PHENOLIC COMPOUNDS IN FOREST SOILS

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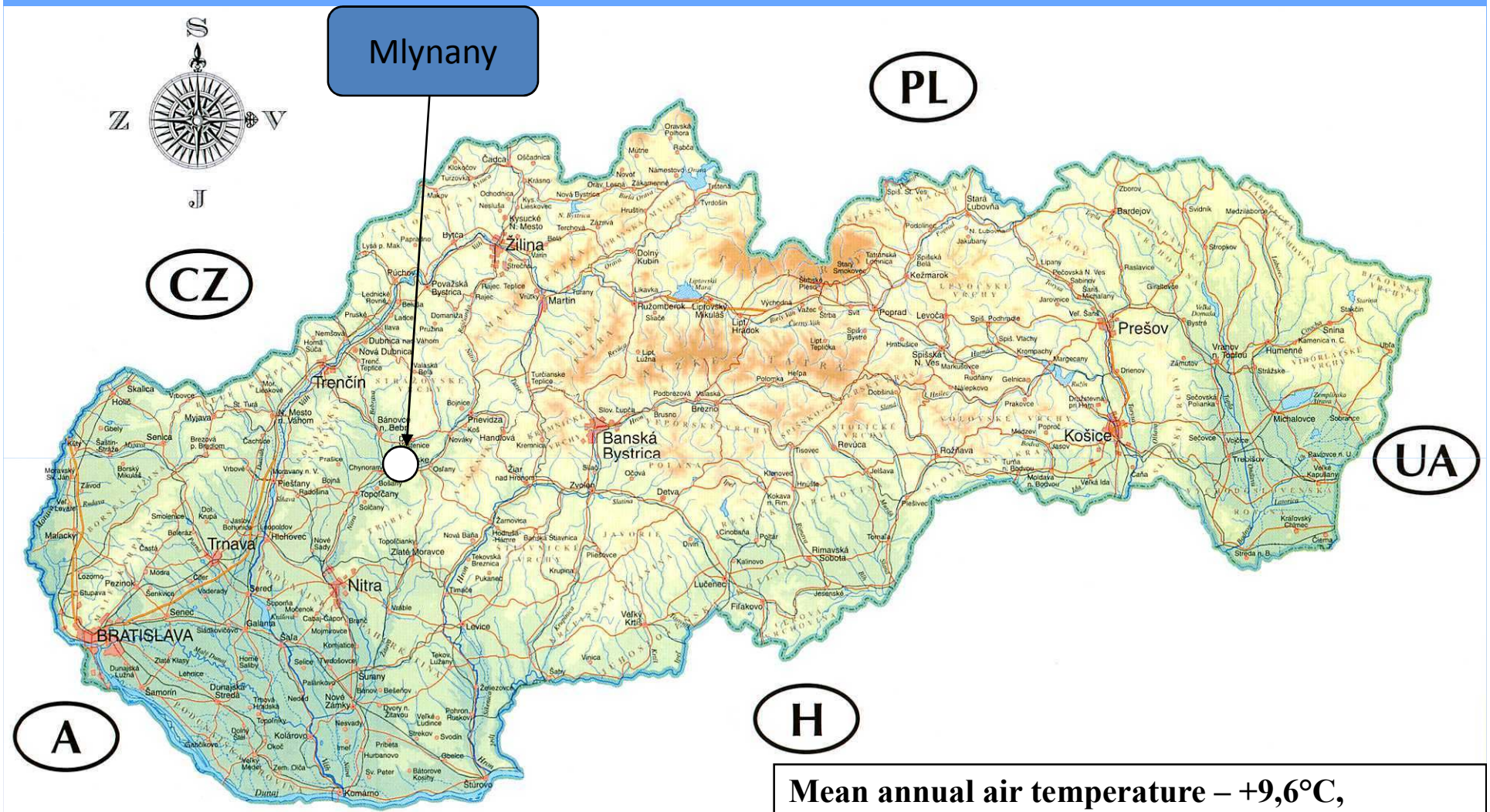
The research involved forest soil sampled from the Arboretum in Mlynany.



Mean annual air temperature – +9,6°C,

Mean July temperature – +19,8°C,

Mean January temperature – -1,3°C,

Mean annual precipitation total – 558 mm

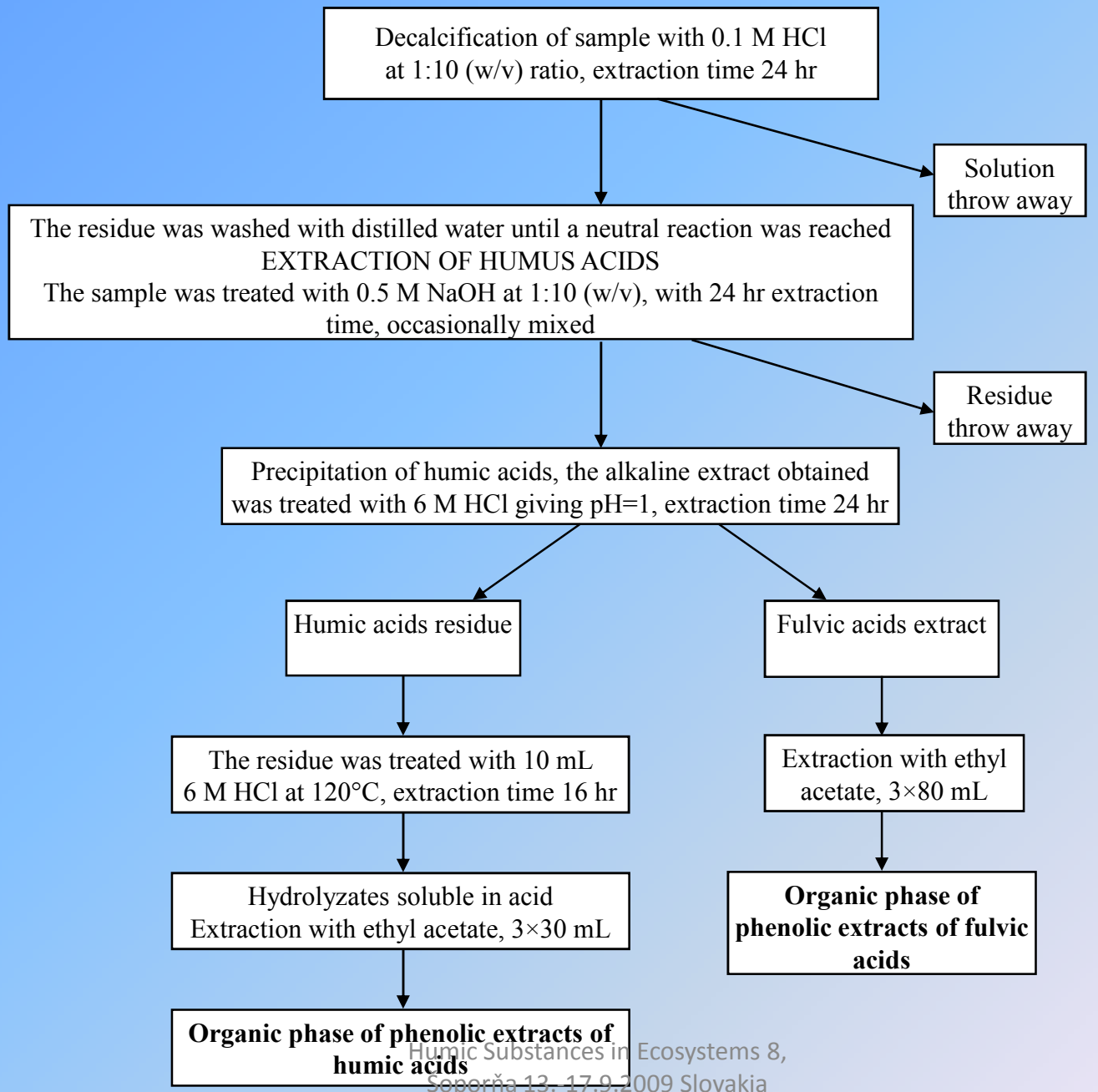
The soil was sampled under the following tree stands:

