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SPECTROSCOPIC CHARACTERISTICS OF HUMIC SUBSTANCES IN RELATION TO LEAD AND CADMIUM LEVELS IN CONTAMINATED SOILS FROM WESTERN CARPATHIANS

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Abstract: The paper deals with the study of the sorption properties of humic substances by using UV-VIS and fluorescence spectroscopy in different soil types on the Slovak Neovulcanites. We observed main spectral peaks by synchronous scan excitation fluorescence spectra of humic substances in soil at $\lambda_{ex}/\lambda_{em}$: 340/360, 404/424, 423/443, 468/488, 490/510, 507/527 with a constant difference $\Delta\lambda = 20$ nm. Fluorescence indexes (F) were calculated from SFS spectra at $\Delta\lambda = 20$ nm (as a ratio RFI_{404}/RFI_{423}). Also, the classical method of humic substances fractionation to assess their quality was applied, because the humic substances can help to improve unfavourable soil properties, plant productivity and nutrient uptake. The comparison is given of the calculated parameters from different spectral regions and humic substances fractionation. The results showed correlation between total organic carbon and fluorescence indexes ($r=-0.590$, $p<0.05$), between humic substances content in soil and fluorescence indexes ($r=0.983$, $p<0.01$), as well as between colour indexes (Q_{HS}) ($r=-0.716$, $p<0.01$). Content of humic substances was in the positive correlation with the bioavailable forms of lead ($r=0.709$, $p<0.01$) and cadmium ($r=0.692$, $p<0.01$), which plants are able to take. On the total content of cadmium and the bioavailable forms lead and cadmium has an impact also the spectral peak at 468/488 nm, which is associated with the presence of five or more fused aromatic rings.

Key words: SFS and UV-VIS spectroscopy, Slovakia, soil, sorption, heavy metals

1. INTRODUCTION

According to Milori et al., (2000) molecular fluorescence spectroscopy is a very sensitive method that conveys information about the properties, origin, chemistry, structure and functionality of humic substances and therefore fluorescence spectroscopy is suitable as a complementary method to other spectroscopic methods. Fluorescence spectroscopy was used to determine the fluorescence index (F) of humic substances. According to Kumke et al., (1995) emission peak at 360 nm corresponds to the presence of phenanthrene derivatives and emission wavelength of 480 to 489 nm is associated with the presence of five or more fused aromatic rings. Pospíšilová et al., (2008) argue that the spectral shape of the curve depends on the fractional composition of humic substances (humic acid (HA) and fulvic acids (FA) content). Humic substances (HS) are a heterogeneous mixture of various organic compounds (aromatic, aliphatic, phenolic) (Chen et al., 2003) with many functional groups and their composition depends on the conditions of humification (Barančíková, 2009). Navarete et al., (2010) argue that the changes in the composition and structure of humic substances occur by change in the land usage. Emission spectra are generally characterized by a unique broad band showing a maximum wavelength and relative fluorescence intensity that depend mainly on the nature and origin of the humic material. Excitation and synchronous-scan excitation spectra generally exhibit a number of peaks and shoulders of relative intensity at wavelengths that also vary according to the type and source of the humic material (Senesi et al., 1991).

According to Chen et al., (1977) absorbance from UV-VIS spectroscopy at a wavelength of 465 nm is corresponding to the absorption of radiation of young humic substances in the early stage of the humification process and the absorbance at a wavelength of 665 nm is corresponding to the absorption of radiation, mature and well humified humic substances. Kumada (1987) states the color quotient indicates the maturity, degree condensation and dispersion of humic substances. Stevenson (1982) argues that value coefficient the humification of $Q_{4/6}$ decreases with increasing molecular weight and degree of condensation. The low ratio of color quotient by Stevenson (1982) suggests to more humified, mature organic matter with high presence of condensed, hence aromatic compounds.

The circulation and migration of metals in the natural environment are mainly related to such processes as rock decay, volcano eruptions and soil formation processes (Szyczewski et al., 2009). Metal mobilisation depends on soil characteristics and is controlled by the soil matrix and the composition of the soil solution (Tóth et al., 2006). Activities between trace elements bound to soil components in various forms, change over time due to the effects of the process of pedogenesis (Belanović et al., 2013). With increasing pH, content of organic matter, and clay the bioavailability of most metals decreases due to their increased adsorption (Zogaj et al., 2014).

