strong in 1997 and 1998, and negligible in 1996 (Fig. 3), resulting in significant year * date interactions (Table 1).

The content of FLAs, in contrast to the two other groups, varied with the sampling year and the study site, but displayed no seasonal variation (Table 1). The multiannual patterns followed those found in other classes of LMWPs. FLA content among the years did not vary in a consistent way along the gradient. The main pattern was a non-linear “wave-shaped” gradient (Fig. 3). It indicates multiple minima and maxima at 7, 9 and 15 km from the smelter. In a sampling site 15 km from the smelter, the content of FLAs in early leaves was lower than that in the control area as indicated by a significant year * date * gradient interaction (Table 1).

3.4. Characteristic individual phenolics

A set of five phenolic compounds (5-p-coumaroylquinic acid (HCA), (+)-catechin (FLA), digalloylgucose (GA), quercetin–glycoside (FLA) and chlorogenic acid (HCA)) was used to illustrate how individual foliar phenolics may vary during the leaf ageing, and how pollution may shape the variation during the leaf maturation (Fig. 4, Table 2). In the sampling year 1997, the contents of the first four compounds showed statistically significant differences in t-tests ($P \leq 0.01$) between the site closest to the smelter and the control one in both sampling dates. For this reason, the data of that year were selected. Instead, chlorogenic acid, the main individual phenolic compound in leaves of B. pubescens species, showed no significant differences in contents along the pollution gradient in 1997, and between the polluted and control areas in the sampling years 1996–1998 (data not shown). Analyses of contrasts revealed that contents of 5-p-coumaroylquinic acid and digalloylgucose differed statistically from those of the control site in the polluted end of the gradient (independent contrasts for 5-p-coumaroylquinic in the sites 7 and 9 km from the smelter $F_{1.114} = 15.54; P < 0.0001$ and...
Fig. 3. Contents of different groups of phenolic compounds: GAs, HCAs and FLAs in mountain birch leaves growing along the pollution gradient; mg g\(^{-1}\) DW, mean ± SE.

\(F_{1,114} = 19.14; P < 0.0001\), respectively, and independent contrasts for digalloylgucose in the sites 7, 9 and 29 km from the smelter \(F_{1,114} = 14.11; P < 0.0001, F_{1,114} = 9.77; P = 0.002\) and \(F_{1,114} = 5.93; P = 0.0165\), respectively. For quercetin–glycoside effects were detected in areas 7, 9, 15 and 20 km from the smelter (independent contrasts: \(F_{1,114} = 17.68; P < 0.0001, F_{1,114} = 11.88; P = 0.0008, F_{1,114} = 9.65; P = 0.0024, F_{1,114} = 5.74; P = 0.018\), respectively). (+)-Catechin content differed significantly from the control in every sampling plot in the study year 1997, and was found to be significantly higher in the most polluted than in the control plot in every sampling set (1996–1998). There were differences in seasonal variation for the contents of individual compounds. Content of digalloylgucose decreased during the leaf development, whereas that of quercetin–glycoside increased (Table 2). For (+)-catechin, a date-dependent response to heavy metal gradient was detected. The analysis of contrasts indicated that the area 7 km from the smelter was responsible for the significant date*gradient interaction \((F_{1,114} = 12.00; P < 0.0001)\).

4. Discussion

The main classes of phenolic compounds in birch leaves, GAs, HCAs and FLAs, responded to pollution in different ways. This indicates that it might be possible to find out compounds, which are suitable for monitoring the effects of environmental changes on biochemical processes in plants growing under pollution, although many of the compounds are not suitable for this purpose. In addition, there is a large background variation in phenolic compounds due to other factors relating to plant growth, for instance because of soil fertility or
climatic variations. Trying to reduce the variation of other than environmental pollutants on the phenolic metabolism, several study sites and along a pollution gradient as well as repeating of samplings within and between study years are needed. The data from the present study satisfy these demands well because six

