

## EVALUATION OF THE QUALITATIVE PARAMETERS OF HUMUS AND SELECTED HEAVY METALS CONTENT

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**Abstract.** Heavy metals get into the soil primarily through human and environmental activity. The effects of heavy metal pollution on land are processes that are long-lasting. To assess the relationship between chemical factors and heavy metal contents in the soil we used Pearson correlation analysis, which was tested at  $p < 0.05$  and  $p < 0.01$ . The content of Pb and Cd heavy metals were compared using a Student t-test to calculate the differences between the averages to a significance level of  $\alpha = 0.01$ .

**Keywords:** Pearson correlation analysis, Student t-test, heavy metals, lead, cadmium, humic substances, Kremnické and Štiavnické mountains

*Mathematics Subject Classification:* Primary 62F; Secondary 92E

### 1 Introduction

Inorganic impurities are primarily introduced into the soil through human activity or the activity of the natural environment. According to Apea and Ephraim (2012) and Perelomova (2011), the distribution of trace elements in the soil is related to the sorption process. The impact of heavy metal soil pollution is long-term due to the relatively strong adsorption of many metals by humus and the impact of anthropogenic environmental factors and rock structure. Volcanic rocks are responsible for the presence of metals in soil and water (Aiuppa et al., 2000; Kelepertsis et al., 2001). According to Delmelle and Stix (2000) and Durand et al. (2004), it is volcanic activity that is responsible for the release of metals such as lead (Pb), aluminium (Al), magnesium (Mg), copper (Cu) and zinc (Zn), among others.

In the case of contaminated soils, it is very important that heavy metals are not absorbed by plants, not only on agricultural land but also on meadows and pastures, due to the fact that heavy metals can then enter the food chain. Humic substances can help improve soil properties, increase production productivity and improve nutrient intake (Tobiášová, 2017). Organic soil inputs such as mature compost containing a high proportion of the aggregate organic matter may help reduce the bioavailability of heavy metals in the soil by adsorbing

them and forming stable complexes with humic substances (Takamatsu & Yoshida, 1978). These procedures and processes allow the restoration of vegetation on contaminated sites (Tordoff et al., 2000).

It is important to examine the mechanisms of sorption and the distribution of heavy metals Pb and Cd in the soil between the fractions of humic substances. Burlakovs et al. (2013) claim that humic substances have a good ability to reduce the content of bioavailable heavy metals, and the ability to form complexes with metal ions depends on the type of soil, the type of metal, and the concentration of humus in the soil.

The aim of this paper is to describe the characteristics of the qualitative parameters of humus and the selected heavy metal content (Pb, Cd) in the soil. To assess the relationship between chemical factors and heavy metal contents in the soil we used Pearson correlation analysis, which was tested at  $p < 0.05$  and  $p < 0.01$  levels. The content of lead and cadmium heavy metals extracted using  $\text{HNO}_3$  ( $c_{\text{HNO}_3} = 2 \text{ mol.dm}^{-3}$ ) and aqua regia was compared using a Student t-test to calculate the differences between the averages to a significance level of  $\alpha = 0.01$ .

## 2 Material and Methods

The geological composition of the monitored area is a set of volcanic rocks represented mainly by andesites, rhyolites, tuffs and volcanic breccia. The geological footwall is composed from the lava flows of pyroxenic and pyroxene-amphibolic andesites.

The analyzed soil samples were collected in the form of open borrow pits in order to determine the profile distribution of total organic carbon, in the autumn of 2011, 2012, 2013 and in the spring of 2012, 2013 and 2014. There was selected area 5 x 5 m on each location and it was realized (conducted) soil sampling in 6 different sampling sites. The opened borrow pits are used for soil for determination of soil characteristics, such as soil profile description, their depth, the stratigraphy (the arrangement of genetic horizons) and morphological characteristics. The study was conducted at Kremnické and Štiavnické vrchy Mountains (Fig. 1.). Kremnické and Štiavnické vrchy Mountains are volcanic mountain ranges located in central Slovakia. Soil samples were collected from 3 main soil types (Eutric Andosol, Eutric Cambisol, Eutric Planosol) of the selected horizons.

Soil was air-dried at room temperature and sieved ( $< 2 \text{ mm}$ ). We used to analysis selected parameters in soil samples and a sample was taken using standard procedures.

Total organic carbon (TOC) was measured using the Tjurin method modified by Nikitina according to Orlov and Grišina (1981) and from that we calculated the humus content ( $\text{Humus} = \text{TOC} \cdot 1.724$ ).

