

Forms of Binding of Copper, Lead, and Cadmium in Carbonate Type Soil Studied by Differential Pulse Anodic Stripping Voltammetry

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A sequential extraction procedure was used for determination of the total and extractable forms of Cu, Pb, and Cd in soil by DPASV for determination of trace metal contents. Detection limits for these heavy metals were about tenth of $\mu\text{g dm}^{-3}$. The total content of Cu, Pb, and Cd, soluble, exchangeable, sorbed, and organically bound forms was determined.

Analysis of a loess-carbonate black soil sample was evaluated. The Cu content bound to humic matter was 47 %, 40 % of it bound to humic matter in originally free form and 7 % to humic matter in originally bound form. The occluded Cu forms represented 41 %, the residual bound Cu 12 %. Lead has a minor ability to form complexes with organic material compared to copper and this was confirmed by the determination of its distribution in the studied soil sample fractions. 35 % of Pb was bound to the humic matter. The occluded form represented 49 % and the residual form 14 % of the total Pb content in the soil. In spite of the very low initial Cd content in the soil sample it was determined that the major part of Cd was bound to the humic matter.

Heavy metals, *e.g.* copper, lead, mercury, nickel, affect the environment [1] due to their toxicity, cumulative character, and biological nondegradability. The action of a given heavy metal on animal or human depends on its mobility, which can be evaluated on the basis of the knowledge of its distribution between solutes and solid compounds [2] in various environmental material, *e.g.* in soil.

A lot of chemical tests on soils are based on the determination of leachable fractions of metals, related to their bioaccessibility [3–7]. Plants can utilize even more strongly bound heavy metals than are those extracted by nonaggressive solvents [8].

The heavy metals react practically with most of the soil components. Important are reactions with organic compounds, especially with the humic acids, ion exchange on the surface of silicates, and chemisorption on minerals [9, 10].

According to the recent knowledge the heavy metals Cu, Pb, and Cd occur in soils in various forms [11] influencing their mobility and plant availability as well.

Heavy metals in environmental material are mostly determined using optical methods of analysis. For the determination of heavy metals Cu, Pb, and Cd the voltammetric (VA) methods have an attractive feature – the possibility of simultaneous determinations from

a single experimental performance using a cheaper equipment [12].

Voltammetric trace metal analysis of the mentioned metals in environmental waters has been reported [13–16]. Substantially less frequently than in water are these metals determined using VA methods in soils. Among the most known reports concerning this problem are the works of *Redy* [17] and *Gregor* [18]. Some of our previous papers are based on these works [19, 20].

The aim of our work was to determine the distribution of Cu, Pb, and Cd between the inorganic and organic parts of soil. A sequential fractionation procedure was carried out to isolate humic matter (HM) from the soil sample according to [21]. For determination of low and trace contents of the mentioned metals in the soil fractionation residues the differential pulse anodic stripping voltammetry (DPASV) method was used.

EXPERIMENTAL

Nitric acid, hydrofluoric acid, and perchloric acid were used for sample destruction and/or the preparation of the leachable forms of the metals (soil extracts). Ammonium hydroxide, hydrogen peroxide, boric acid (saturated solution), and ascorbic acid were

used to adjust the pH value and/or to eliminate the excess of HF. All reagents were of anal. grade used without any further purification (Lachema, Brno).

Mixed standard solution of Cu, Pb, and Cd was prepared by dilution of the given metal stock solution with certified amount 1.0000 g dm^{-3} at $(20.0 \pm 0.1)^\circ\text{C}$ (Slovak Metrologic Institute, Bratislava). The mass concentration of the mixed standard solution was $\rho(\text{Pb}) = 0.1 \text{ mg dm}^{-3}$, $\rho(\text{Cu}) = 0.1 \text{ mg dm}^{-3}$, $\rho(\text{Cd}) = 0.01 \text{ mg dm}^{-3}$.

For complete soil sample digestion PTFE crucibles with clothing and sand bath were used. To prepare soil fractions a horizontal laboratory shaker LT 1 (Kavaliar, Votice) was used. The soil samples and the soil fractionation residues were dried in a drying box and heated in a muffle furnace in Pt crucibles. All VA determinations were carried out using an electrochemical analyzer EP-100 (HSC Servis, Bratislava), set to pH-metric and VA modes. The electrochemical system applied for VA determination had the following electrode configuration: a mercury microelectrode UME $\mu\text{E}2$ (HMDE), a silver chloride electrode RAE 113, and a Pt electrode. A combined glass electrode (Crytur, Turnov) for determination of the pH value together with an electromagnetic stirrer MM 6 (Labora, Prague) were applied.