Table 2
Results for repeated measures analysis of variance for contents of four individual phenolic compounds representing major classes of phenolic compounds

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>5-p-Coumaroylquinic acid (HCA)</th>
<th>(+)-Catechin (FLA)</th>
<th>Digalloylglucose (GA)</th>
<th>Quercetin-glycoside (FLA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date</td>
<td>$F_{1,114} = 3.61$;</td>
<td>$F_{1,114} = 1.20$;</td>
<td>$F_{1,114} = 11.80$;</td>
<td>$F_{1,114} = 83.49$;</td>
</tr>
<tr>
<td>(within subject)</td>
<td>$P = 0.06$</td>
<td>$P = 0.28$</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Gradient (between</td>
<td>$F_{3,114} = 6.79$;</td>
<td>$F_{3,114} = 31.77$;</td>
<td>$F_{3,114} = 2.90$;</td>
<td>$F_{3,114} = 6.06$;</td>
</tr>
<tr>
<td>subject factor)</td>
<td>$P &lt; 0.0001$</td>
<td>$P &lt; 0.0001$</td>
<td>$P = 0.017$</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Date * gradient</td>
<td>$F_{3,114} = 1.14$;</td>
<td>$F_{3,114} = 4.39$;</td>
<td>$F_{3,114} = 1.67$;</td>
<td>$F_{3,114} = 1.57$;</td>
</tr>
<tr>
<td></td>
<td>$P = 0.34$</td>
<td>$P = 0.0011$</td>
<td>$P = 0.15$</td>
<td>$P = 0.17$</td>
</tr>
</tbody>
</table>

$^a$ $F$ = test statistic for ANOVA with respective degrees of freedom; $P$ = significance level.
study sites were sampled during three years and possible responses of the phenolic compounds – products of the shikimate pathway were monitored.

On the basis of high-performance liquid chromatography incorporated with UV-spectrophotometric detection, mountain birch leaves from Kola Peninsula had a similar composition of individual low-molecular weight phenolic compounds to that have been earlier found in B. pubescens (Ossipov et al., 1995, 1996, 1997; Loponen et al., 1997, 1998). Additionally, with the negative-ion mass spectral analysis for the components by HPLC–ESI–MS, it was possible to determine the deprotonated molecules of the compounds and conclude directly the characteristic molecular weights of phenolic compounds. Different biosynthetic origins, or biosyntheses of different groups of phenolic compounds in different steps of the shikimate pathway, are one of the factors of variation for their environmental response. Precursor of hydrolysable tannins (GAs) is gallic acid, which is synthesised through an intermediate of the shikimate pathway, dehydroshikimic acid (Haslam, 1989; Gross, 1992; Waterman and Mole, 1994). Hydroxycinnamic acid derivatives are formed from one of the terminal products of shikimate pathway, L-phenylalanine, through the phenylpropanoid pathway. At the same time, coumaroylquinic and chlorogenic acids are products derived from both hydroxycinnamic and quinic acids (Zenk, 1979; Gross, 1981). Direct precursor of quinic acid is also an intermediate compound of the shikimate pathway, dehydroquinic acid, and the reaction of quinic acid formation from dehydroquinic acid is catalysed by quinate dehydrogenase (Boudet, 1973; Boudet et al., 1985; Herrmann and Weaver, 1999). FLAs are the results of the combination of precursors from acetate–malonate pathway and phenylpropanoid pathway (Waterman and Mole, 1994). Further, some enzymes, which take part in these biosynthetic pathways, may be more sensitive than others in relation to pollutants (including primarily heavy metals and SO₂). Native enzymes can contain different amounts of metals, originating from the growth environment of the plant. Metals probably have an effect on the structure and catalytic level of the enzyme (Herrmann and Weaver, 1999).

