Influence of vegetation on phenolic acid contents in soil

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ABSTRACT: The study described in this paper was undertaken in order to assess the impact of different woody plants on the dynamics of phenolic acids in soil. The influence of plant litter on the amount of phenolic compounds occurring in soils beneath several deciduous and coniferous tree species was examined in pot experiments. The contents of endogenous methanol soluble free and conjugated phenolic acids in Norway spruce, larch, rowan and two species of willow were determined. We focused on the dynamics of water-soluble and reversibly bound phenolic acid contents in the upper layers of soil substrates in pots after the cultivation of studied woody plants.

Keywords: Norway spruce; larch; rowan; willow; phenolic compounds; pot experiment

Phenolics, arising mainly from decomposing plant materials and soil microbial activity, represent an important group of soil organic matter. Phenolic compounds in soils play an important role in controlling many aspects of plant-soil interactions, e.g. allelopathic interactions (Muscolo et al. 2001), and they are important intermediates in humus formation.

The hypothesis was proposed that highly polymeric humic substances originate from polymerization of water-soluble phenolic precursors under the catalytic effect of immobilized phenoloxidases (heterophasic biocatalysis). This hypothesis was confirmed by a laboratory experiment using a mixture of monomeric phenols and nitrogenous compounds as structural precursors and fungal laccase as a biotic catalyst (Zavarzina 2006).

In soil, phenolic acids occur rarely as free molecular bases. In overall majority they are covalently linked to high molecular-mass organic substances and on mineral surfaces. Phenol composition of the soil is largely influenced by the vegetation and soil type (Whitehead et al. 1982, reviewed in Strobel 2001). The quantitative and qualitative differences in phenolic contents in soil, depending on the character of vegetation, may subsequently influence the phenolic concentration in sources of drinking water.

In spite of intensive studies, contamination of drinking water by humic substances is still a frequent problem. From the water management point of view they are harmful due to the formation of toxic haloforms (trihalogenmethanes) during water chlorination (Vlková, Církva 2005). In the course of water chlorination plant-derived phenolic acids form chlorophenolic mixtures whose toxicity was assessed on an *Escherichia coli* strain similar to that found in the human intestine (Borlakoglu, Kickuth 1986).

The study described in this paper was undertaken in order to assess the impact of different woody plants on the dynamics of phenolic acids in soil. To eliminate the impact of phenolic acids released from the humus enriched horizon we removed

the soil layer 0–20 cm from the soil before the use in the experiment. The study was realized in the framework of the project focused on the research of the possibilities of reducing the content of organic pollutants in the water sources in the Krušné hory Mts.

The objective of this study was:

(i) to determine the contents of phenolic acids in plant material of woody plants growing in the studied locality Krušné hory Mts.,

(ii) to determine water-soluble and reversibly bound phenolic contents in the upper layer of soil substrates in pots after the cultivation of Norway spruce, larch, rowan and two species of willow.

MATERIAL AND METHODS

Study area and vegetation sampling

The Fláje locality (Krušné hory Mts., at a height of ca 800 m a.s.l.) used in this study was dominated by broadleaves *Sorbus aucuparia, Salix arita* and *Salix pentandra* and conifers *Picea abies* and *Larix decidua*. Soil type in this locality is modal cryptopodzols. The samples of leaves and needles were collected from 4–5 trees of each species growing in the studied locality in September 2010 and transferred to a laboratory for phenolic acid analyses.

Pot experiments

The soil for pot experiments originated from the above described locality Fláje. After removing the humus enriched upper soil layer, the soil was taken from a depth of 20 to 100 cm, transferred to the laboratory and filled into ten-litre pots.

These values were determined in the soil substrate used in the experiment: pH_{CaCl_2} 4.29, 21.8 mg of total organic C per 100 mg dry soil, 0.96 mg of total organic N per 100 mg dry soil, 19.8 mg per kg dry soil of so called "mobile" humic substances (HS) extracted with 0.001M calcium chloride and 175.3 g per kg dry soil of HS extracted with 0.1M sodium pyrophosphate.