Soil reaction was determined in distilled water as active soil reaction ( $\text{pH}_{\text{H}_2\text{O}}$ ) and in a solution of  $1 \text{ mol.dm}^{-3}$  KCl as exchange soil reaction ( $\text{pH}_{\text{KCl}}$ ). The ratio of soil and solution was 1 : 2.5 (van Reeuwijk, 2002).

The content of humic substances (HS), as well as the ratio humic acid : fulvic acid (HA : FA) were determined by group composition of humic substances using the Belčíková-Kononová method (Kononová & Belčíková, 1962). The humification degree (DH) of humified

substances was calculated from the relation  $DH = \text{CHA} / \text{TOC} \cdot 100$  [%] (Grišina, 1986). Humic substances were extracted into solution  $\text{Na}_4\text{P}_2\text{O}_7$  ( $c = 0.1 \text{ mol} \cdot \text{dm}^{-3}$ ) and adjusted to  $\text{pH} = 13$  with  $\text{NaOH}$  ( $c = 1.0 \text{ mol} \cdot \text{dm}^{-3}$ ) 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.

Quantitative determination of heavy metals (lead, cadmium) was performed by ET-AAS technique on atomic absorption spectrometer Spectra AA-200 (Varian, Mulgrave Virginia, Australia) equipped with deuterium background correction with GTA-100 module.

Potentially bioavailable forms of lead (Pb) and cadmium (Cd) were extracted with the solution  $2 \text{ mol} \cdot \text{dm}^{-3}$  nitric acid ( $\text{HNO}_3$ ) in a 1:10 (soil/ nitric acid).

Total concentration of heavy metals was determined using aqua regia (aqua regia =  $\text{HCl} : \text{HNO}_3$  in a 3 : 1) extraction method. 3 g of soil sample had been digested for 2 h. at  $180 \text{ }^\circ\text{C}$  (ISO 11466:1995).

The measured data was statistically processed using the software STATISTICA.

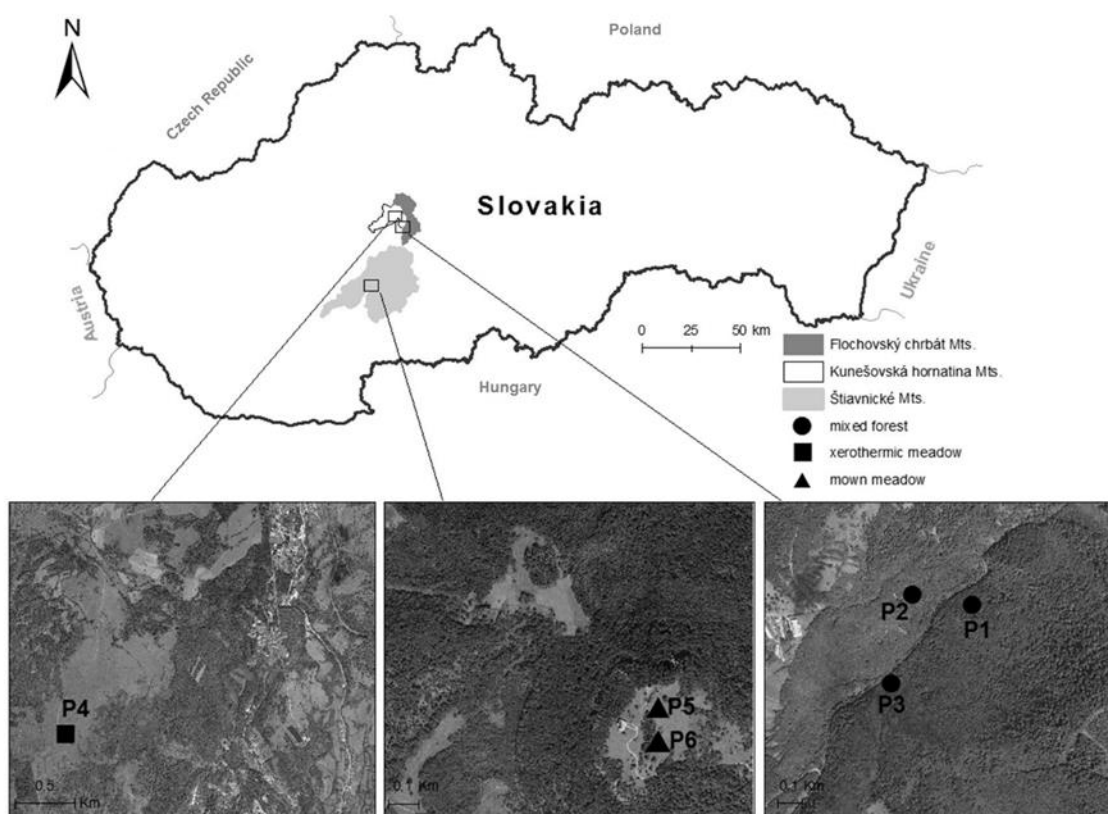


Fig. 1. Localisation of sampling sites.