Voltammetric Operation Parameters

Accumulation potential	-900 mV
Preconcentration time	240 s
Starting potential	-900 mV
Terminating potential	150 mV
Scanning rate (potential expansion)	50 mV s^{-1}
Extent of current	$40 \mu\text{A}$
Pulse duration time	60 ms
Pulse amplitude	30 mV
Size of drop	200 ms
Stoptime	10 s

Procedures

Soil sample, taken according to usual procedure [22] in the Agricultural Research Institute (Bratislava) from surroundings of Trnava town from the 1st horizon 5–15 cm was chosen. The carbonate type black soil was abbreviated as fraction 0 (unpolluted soil sample). The sample was homogenized, sieved to 0.2 mm and dried at 105°C for 3 h. The soil fractions were obtained by six extraction steps briefly described in the paper [21].

The oven-dry sample (90 g) was extracted with 2000 cm^3 of benzene–methanol (1:2) solution in a Soxhlet extractor for 8 h to remove free lipids. The dried soil residue was washed with 0.1 M-HCl solution until all carbonates were dissolved. With centrifuge separated soil residue (2nd fractionation step) was suspended in 900 cm^3 of neutral 0.1 M- $\text{Na}_4\text{P}_2\text{O}_7$ solution

to extract that fraction of humic matter present as free metal complexes. This extraction was repeated two times (3rd fractionation step). The soil residue after the third step was hydrolyzed in 6 M-HCl at 90°C for 12 h (4th fractionation step).

The hydrolyzed soil residue was washed with water, oven-dried, and extracted with chloroform–methanol (5:1) solution in a Soxhlet extractor for 12 h. The soil residue air- and oven-dried was suspended in 0.5 M-NaOH solution and mixed for 12 h to extract the remaining alkali-soluble humic substances. This extraction was repeated two times.

The isolated soil fractionation residues were dried at 105°C for 3 h. Oxidable carbon was determined in the whole soil sample and in the residues of the fractionation steps according to the procedure [22] as well.

For determination of the total Cu, Pb, and Cd content in the soil samples, the soil and the fractionation residues were burned in Pt crucibles in a muffle furnace at 450°C for 2 h. Total decomposition of soil and soil fractionation residues as well as the fractionation processes were carried out according to [19]: 15 cm^3 of soil extract and/or the soil sample after total decomposition was taken and treated with 10 mg of solid ascorbic acid. The pH value of the solution was adjusted by concentrated aqueous NH_3 to 2.0–2.2; 15 cm^3 were pipetted for a single determination.

The multiple standard addition technique was used to evaluate the results. Blank sample was evaluated using the calibration technique as well. For calculation and evaluation of the calibration curves a statistical program "CHEMSTAT" equipped with "linear regression" and "calibration" programs was used [23]. Calibration curves were constructed from seven independent values. Each value is an average of two parallel measurements. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the results of the work [24].

Results of heavy metal determination (Tables 3, 5, and 6) are the average of five parallel determinations and the confidence interval was computed from the dispersion variance. The coincidence results were tested using the Moore *U* test, the relative standard deviation results were computed from the dispersion variance as well [25]. All results were calculated for the significance level 0.05.

RESULTS AND DISCUSSION

According to the works [20] and [26], VA trace heavy metal analysis of soil extracts shows differences for irradiated (UV) and nonirradiated samples. This is in agreement with the assumption that a part of heavy metals is bound in complexes with the humic matter. It can be expected that during isolation of humic soil matter the metals bound on this material will be isolated.

Table 1. Calibration Curve Parameters for the Determination of Cu, Pb, and Cd

	Cu		Pb		Cd	
	soil ex.	t. dec.	soil ex.	t. dec.	soil ex.	t. dec.
$b_0 \cdot 10^{-1}$	-13.88	-16.15	-9.46	-7.10	-0.73	-4.27
$b_1 \cdot 10^1$	11.40	9.47	5.32	3.63	8.23	4.79
r	0.9991	0.9988	0.9986	0.9982	0.9991	0.9939
$H_0 : b_0 = 0$			accepted			
RSQ	10.80	5.73	3.75	1.27	0.58	0.21
LOD	71.2	90.6	88.6	109.0	7.2	2.0
LOQ	210.0	229.0	267.0	279.0	20.8	72.5

Regression model $y = b_0 + b_1x$, r – correlation coefficient.

RSQ – residual squares amount.

LOD – limit of detection ($\mu\text{g dm}^{-3}$) determined from the confidence belt.

LOQ – limit of quantification ($\mu\text{g dm}^{-3}$) determined from the confidence belt.