- **oak (*Quercus cerris* L.);**
- **spruce (*Picea abies* L. Karsten),**
- **thuja (*Thuja plicata* D.Don.ex. Lamb.)**

Sample	Depth (cm)	Horizon	Type of soil
Oak stand			
MD01	5-4	Ol	Stagnic Luvisols
MD02	4-0	Ofh	
MD1	0-3	A	
Thuja stand			
MT01	4-3	Ol	Stagnic Luvisols
MT02	3-1	Of	
MT03	1-0	Oh	
MT1	0-5	A	
Spruce stand			
MS01	6-4.5	Ol	Stagni-Albic Luvisols
MS02	4.5-2	Of	
MS03	2-0	Oh	
MS1	0-5	A	

Extraction of phenolic compounds from soil

- To isolate phenolic compounds from soils, soil samples were exposed to acid hydrolysis (6M HCl) in the ratio of 1:10 (w/v), and then phenolic compounds (fluid-fluid) were extracted applying ethyl acetate as the extraction solvent.
- The obtained organic phase of phenolic compounds was vaporized completely and then solved in 5 mL of CH_3OH. The chromatographic separation of solutions containing phenolic compounds was made with HPLC equipped with the DAD detector.


Decalcification of sample with 0.1 M HCl at 1:10 (w/v) ratio, extraction time 24 hr
Solution throw away
The residue was washed with distilled water until a neutral reaction was reached
EXTRACTION OF HUMUS ACIDS
The sample was treated with 0.5 M NaOH at 1:10 (w/v), with 24 hr extraction time, occasionally mixed
Residue throw away
Precipitation of humic acids, the alkaline extract obtained was treated with 6 M HCl giving pH=1, extraction time 24 hr
Humic acids residue
Fulvic acids extract
The residue was treated with 10 mL 6 M HCl at 120°C, extraction time 16 hr
Extraction with ethyl acetate, 3×80 mL
Hydrolyzates soluble in acid Extraction with ethyl acetate, 3×30 mL
Organic phase of phenolic extracts of fulvic acids
Organic phase of phenolic extracts of humic acids

Conditions of chromatographic separation:

Column: C18, particle size 5 μm

Eluent A: H_2O:CH_3CN:CH_3COOH (84:14:2)

Eluent B: CH_3CN

Injection volume: 20 μL

Detection: 254 nm

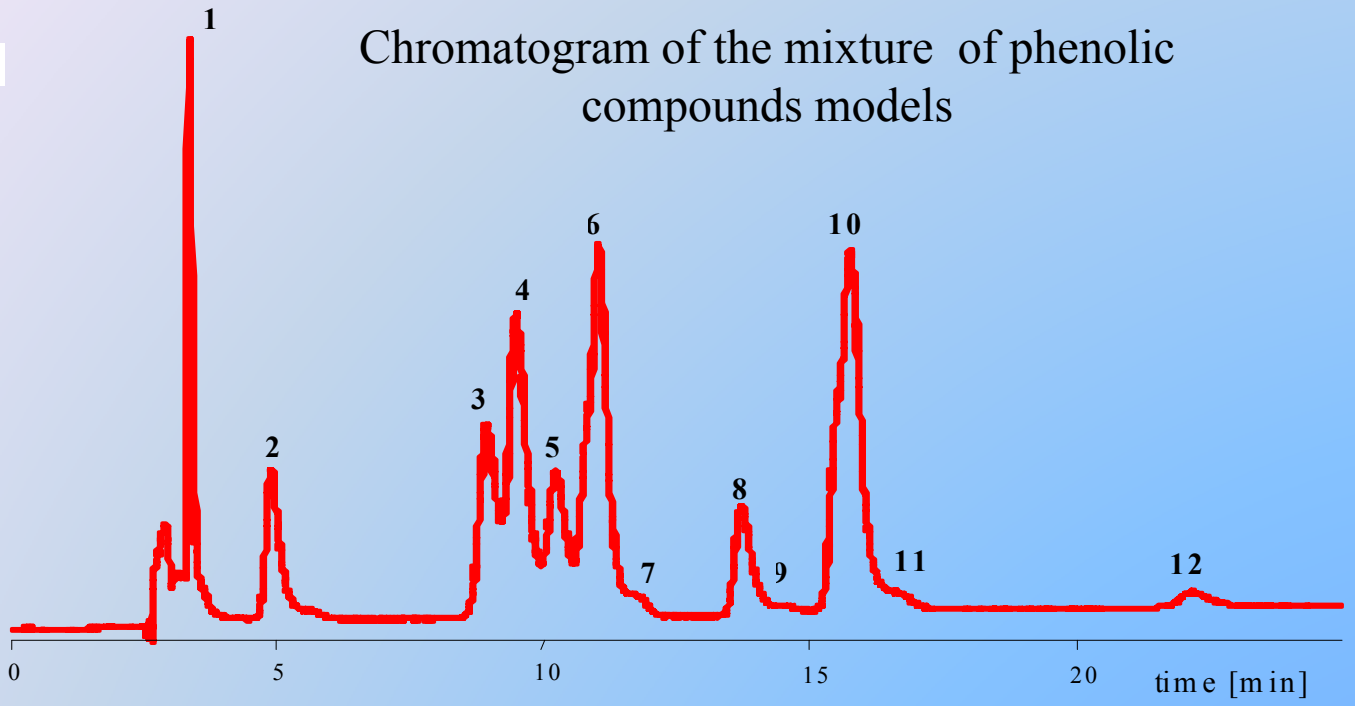
The gradient division program was applied at the rate flow of 1 mL/min

The initial composition of the mobile phase accounted for 100% of eluent A, the concentration of eluent B was increasing linearly during the analysis. The gradient was completed after 49 min. when the content of eluent B was 10%.