### 3 Results and Discussion

Biologically available forms of lead and cadmium in the extract of  $2 \text{ mol} \cdot \text{dm}^{-3}$   $\text{HNO}_3$  are assessed in to the decision of the Ministry of Agriculture No. 531/1994-540 “Limit values of hazardous inorganic substances in soil”. The limit with regard to lead content is  $30 \text{ mg} \cdot \text{kg}^{-1}$  while for cadmium it is  $0.3 \text{ mg} \cdot \text{kg}^{-1}$  of dry matter. The total lead content in the soil samples

ranged from 30.1 mg kg<sup>-1</sup> to 65.3 mg kg<sup>-1</sup>, while the cadmium content ranged from 1.4 mg kg<sup>-1</sup> to 5.3 mg kg<sup>-1</sup> (Tab. 1.). This means that the lead and cadmium content was above the limit values in the soil samples.

In the case of the total lead content in the aqua regia extract, the limit values were not exceeded. The limit value for lead in the a extract is, according to the Act of the National Council of the Slovak Republic No. 220/2004 Coll. on the Protection and Use of Agricultural Land and on the Amendment of Act No. 245/2003 Coll. on Integrated Prevention and Control of Environmental Pollution and on amendments to certain acts for sand-clay and clay soil, 70 mg.kg<sup>-1</sup>. Also the limit value of cadmium in the aqua regia effluent for sand-clay and clay soils - 0.7 mg.kg<sup>-1</sup> - was not exceeded (Tab. 1.).

The content of potentially bioavailable forms of lead in the soil samples ranged from 10.8 mg.kg<sup>-1</sup> to 38.1 mg.kg<sup>-1</sup>, while the cadmium content ranged from 0.05 mg.kg<sup>-1</sup> to 0.27 mg.kg<sup>-1</sup> (Tab. 1.). The content of potentially bioavailable forms of lead and cadmium in the soil samples are low, and they do not exceed the limit values.

Despite the higher content of total heavy metal in the soil, the share of bioavailable lead and cadmium forms is low. This means that there is a low entry risk of these elements in plant biomass. According to Tomaškin et al. (2013), the content of heavy metals in the rays of ecosystems has changed significantly over recent years. Altitude has a great effect on the concentrations of heavy metals in the soil as their content increases with increasing altitude (Tomaškin et al., 2013).

Profile	Soil types	Lead [mg.kg <sup>-1</sup> ]		Cadmium [mg.kg <sup>-1</sup> ]	
		total content	bioavailable forms	total content	bioavailable forms
P1	Eutric Andosols	50.07	29.23	4.83	0.24
P2	Eutric Cambisols	45.23	16.23	2.78	0.12
P3	Eutric Planosols	45.85	14.25	1.95	0.09
P5	Eutric Cambisols	51.53	22.83	4.80	0.16
P6	Eutric Cambisols	50.60	19.07	3.13	0.19

Tab. 1. Average heavy metals content in soil.

The average content of cadmium in the Slovak soil is below 1.0 mg.kg<sup>-1</sup>. Compared to lead, cadmium belongs among the elements that are strongly mobile in acidic soil, whereas lead is not very mobile (Árvay et al., 2012).

	<b>Pb<sub>HNO3</sub></b>	<b>Cd<sub>HNO3</sub></b>	<b>Pb<sub>AR</sub></b>	<b>Cd<sub>AR</sub></b>
<b>pH<sub>H2O</sub></b>	-0.443	-0.450	-0.437	-0.374
<b>pH<sub>KCl</sub></b>	0.309	0.082	0.364	0.287

p < 0.05 pH<sub>H2O</sub> – active soil reaction, pH<sub>KCl</sub> – exchange soil reaction, Pb<sub>HNO3</sub> – lead in leachate 2 mol.dm<sup>-3</sup> HNO<sub>3</sub>, Cd<sub>HNO3</sub> – cadmium in leachate 2 mol.dm<sup>-3</sup> HNO<sub>3</sub>, Pb<sub>AR</sub> – lead in the aqua regia extract, Cd<sub>AR</sub> – cadmium in the aqua regia extract

Tab. 2. Correlation between heavy metal content and soil reaction.