$H_0 : b_0 = 0$ – if accepted – abscissa on y is negligible, the curve passes the zero.

soil ex. – soil extract, t. dec. – total decomposition.

Table 2. Fractionation Procedure for Humic Matter Isolation from Soil

Fraction	Summation of species		Decrease of solid phase	C in solid phase
	Solution	Solid phase	$w/\%$	$w/\%$
0	–	IS, HM, NOM	–	1.82
1	NOM 1	IS, HM, NOM 2	0.44	1.79
2	MeCl_x	IS, HM, NOM 2	1.56	1.75
3	NaHM 1	IS, HM 2, NOM 2	10.78	1.02
4	SIC	ISR, HM 2, NOM 2	9.44	0.74
5	NOM 2	ISR, HM 2	0.56	0.70
6	NaHM 2	ISR	8.00	0.14

IS – original inorganic skeleton, HM – sum of humic matter, HM 1 – humic matter 1, originally free, HM 2 – humic matter 2, originally bound, NaHM 1, NaHM 2 – the sodium salts, NOM – sum of nonhumic organic matter, NOM 1 – nonhumic organic matter 1, originally free, NOM 2 – nonhumic organic matter 2, originally bound, SIC – soluble inorganic compounds in HCl, *e.g.* oxides of Fe and Mn, ISR – inorganic skeleton residue.

The aim of our investigation was to determine and compare the various extractable amounts of chosen Cu, Pb, and Cd metals with their total content in the soil sample. The results are a contribution to a higher information level in search for the real, occurring forms of the mentioned metals in soil.

Trace Cu, Pb, and Cd contents were determined in the original soil sample and the residues of fractionation steps after their total decomposition and in the extracts as well. DPASV was used for metal determination and evaluation using standard addition technique.

To justify this mode of evaluation of Cu, Pb, and Cd contents for all the above-mentioned samples, metal spiked calibration curves of total soil destruction blank solutions and those of fractionation solutions as well were tested. Calibration curve parameters are in Table 1.

Fractionation procedure together with the content of oxidable carbon in soil fractionation residues are summarized in Table 2. During the 1st fractionation step the free nonhumic organic matter (NOM 1) such as hydrocarbons, lipids, peptides, saccharides, and some others was eliminated. In the 2nd step the

carbonates were extracted. In the 3rd fractionation step the neutral 0.1 M- $\text{Na}_4\text{P}_2\text{O}_7$ solution affected the isolation of humic matter (HM 1) as soluble sodium salts (NaHM 1) and of the metals bound to HM 1 as their soluble dihydrogenphosphate complexes. In the 4th fractionation step (heating of the soil fractionation residue in 6 M-HCl at 90°C) destructive changes occurred in the inorganic skeleton (IS) and these made it possible to isolate the nonhumic organic matter (NOM 2) and the humic matter (HM 2) as well during the following 5th and 6th fractionation steps as the sodium salt (NaHM 2). Finally the soil residue contained products of the inorganic skeleton degradation (ISD) together with the residual humic matter insoluble even in acids or bases.

Determination of Copper

Totally digested soil samples and the fractionation residues extracted with 2 M- HNO_3 solution were determined using the DPASV method [19]. Results are in Table 3. Values are average of five parallel determinations in totally decomposed soil samples and of two parallel determinations in the respective fractionation

Table 3. Copper Content Determined in Soil and Soil Fractionation Residues by DPASV

Fraction	Total decomposition		Extract		Extracted form w/%
	$L_{1/2}$ /ppm	s_r /%	\bar{x} /ppm	s_r /%	
0	35.64 ± 3.06	9.61	10.79	10.10	30.27
1	36.60 ± 2.01	2.50	13.21	7.24	36.09
2	43.11 ± 3.79	4.64	15.90	0.56	36.88
3	25.97 ± 3.03	3.55	10.17	10.27	39.16
4	7.89 ± 0.27	1.83	1.15	7.76	14.56
5	8.25 ± 2.13	13.64	1.63	1.52	19.81
6	5.20 ± 0.87	7.57	0.64	9.19	12.36

Table 4. Testing of Statistically Relevant Differences between Amount of Cu, Pb, and Cd Determined in Soil and Soil Fractions

Compared solid phases	Results of testing			Fractionation step
	Cu	Pb	Cd	
0th - 1st	-	-	-	NOM 1 removed
1st - 2nd	+	+	+	carbonates removed
2nd - 3rd	+	+	+	HM 1 isolated
3rd - 4th	+	+	*	acid hydrolysis
4th - 5th	-	-	*	NOM 2 removed
5th - 6th	+	+	*	HM 2 isolated

+ - statistically different results.

-- statistically irrelevant difference between results.