List of models applied in qualitative and quantitative analysis of phenolic compounds

No.	Compound	Symbol
1.	Gallic acid (3,4,5-trihydroxybenzoic acid)	GA
2.	Protocatechuic acid (3,4-dihydroxybenzoic acid)	PA
3.	Vanillic acid (4-hydroxy-3-methoxybenzoic acid)	VA
4.	Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)	SYA
5.	p-Hydroxybenzoic acid (4-hydroxybenzoic acid)	p-HBA
6.	Caffeic acid (3,4-dihydroxy-trans-cinnamic acid)	CA
7.	Vanillin (4-hydroxy-3-methoxybenzaldehyde)	VAN
8.	Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)	SYAL
9.	2,4-Dihydroxybenzoic acid	DHBA
10.	Ferulic acid (4-hydroxy-3-methoxy-trans-cinnamic acid)	FERA
11.	Salicylic acid (2-hydroxybenzoic acid)	SA
12.	p-Coumaric acid (4-hydroxy-trans-cinnamic acid)	p-CA

Chromatogram of the mixture of phenolic compounds models



1. GA – Gallic acid (3,4,5-trihydroxybenzoic acid)
2. PA – Protocatechuic acid (3,4-dihydroxybenzoic acid)
3. VA – Vanillic acid (4-hydroxy-3-methoxybenzoic acid)
4. SYA – Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)
5. p-HBA – p-Hydroxybenzoic acid (4-hydroxybenzoic acid)
6. CA – Caffeic acid (3,4-dihydroxy-trans-cinnamic acid)
7. VAN – Vanillin (4-hydroxy-3-methoxybenzaldehyde)
8. SYAL – Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)
9. DHBA – 2,4-Dihydroxybenzoic acid
10. FERA – Ferulic acid (4-hydroxy-3-methoxy-trans-cinnamic acid)
11. SA – Salicylic acid (2-hydroxybenzoic acid)
12. p-CA – p-Coumaric acid (4-hydroxy-trans-cinnamic acid)

The content of vanillyl (V), syringyl (S) and cinnamyl compounds (C) was calculated as follows:

V – total content of vanillin (VAN) and vanillic acid (VA), (VAN + VA),
S – total content of syringaldehyde (SYAL) and syringic acid (SYA), (SYAL + SYA),
C – content of ferulic acid (FERA), caffeic acid (CA) and coumaric acid (p-CA).

The following parameter was calculated:

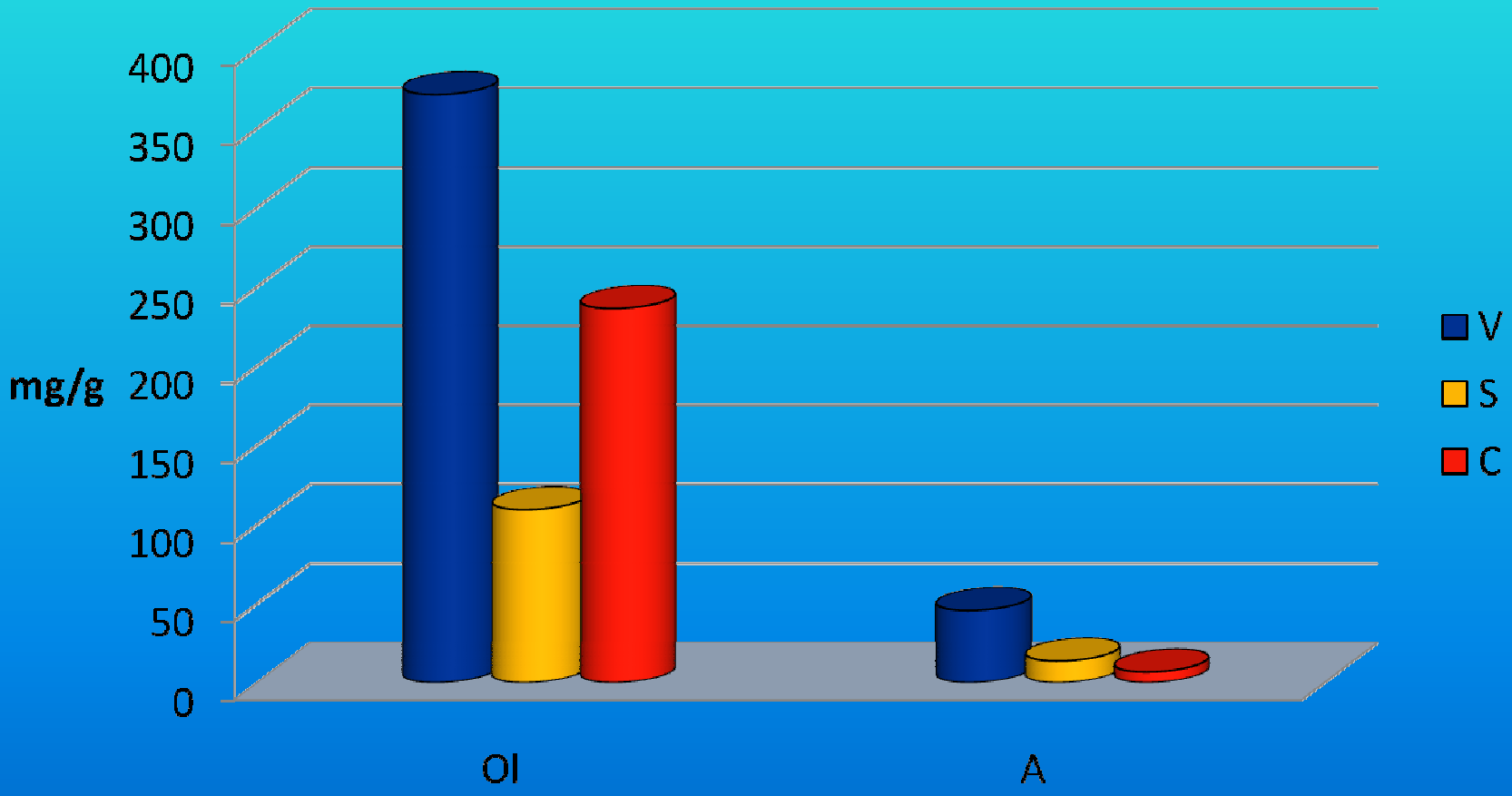
V:S:C – ratio of the share of respective compounds