This assertion is consistent with the results of the correlation analysis. There was a negative correlation that was not statistically significant (Tab. 2.) between the values of the active soil reaction and lead and cadmium content in the aqua regia extract and in the HNO<sub>3</sub> solution (C<sub>HNO3</sub> = 2 mol.dm<sup>-3</sup>). The negative influence of the acidic soil reaction on the increased mobility of heavy metals was confirmed by Styk (2000).

We have found a positive correlation between the chemical factors of humic substances and the heavy metal content in soil (Tab. 3.) using Pearson correlation. The bond formed between heavy metals and organic matter in the soil tends to be relatively strong, with its strength decreasing in the order of Cr<sup>3+</sup> > Pb<sup>2+</sup> > Cu<sup>2+</sup> > Ni<sup>2+</sup> > Cd<sup>2+</sup> > Zn<sup>2+</sup> > Mn<sup>2+</sup> (Kováčik, 2000). According to Tomáš et al. (2001), the mobility and immobility of metals in soils influences the soil reaction and the make up of humic substances. The total amount of organic carbon is positively correlated with the total cadmium content (r = 0.493, p < 0.05) and with bioavailable lead forms (r = 0.708, p < 0.01) and with cadmium (r = 0.734, p < 0.01). (Tab. 3.) The content of humus substances was positively correlated with the bioavailable forms of lead (r = 0.709, p < 0.01) and with cadmium (r = 0.692, p < 0.01). We found a positive correlation between the content of humic acids and the bioavailable form of cadmium (r = 0.499, p < 0.05), and between the content of fulvic acids and bioavailable forms of lead (r = 0.743, p < 0.01) and cadmium (r = 0.700, p < 0.01) (Tab. 3). According to Tobiášová (1996), the stability of metal complexes with humic substances decreases in the order Cd<sup>2+</sup> > Zn<sup>2+</sup> > Ni<sup>2+</sup> > Cu<sup>2+</sup> > Pb<sup>2+</sup>. The stability of the complexes depends strongly on pH values, with more stable complexes occurring in weakly acidic and neutral areas (Mestek & Volka, 1993).

Parameters	pH <sub>H2O</sub>	pH <sub>KCl</sub>	Pb	Cd	Pb	Cd
			Total content		Bioavailability forms	
<b>TOC</b>	..-0.757**	-.0521*	.0344	...-0.493*	0.708**	0.734**
<b>HS</b>	..-0.795**	-.0568*	.0299	.0448	0.709**	0.692**
<b>HA</b>	-0.507*	-.0496*	.0299	.0214	0.352	0.499*
<b>FA</b>	..-0.813**	-.0559*	.0290	.0471	0.743**	0.700**
<b>Q<sub>HL</sub></b>	..-0.804**	-.0585*	.0343	.0429	0.734**	0.664**
<b>HA:FA</b>	0.140	.0153	.0075	-0.443	-0.448	-0.228
<b>DH</b>	0.087	-0.090	-0.057	...-0.774**	-0.635**	-0.449

\* p < 0.05 \*\* p < 0.01 pH<sub>H2O</sub> – active soil reaction, pH<sub>KCl</sub> – exchange soil reaction, Pb – lead, Cd – cadmium, TOC – total organic carbon, HS – humic substances, HA – humic acids, FA – fulvic acids, Q<sub>HL</sub> – color quotient of humic substances, DH – humification degree

Tab. 3. Correlation between carbon parameters, soil reaction and heavy metal content.

Figure 2. shows the impact of the exchange soil reaction on the concentration of lead and cadmium heavy metals. Dependency is represented by point graphs translated using polynomial with regression equation. From the data obtained, it can be deduced that lead shows higher phytotoxicity at low values of the exchange soil reaction (pH<sub>KCl</sub> = 4.1 - 5.3). The results found correspond with the results of Árvay et al. (2012), indicating an interchange interval from 4.4 to 5.6. In the case of cadmium, its phytotoxicity is fairly equal at all exchange soil response values. For all elements, we recorded positive correlations between

the comparison parameters, which were not statistically significant (Tab. 2.). This does not correspond to the results of Huynh et al. (2008) and Pehlivan et al. (2009). However, a negative correlation was found in the case of an active soil reaction. However, this was also not statistically significant (Tab. 2.).