The aim of the paper is characterization of heavy metal sorption in *Eutric Andosol*, *Eutric Cambisol* and *Eutric Planosol* formed on Neovulvanites in the Western Carpathians (Kremnicke vrchy Mts., Slovak republic). UV-VIS and fluorescence spectroscopy were applied to give basic soil properties.

2. MATERIAL AND METHODS

Soil samples were collected from selected sites in Kremnické vrchy Mountains from the xerothermic meadow and mowed meadow in which we have identified *Eutric Cambisols, Eutric Andosols* and *Eutric Planosols* (Table 1, Fig. 1). It is region long burdened mining activity in the past, which causes high levels of heavy metals until today.

Soil reaction was determined in distilled water as active soil reaction (pH_{H2O}) and in a solution of 1 mol l^{-1} KCl as exchange soil reaction (pH_{KCl}). The ratio of soil and solution was 1:2.5 (van Reeuwijk, 2002). Organic carbon content (TOC) was measured via Turin method modified by Nikitina according to Orlov & Grišina (1981). The content of humic substances, as well as the ratio HK:FK were determined by group composition of humic substances using the Belčiková - Kononová method (Kononová & Belčiková, 1962). The humification degree of humified substances was calculated from the relation $DH=C_{HA}/TOC.100$ [%] (Grišina, 1986).

Excitation fluorescence spectrum of humic substances was measured in A-horizon in 0.1 mol l^{-1} solution $Na_4P_2O_7$ + NaOH with the concentration c=0.1 mol l^{-1} (pH =13) on the Cary Eclipse Spectrofluorimeter (voltage 600 V, band pass of both monochromators 5 nm, constant difference $\Delta\lambda$=20 nm and $\Delta\lambda=\lambda_{em}-\lambda_{ex}$) in the range from 300 nm to 600 nm as synchronous scan excitation fluorescence spectra. Fluorescence indexes (F) were calculated as the ratio of fluorescence intensities at an excitation wavelength of 404 nm and at emisson 423 nm (F=404/423).

Color quotients of humic substances (Q_{HS}) were determined as the ratio of the absorbance values at 465 nm and 665 nm from VIS spectra (Orlov & Grišina, 1985). Humic substances were extracted into solution $Na_4P_2O_7$ c=0.1 mol l^{-1} and adjusted to pH 13 with NaOH c=1.0 mol l^{-1} and the samples were left for infusion for 24 hours. UV/VIS spectra were measured by Varian Cary 50 spectrophotometer in the range from 300 nm to 700 nm.

Table 1. Characteristics of sampling places in soil samples

Profile	**Soil types**	**Altitude [m n. m.]**	**Position**	**Exploitation**	**Slope relief**	**Cadaster territory**	**Geomorphological division**
P1	Eutric Cambisols	486	slope	mowed meadow	12 - 17°	Jastrabá	Jastrabská vrchovina Mts.
P2	Eutric Cambisols	430	slope	mowed meadow	17 - 25°		
P3	Eutric Andosols	944	saddle	xerothermic meadow	12 - 17°	Kremnické Bane	Kunešovská hornatina Mts.
P4	Eutric Cambisols	872	slope	mowed meadow	7 - 12°		
P5	Eutric Planosols	808	slope	mowed meadow	3 - 7°		

