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Dynamics of Phenolic Composition and Content of Representatives of the Genus *Spiraea* L. under the Conditions of Transport and Industrial Pollution in Novosibirsk

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Abstract

The annual dynamics of composition and content of groups of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acids and flavonols) and individual phenolics (gallic, protocatehuic, chlorogenic, caffeic, *p*-coumaric and cinnamic acids, hyperoside, isoquercitrin, rutin and avicularin) in the leaves of *Spiraea media* Fr. Schmidt, *S. chamaedryfolia* L. и *S. hypericifolia* L., growing in Novosibirsk in the areas with high and background levels of transport and industrial pollution were studied. The peculiarities of effects of environmental contamination and meteorological conditions on the content of phenolic compounds and their groups are revealed. The concentrations of the major constituents in leaves of the species decreased significantly in the urban area in comparison with the background one. The most stable annual decrease in the concentrations was found in the leaves of *S. hypericifolia*. The level of the annual variability of the number of phenolic components under the conditions of pollution increased (*S. media*, *S. chamaedryfolia*) or slightly changed (*S. hypericifolia*) compared with the control, and the level of variation in the concentrations of most constituents, on the contrary, decreased.

Key words: *Spiraea media*, *S. chamaedryfolia*, *S. hypericifolia*, flavonoids, phenolic acids, annual dynamics, industrial pollution

INTRODUCTION

Ornamental shrubs of the *Spiraea* L. genus (Rosaceae Juss. family) possessing high ecological plasticity and playing an important part in settlement gardening in industrial centres are of substantial interest as the model objects for the studies of plant adaptation to the conditions of industrial pollution. The high content and diversity of phenolic compounds in the plants of this genus give the possibility to study the reactions of separate components and fractions of the phenol complex [1].

The role of phenolic compounds in the adaptive reactions of plants was demonstrated for various taxons and stress factors. A large number of works deal with the studies of the metabolism of phenolic compounds, including flavonoids, under stress with controllable conditions [2–4]. The attention of researchers to the studies of the effect of long-term action of technogenic factors on the composition of phenolic compounds has also increased [5–8]. It was demonstrated that under the conditions of long-term industrial pollution not only the biochemical parameters of adapting plants change but also the nature of their variability [9].

The species *Spiraea media* Fr. Schmidt, *S. chamaedryfolia* L. and *S. hypericifolia* L. have substantial differences in the systematic position and in the composition of phenolic compounds [10]. For instance, in the leaves of *S. media* quercetin glycosides dominate, while in the leaves of *S. chamaedryfolia* and *S. hypericifolia* – the glycosides of cinnamic acid and the glycosides of quercetin, respectively, which go with a substantial number of oxycynnamic acids present in small amounts.

The goal of the study was to carry out a comparative investigation of the annual dynamics of the composition and content of phenolic compounds in the leaves of plants: *S. media*, *S. chamaedryfolia* and *S. hypericifolia*, growing under the conditions of transport and industrial pollution and under relatively favourable (background) ecological conditions.

EXPERIMENTAL

To carry out the investigation, we sampled the leaves of *S. media*, *S. chamaedryfolia* and *S. hypericifolia* in the Leninsky and Sovetsky districts of Novosibirsk in July 2012–2014. These two districts differ in the level of the transport and industrial pollution. In the Leninsky district, which is characterized by unfavourable ecological parameters, the samples were collected in the Slava Park situated near the city road (urban conditions, UC). The major pollutants of the air in this district are nitrogen dioxide, ammonia, carbon (II) oxide, formaldehyde and 3,4-benz(a)pyrene [11]. The samples from the background conditions (BC) were collected in the arboretum at the CSBG SB RAS situated amidst the forest forestland under relatively favourable ecological conditions (reference).

The years during which the studies were carried out had definite differences in the climatic characteristics during the period of the most active vegetation (June – July). July 2013 was characterized by lower maximal and average diurnal temperature and higher air humidity, which depicts the increased amount of atmospheric precipitation in comparison with July 2012 and 2014 (Table 1) [12].

During the period from July 20 to July 25, ten annual shoots were taken uniformly over the whole bush crown from each plant of each species simultaneously from UC and BC. The leaves of a strongly different size of damages leaves were discarded. Sample volume for each region was 3–10 plants at the age of 25–30 years.

The composition and content of phenolic compounds in the leaves were studied before and after hydrolysis of water-alcohol extracts by means of high-performance liquid chromatography (HPLC). The regimes of extraction, acid hydrolysis and chromatographic separation were described in detail in [13]. In the native water-alcohol extract, the content of glycosides and free aglycons of flavonoids, free phenolic acids, their esters and glycosides was determined, while the content of the aglycons of flavonoids and phenolic acids was determined in the hydrolyzed extract.

Chromatographic analysis was carried out with an Agilent 1200 chromatograph with a diode matrix detector and the system for processing the chromatographic data ChemStation (Agilent Technologies, USA). The separation was carried out with a Zorbax SB-C18 column 4.6 × 150 mm in size, with particle diameter 5 μm.