The contents of phenolic acids were determined in the soil substrates of pots prior to the tree cultivation. Two years old seedlings of *Picea abies, Larix decidua* and *Sorbus aucuparia* and one year old cuttings of *Salix aurita* and *Salix pentandra* (five plants of each species) were planted in the pots in the greenhouse. For the experiment each tree spe-

cies was planted into 10 pots. Water soluble and alkali-soluble phenolic acids were extracted from the upper layers of soil substrates after the annual cultivation of spruce, larch and rowan and two-year cultivation of the two species of willow in the pots. At the end of the experiment the trees were three years old. The root system of all experimental trees was well developed.

Extraction and determination of phenolic acids in plant material

Methanol-soluble phenolic acids (represented by the sum of methanol soluble free, ester and glycoside bound phenolic acids) were extracted from needles and/or leaves of spruce, larch, rowan and two species of willow and HPLC analysed by the method described by Cvikrová et al. (2008).

Extraction and determination of phenolic acids in soil

Water soluble and alkali-soluble phenolic acids (extracted with 1M NaOH) were extracted from soil samples according to the method of Bruckert et al. (1967) and HPLC analysed by the method described by Cvikrová et al. (2008).

RESULTS

Content of phenolic acids in needles and/or leaves of woody plants

The total contents of phenolic acids (represented by the sum of methanol soluble free, ester and glycoside bound phenolic acids) in spruce, larch, rowan and two species of willow are presented in Table 1. In needles of Norway spruce were detected 6 phenolic acids, 3 derivatives of cinnamic acid and 3 benzoic acid derivatives.

In higher concentrations were found *p*-coumaric, *p*-hydroxybenzoic, ferulic and vanillic acids. In needles of larch were found 8 phenolic acids (4 derivatives of cinnamic acid and 4 benzoic acid derivatives). The highest concentrations were determined in vanillic and *p*-hydroxybenzoic acids, followed by concentrations of *p*-coumaric, gallic and ferulic acids.

In rowan leaves the constituent of extracts caffeic acid was dominant, followed by the contents of *p*-coumaric, ferulic, chlorogenic and *p*-hydroxy-

Table 1. Contents of methanol-soluble phenolic acids (represented by the sum of methanol soluble free ester and glycoside bound phenolic acids) extracted from needles and/or leaves of spruce (*Picea abies*), larch (*Larix decidua*), rowan (*Sorbus aucuparia*) and two species of willow (*Salix aurita, Salix pentandra*). Means ± S.E. of two independent experiments with two replicates using material from 4 to 5 trees in one experiment

	GA	PA	2.5-HBA	*p*-HBA	2.3-HBA	VA	CaA	ChA	SA	*p*-CA	FA
P. abies	3.20 ± 0.74	n.d.	n.d.	51.54 ± 2.16	n.d.	10.13 ± 0.29	1.64 ± 0.22	n.d.	n.d.	57.06 ± 1.97	9.76 ± 0.10
L. deciduas	25.31 ± 1.30	0.10 ± 0.02	n.d.	39.38 ± 3.26	n.d.	43.24 ± 1.20	8.25 ± 1.82	3.60 ± 2.38	n.d.	36.97 ± 3.93	42.40 ± 0.98
S. aucuparia	n.d.	0.13 ± 0.01	n.d.	43.96 ± 6.18	n.d.	0.94 ± 0.21	1023.80 ± 109.83	80.87 ± 6.70	n.d.	488.60 ± 36.18	193.74 ± 18.60
S. aurita	19.10 ± 0.04	0.35 ± 0.03	54.93 ± 7.57	28.57 ± 1.23	4.86 ± 0.08	22.84 ± 1.31	315.96 ± 13.15	12.30 ± 0.03	506.41 ± 22.52	200.74 ± 6.38	23.39 ± 0.72
S. pentandra	4.24 ± 0.04	n.d.	49.13 ± 0.22	44.04 ± 0.08	36.21 ± 0.03	26.76 ± 0.05	766.32 ± 41.88	32.25 ± 0.03	1787.19 ± 0.13	130.72 ± 1.70	45.99 ± 0.07