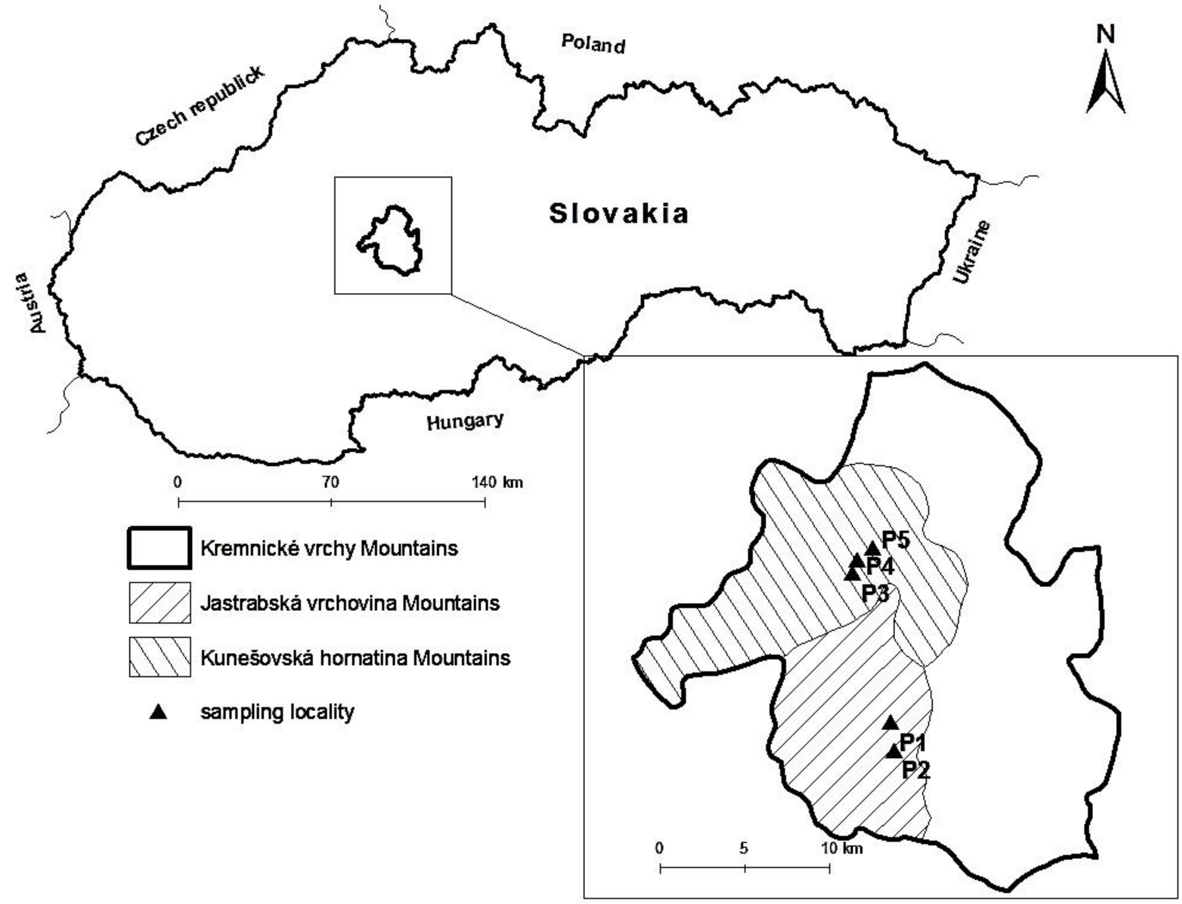

Figure 1. Sampling locality.

Quantitative determination of heavy metals (lead, cadmium) was performed by ET-AAS technique on atomic absorption spectrometer SpectrAA–200 (Varian, Mulgrave Virginia, Australia) equipped with deuterium background correction with GTA-100 module. Potentially bioavailability forms of lead (Pb) and cadmium (Cd) were extracted with the solution 2 mol l^{-1} nitric acid in a 1:10 (soil/ nitric acid). Total concentration of heavy metals was determined using Aqua Regia (HCl/HNO_3 in a 3/1) extraction method. 3g of soil sample had been digested for 2h at 180 °C (ISO 11466:1995).

The obtained data were analyzed using statistical software Statistics Portable. Correlation analysis (Spearman correlation) was used to determine the relationships between the chemical properties and content of heavy metals in the soil. Significant correlation coefficients were tested at $p<0.05$ and $p<0.01$. One-way Anova model was used for detection depending of heavy metals content from the measured spectral peaks at $p<0.05$ and $p<0.01$.

3. RESULTS AND DISCUSSION

The highest average content of humic substances was present in *Eutric Andosol* soil type (xerothermic meadow) with the average value of 3.6 % and the lowest value was in the cambisol soil type. Humic substances extracted from the monitored soils indicate the low quality of humus, considering that the soils contain high quantity of fulvic acids (Table 2). In Figure 2 you can see the high fluorescence of HS isolated from *Eutric Andosol*. The prevalence of fluorescence bands and peaks with high relative intensity were found at the short wavelength, which confirmed high content of FA in fractional composition of HS (Table 2).