The compounds were identified by comparing with the standard samples of quercetin, kaempferol, chlorogenic acid (Sigma-Aldrich, USA), hyperoside, rutin, isoquercitrin, avicularin (Fluka, Sigma-Aldrich Chemie GmbH, Germany), acids: gallic, protocathechuic, *p*-coumaric, caffeic, cinnamic (Serva, Germany). The content of non-identified components was calculated depending on the spectral characteristics in the equivalents of gallic acid (for phenolic acids), hyperoside (for flavonol glycosides) or quercetin (for the aglycons of flavonols). The components having one absorption maximum λ_{max} within the range 250–270 nm were assigned to the fraction of hydroxybenzoic acids, cinnamic acid and the components with two λ_{max} within the ranges 250–270 and 290–325 nm were assigned to the fraction of hydroxycinnamic acids, the components with two λ_{max}

TABLE 1

Major climatic characteristics in Novosibirsk in 2012–2014 (June–July)

Parameter	2012		2013		2014	
	June	July	June	July	June	July
Air temperature (monthly average), °C	17	21	20	19	16	21
Air temperature (maximal), °C	33	33	29	30	33	37
Relative air humidity (monthly average), %	58	66	69	76	62	68

within the ranges 250–270 and 350–370 nm were related to the fraction of flavonol glycosides. The content of the components was expressed in per cent of absolutely dry leaf mass.

Statistical treatment of the data was carried out with the help of Statistica 7.0 software (Statsoft Inc., USA). Using the results of the experiment that lasted for three years, we calculated the maximal and minimal values (Lim), arithmetic mean (*M*), the error of arithmetic mean (*m*) and variation coefficient (CV). The level of variability (LV) of the features was estimated with respect to S. A. Mamaev's scale [14]. Depending on the value of variation coefficient, we distinguished very low (CV < 7 %), low (CV = 8–12 %), medium (CV = 13–20 %), increased (CV = 21–30 %), high (CV = 31–40 %) and very high (CV > 40 %) LV.

RESULTS AND DISCUSSION

Composition and content of aglycons and native phenolic compounds in the leaves of S. media, S. chamaedryfolia, S. hypericifolia and their variability

In the hydrolyzed extracts of *S. media*, 13 to 19 compounds were detected. In agreement with the spectral and chromatographic characteristics of these compounds, they were assigned to phenolic acids and aglycons of flavonols. A detailed description of the components of the studies species was reported in [10]. The dominating aglycon component of the leaves of *S. media* is quercetin (Table 2).

The total number of aglycon components and the number of components in the fractions of phenolic acids and flavonoids for the urban and background conditions did not differ from each other substantially. The variability of the number of aglycons over the years was insignificant.

The number of phenolic acids for the BC varied at a very low level (3 %), while for the UC at the medium level (20 %). The level of variability of the number of aglycons of flavonoids in both versions of conditions was increased (22 %).

The content of the majority of components and fractions, as well as the total sum of aglycons for UC were lower than for BC. Quite the contrary, kaempferol content increased for UC. Variation of the content of components and fractions over the years for BC was high, very high and anomalously high, for the majority of components it was higher than that for UC. The level of variability of the total content of the aglycons of phe-

TABLE 2

Composition and content of aglycons in the leaves of *Spiraea media* during the years 2012–2014

Parameter	*M*±*m** / Lim		CV**, %	
	1	2	1	2
Number of aglycon components, including:	18.3±0.7 17–19	17.0±2.0 13–19	6	20
phenolic acids	15.7±0.3 15–16	14.3±1.7 11–16	3	20
flavonoids	2.7±0.3 2–3	2.7±0.3 2–3	22	22
Content of aglycon components and fractions, % of the dry mass of leaves:				
quercetin	0.78±0.19 0.56–1.17	0.53±0.15 0.23–0.70	42	49
kaempferol	0.09±0.02 0.06–0.13	0.15±0.03 0.11–0.20	45	34
isorhamnetin	0.10±0.01 0.00–0.29	0.02±0.01 0.00–0.04	160	90
phenolic acids	1.6±0.5 0.9–2.5	1.4±0.3 1.0–1.9	47	32
Total content of aglycons	2.6±0.6 1.6–3.7	2.1±0.1 1.9–2.2	40	9

Note. Here and in Tables 3–7: 1 – background, 2 – pollution.

* Numerator: arithmetic mean over three years (*M*)±error of mean (*m*) (g/100 g of abs. dry mass), denominator: variation limits (Lim).

** CV – coefficient of variation.

TABLE 3

Composition and content of phenolic compounds in the leaves of *Spiraea media* during the years 2012–2014