Content of phenolic compounds in the soils

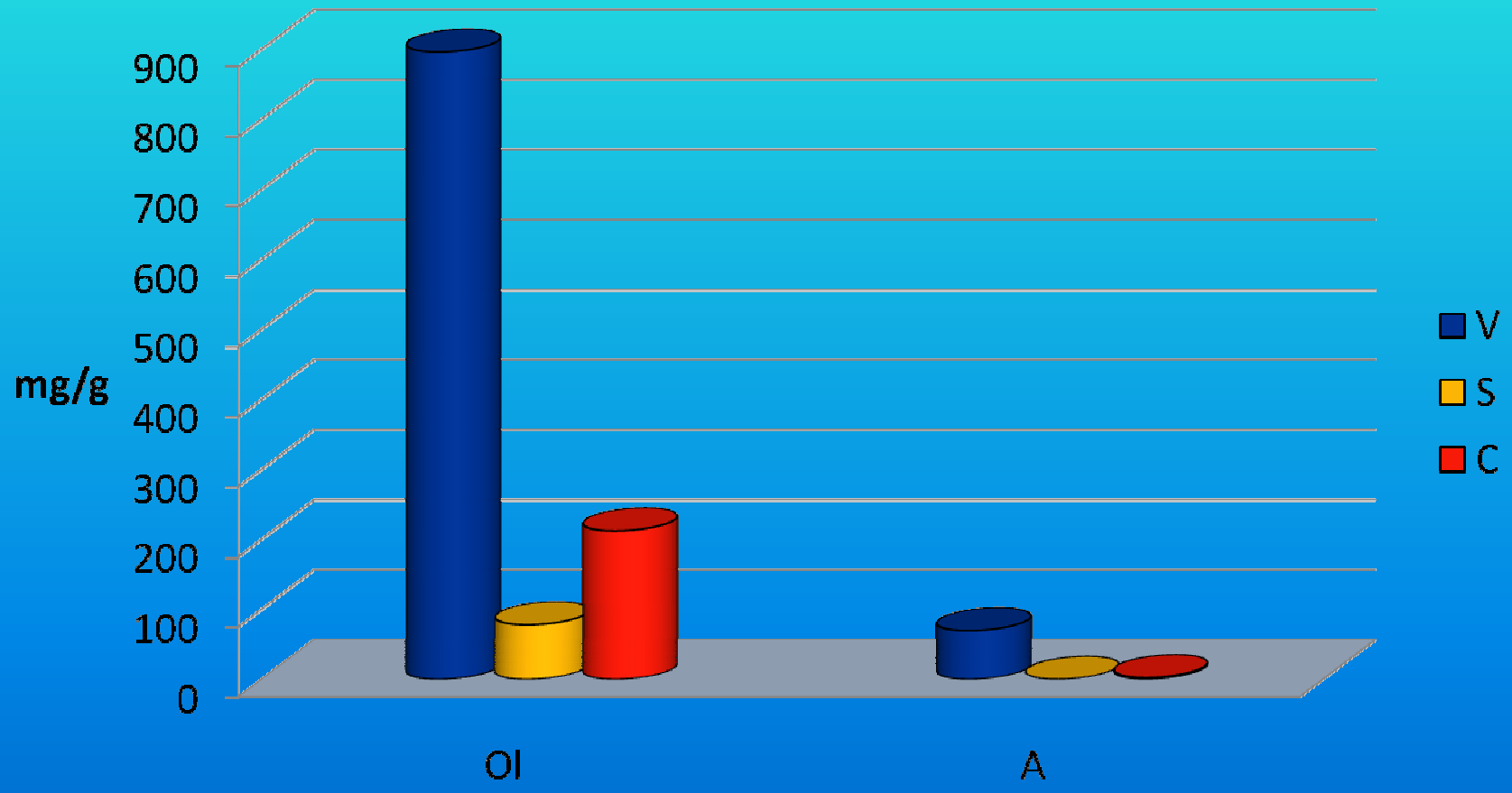
Sample	GA	PA	VA	SYA	p-HBA	VAN	SYAL	DHBA	CA	FERA	SA	p-CA	SUM
	mg·g^{-1} soil												
Oak stand													
MD01	337	500	320	98.3	363	51.5	10.8	51.9	nd	10.7	617	225	2585
MD02	253	567	55.2	74.4	374	32.3	7.7	63.3	nd	7.06	681	10.4	2125
MD1	101	85.6	25.6	4.82	199	19.9	8.8	13.4	nd	3.41	74.1	3.19	539
Thuja stand													
MT01	253	371	424	57.0	75.2	155	114	343	nd	20.7	1037	33.7	2884
MT02	220	341	436	58.3	78.9	209	109	480	nd	19.6	1003	29.4	2984
MT03	215	294	233	3.57	7.49	17.9	17.0	348	nd	4.20	50.0	20.3	1211
MT1	195	227	15.8	2.71	nd	1.58	8.00	154	nd	5.45	13.8	18.7	642.0
Spruce stand													
MS01	205	362	817	48.1	718	79.0	29.7	nd	90.4	153	534	59.4	3096
MS02	144	321	655	32.1	741	78.4	60.4	nd	97.3	46.5	343	40.2	2559
MS03	169	51.0	521	26.4	823	57.1	48.3	nd	8.02	65.7	44.6	9.61	1824
MS1	112	nd	63.9	nd	68.8	5.52	nd	nd	nd	2.41	nd	nd	252.6

nd – not detected

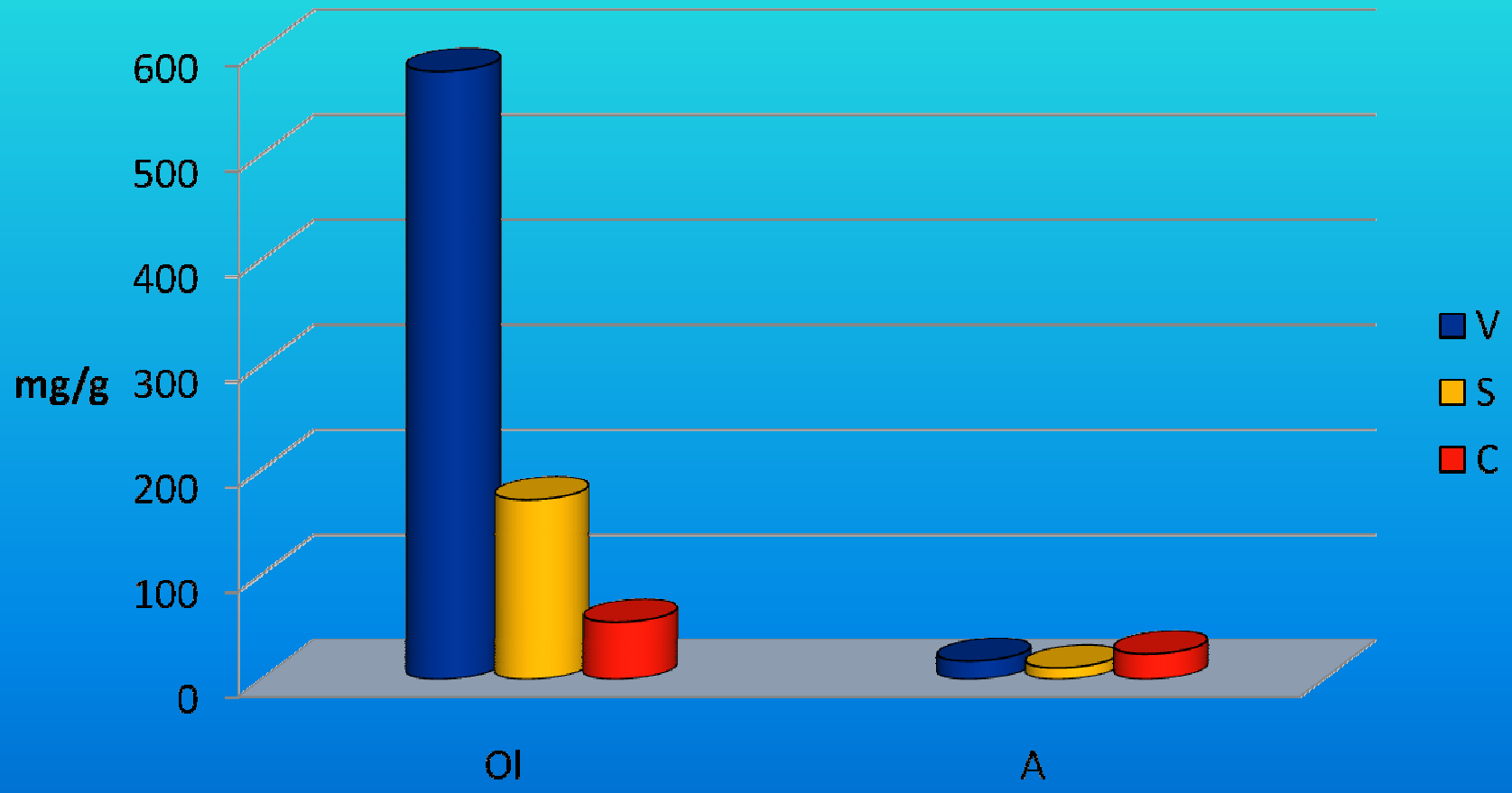
Content of vanillyl, syringyl and cinnamyl compounds in soil – oak stand