The results of this study revealed that the total content of phenolic compounds (as a sum of individual phenolics) did not change much with the distance from the smelter, unlike for example GAs. In agreement with earlier studies, the content of GAs was constantly higher in young leaves compared to the mature leaves at the late season samples. The decreased levels of GAs in mature leaves might result from transformation of these compounds from soluble form into insoluble cell wall-bound form (Ossipov et al., 1997). There was no significant negative correlation in the content of FLAs with the gradient, parallel to the earlier study (Loponen et al., 1997). Furthermore, distance from the smelter did not modify patterns in contents of HCA in the present study. Contents of FLAs and HCA showed in the heavy metal gradient year-to-year and seasonal variation, most often displaying “wave-like” patterns with more than one peak and through. The maximum of phenolic contents was not necessarily found from leaf samples near the smelter (Figs. 2–4).

Interestingly, among the individual compounds, the (+)-catechin content showed a significant increase towards the smelter. This increase started already in the plot 29 km from the smelter, where the amount of (+)-catechin was significantly higher compared to background site (65 km). The finding is consistent with other studies indicating that (+)-catechin tends to accumulate in tissues of plants affected by pollutants (Kettrup et al., 1991; Masuch et al., 1992; Hartling and Schultz, 1998; Loponen et al., 1998). Functionally, (+)-catechin monomers (flavan-3-ol) serve as precursors in a chain of steps towards oligomeric and polymeric proanthocyanidins (condensed tannins) (Swain, 1979; Hemingway, 1989; Waterman and Mole, 1994; Haslam, 1996). Within FLAs, also contents of myricetin-, quercetin- and kaempferol-glycosides (flavonol-glycosides) were measured in this work. Flavonol-glycosides are products of the reaction chain from malonate and ρ-coumarate precursors via the chalcone and dihydroflavonol types of compounds (Hahlbrock, 1981; Ebel and Hahlbrock, 1982; Harborne, 1989). Dihydroflavonols can be supposed to be the common precursors for flavonols and flavan-3-ols (catechins and proanthocyanidins) (Markham, 1982). Our results hint at an inhibitory effect in the step of dihydroflavonol transformation into flavonols, which can be indicated as lower or invariable sum contents of myricetin-, quercetin- and kaempferol-glycosides in leaves from polluted areas. (+)-Catechin production follows similar early steps in this pathway. However, flavonol-glycosides are probably terminal products, while (+)-catechin is an intermediate compound on the pathway of proanthocyanidin synthesis (Hemingway, 1989). Because, the relatively higher level of (+)-catechin in the leaves from polluted plots may be the result not only from acceleration of its biosynthesis. The investigation of the step of (+)-catechin transformation towards proanthocyanidin polymers might call for an additional explanation, as to why its content regularly increased in the leaves growing in highly polluted stressed areas.

The above-mentioned change in phenolic contents in relation to pollution level was detectable despite large annual and seasonal variations. However, it is worth emphasising that annual variation in total content of phenolic compounds in mountain birch leaves explained about 45% of total variance, being the largest source of variation. These data indicate that one-year studies may not be sufficient for making conclusions on the envi-
vironmental effects. However, most biochemical studies of pollution effects are based on the single-year data, casting doubt on the generality of conclusions. Another potential source of uncertainty is introduced by reporting pooled contents of individual phenolics. Such information may be inefficient in detecting pollution-induced changes in phenolic metabolism. More detailed data concerning different main groups of phenolics and individual phenolic compounds are needed. On the basis of the results in this study, the contents of GAs and (+)-catechin in leaves of the mountain birch seemed to be the possible candidates to detect increased pollution load from a biochemical point of view. The accumulation of gallic acid derivatives and (+)-catechin indicated activation of the shikimate pathway in mountain birch leaves growing under the effect of air pollution, and they responded in a similar way through the sampling years and dates.

Acknowledgements

The study was funded by Academy of Finland, Emil Aaltonen Foundation and Kone Foundation for JL. The assistance of V. Zverev and E. Melnikov is acknowledged.

References