Parameter	t_r, min	*M*±*m* Lim		CV, %	
		1	2	1	2
Number of phenolic compounds, including:		37.3±5.3 32–48	27.3±4.8 22–37	25	31
phenolic acids		24.7±3.2 21–31	17.0±3.1 13–23	22	31
flavonol glycosides		12.7±2.2 10–17	10.3±1.9 8–14	30	31
Content of components and fractions, % of abs. dry mass of leaves:					
gallic acid	1.82	0.32±0.13 0.11–0.57	0.29±0.04 0.21–0.35	73	25
protocathechuic acid	2.56	0.05±0.03 0.006–0.12	0.14±0.10 0.00–0.35	107	139
sum of hydroxybenzoic acids		0.67±0.21 0.24–0.89	0.88±0.19 0.56–1.21	56	37
chlorogenic acid	3.22	0.11±0.03 0.06–0.17	0.14±0.04 0.09–0.23	53	53
caffeic acid	4.95	0.01±0.003 0.008–0.02	0.01±0.007 0.00–0.03	44	122
p-coumaric acid	7.90	0.08±0.05 0.01–0.18	0.06±0.02 0.03–0.10	119	65
component 15 (hydroxycinnamic acid) (λ_{max} 225, 325 nm)	10.9	0.09±0.05 0.02–0.19	0.24±0.18 0.03–0.59	100	129
sum of hydroxycinnamic acids		0.81±0.20 0.59–1.22	0.80±0.28 0.51–0.35	43	61
sum of flavonol glycosides		1.48±0.02 1.45–1.52	1.68±0.15 1.39–1.92	2	26
hyperoside	18.4	0.39±0.20 0.11–0.79	0.19±0.09 0.08–0.38	91	83
rutin	20.0	0.38±0.31 0.02–1.00	0.02±0.02 0.00–0.05	142	173
component 37 (O-glycoside of flavonol) (λ_{max} 255, 360 nm)	41.6	0.51±0.40 0.08–1.32	0.33±0.11 0.14–0.55	138	63
sum of flavonol glycosides		2.38±1.34 0.65–5.02	1.17±0.23 0.87–1.61	98	34
Sum of phenolic compounds		3.88±1.33 2.10–6.48	2.85±0.31 2.26–3.33	60	19

Notes. 1. t_r – retention time. 2. For designations, see Table 2.

nolic compounds for UC was substantially lower (9 %) than for BC (40 %).

The number of compounds found in the extracts of *S. media* was 23 to 48; about a half of them were flavonol glycosides. The permanent components of the leaves during the three years of the investigations under the urban and background conditions were six glycosides, in particular hyperoside and rutin accounting for about 70 % of the sum of flavonoids (Table 3). The number of phenolic acids and flavonol glycosides was lower for UC, while the LV of these parameters was higher (31 %) than for BC (22 and 30 %, respectively). The number of flavonol glycosides for BC (30 %) was changing stronger in comparison with phenolic acids (22 %). The content of some phenolic components and fractions was higher for UC than for BC. The most clearly pronounced differences were revealed for the sum of phenolic acids and the sum of hydroxybenzoic acids. Quite the contrary, the sum of phenolic compounds, the sum of flavonol glycosides and the major glyco-

TABLE 4

Composition and content of aglycons in the leaves of *Spiraea chamaedryfolia* during 2012–2014

Parameter	$\frac{M \pm m}{\text{Lim}}$		CV, %	
	1	2	1	2
Number of aglycon components, including:	24.00±0.58 23–25	24.33±0.67 23–25	4	5
phenolic acids	22.33±0.67 21–23	22.67±0.88 21–24	5	7
flavonoids	1.67±0.33 1–2	1.67±0.33 1–2	35	35
Content of aglycon components and fractions, % of abs. dry mass of leaves				
cinnamic acid	0.87±0.14 0.60–1.09	0.80±0.21 0.43–1.17	28	47
chlorogenic acid	0.18±0.09 0.02–0.35	0.21±0.03 0.17–0.28	94	28
sum of phenolic acids	2.75±1.14 0.99–4.89	3.35±0.17 3.08–3.66	72	9
quercetin	0.18±0.09 0.03–0.33	0.27±0.11 0.14–0.49	84	74
isorhamnetin	0.19±0.15 0.00–0.58	0.03±0.01 0.00–0.05	168	94
Total sum of aglycons	3.13±1.02 1.60–5.08	3.64±0.08 3.52–3.80	57	4

Note. For designations, see Table 2.

side component hyperoside were nearly two times higher for BC than for UC, while rutin content was more than an order of magnitude higher than under the urban conditions (Table 3).

The level of variability of the content of individual components was within the range from increased (25 %) to anomalously high (173 %). This parameter of fraction content and total content of phenolic compounds was substantially lower (2–98 %). The content of flavonol glycosides varied more substantially (34–98 %) than the content of phenolic acids and the sum of phenolic compounds (2–61 %). Under the urban conditions, LV of different components and fractions either increased (for example, for caffeic acid and rutin) or decreased (for example, for gallic and *p*-coumaric acids) in comparison with BC. The level of variability of the sum of hydroxybenzoic acids and the sum of phenolic compounds was lower for UC (37, 34 and 19 %) than for BC (56, 98 and 60 %, respectively). Variation of the sum of hydroxycinnamic acids and the total sum of phenolic acids was higher for UC (61 and 26 %) than for BC (43 and 2 %).

So, during the period of observation, the leaves of *S. media* from UC differed from the reference plants by the decreased content of the sum of phenolic compounds, hyperosid and rutin, and by the increased content of phenolic acids, the sum of hydroxybenzoic acids and some acid components. Variation of the sum of phenolic compounds was lower for UC than for BC.