The peaks in the long wavelength (468/488 and 490/510 nm) indicated the presence of condensed aromatic rings, and other unsaturated bond systems capable for conjugation.

The detected values of color quotient of humic substances indicate more humified and mature organic matter with a high presence of condensed compounds in the cambisol and planosol soil types. Between these two soil types there was no significant difference in color values of the quotient of humic substances. The average value of the color quotient of observed humic substances in cambisol was from 1.78 to 3.21 and planosol from 2.65 to 3.67. Color quotient of andosols humic substances was in the range from 4.68 to 7.11.

Table 2. The values of carbon parameters in soil profiles

Profile	Soil types	TOC	HS	HA	FA	HA:FA	Q_{HS}	DH [%]	F
		[%]							
P1	Eutric Cambisols	0.52	0.44	0.10	0.35	0.27	2.27	6.25	1.40
P2	Eutric Cambisols	2.30	0.91	0.20	0.71	0.54	2.63	13.00	1.41
P3	Eutric Andosols	10.49	5.20	0.65	4.56	0.14	6.02	5.93	1.05
P4	Eutric Cambisols	2.39	0.95	0.22	0.72	0.35	2.71	10.10	1.54
P5	Eutric Planosols	2.00	1.04	0.35	0.70	0.49	3.11	17.02	1.46
Min		0.13	0.14	0.01	0.12	0.04	1.78	3.59	0.84
Max		14.77	6.84	1.22	5.91	1.18	7.11	21.37	1.62
Average for all soils		3.56	1.62	0.30	1.32	0.36	3.30	10.82	1.39
SD		4.10	1.92	0.27	1.70	0.28	1.42	5.24	0.20

TOC – total organic carbon content, HS – humic substances content, HA – humic acids content, FA – fulvic acids content, Q_{HS} – color quotient of humic substances, DH – humification degree, F – fluorescence index of humic substances (F=404/423), SD – standard deviation