GA – gallic acid; PA – protocatechuic acid; 2.5-HBA – 2.5 di-hydroxybenzoic acid (gentisic); *p*-HBA – *p*-hydroxybenzoic acid; 2.3- HBA – 2.3 di-hydroxybenzoic acid; VA – vanillic acid; CaA – caffeic acid; SyA – syringic acid; ChA – chlorogenic acid; SA – salicylic acid; *p*-CA – *p*-coumaric acid; FA – ferulic acid; CA – cinnamic acid; n.d. – not detected

benzoic acids. Content of vanillic acid in rowan leaves was very low. The broadest spectrum of phenolic acids was detected in willow leaves. In the leaves of both *Salix* species were determined 11 acids (7 derivatives of benzoic acid and 4 cinnamic acid derivatives) (Table 1). Salicylic acid showed the highest concentration, followed by concentrations of caffeic, *p*-coumaric, gentisic, *p*-hydroxybenzoic and vanillic acids.

Content of phenolic acids extracted from the upper soil layers of pots

Extraction with distilled water

The amount of water-soluble phenolics extracted with distilled water from the upper layers of soil substrates in pots before the experiment (before the tree cultivation) was rather low. The contents of gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric and ferulic acids are presented in Table 2.

In soil samples after one year of spruce, larch and rowan cultivation gallic and protocatechuic acids were not detected as they can be easily oxidized, and syringic acid was not detected either. On the contrary, the spectrum of water-soluble phenolics comprised caffeic and cinnamic acids (Tables 2 and 3). The concentrations of phenolic acids in water extracts of the soil upper layers after two years of willow cultivation were very low, almost at the detection level of the HPLC method (Table 3).

Alkaline extraction

The content of phenolic acids reversibly bound to soil organic matter, determined in 5 samples of the upper layers of soil substrates in pots before the experiment (extraction with 1M NaOH), was substantially higher in comparison with the content of phenolics in water extracts. In alkaline extract were detected 7 phenolic acids. High concentrations were determined in vanillic, *p*-hydroxybenzoic and *p*-coumaric acids, followed by concentrations of ferulic, syringic, protocatechuic and gallic acids (Table 2).

Lower levels of phenolic acids were detected in soil substrates after one year of spruce cultivation (Table 4). On the contrary, in soil substrates after one year of larch cultivation the content of vanillic acid was increased and a slight decrease in *p*-hydroxybenzoic and *p*-coumaric acids was observed (in comparison with the values prior to the experiment). Similarly, in soil samples after one year of rowan cultivation an increase in vanillic acid content and a decrease in

Table 2. Contents (in µg·g^{-1} dry soil) of phenolic acids extracted with solution from the upper layers of soil substrates in pots before the tree cultivation. The data represent the means ± S.E. of results from 5 pots

GA	PA	*p*-HBA	VA	SyA	*p*-CA	FA
With water						
0.004 ± 0.001	0.008 ± 0.002	0.016 ± 0.007	0.023 ± 0.01	0.064 ± 0.03	0.019 ± 0.04	0.003 ± 0.001
With 1M NaOH						
0,59 ± 0,08	9,00 ± 0,90	121,10 ± 21,90	137,92 ± 22,71	11,18 ± 1,82	120,88 ± 16,97	31,35 ± 4,50

GA – gallic acid; PA – protocatechuic acid; *p*-HBA – *p*-hydroxybenzoic acid; VA – vanillic acid; SyA – syringic acid; *p*-CA – *p*-coumaric acid; FA – ferulic acid

p-hydroxybenzoic and *p*-coumaric acids were detected (Table 4). A decline in phenolic contents determined in the alkaline extract of soil substrates was observed after two-year cultivation of willow species (Table 4). Syringic acid (3,5-methoxy 4-hydroxy benzoic acid) was not found in soil substrates after woody plant cultivation.

DISCUSSION

Phenolic compounds are naturally present in soils due to the biochemical degradation and transformation of organic substances, and they also derive from anthropogenic activities. It is proposed that phenolic compounds, released into the soil by root exudates and litter inputs, have important implications for plant-litter-soil interactions (Muscolo et al. 2001).

However, this study was undertaken in order to assess the impact of different woody plants on the dynamics of phenolic acids in soils and subsequently on their concentrations in sources of drinking water.