The number of compounds detected in the hydrolyzed extracts of *S. chamaedryfolia* was 23 to 25, and the major part of these compounds were phenolic acids. The dominating aglycon component in *S. chamaedryfolia* is cinnamic acid [10] (Table 4). The parameters of the composition of aglycons did not differ substantially under the urban and background conditions during the time of the investigation. The total number of aglycons of phenolic compounds and the number of phenolic acids in the leaves of *S. chamaedryfolia* varied at a very low level, while the number of the aglycons of flavonoids varied at a high level. The level of variability of the number of flavonoids (35 % in both versions of conditions) exceeded the LV of phenolic acids (5 and 7 % for BC and UC, respectively). The content of cinnamic acid in the leaves of the samples from UC was insignificantly lower than in the samples from background conditions. The content of some aglycon components (quercetin and chlorogenic acid), the fractions (the sum of phenolic acids) and the sum of aglycons in the sample of *S. chamaedryfolia* from the urban plantations was higher in comparison with BC. Quite contrary, the content of the minor aglycon isorhamnetin was substantially lower for the UC.

TABLE 5

Composition and content of phenolic compounds in the leaves of *Spiraea chamaedryfolia* during 2012–2014

Parameter	t_r, min	M±m Lim		CV, %	
		1	2	1	2
Number of phenolic compounds, including:		37.67±4.18 33–46	36.67±4.26 31–45	19	20
phenolic acids		36.33±4.33 32–45	35.33±4.48 29–44	21	22
flavonol glycosides		1.33±0.33 1–2	1.33±0.33 1–2	43	43
Content of components and fractions, % of abs. dry mass of leaves:					
gallic acid	1.82	0.18±0.08 0.08–0.34	0.17±0.01 0.15–0.20	77	15
protocathechuic acid	2.56	0.08±0.01 0.06–0.10	0.09±0.02 0.06–0.12	26	36
sum of hydroxybenzoic acids		1.15±0.05 1.06–1.21	0.76±0.06 0.68–0.88	7	14
chlorogenic acid	3.22	0.17±0.12 0.15–0.19	0.12±0.06 1.07–2.45	11	82
caffeic acid	4.95	0.08±0.03 0.02–0.11	0.08±0.04 0.03–0.15	65	75
p-coumaric acid	7.90	0.10±0.06 0.03–0.22	0.03±0.007 0.02–0.05	102	37
sum of hydroxycinnamic acid		2.27±1.02 1.13–4.30	1.82±0.40 1.07–2.45	78	38
component 16 (glycoside of cinnamic acid) (λ_{max} 225, 325 nm)	10.9	1.14±0.35 0.48–1.68	0.91±0.21 0.52–1.21	53	40
component 26 (glycoside of cinnamic acid) (λ_{max} 228, 290 nm)	24.3	2.17±1.00 0.81–4.23	0.69±0.26 0.19–1.03	84	65
hyperoside	18.4	0.27±0.03 0.21–0.33	0.14±0.008 0.13–0.16	21	10
isoquercitrin	19.7	0.03±0.01 0.00–0.07	0.11±0.11 0.00–0.32	159	173
sum of flavonol glycosides		0.30±0.01 0.28–0.33	0.25±0.10 0.13–0.46	9	74
Sum of phenolic compounds		7.02±2.33 3.89–11.59	4.43±0.16 4.12–4.66	58	6

Note. For designations, see Table 2.

The variability of aglycon components over the years was high and anomalously high. For UC, LV of the majority of the parameters of component content was much lower (28–94 %) than for BC (28–168 %), except for the major component – cinnamic acid, for which the LV was higher for UC than for background (47 and 28 %, respectively). The sum parameters – the sum of phenolic acids and the total sum of aglycons – exhibited smaller variations. The lowest LV was revealed for UC (9 and 4 %, respectively).

In the native extracts of the leaves of *S. chamaedryfolia*, 31 to 45 compounds were detected. The major part of them were glycosides of cinnamic acid (components 16 and 26, see Table 5) and hydroxycinnamic acids. The relative content of flavonoids (hyperosid, isoquercitrin) was insignificant (about 5 %) (see Table 5). The parameters of the composition of native compounds in the leaves of *S. chamaedryfolia* under the background and urban conditions did not differ substantially and varied at the levels from medium (19 %) to very high (43 %). The LV for these parameters, similarly to the parameters of the composition of aglycons, were very close in value for the background and urban conditions. The number of flavonol glycosides varied stronger (43 % under the studied conditions) than the number of phenolic acids (21 and 22 %, respectively).

TABLE 6

Composition and content of aglycons in the leaves of *Spiraea hypericifolia* during 2012–2014

Parameter	$\frac{M \pm m}{\text{Lim}}$		CV, %	
	1	2	1	2
Number of aglycon components, including:	25.33±0.41 25–26	25.00±0.71 24–26	2	4
phenolic acids	24.00±0.00 24–24	23.67±0.41 23–24	0	2
flavonoids	1.33±0.41 1–2	1.33±0.41 1–2	43	43
Content of aglycon components and fractions, % of abs. dry mass of leaves:				
quercetin	0.81±0.18 0.63–1.09	0.31±0.10 0.16–0.44	30	46
caffeic acid	0.22±0.10 0.04–0.32	0.09±0.05 0.02–0.15	70	68
p-coumaric acid	0.29±0.16 0.05–0.51	0.12±0.06 0.03–0.20	79	75
cinnamic acid	0.04±0.006 0.03–0.05	0.02±0.008 0.01–0.03	26	47
sum of phenolic acids	3.75±1.25 2.20–5.67	1.85±0.37 1.32–2.37	47	28
Total sum of aglycons	4.49±1.44 2.83–6.70	2.17±0.27 1.76–2.53	45	18

Note. For designations, see Table 2.