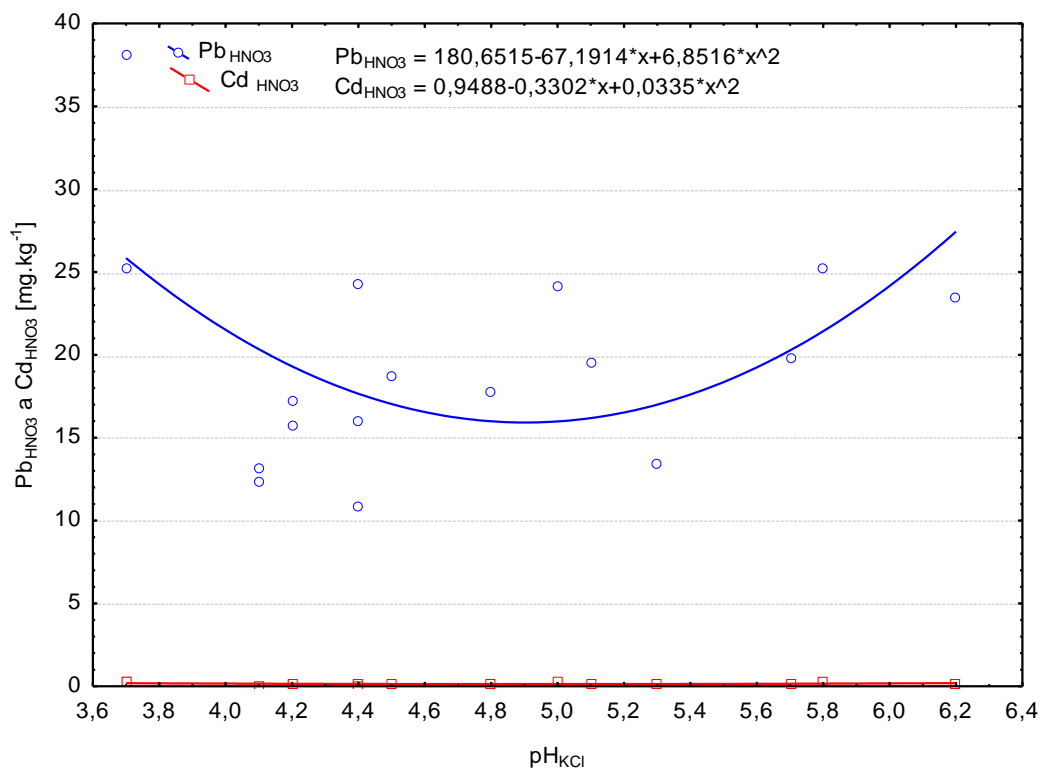


Fig. 2. Concentration of mobile forms Pb, Cd in the soil of the monitored sites depending on the exchange soil reaction values.

A correlation relationship was also found between the bioavailable forms of heavy metals and the color quotient of humic substances. (Tab. 3.)

The heavy metal contents in the extracted fractions are shown in Tab. 4. By statistical testing, significant differences were found between the cadmium and lead contents extracted with HNO<sub>3</sub> (C<sub>HNO3</sub> = 2 mol.dm<sup>-3</sup>) and that extracted with aqua regia, with Cd (t = -4.516, p = 0.001) and Pb (t = -10.086, p = 0.000).

The results also show the high sorption capacity of heavy metals to humic acids. The heavy metal distribution in the humic acid fraction (HA) was in the order of Pb > Cd, and in the fulvic acid fraction (FA) in the order Cd > Pb, with no statistically significant difference between the lead contents in HA and FA. The complexes Pb and Cu with humic acids are more stable than the Cd and Zn complexes (Waller & Pickering, 1993; Pinheiro et al., 1994; Gao et al., 1999). Higher Cd content bound to FA shows that this fraction is affected by the level of pollution (the Cd content to HA was lower). This might indicate a not very different sorption mechanism of Pb to FA or the presence of specific sorption sites on FA.