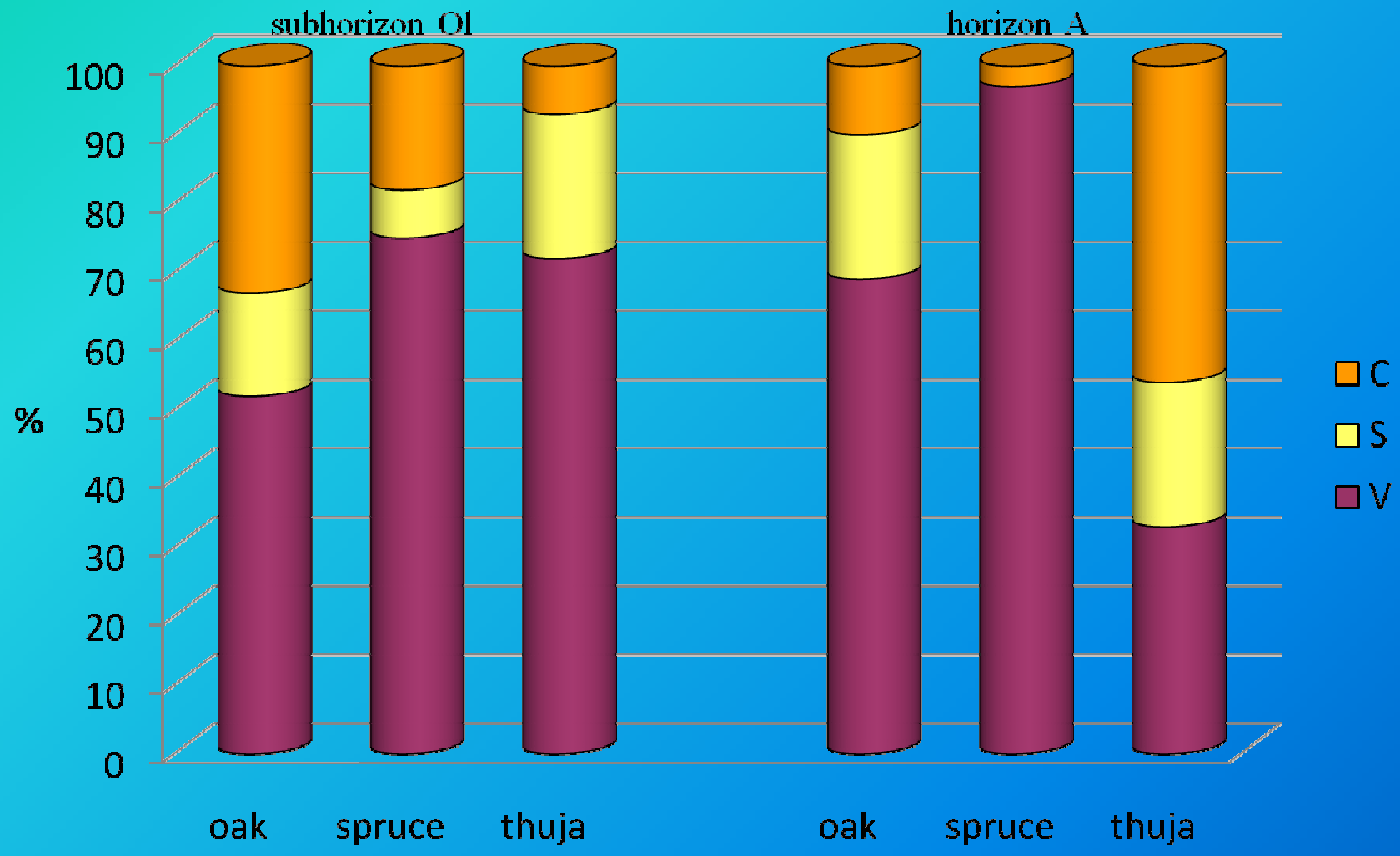
Content of vanillyl, syringyl and cinnamyl compounds in soil – spruce stand



Content of vanillyl, syringyl and cinnamyl compounds in soil – thuja stand



Proportions of vanilyl, syringyl and cinnamic compounds – in soil



Content of phenolic compounds in the extracts of fulvic acids

Sample	Horizon (Depth) (cm)	GA	PA	VA	SYA	p-HBA	VAN	SYAL	DHBA	CA	FERA	SA
		µg/mL										
Oak stand												
MD01	Ol	27.7	123	22.9	nd	37.4	8.02	16.9	nd	nd	24.5	8.70
MD02	Ofh	41.6	101	17.6	nd	14.0	5.41	7.01	nd	nd	7.31	6.46
MD1	A (0-3)	16.9	139	1.47	nd	1.23	0.477	0.597	nd	nd	0.529	nd
Spruce stand												
MS01	Ol	22.3	14.7	0.682	0.600	39.7	10.3	6.20	24.0	nd	0.607	99.1
MS02	Of	14.0	8.29	0.119	0.413	40.7	8.36	3.93	11.5	nd	0.770	62.6
MS03	Oh	22.0	7.54	1.25	0.247	44.1	2.42	2.87	3.48	nd	nd	23.4
MS1	A (0-5)	4.40	5.23	2.17	0.161	45.8	0.242	0.339	5.36	nd	nd	17.6
Thuja stand												
MT01	Ol	17.1	11.7	17.7	nd	nd	6.85	1.07	0.742	nd	4.14	15.1
MT02	Of	11.8	4.12	13.4	nd	nd	4.32	0.864	0.508	nd	3.82	12.2
MT03	Oh	2.49	1.15	4.75	nd	nd	1.68	0.497	0.366	nd	2.77	11.6
MT1	A (0-5)	0.891	0.411	2.79	nd	nd	1.16	0.304	nd	nd	0.059	10.8