The content of chlorogenic, *p*-coumaric acids and hyperoside in the leaves from UC decreased in comparison with the background. On the contrary, isoquercitrin was either absent from the BC samples or was present in minimal amounts, while its content under urban conditions was much higher. The total content of flavonol glycosides for UC was substantially lower than under the background conditions due to a decrease in the content of the major component – hyperoside, and the sum of the glycosides of cinnamic acid and the total sum of phenolic compounds decreased almost by a factor of two.

So, during the period of observation, the lower content of major components was revealed in the leaves of *S. chamaedryfolia* from UC: glycosides of cinnamic acid, flavonol glycosides and the total sum of phenolic compounds, in comparison with the plants from BC. The number of phenolic components and its LV exhibited no substantial differences for UC and for BC. The level of variability of the number of phenolic acids was lower (increased) in comparison with flavonoids (very high).

In the hydrolyzed extracts of *S. hypericifolia*, 24 to 26 compounds were detected (Table 6) [10]. Similarly to the leaves of *S. media*, the dominating aglycon in the leaves of *S. hypericifolia* was quercetin. However, its relative content was substantially lower than for *S. media*. The parameters of the composition of aglycon components, similarly for the case of *S. chamaedryfolia*, exhibited no substantial differences for the urban and background conditions. The level of variability of the number of phenolic acids was very low (0 and 2 %, respectively), while LV of the number of aglycons of flavonoids was very high (43 % in both cases).

The total content of aglycons for UC was more than two times lower than for BC. Both the content of phenolic acids and the content of quercetin were substantially decreased. The variability of the total sum of aglycons was much lower for UC (18 %) than for BC (45 %), though the variation of the content of some components (including quercetin) was higher for UC (46 and 30 %, respectively).

In the native extracts of *S. hypericifolia*, 28 to 38 compounds were detected. The major part of them were hydroxycinnamic acids and flavonol glycosides (Table 7). One can see that components 26 and 28 dominated in the composition of hydroxycinnamic acids, while the dominating components of flavonol glycosides were hyperoside, isoquercitrin and avicularin. The composition of native components in the leaves of *S. hypericifolia* and the LV of these parameters, similarly to

TABLE 7

Composition and content of phenolic compounds in the leaves of *Spiraea hypericifolia* during 2012–2014

Parameter	t_r, min	$M \pm m$ Lim		CV, %	
		1	2	1	2
Number of phenolic compounds, including:		33.00±3.54 28–38	31.67±2.86 28–36	15	13
phenolic acids		25.33±3.49 22–31	24.33±2.94 21–29	19	17
flavonol glycosides		6.33±0.41 6–7	6.33±0.41 6–7	9	9
Content of components and fractions, % of abs. dry mass of leaves:					
gallic acid	1.82	0.14±0.03 0.10–0.18	0.10±0.03 0.06–0.15	29	46
sum of hydroxybenzoic acids		0.29±0.07 0.19–0.38	0.24±0.008 0.23–0.25	33	4
chlorogenic acid	3.22	0.15±0.10 0.00–0.25	0.06±0.04 0.00–0.10	88	88
caffeic acid	4.95	0.09±0.05 0.01–0.14	0.04±0.02 0.01–0.07	75	68
p-coumaric acid	7.90	0.03±0.01 0.01–0.05	0.05±0.008 0.04–0.05	73	21
component 26 (acylated O-glycoside of flavonol) (λ_{max} 315, 355 nm)	38.2	0.37±0.19 0.08–0.63	0.09±0.03 0.04–0.13	75	52
component 28 (hydroxycinnamic acid) (λ_{max} 250, 325 nm)	40.4	0.76±0.40 0.23–1.36	0.16±0.05 0.11–0.25	74	44
sum of hydroxycinnamic acids		3.80±1.57 1.54–5.96	1.82±0.64 0.83–2.59	58	50
sum of phenolic acids		4.03±2.31 1.73–6.34	1.96±0.88 1.08–2.84	81	64
hyperoside	18.4	0.82±0.39 0.27–1.37	0.54±0.22 0.24–0.87	67	58
isoquercetrin	19.7	0.46±0.22 0.17–0.79	0.30±0.18 0.05–0.55	68	83
avicularin	28.2	0.22±0.06 0.14–0.30	0.27±0.14 0.06–0.45	36	73
Sum of flavonol glycosides		2.01±0.68 1.04–2.97	1.56±0.66 0.61–2.47	48	60
Sum of phenolic compounds		6.11±2.33 2.77–9.35	3.68±1.30 1.69–5.33	54	50

Note. For designations, see Table 2.

the parameters of the leaves of *S. chamaedryfolia*, did not differ substantially under urban and background conditions. However, unlike for other studied species, the LV of the number of flavonol glycosides (9 % in both versions of conditions) in *S. hypericifolia* was lower than that for phenolic acids (19 and 17 %, respectively).

The content of the majority of components (including the major flavonol glycosides – hyperoside and isoquercitrin) in the urban samples was lower in comparison with the reference samples. The sum of phenolic compounds decreased substantially (3.68 and 6.11 %, respectively), mainly due to a decrease in the content of hydroxycinnamic acids (1.82 and 3.80 %) and flavonol glycosides (1.56 and 2.01 %). The content of hydroxybenzoic acids exhibited no substantial differences under urban and background conditions.