Fractions of heavy metals	Parameters	Pb	Cd
Aqua regia (total content heavy metals)	Average	48.49	3.46
	SD	9.69	1.25
2 mol.dm <sup>-3</sup> HNO <sub>3</sub> extract (content of bioavailability forms of heavy metals)	Average	20.13	0.16
	SD	6.25	0.06
NaOH/Na <sub>2</sub> P <sub>2</sub> O <sub>7</sub> extract	Average	2.56	0.02
	SD	3.46	0.01
Humic acids	Average	0.88	0.01
	SD	1.03	0.02
Fulvic acids	Average	0.56	0.07
	SD	0.33	0.05

Tab. 4. Average values and standard deviation (SD) of the extracted fractions of heavy metals in soil (mg.kg<sup>-1</sup>).

The results according to Adekunle et al. (2007) show that the concentration of the metals bound to humic acids was in the order Pb > Cd, which corresponds to our results. Angehrn-Bettinazzi et al. (1989) report the high ability of lead to form complexes with insoluble humus substances, whereas cadmium and zinc form complexes with low molecular weight organic molecules.

The cadmium content for HA and FA was very low in most of the analyzed soil samples. This may cause an error in interpreting the results. Árvay et al. (2012) report the ability of humus to bind and favour Cd rather than Pb, which could affect its accumulation and the binding strength of humus substances. In her study, Barančíková (2009) found significant differences in the sorption of Cd to humic acids for different soil types and pH values. The lead content in the FA fraction correlated positively with the fulvic acid content in the soil ( $r = 0.582$ ,  $p < 0.05$ ) and correlated negatively with the values of the active soil reaction ( $r = -0.599$ ,  $p < 0.05$ ) (Tab. 5.). These results are confirmed by Adriano (2001), who states that lead forms stable compounds with soil organic matter at low pH values. Low pH values increase the lead sorption on both fractions of humus substances. The strength of the metals that are complex-bound to humic acids is influenced by soil pH values and ionic strength (Ladonin & Margolin, 1997; Adriano, 2001; Evangel & Marsi 2001).

	Pb	Cd	Pb <sub>HA</sub>	Cd <sub>HA</sub>	Pb <sub>FA</sub>	Cd <sub>FA</sub>
	NaOH/Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> extract		Humic acids		Fulvic acids	
TOC	0.657*	0.715*	0.728*	0.065	0.534*	0.571*
HA	0.695*	0.724*	0.730*	-0.088	0.269	0.643*
FA	0.637*	0.725*	0.731*	0.066	0.582*	0.564*
pH <sub>H2O</sub>	-0.260	-0.263	-0.220	0.081	-0.599*	-0.170
pH <sub>KCl</sub>	-0.126	-0.048	0.297	0.508	0.571*	0.168

Tab. 5. Correlation between the heavy metal contents bound in HA and FA and the chemical properties of the soil.

Soil contamination involving heavy metals is long-term due to the relatively strong adsorption of many metals to humus, and also to the influence of anthropogenic factors of the environment and the structure of rocks. The TOC content was positively correlated with all forms of heavy metals with the exception of cadmium in the humic acid fraction ( $r = 0.065$ ;  $p < 0.05$ ). This was because the cadmium content in humic acids was very low. This could increase the relative error and the interpretation of the results (Tab. 5.).

The contents of both heavy lead and cadmium metals in the FA fraction are closely correlated with the total metal content in the extract of aqua regia, Pb ( $r = 0.662$ ,  $p < 0.05$ ) and Cd ( $r = 0.644$ ;  $p < 0.05$ ). The content of Pb and Cd in the aqua regia extract was positively correlated with the content of metals found in humic substances, Pb ( $r = 0.638$ ;  $p < 0.05$ ) and Cd ( $r = 0.717$ ,  $p < 0.05$ ). There was no significant correlation at  $p < 0.05$  between the heavy metal content in the aqua regia and Pb ( $r = 0.474$ ) and Cd ( $r = 0.305$ ) content in humic acids. A correlation was also found between the lead content extracted with  $\text{HNO}_3$  ( $c_{\text{HNO}_3} = 2 \text{ mol.dm}^{-3}$ ) and the content of the lead extracted with aqua regia ( $r = 0.985$ ) and tetrasodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ) ( $r = 0.674$ ) at the  $p < 0.05$  level. A positive correlation was also found between the lead content in the aqua regia and in the tetrasodium pyrophosphate ( $r = 0.638$ ;  $p < 0.05$ ). The cadmium content found in the tetrasodium pyrophosphate did not correspond to the cadmium content in a solution of  $2 \text{ mol.dm}^{-3} \text{ HNO}_3$  ( $r = 0.126$ ) and in aqua regia ( $r = 0.305$ ) for  $p < 0.05$ . A positive correlation was found only between the cadmium extracted with  $\text{HNO}_3$  ( $c_{\text{HNO}_3} = 2 \text{ mol.dm}^{-3}$ ) and aqua regia ( $r = 0.907$ ;  $p < 0.05$ ).