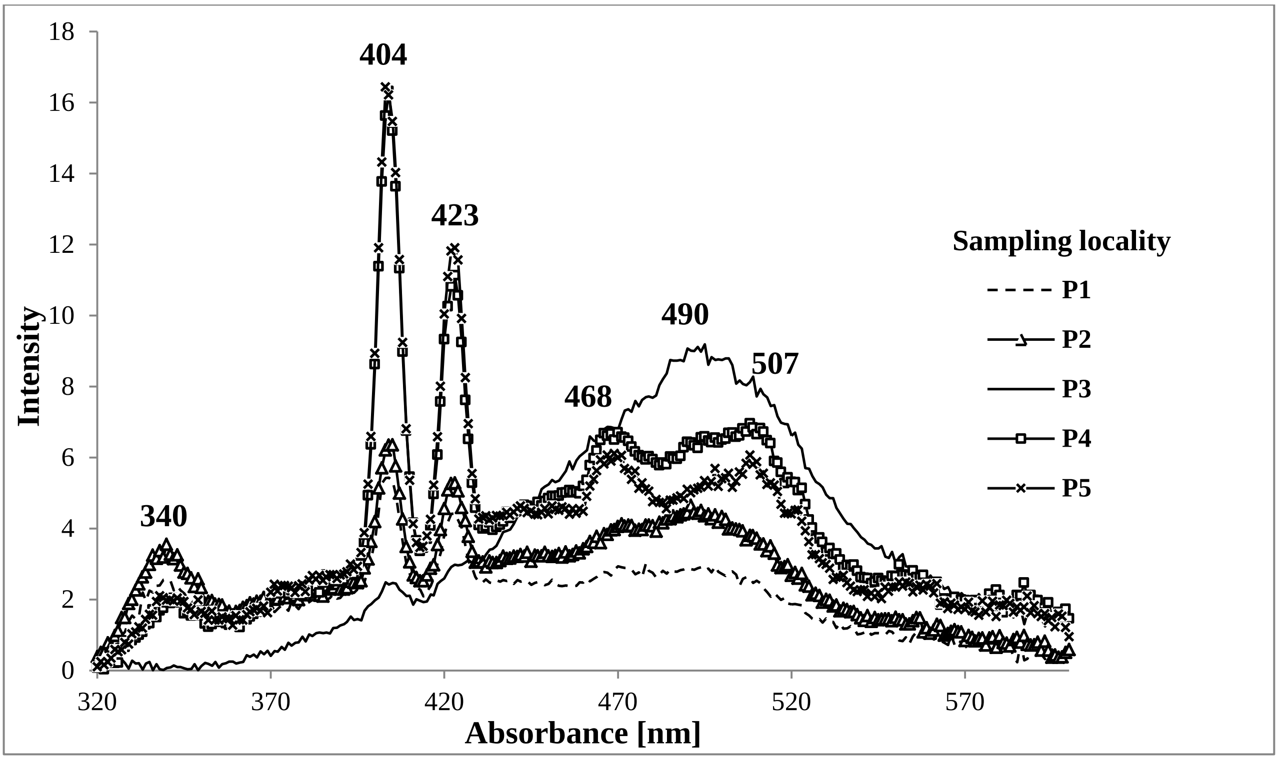

Figure 2. Excitation fluorescence spectrum of humic substances in A-horizons of soil.

The scan excitation fluorescence spectra of humic substances in the soil samples showed the main spectral peaks at $\lambda_{ex}/\lambda_{em}$: 340/360, 404/424, 423/443, 468/488, 490/510, 507/527, with a constant difference $\Delta\lambda$=20 nm (Table 3). In all studied soils we found two dominant peaks at $\lambda_{ex}/\lambda_{em}$: 404/424 and $\lambda_{ex}/\lambda_{em}$: 423/443. According to Pospíšilová et al. (2008) in simple phenolic compounds is dominated by the peak emission wavelength of 359 nm and 419 nm, which are characterized by a higher content of fulvic acids. Fusarová & Pospíšilová (2010) affirmed a wavelength of 360 nm and 420 nm. In the case of wavelength 360 nm was measured spectral peak at $\lambda_{ex}/\lambda_{em}$: 340/360, in the soil type of cambisol and andosol. The peak for the emission wavelength of 419 nm or 420 nm was not observed, but we measured a similar peak at $\lambda_{ex}/\lambda_{em}$: 404/424. According to Pospíšilová et al., (2008) the peak at 492/512 nm is characteristic for the presence of lignite humic acids. We measured a similar peak at 490/510 nm in the eutric subtype of andosol soil type in xerothermic meadow and in the eutric subtype of cambisol soil type in mowed meadow in Kremnické vrchy Mountains (Table 3). Figure 2 shows the excitation fluorescence spectra of humic substances.

Table 3. Determined F indexes in studied soils

F wavelength indexes		$\lambda_{ex}/\lambda_{em}$, $\Delta\lambda$=20 nm					
Profile	**Soil types**	**340/360**	**404/424**	**423/443**	**468/488**	**490/510**	**507/527**
P1	Eutric Cambisols	1.76	1.28	1.74	ND	ND	ND
P2	Eutric Cambisols	1.21	1.26	1.64	ND	1.19	ND
P3	Eutric Andosols	ND	0.82	0.66	ND	1.18	1.51
P4	Eutric Cambisols	1.48	1.54	2.39	1.10	ND	1.81
P5	Eutric Planosols	1.28	1.40	2.61	1.20	ND	1.79

ND - not detected

The peak at $\lambda_{ex}/\lambda_{em}$: 468/488 was recorded only in *Eutric Cambisols* and *Eutric Planosols* (P4 and P5), which is according to Kumke et al., (1995) associated with the presence of five or more fused aromatic rings. In the case of andosol soil type with the fresh supply of organic matter with the highest content of total organic carbon and humic substances, we found the lowest degree of humification, which is characterized by a lower degree of aromaticity of humic acids (Table 2). Humic substances of Andosol mainly on volcano substrate are characteristic by high degree of humification. Data of Andosol humic substances parameters indicate low degree of humification for this soil type. Such values could be for forest floor (Ol diagnostic horizon) and not for mineral soils. Higher values degree of humification of humic substances (HS) are associated with higher ratio of HK:FK (Table 2).