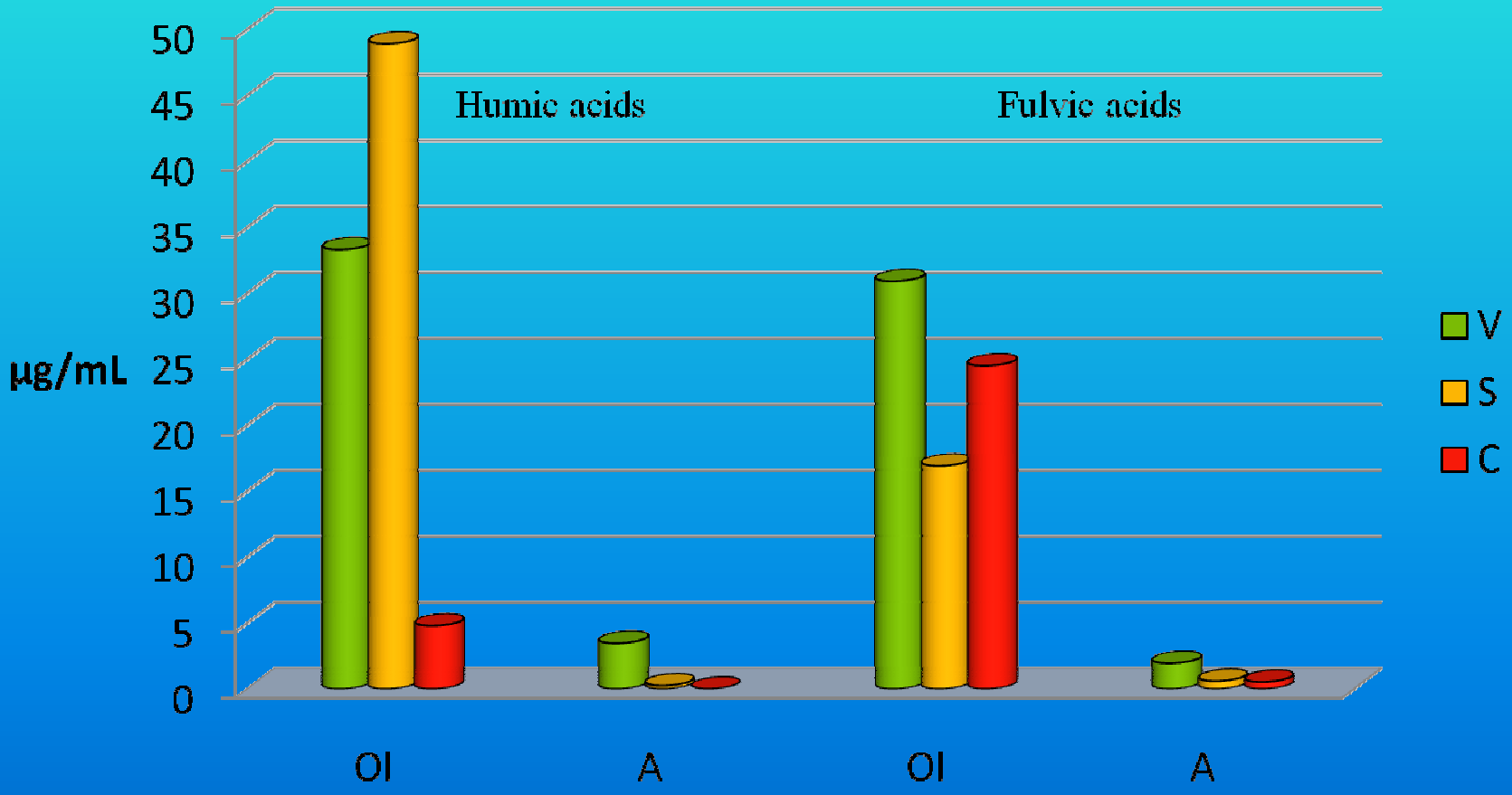
nd – not detected

Content of phenolic compounds in the extracts of humic acids

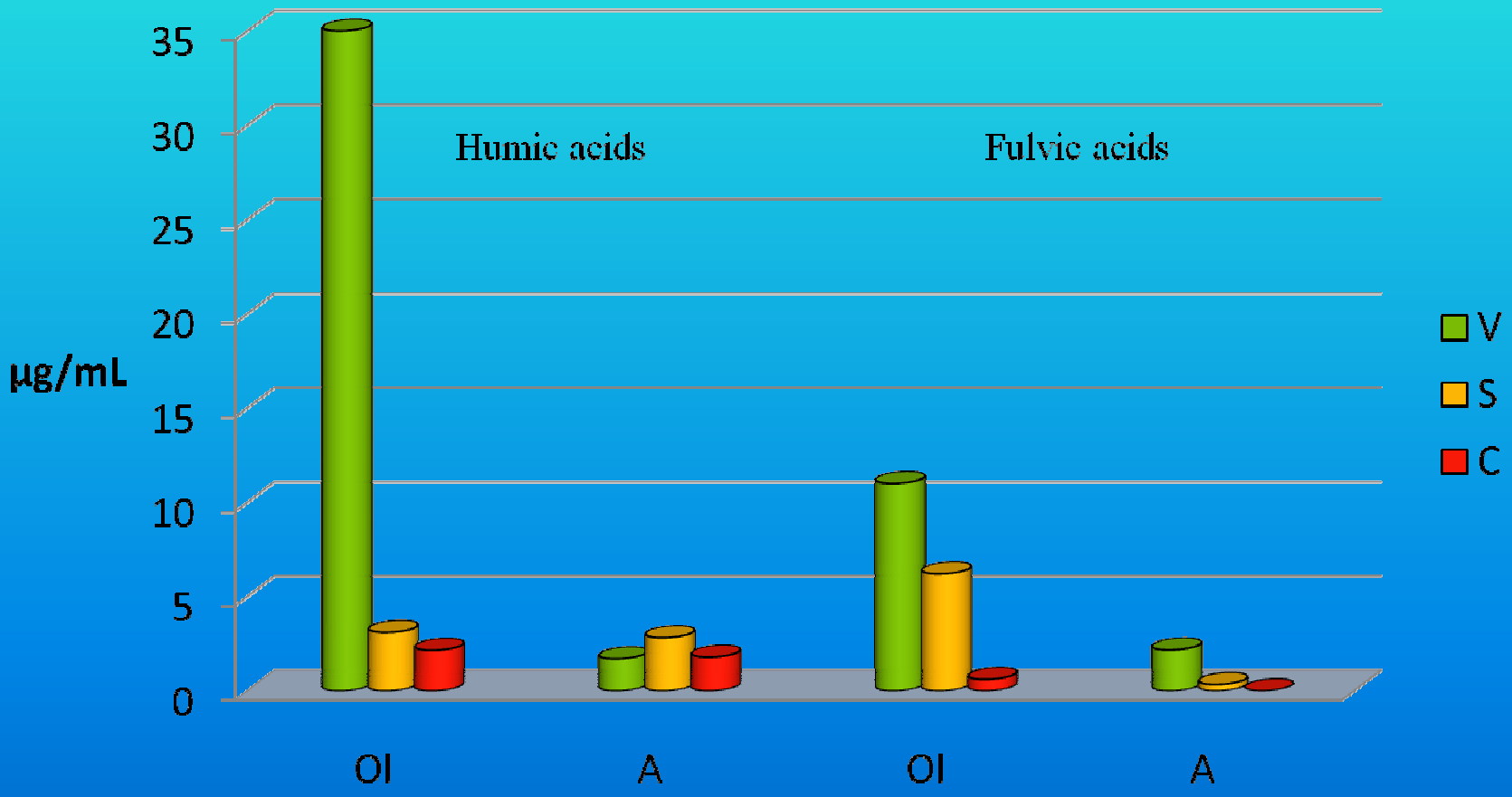
Sample	Horizon (Depth) (cm)	GA	PA	VA	SYA	p-HBA	VAN	SYAL	DHBA	CA	FERA	SA
		µg/mL										
		Oak stand										
MD01	Ol	43.5	33.6	20.2	21.5	38.6	13.1	27.4	nd	nd	4.79	nd
MD02	Ofh	9.36	13.8	23.1	1.35	2.92	3.77	3.40	nd	nd	0.699	nd
MD1	A (0-3)	1.70	0.281	3.21	nd	0.195	0.248	0.249	nd	nd	nd	nd
		Spruce stand										
MS01	Ol	14.2	3.13	34.3	0.733	1.05	0.714	2.37	0.324	0.663	1.49	4.10
MS02	Of	16.2	5.79	58.8	0.781	2.07	1.22	1.56	0.796	2.32	1.73	7.23
MS03	Oh	10.9	5.23	54.8	0.789	1.52	1.11	1.11	1.63	0.667	0.614	13.2
MS1	A (0-5)	5.61	2.10	0.871	0.341	2.67	0.833	2.72	nd	1.79*b*	nd	1.07
		Thuja stand										
MT01	Ol	1.32	4.46	21.2	0.731	nd	0.778	0.147	nd	nd	nd	2.01
MT02	Of	2.09	6.31	27.0	1.03	nd	0.961	0.164	nd	nd	0.108	1.90
MT03	Oh	0.397	0.720	8.9	0.342	nd	0.511	0.092	nd	nd	nd	nd
MT1	A (0-5)	0.245	nd	1.02	0.103	nd	nd	0.057	nd	nd	nd	nd