The annual variability of the content of the majority of components and fractions was high and very high, under UC lower than under BC.

However, the variation of some components for UC was higher (gallic acid, isoquercitrin, avicularin). The level of variability of the sum of phenolic compounds did not exhibit substantial differences under the considered conditions.

Features of the annual dynamics of the composition and content of phenolic compounds and their fractions in the leaves of S. media, S. chamaedryfolia and S. hypericifolia

Results of the studies provide evidence that, with the substantial level of annual variability, the composition and content of phenolic compounds in the leaves of the studied species of genus *Spiraea* under the conditions of transport and industrial pollution exhibit some differences in comparison with BC. The composition of phenolic compounds in the leaves of urban plants changed to the highest extent for *S. media*, where the number of native phenolic acids and flavonol glycosides was every year lower in comparison with BC (Fig. 1). In the leaves of other studied species, the number of compounds for UC did not change substantially but did not exceed the background values.

The data of the studies three years long generally confirm our previous results providing evidence of a decrease in the content of phenolic compounds in the leaves of *Spiraea* under the conditions of technogenic pollution [10]. In the leaves of *S. hypericifolia*, the content of the major part of phenolic compounds was lower under UC than under BC, independently of the year when sampling was carried out (Fig. 2, *a*), while the content of the majority of components and fractions in the leaves of two other species in the most humid and cool year 2013 was somewhat higher for UC than for BC (see Fig. 2, *b*, *c*).

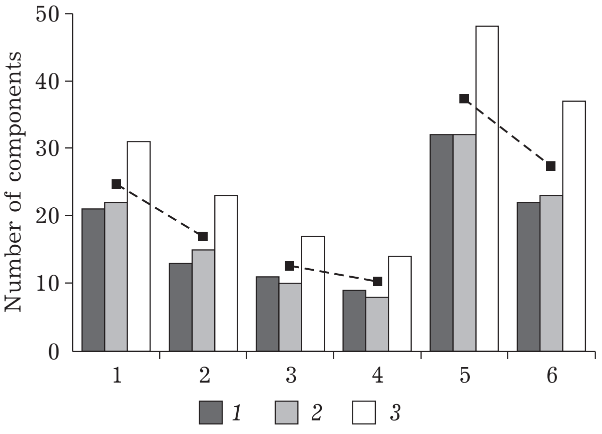

Fig. 1. Number of phenolic acids (1, 2), flavonol glycosides (3, 4) and total number of phenolic compounds (5, 6) in the leaves of *Spiraea media* under the conditions: background (1, 3, 5) and pollution (2, 4, 6). Here and in Fig. 2–4: years *1* – 2012, *2* – 2013, *3* – 2014; dashed line with marker – arithmetic mean over three years of investigation.

So, the results demonstrate the effect of not only ecological but also meteorological conditions on the content of the majority of phenolic compounds in the studied species. The significance of this factor was demonstrated in many works [15]. A decrease in the content of phenolic compounds in the leaves of *S. media* and *S. chamaedryfolia* in urban plants was less clearly pronounced, while in the leaves of *S. hypericifolia* is was stronger expressed in 2013 than in the years 2012 and 2014. However, the content of some com-