Pseudo-total content of lead in the soil was in the range from 30.1 mg kg^{-1} to 65.3 mg kg^{-1} and cadmium was in the range from 1.4 mg kg^{-1} to 5.3 mg kg^{-1} (Table 4). The limit value was not exceeded in all samples (Table 4). Evaluation the content of Pb and Cd in the extract 2 M HNO_3, however, points out the contents of potentially bioavailable forms of lead and cadmium in soil is low, not exceeding the limit values, which means that despite the high total content of monitored elements in the soil, plant the proportion of acceptable forms of Pb and Cd is low and implies a low risk entry of these elements in the soil to plant biomass. According to Tomaškin et al., (2013) content of heavy metals in grass ecosystem is significantly changing during the years. A different altitude of locations has a big impact on concentration of heavy metals. The content of heavy metals is increasing with growing of altitude.

Inorganic contaminants are brought into the soil mostly by human activity or activity of the natural environment, since according to Apea & Ephraim (2012) and Perelomov et al., (2011) trace metal distribution in terrestrial environments is related to the sorption process. Geological composition of monitored area represents a set of volcanic rocks represented mainly andesites, rhyolites, tuffs and volcanic breccia. The geological footwall consists of lava flows of pyroxenic and pyroxene-amphibolic andesites. The detected values of lead´s and cadmium´s bioavailability forms in leachate 2 mol l^{-1} HNO_3 are judged by a decision of the Ministry of Agriculture of the Slovak Republic nb. 531/1994-540 „Limitarian values of danger inorganic substances in soil“. The limitarian value for content of lead is 30 mg kg^{-1} and of cadmium is 0.3 mg kg^{-1} of dry solid, based on it can be said that content of lead was less limited in every sample of soil (Table 4).

Effects of heavy metal pollution are most long lasting in soils due to relatively strong adsorption of many metals onto the humus and influence of anthropogenic factors of environment and structure of rocks. Rocks and volatiles of volcanic origins are responsible for the presence of metals in soils and waters (Aiuppa et al., 2000; Kelepertsis et al., 2001). According to Delmelle & Stix (2000), Durand et al., (2004) volcanic activity is responsible for the release of metals such as lead (Pb), aluminum (Al), magnesium (Mg), copper (Cu) and zinc (Zn), among others.

In the case of contaminated soils is very important so that the heavy metals have not got into the plants not only on the agricultural use of soils but also on the meadows and pastures, thus can get into the food chain. HS can help to improve unfavourable soil properties, plant productivity and nutrient uptake. Organic amendments such as mature compost, which contain a high proportion of humified organic matter, can decrease the bioavailability of heavy metals in soil by adsorption and by forming stable complexes with humic substances (Takamatsu & Yoshida, 1978), permitting the reestablishment of vegetation on contaminated sites (Tordoff et al., 2000). It is important to examine mechanisms sorption and distribution of heavy metals of Pb and Cd in soils between fractions of humic substances, because by Burlakovs et al., (2013) humic substances have good ability for diminishing the content of biologically available heavy metals and the ability to form complexes with metal ions depends on the type of soil, type of metal, as well as concentrations of HS in soil. During statistical testing of the correlation relationship by Spearman correlation, we found a positive correlation between chemical factors of humic substances and content of heavy metals in the soil (Table 5). Total organic carbon was in the positive correlation with the total content of cadmium (r=0.493, p<0.05) and with the bioavailable forms of lead (r=0.708, p<0.01) and cadmium (r=0.734, p<0.01) (Table 5). Content of humic substances was in the positive correlation with the bioavailable forms of lead (r=0.709, p<0.01) and cadmium (r=0.692, p<0.01).

Table 4. Some characteristics of soils

Profile	**Soil types**	**pH$_{\mathrm{H2O}}$**	**pH$_{\mathrm{KCl}}$**	**Lead [mg kg^{-1}]**		**Cadmium [mg kg^{-1}]**	
				total content	**bioavailability forms**	**total content**	**bioavailability forms**
P1	Eutric Cambisols	7.00	5.90	51.53	22.83	4.80	0.16
P2	Eutric Cambisols	5.77	5.13	50.60	19.07	3.13	0.19
P3	Eutric Andosols	5.00	3.93	50.07	29.23	4.83	0.24
P4	Eutric Cambisols	6.15	4.25	45.23	16.23	2.78	0.12
P5	Eutric Planosols	6.18	4.43	45.85	14.25	1.95	0.09
Min		3.90	3.70	30.10	10.80	1.40	0.05
Max		7.30	6.20	65.30	38.10	5.30	0.27
Average for all soils		6.02	4.71	48.49	20.13	3.46	0.16
SD		0.73	0.72	9.67	6.25	1.25	0.06

pH$_{\mathrm{H2O}}$ – active soil reaction, pH$_{\mathrm{KCl}}$ – exchange soil reaction, SD – standard deviation