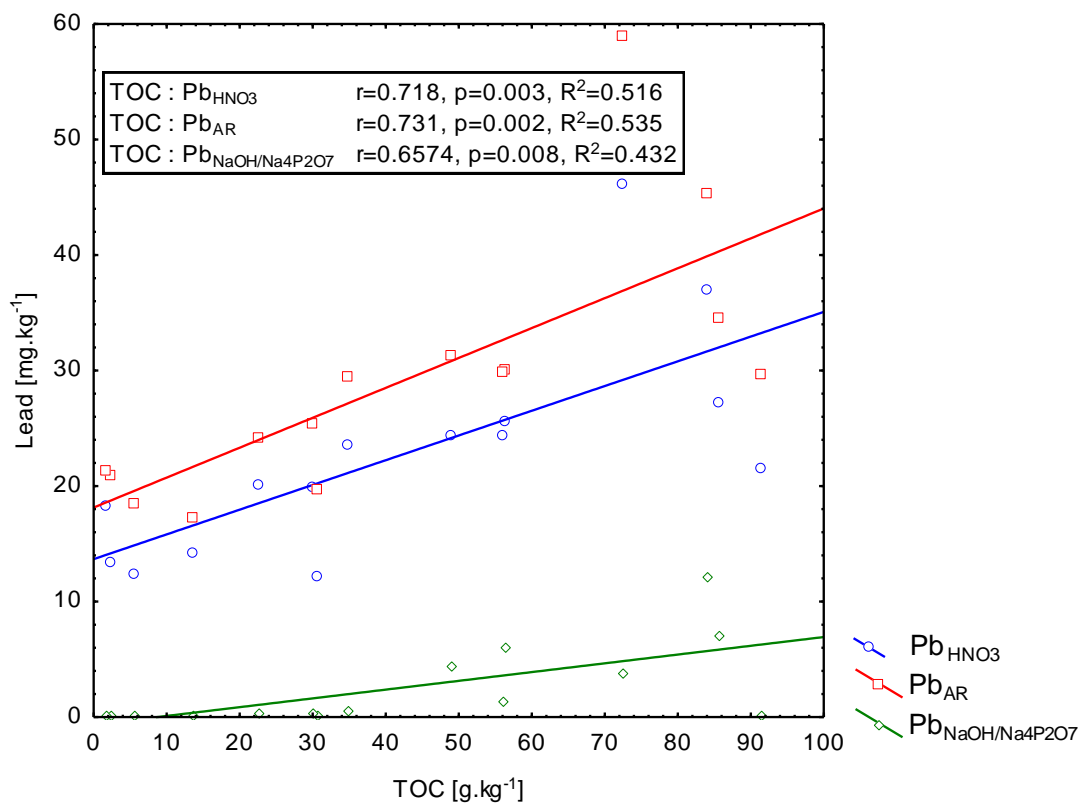


Fig. 3. Correlation between TOC and lead content in different extracts.



The contents of selected heavy metals (Pb, Cd) found in leachate 2 mol.dm<sup>-3</sup> HNO<sub>3</sub> and in leachate aqua regia and tetrasodium pyrophosphate leachate (NaOH / Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>) also had a positive effect on the total organic carbon content of the soil (Fig. 3. and Fig. 4.). Several studies show that organic matter can be used as a biosorbent due to its high sorption capacity, and the formation of insoluble complexes which prevent the intake of heavy metals by plants (Wang et al., 2007; Bose & Bhattecharyya, 2008).