* - no testing.

NOM 1 - nonhumic organic matter 1, originally free.

HM 1 - humic matter 1, originally free.

NOM 2 - nonhumic organic matter 2, originally bound.

HM 2 - humic matter 2, originally bound.

extracts. The coincidence of the determined amount of heavy metals in the soil and its fractionation residues was tested using the Moore U test [25]. The hypothesis $H_0 : x_A = x_B$ was tested; x_A , x_B are the average values of determined amount of heavy metals in two various solid phases. Results from the test are summarized in Table 4. Comparing the results in Table 3 with the fractionation scheme (Table 2) and using Table 4 it can be concluded:

Results of the Cu determination in the soil (0th fractionation residue) and the 1st fractionation residue show that they do not differ statistically in any of the samples. This indicates that in the 1st fractionation step only NOM 1 unable to bind Cu was removed.

The content of Cu in the 2nd fractionation residue was higher than in the initial soil sample or in the 1st fractionation residue. This fact, verified using the Moore U test, leads to the conclusion that Cu remains bound to the inorganic skeleton and the humic matter as well. The higher amount of Cu in this fractionation residue can be explained by Cu preconcentration on the solid phase as in this step the free organic materials and the carbonates are successively removed. The fact that Cu is not bound to carbonates is indicated by the equal mass fraction of extractable Cu (2 M-HNO₃) found in the 1st and 2nd fractionation residue.

Treating the 2nd soil fractionation residue with Na₄P₂O₇ the free and complex forms of HM 1 were removed. In this fraction a decrease of Cu content was determined as statistically significant difference between the Cu content in the 2nd and 3rd fraction. The results (Table 3) and the statistical evaluation show that relatively large amount (40 %) of Cu is bound to humic organic matter, $U_{\text{calc}} < U_{\text{tab}}$. This is in good agreement with the published 40–65 % content of organically bound Cu in sandy soils [11].

A further significant loss of Cu occurs (Table 3) due to acidic hydrolysis of the soil sample. It can be considered that in this step of fractionation the inorganic skeleton was fractured and less soluble inorganic compounds as Fe and Mn oxides passed into the extracted sample solution. This led to breaking of the remaining organically bound and occluded Cu content as well. The so-called residual Cu bound into the crystal lattice of primary and secondary minerals remained in the soil residue. Our findings taking into account the total Cu content yielding about 41 % of Cu bound to free Fe and Mn oxides are in good agreement with published data [11] that the occluded Cu content represents about 40 % of the total Cu in soil samples.

Between the 4th and 5th fractionation residue there is a statistically irrelevant difference of the deter-

Table 5. Lead Content Determined in Soil and Soil Fractionation Residues by DPASV

Fraction	Total decomposition		Extract		Extracted form w/%
	$L_{1/2}$ /ppm	s_r /%	\bar{x} /ppm	s_r /%	
0	29.69 ± 3.06	11.56	13.06	1.02	43.99
1	31.17 ± 0.58	0.85	15.58	2.50	49.98
2	36.09 ± 2.32	7.19	18.81	6.64	52.12
3	28.29 ± 2.83	4.55	10.72	11.37	37.89
4	10.60 ± 2.33	11.62	less than LOQ		–
5	9.80 ± 1.44	7.77	less than LOQ		–
6	5.15 ± 1.70	17.49	less than LOQ		–

LOQ – limit of quantification.

mined Cu contents (Tables 3 and 4). This shows that in the 5th fractionation step only the organic matter NOM 2 was extracted and that it does not form stable Cu complexes.

The residue of the 5th fractionation released by NaOH from the inorganic skeleton HM 2 changed to water-soluble alkaline humates and fulvates (NaHM 2). Statistically significant difference of the results of Cu determination in the 5th and 6th soil fractionation residue proved that a part of the alkaline humates and fulvates probably create Cu bound forms. They represent 7 % of the total Cu content.

The fact that the most insoluble, residual Cu forms are present in the 6th soil fractionation residue is proved by the lowest content of extractable Cu in the 6th soil residue. Related to the total Cu content in the soil sample the residual Cu represented about 12 %.

Determination of Lead

Similarly as for copper the DPASV method was applied for lead determination in soil and soil fractionation residues as well. The results are summarized in Table 5. The coincidence of Pb determination in soil and soil fractionation residues was also statistically tested (Table 4). From the tested results (Tables 4 and 5) the following conclusion can be drawn:

There is no statistically significant difference between the determined Pb content in the soil (0th fractionation residue) and the 1st fractionation residue ($U_{\text{calc}} < U_{\text{tab}}$) which confirms that the whole amount of Pb remains bound in the solid phase, as it was in the case of Cu as well.