Table 5. Correlation relationships between chemical factors of humic substances and content of heavy metals

	pH$_{\mathrm{H2O}}$	**pH$_{\mathrm{KCl}}$**	**Pb**	**Cd**	**Pb**	**Cd**
			total content		**bioavailability forms**	
TOC	-0.757**	-0.521*	0.344	0.493*	0.708**	0.734**
HS	-0.795**	-0.568*	0.299	0.448	0.709**	0.692**
HA	-0.507*	-0.496*	0.299	0.214	0.352	0.499*
FA	-0.813**	-0.559*	0.290	0.471	0.743**	0.700**
Q$_{\mathrm{HS}}$	-0.804**	-0.585*	0.343	0.429	0.734**	0.664**
HK:FK	0.140	0.153	0.075	-0.443	-0.448	-0.228
DH	0.087	-0.090	-0.057	-0.774**	-0.635**	-0.449
F	0.436	0.132	-0.285	-0.716**	-0.750**	-0.676**

* $p<0.05$ ** $p<0.01$ pH$_{\mathrm{H2O}}$ – active soil reaction, pH$_{\mathrm{KCl}}$ – exchange soil reaction, Pb – lead, Cd – cadmium, TOC – total organic carbon content, HS – humic substances content, HA – humic acids content, FA – fulvic acids content, Q$_{\mathrm{HS}}$ – color quotient of humic substances, DH – humification degree, F – fluorescence index of humic substances.

Table 6. Statistical evaluation of heavy metals content from spectral peaks of humic substances

		Spectral peaks of humic substances $\lambda_{ex}/\lambda_{em}$, $\Delta\lambda$=20 nm			
		340/360	**468/488**	**490/510**	**507/527**
Pb	**total content**	F=1.906 p=0.261	F=0.003 p=0.957	F=0.944 p=0.403	F=0.875 p=0.419
Cd		F=0.126 p=0.746	F=16.844 p=0.026*	F=0.741 p=0.453	F=4.035 p=0.138
Pb	**bioavailability forms**	F=0.826 p=0.430	F=139.040 p=0.001**	F=1.897 p=0.262	F=1.778 p=0.275
Cd		F=0.820 p=0.432	F=48.572 p=0.006**	F=4.018 p=0.139	F=1.620 p=0.293

* $p<0.05$ ** $p<0.01$

Correlation was found also between the content of humic acids and the bioavailable content of cadmium (r=0.499, p<0.05) and between the content of fulvic acids and the bioavailable forms of lead (r=0.743, p<0.01) and cadmium (r=0.700, p<0.01) (Table 5). Correlation relationships were found between the bioavailable forms of heavy metals and color quotients of humic substances (Q_{HS}) (Table 6).

On the total content of cadmium and the bioavailable forms lead and cadmium has an impact also the spectral peak at 468/488 nm (Table 6), which is associated with the presence of five or more fused aromatic rings.

4. CONCLUSIONS

In the soil types (andosol, cambisol, planosol) in selected locations Kremnické vrchy Mountains, we identified six main spectral peaks at $\lambda_{ex}/\lambda_{em}$: 340/360, 404/424, 423/443, 468/488, 490/510, 507/527 via synchronous scan excitation fluorescence spectra of humic substances with a constant difference $\Delta\lambda=20$ nm. In the monitored soils was high content of lead found, possibly due to human activity or activity of the natural environment. Humic substances can help to improve unfavourable soil properties and therefore ability of humic substances to form stable complexes with soil or water inorganic and organic components plays a key role in formation of the level of environmental pollution and the migration and transformation of pollutants.

On the total content of cadmium and the bioavailable forms lead and cadmium has an impact also the spectral peak at 468/488 nm, which is associated with the presence of five or more fused aromatic rings of humic substances. We found correlation between total organic carbon and fluorescence indexes (r=-0.590, p<0.05), between humic substances content in soil and fluorescence indexes (r=0.983, p<0.01), as well as between colour indexes (Q_{HS}) (r=-0.716, p<0.01).

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