nd – not detected

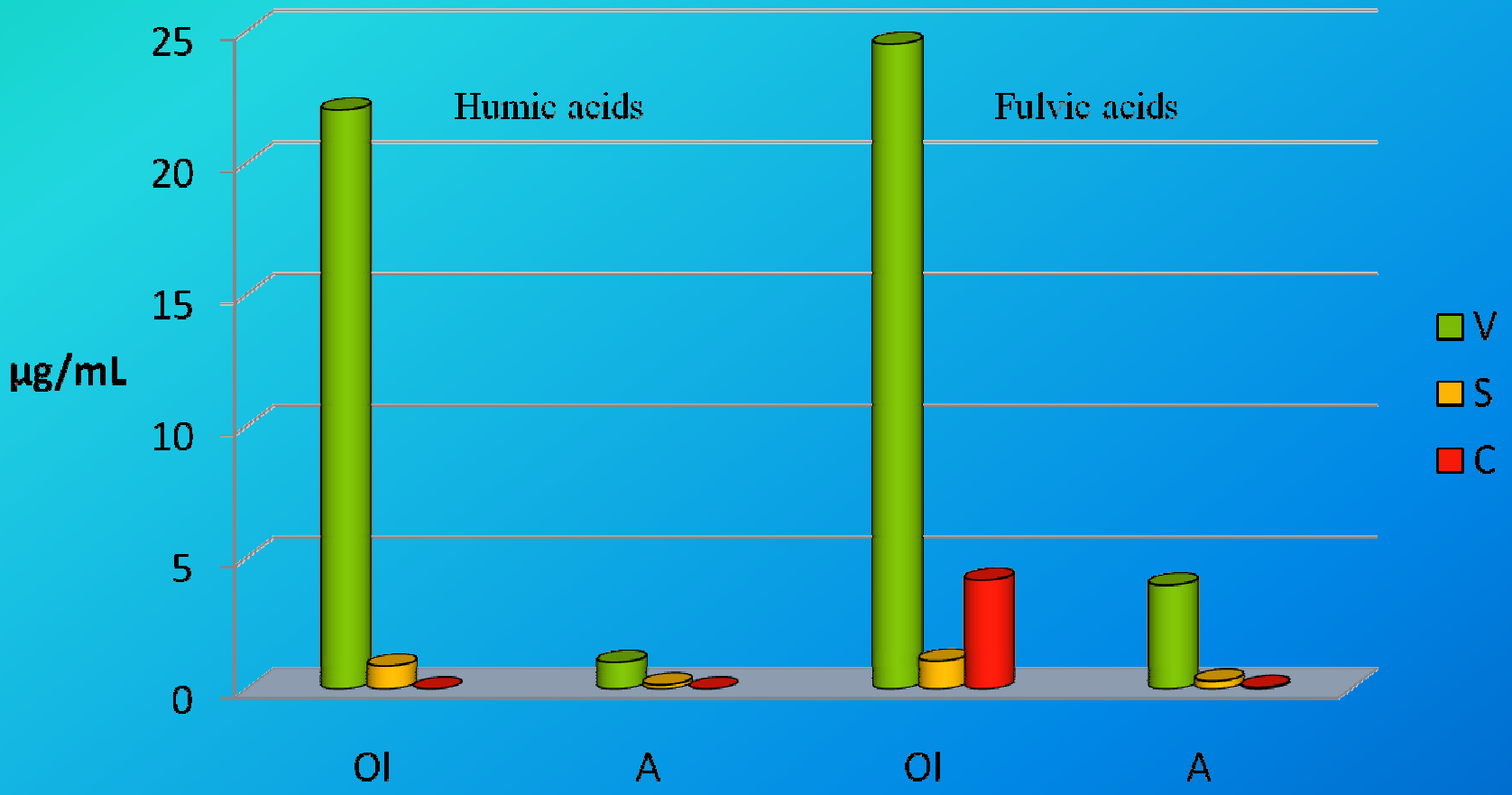
Content of vanillyl, syringyl and cinnamyl compounds in humus acids – oak stand