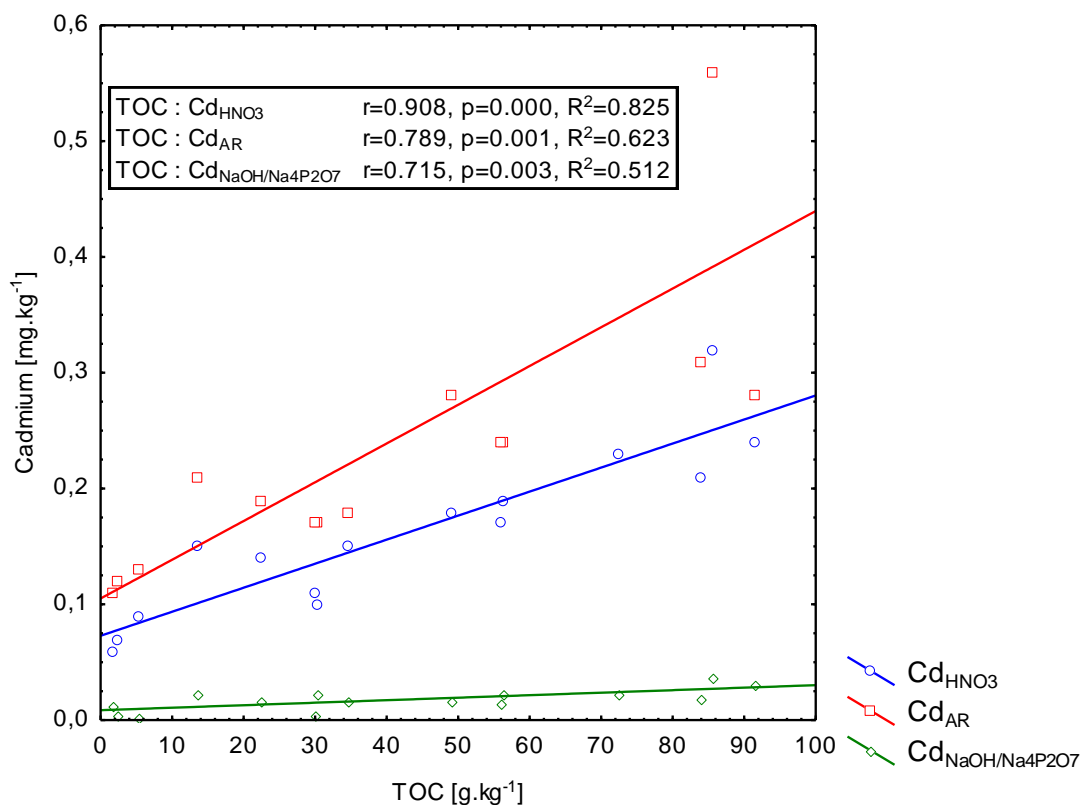


Fig. 4. Correlation between TOC and cadmium content in different extracts.

The strong bond between soil organic matter and heavy metals in soil is described by Deka and Sarma (2012). According to McBride et al. (1999), the higher the amount of dissolved organic matter in the soil the greater the increase in the mobility of metals in the soil / soil solution. Ashworth and Alloway (2004) claim that soluble organic matter reduces sorption but increases the mobility of heavy metals in the soil. According to Sherene (2010), soil organic matter is important for the absorption of heavy metals into the soil. This agrees with the results we have achieved. The increase in solubility of Pb in soil relates to the dissolution of humic acids (HA) as components of organic matter. This means that when the organic material solution is specified, the distribution of metals at a higher pH is dominant This is because, at a high pH values, the TOC in the soil increases due to the presence of dissolved humic acids (Sherene, 2010).

#### 4. Conclusion

High content of lead were found in the studied soils, presumably due to human activities or the activities of the natural environment. Humic substances can contribute to improving the adverse soil properties. The ability of humic substances to form stable complexes with soil inorganic and organic compounds plays a key role in environmental pollution and also in migration and transformation of pollutants. By statistic tests significant differences of Cd ( $t = -4.516$ ,  $p = 0.001$ ) and Pb ( $t = -10.086$ ,  $p = 0.000$ ) were found between the cadmium and lead content extracted with  $2 \text{ mol.dm}^{-3} \text{ HNO}_3$  and extracted from the aqua regia. TOC has a positive effect on all forms of heavy metals extracted with aqua regia  $2 \text{ mol.dm}^{-3} \text{ HNO}_3$  and NaOH /  $\text{Na}_4\text{P}_2\text{O}_7$ . The highest content of lead and cadmium was found in andosol soil with the highest content of TOC, and humic substances, as well as the lowest degree of humification. The results also show a high sorption capacity of heavy metals to the humic acids. The heavy metal distribution in the humic acids fraction (HA) was in the order of  $\text{Pb} > \text{Cd}$  and in the fulvic acids fraction (FA) in the order  $\text{Cd} > \text{Pb}$ , taking between contents of lead in HA and FA we found no statistically significant difference. Higher content of Cd bound to FA shows that this fraction is affected by the level of pollution (Cd content to HA was lower), which may indicate a slightly different mechanism of Pb sorption on FA or the presence of specific sorption sites on FA.

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