The influence of forest litter on the amount of phenolic compounds occurring in soils beneath several deciduous and coniferous tree species was examined. We have compared Norway spruce, larch, rowan and two species of willow for their spectrum of phenolic substances in needles and/or leaves and determined the differences in the levels of water-soluble and reversibly bound phenolics in the upper layers of soil substrates in pots after one- and two-year cultivation of these woody plants.

According to the results of Palm et al. (1996) up to 40% of the initial dry weight of litter material can be lost by leaching of inorganic as well organic compounds within a period of 4 months from litter

Table 3. Contents (in µg·g^{-1} dry soil) of phenolic acids extracted with water from the upper layers of soil substrates in pots after cultivation. The data represent the means ± S.E. of results from 5 pots of each tree from two independent experiments

	p-HBA	VA	CaA	*p*-CA	FA	CA
After the annual cultivation of spruce, larch and rowan						
P. abies	0.018 ± 0.002	0.015 ± 0.001	0.004 ± 0.001	0.027 ± 0.010	0.002 ± 0.001	0.013 ± 0.001
L. decidua	0.019 ± 0.006	0.015 ± 0.006	0.001 ± 0.000	0.011 ± 0.0003	n.d.	0.004 ± 0.001
S. aucuparia	0.023 ± 0.010	0.028 ± 0.007	0.002 ± 0.000	0.016 ± 0.002	0.001 ± 0.000	0.012 ± 0.002
Two-year cultivation of two willow species						
S. rubra	0.013 ± 0.003	0.014 ± 0.002	0.008 ± 0.005	0.008 ± 0.001	n.d.	0.002 ± 0.000
S. pentandra	0.009 ± 0.003	0.007 ± 0.001	0.001 ± 0.000	0.005 ± 0.003	n.d.	0.001 ± 0.000

p-HBA – *p*-hydroxybenzoic acid; VA – vanillic acid; CaA – caffeic acid; *p*-CA – *p*-coumaric acid; FA – ferulic acid; CA – cinnamic acid; n.d. – not detected

Table 4. Contents (in µg·g^{-1} dry soil) of phenolic acids extracted with 1M NaOH from the upper layers of soil substrates in pots after the cultivation. The data represent the means ± S.E. of results from 5 pots of each tree from two independent experiments

	GA	PA	*p*-HBA	VA	*p*-CA	FA
After the annual cultivation of spruce, larch and rowan						
P. abies	0.31 ± 0.01	6.16 ± 0.16	75.57 ± 1.35	87.14 ± 1.11	70.36 ± 2.29	27.58 ± 1.03
L. deciduas	0.78 ± 0.12	12.86 ± 1.41	114.86 ± 9.32	158.75 ± 27.70	98.60 ± 9.49	30.56 ± 4.00
S. aucuparia	0.57 ± 0.17	9.87 ± 1.43	93.32 ± 19.05	157.39 ± 29.32	71.58 ± 10.53	33.16 ± 5.53
After two-year cultivation of two willow species						
S. rubra	0.41 ± 0.12	5.57 ± 1.74	75.23 ± 18.51	87.10 ± 12.00	72.58 ± 11.18	24.24 ± 3.36
S. pentandra	0.53 ± 0.002	7.55 ± 0.03	112.05 ± 0.03	108.62 ± 0.02	92.12 ± 0.01	30.13 ± 0.02

GA – gallic acid; PA – protocatechuic acid; *p*-HBA – *p*-hydroxybenzoic acid; VA – vanillic acid; *p*-CA – *p*-coumaric acid; FA – ferulic acid

fall. The analyses of phenolics in our experiments were performed in April, i.e. in the period when we proposed a higher level of phenolic substances in the upper layer of soil substrate. As the process of lignin degradation proceeds slowly (Flaig 1971), the dynamics of observed changes was mainly influenced by leaching of different litter types and microbial activity of soil.