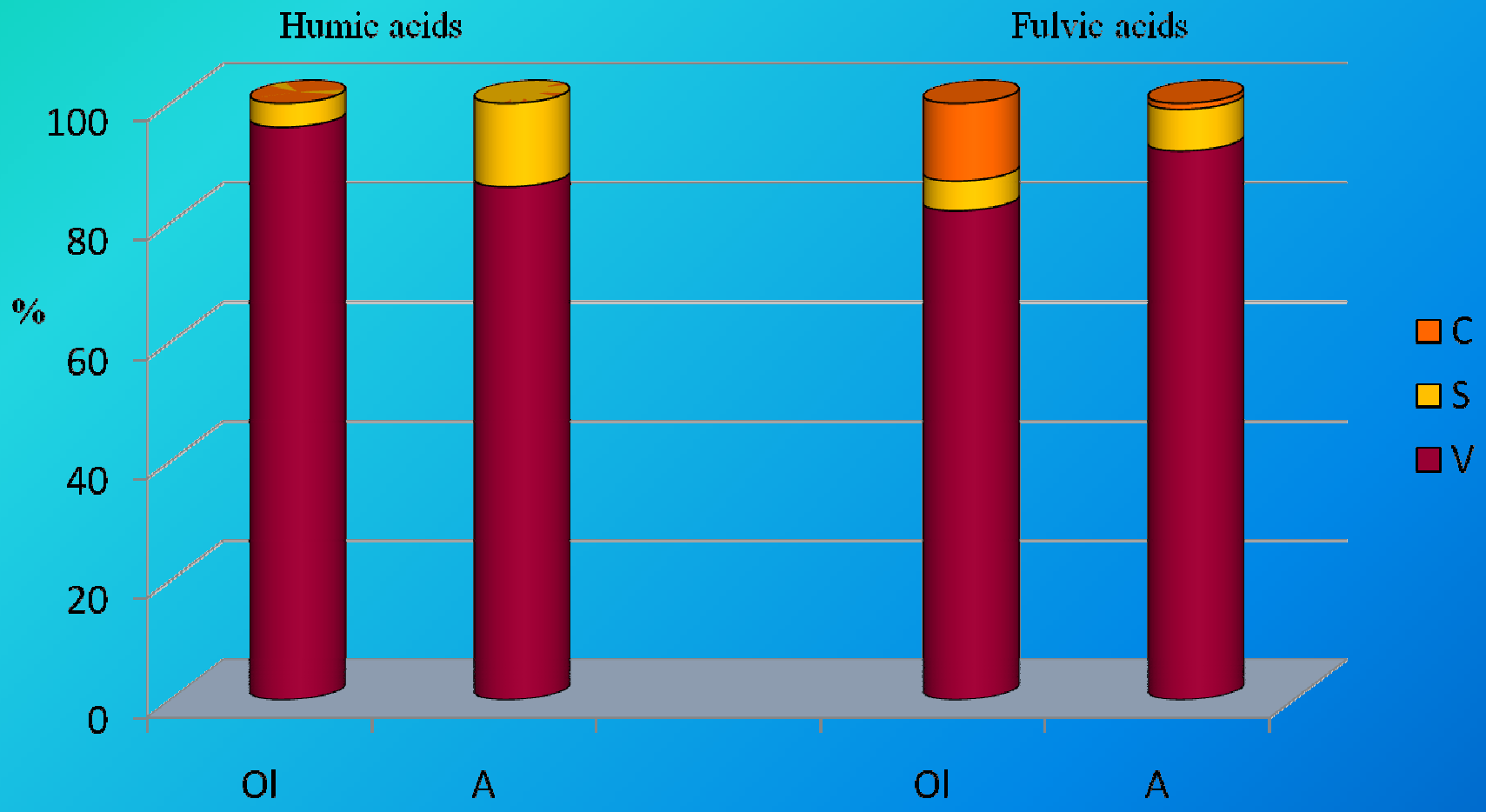
Content of vanillyl, syringyl and cinnamyl compounds in humus acids – spruce stand



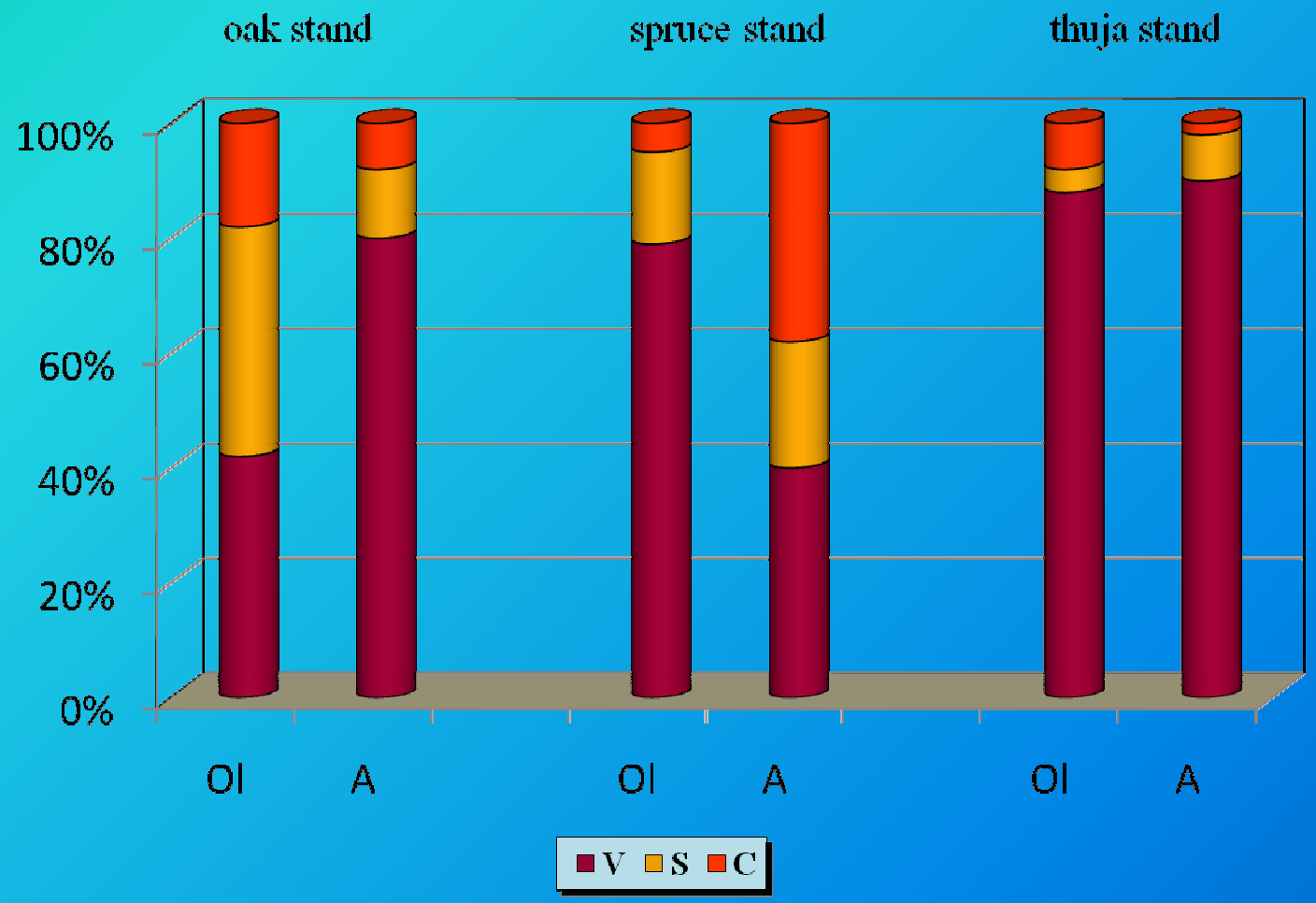
Content of vanillyl, syringyl and cinnamyl compounds in humus acids – thuja stand



Proportions of vanilyl, syringyl and cinnamic compounds – thuja stand



Proportion of vanilyl, syringyl and cinnamic compounds in humus acids (HAs+FAs)



Conclusion

4.2. The highest content of vanillyl, syringyl and cinnamyl compounds was noted in subhorizon Of on the spruce stand and the lowest – on the oak stand.

4.3. The highest content of vanillyl, syringyl and cinnamyl compounds was noted in subhorizon Of on the spruce stand and the lowest – on the oak stand.

4.4. The highest content of vanillyl, syringyl and cinnamyl compounds was noted in subhorizon Of on the spruce stand and the lowest – on the oak stand.

(spruce, pine) considerable advantage of vanillyl compounds.