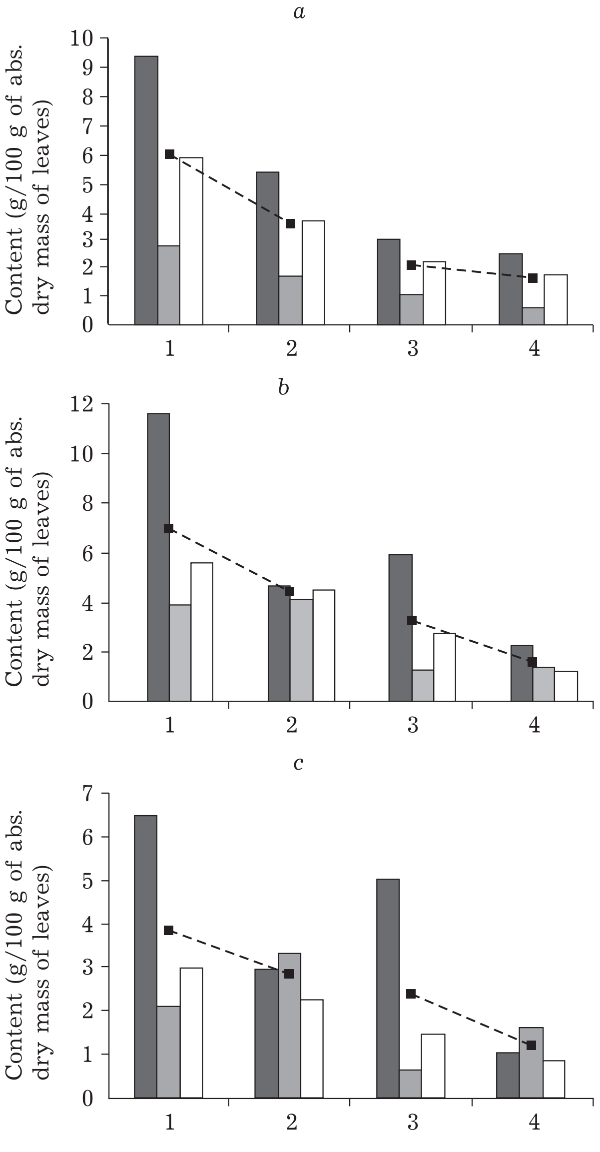

Fig. 2. Content of the sum of phenolic compounds (1, 2) and the sum of flavonoids (3, 4) in the leaves of *Spiraea hypericifolia* (*a*) and *Spiraea media* (*b*), and glycosides of cinnamic acid (3, 4) in the leaves of *Spiraea chamaedryfolia* (*c*) under background conditions (1, 3) and under pollution (2, 4). Designations: see Fig. 1.

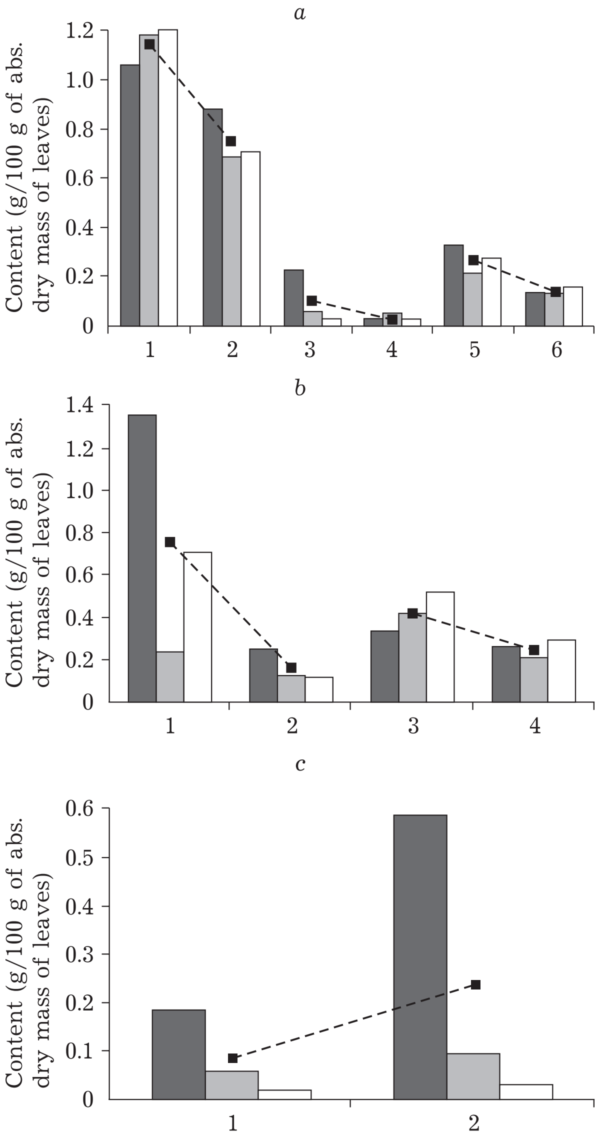

Fig. 3. Content of the sum of hydroxybenzoic acids (1, 2), *p*-coumaric acid (3, 4) and component 22 (hydroxybenzoic acid, t_r = 17.6 min) (5,6) in the leaves of *Spiraea chamaedryfolia* (*a*), component 28 (hydroxycinnamic acid, t_r = 40.4 min) (1, 2), sum of gallic, chlorogenic, caffeic and *p*-coumaric acids (3, 4) in the leaves of *Spiraea hypericifolia* (*b*), and component 15 (hydroxycinnamic acid, t_r = 15.7 min) in the leaves of *Spiraea media* (*c*) under background conditions (1, 3, 5) and under pollution (2, 4, 6). Designations: see Fig. 1.

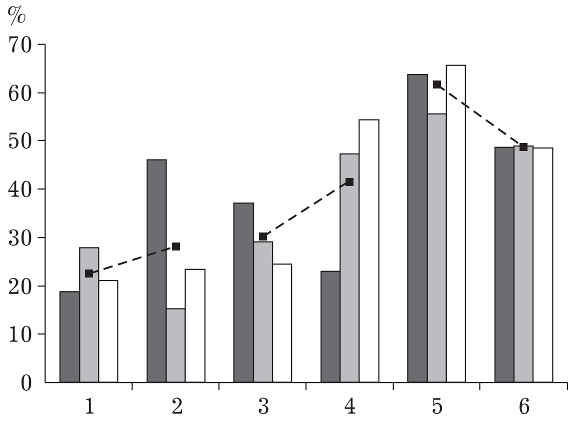

Fig. 4. Fraction of hydroxycinnamic acids in the sum of phenolic compounds in the leaves of the plants of *Spiraea media* (1, 2), *Spiraea chamaedryfolia* (3, 4) and *Spiraea hypericifolia* (5, 6) under background conditions (1, 3, 5) and under pollution (2, 4, 6) Designations: see Fig. 1.

ponents was insignificantly dependent on the weather conditions and exhibited a substantial difference from the corresponding background conditions in 2013. These parameters for UC may be considered as characteristic for the adaptation to technogenic pollution.