The total Pb content increased in the 2nd fractionation residue compared to the 1st fractionation. There are the same reasons for this behaviour as were for Cu distribution, though the portion of extractable Pb was in the 1st and 2nd extraction residue approximately equal and higher than it was for Cu.

Treating the 2nd extraction residue with $\text{Na}_4\text{P}_2\text{O}_7$ caused that the amount of Pb decreased in the 3rd fractionation (Tables 4 and 5), although in a lesser extent than it decreased for Cu. It can be assumed from the results obtained that the content of Pb bound

to organic humic matter HM 1 is approximately 22 % while for Cu it was 40 %. This fact can be explained by a generally lesser tendency of Pb^{2+} ions to form complexes or chelates in comparison with Cu^{2+} ions. The total content of organically bound Pb in similar soils was found to be 30 % [11].

Balancing the whole Pb content it can be concluded that the content of Pb bound to the free Fe and Mn oxides is about 49 % compared to the total Pb content (100 %). The decrease was determined in the 3rd fractionation soil residue (Table 4).

After separation of the 5th fraction the Pb content in the residue was the same as in the 4th fractionation residue (Table 4). The explanation can be the same as it was for the distribution forms of Cu in this fractionation step; Pb is not bound to nonhumic organic matter NOM 2.

Treating the 5th fractionation residue with sodium hydroxide resulted in dissolution of the humates and fulvates; of a part of them probably with the bound Pb as it was confirmed (Tables 4 and 5). The Pb content bound to the above-mentioned compounds represented 13 % of the total content.

The portion of mineral bound Pb (residual Pb) was a little higher than the residual Cu and represented 14 % of the total Pb content in the studied soil.

Determination of Cadmium

Similarly to Cu and Pb the results of Cd determination in soil and soil fractionation residues are summarized (Table 6); the coincidence of the values obtained by the solid-phase analysis was statistically tested. From the results in Table 6 it follows:

Similarly to the above described Cu and Pb speciations the highest content of the determined Cd was in the 2nd soil fractionation residue and the lowest in the 6th residue. Taking into account the relatively very low Cd level in the studied soil the values of Cd determination especially in the 4th, 5th, and 6th fractionation residue are loaded with relatively great error. Statistical evaluation of the experiments showed (Table 1) that the limit of quantification for Cd was

Table 6. Cadmium Content Determined in Soil and Soil Fractionation Residues by DPASV

Fraction	Total decomposition		Extract		Extracted form w/%
	$L_{1/2}$ /ppm	s_r /%	\bar{x} /ppm	s_r /%	
0	0.366 ± 0.027	3.93	0.088	5.25	24.04
1	0.393 ± 0.037	4.24	0.106	22.51	26.97
2	0.578 ± 0.028	2.20	less than LOQ		—
3	0.202 ± 0.077	17.25	less than LOQ		—
4	0.052 ± 0.019	19.02	less than LOQ		—
5	less than LOQ		less than LOQ		—
6	less than LOQ		less than LOQ		—

LOQ – limit of quantification.

Table 7. Results of Environmental Speciation of Cu, Pb, and Cd in Carbonate Type Soil

Metal	Mass fraction w/%						
	NOM 1	Carbonate	HM 1	Occluded	HM 2	NOM 2	Residual
Cu	0	0	40	41	7	0	12
Pb	0	0	22	49	13	0	14
Cd	0	0	65	—	—	—	—

NOM 1 – nonhumic organic matter 1, originally free.

HM 1 – humic matter 1, originally free.

NOM 2 – nonhumic organic matter 2, originally bound.

HM 2 – humic matter 2, originally bound.

72.5 $\mu\text{g dm}^{-3}$ using the total decomposition procedure and 20.8 $\mu\text{g dm}^{-3}$ in the extracts, respectively. The determined values, e.g. in the 4th soil fractionation residue, were 2 $\mu\text{g dm}^{-3}$ (total decomposition) and 1 $\mu\text{g dm}^{-3}$ (extract). As these values were under the quantification limit of Cd, a complete quantitative balance of the individual forms bound in the soil was not provided. Only the portion of the HM 1 bound Cd representing 65 % of the total Cd determined in the soil samples was evaluated.

The results of environmental speciation of Cu, Pb, and Cd in carbonate type soil determined using the sequential fractionation and DPASV method are summarized in Table 7.

In conclusion, the presented method of determination of the trace heavy metals Cu, Pb, and Cd in soil and soil fractions enables the metal pollutants control in soil in respect to their uptake by plants and other ecological aspects as well. It is well suitable to determine the metals bound to the inorganic and organic soil components.

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