Although the contents of phenolic substances among the studied woody plants were rather diverse (Table 1), these differences did not appear in the spectrum of water-soluble phenolics in soil substrates. In soil samples after the annual cultivation of spruce, larch and rowan gallic and protocatechuic acids were not detected, i.e. phenolic acids that can be easily oxidized. On the contrary, the spectrum of water-soluble phenolics comprised a low concentration of caffeic and cinnamic acids (Tables 2 and 3). The contents of phenolic acids in water extracts of the soil upper layers after two years of willow cultivation were very low, almost at the detection limit of the used HPLC method (Table 3). There is little evidence that higher plants can cleave the aromatic ring and use phenolics as food reserves.

However, by contrast, many species of soil microorganisms are able to degrade aromatic compounds and use the phenolics as a source of carbon. Free phenolic acids generally have a short half-live in most soils due to their microbial utilization (Dalton 1989).

Characterization of microbial production in soil and subsequent utilization of phenolic acid breakdown products were described by Blum (1998).

In consequence of the diverse microbial activity in the course of the year seasonal variations were observed in levels of water-soluble and mild alkali-soluble phenolics (Kuiters, Denneman 1987). We propose on the basis of the above-mentioned studies that observed decreases in the levels of water-soluble phenolics, much more evident in the soil samples after two-year cultivation of willow, resulted from microbial degradation (Table 3).

It was found out that reversible sorption of phenolic acids may provide some protection from microbial degradation (Dalton 1989).

The contents of alkali extracted phenolic acids reversibly bound to soil organic matter determined in our experiment were substantially higher in comparison with the content of phenolics in water extracts.

Depending on the type of woody plants there were observed considerable differences in the quantity of individual phenolic acids in soil substrates under the trees. It is proposed that phenolic acids extracted with cold 1M NaOH are predominantly bound as ester linked compounds and originate from leaching of litter types. A release of phenolics from lignin requires much stronger oxidizing conditions (Provan 1994).

The research focused on microbial metabolism of free and reversibly bound phenolics in soil, with emphasis on the initial phenolic acid breakdown products generated by microbes, was described by Blum (1998).

In our experiment the levels of gallic acid in the upper layers of soil substrates did not change after one-year cultivation of rowan, slightly decreased

and on the contrary increased after spruce and larch cultivation, respectively (Table 4). A slight increase in protocatechuic acid content was observed in the soil substrate after larch cultivation only. No difference in ferulic acid concentrations after woody plant cultivation and before the tree cultivation ($P > 0.05$, Student's t-test) was determined.

This fact might result from the microbial utilization of simple phenolic acids, such as ferulic and p-coumaric acids, which leads to the production of p-hydroxybenzoic, vanillic and/or protocatechuic acids before the aromatic ring structure is broken (Blum, Shafer 1988).

Regardless of the cultivated tree, a decline in the contents of p-coumaric and p-hydroxybenzoic acids was observed ($P > 0.05$, Student's t-test).

The amount of vanillic acid decreased after spruce and willow cultivation ($P > 0.05$, Student's t-test) and on the contrary it increased in soils beneath larch and rowan.

The comparison of the total amounts of phenolics in the upper soil layers after one year of larch and spruce cultivation showed a significant difference ($P > 0.05$, Student's t-test), probably due to the massive fallout of larch needles, i.e. the high litter input into the soil.

Although the leaves of rowan contained very high concentrations of both ferulic and p-coumaric acids (Table 1), which after the fallout might enrich the phenolic acids in the soil substrate, no marked increase in the levels of these phenolics was observed in soil after rowan cultivation.

However, the increase in vanillic acid content in soil after one year of rowan cultivation might result from its microbial production from ferulic acid as proposed by Blum (1998). Subsequent differential utilization of cinnamic and benzoic acids by soil microbes and incorporation into humic substances might also influence the composition and concentration of soil phenolic compounds.

Considerable amounts of phenolic residues bound to humic acids still contained the unchanged aromatic ring as revealed by C^{-13} NMR analysis, indicating that the residues were probably bound via ester or ether bonds (Li et al. 2012).

Although the contents were significantly higher in comparison with the contents of phenolics in water extracts, their levels did not change significantly in soils beneath several deciduous and coniferous tree species in the course of the experiment. However, to follow the dynamics of changes in soil phenolic acids, e.g. their migration in the soil profile along with the water leaching regime and their incorporation into humic substances, requires a longer time period of study.

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