As far as the species *S. chamaedryfolia* is concerned, these parameters include the low content of hydroxybenzoic acids (< 1 %) (Fig. 3, *a*), for *S. hypericifolia* – low content of hydroxycinnamic acid (component 28, see Table 7) (λ_{max} 250, 325 nm) and the sum of gallic, chlorogenic, caffeic and *p*-coumaric acids (see Fig. 3, *b*). For *S. media*, such a component is hydroxycinnamic acid (component 15, see Table 3) (λ_{max} 225, 325 nm): the high content (>0.19 %) of this component may be the evidence of the state of adaptation to the unfavourable ecological conditions (see Fig. 3, *c*).

There are published data both on an increase [5, 6, 16] and a decrease [17] in the content and number of flavonoids and phenolic acids under the conditions of technogenic pollution. J. Loponen with co-authors [18] revealed a significant increase in the content of 7 individual phenolic compounds of 30 in the leaves of birch growing in the zone affected by the nickel-copper smelt, in comparison with background conditions. Investigation of birch in another zone of intense industrial pollution demonstrated substantial increase in the content of (+)-catechine and the derivatives of gallic acid. However, the changes in the fraction of hydroxycinnamic acids were minimal [19].

The results obtained by us provide evidence of a decrease in the content of the majority of phenolic compounds under the conditions of chronic transport and industrial pollution. Under these conditions, an increase in the content was revealed only for the fraction of phenolic acids in the leaves of *S. media*. The number of phenolic acids and flavonol glycosides was smaller than for BC. In the leaves of the plants of other studied species, both the number of the majority of phenolic compounds and their content were lower under the conditions of pollution than under background conditions. The absence of any increase in the content and number of phenolic compounds in the leaves of *Spiraea* in the zone of chronic pollution may be connected with attenuation of adaptive reactions.

In the leaves of *S. chamaedryfolia* and *S. hypericifolia*, the components exhibiting the highest sensitivity to pollution turned out to be the glycosides of cinnamic acid and hydroxycinnamic acids: their content decreased substantially under the conditions of technogenic action. At that, in the leaves of *S. hypericifolia* we also observed yearly decrease in the fraction of hydroxycinnamic acids in the sum of phenolic compounds for UC (48 %) in comparison with BC (56–64 %). In the leaves of other species, no definite trends of the relative content of fractions were detected (Fig. 4).

So, the detected decrease in the concentrations of a number of phenolic compounds not quite corresponds to an increase in the concentrations of components detected by some authors. This allows us to conclude the absence of universal regularities in the response of plants to the chronic pollution of soil and air. The reaction of the phenolic complex and the content of the most sensitive components vary depending on the genetically fixed features of the metabolism of phenolic compounds, meteorological conditions, technogenic action, its intensity etc. The effect of some factors on the content of the fractions of phenolic compounds was studied in [19].

The results of the investigation are in agreement with the literature data concerning substantially lower variability of the sum parameters in comparison with the variability of individual compounds [7] and a decrease in the intensity of the response to growing conditions in the case of plant adaptation to the chronic environmental pollution [9]. It is demonstrated that long-term existence of the populations under the conditions of stress may lead to the weakening of their sensitivity to acute actions, while in the intact populations, as a rule, the contribution from the environmental component is determinative in the case when the samples are placed in contrast conditions [20].

CONCLUSION

The features of the action of environmental pollution and climatic parameters of vegetation seasons on the composition and content of phenolic compounds were revealed as a result of the studies of the annual dynamics of the content of phenolic compounds in the leaves of *S. media*, *S. chamaedryfolia* and *S. hypericifolia* growing under the conditions and transport and industrial pollution in Novosibirsk and under more favourable (background) conditions at the introduction ground of the CSBG SB RAS.

Under the conditions of pollution, the composition of phenolic compounds changed to the most substantial degree in the leaves of *S. media*, where the number of phenolic acids (up to 23) and flavonol glycosides (up to 14), independently of the year of observation, was lower in comparison with urban conditions (to 31 and 17, respectively). In the leaves of other studied species (*S. chamaedryfolia* and *S. hypericifolia*), the number of compounds did not change substantially under the conditions of pollution but did not exceed the background values. The content of the major phenolic components decreased substantially in all these species. The most stable annual decrease in the content of the major group of phenolic compounds (hydroxycinnamic acids) and a decrease in the fraction of this group in the sum of phenolic compounds in urban plants with respect to the reference was detected in the leaves of *S. hypericifolia* – 1.82 and 3.80 % (48.7 and 65.6 % of the sum of phenolic compounds), respectively.

Changes were detected not only in the composition and content but also the level of their annual variability, which was substantial in both versions of conditions. The level of variability of the number of aglycon and native components under the conditions of pollution increased (*S. media* и *S. chamaedryfolia*) or exhibited no significant changes (*S. hypericifolia*) in comparison with the background conditions, and the level of variability of the content of the majority of compounds decreased.

The annual dynamics provides evidence of a definite effect of the climatic parameters of vegetation periods on the content of the majority of phenolic compounds. A decrease in the content of phenolic compounds was less clearly expressed in the leaves of *S. media* and *S. chamaedryfolia* in urban plants, while in the leaves of *S. hypericifolia* it was more clearly pronounced in 2013 than under the conditions of 2012 and 2014.

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