

Copper Fractionation from Cambisols and Luvisols Using the BCR Procedure

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Abstract

We evaluated copper content and its distribution in the exchangeable fraction (F1), the reducible fraction (F2), the oxidizable fraction (F3), and the residual fraction (the residues after extraction – F4) extracted with the optimized sequential BCR procedure in the particular horizons of 11 profiles of arable Cambisols and Luvisols of the Siedlce Upland region. In these soils, the varied natural, uncontaminated levels of total copper were determined. For the complete profiles, the average percentage share of copper fractions in the total copper content was arranged in the following decreasing order: $F4 > F3 > F2 > F1$ (Cambisols) and $F4 > F2 > F3 > F1$ (Luvisols). In the humus horizons of the tested soils, the highest percentage share of copper was found in F1 and F3 fractions, whereas the enrichment horizons and in the parent material horizons – in the F4 fraction. The highest Cu percentage share in the F2 fraction was measured in the humus horizon of Cambisols and in the enrichment horizons and in the parent material horizons of Luvisols. A statistical analysis revealed the significant impact of selected soil properties (pH, CEC, C_{org} , clay fraction $\phi < 0.002$ mm) on copper fractionation in investigated soils. The low total content of copper and low percentage of its bioavailable forms indicated deficit of these elements for plants. Taking into account the normal growth of crops, it is advisable to supplement copper deficiency by fertilization.

Keywords: sequential extraction, copper, arable soils, vertical distribution

Introduction

Soil is involved in the biogeochemical circulation of elements in the environment. The natural content of copper in soil is related to the type of parent material. Copper accumulates in the surface horizons of soil and can be associated with various components: adsorbed on surfaces of clays, iron and manganese oxyhydroxides, complexed with organic matter, and be present in the lattice of secondary minerals like carbonates, phosphates, sulfates or oxides, or the remains of biological organisms. The chemical behaviour and profile diversification of copper depends directly on geological and pedogenetic processes and on properties

of the soil environment (pH, red-ox conditions, sorption) [1]. The pH influences adsorption and desorption, precipitation, dissolution, and speciation reactions of copper. Copper is strongly bound to soil under $pH > 5.0$. For most agricultural soils, bioavailability of copper is controlled by the adsorption–desorption process. Soil components vary greatly in their sorption capacity for Cu. Adsorption capacity decreases in the order: soil organic matter $>$ Fe-Mn oxides $>$ clay minerals. Both mechanisms (i.e., increased binding sites and lower labile fraction) may partly offset the risk of increasing the availability of Cu concentrations in soil [2, 3].

Copper is one of the most important essential elements in the nutrition of plants and animals. It occurs naturally in soils but is potentially toxic at high concentrations for living organisms (as a result of intensive pollution processes)

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and may produce deficiency symptoms at very low concentrations in the environment. Plants take up copper from the soil solution mainly as Cu^{2+} . Both the absence ($< 5 \text{ mg}\cdot\text{kg}^{-1}$ dry weight) and excess ($> 20 \text{ mg}\cdot\text{kg}^{-1}$ dry weight) of copper in plants tissues inhibits plant growth and impairs important cellular processes. Therefore, optimum copper concentration ($5\text{-}20 \text{ mg}\cdot\text{kg}^{-1}$ dry weight) ensures normal growth and development of plants. This microelement conditions the proper development of organisms, increases the biological activity of the soil, and has both a direct and indirect impact on the quantity and quality of plant crops [3, 4].

The average copper content in surface horizons of Polish soil is $6.6 \text{ mg}\cdot\text{kg}^{-1}$ (ranged $1\text{-}140 \text{ mg}\cdot\text{kg}^{-1}$ generally [1, 5]). There is a copper deficit in the majority of cultivated soils in Poland, resulting from the low total content of this element and a low percentage of bioavailable forms in the soil parent material (absolute deficiency) and its immobilization by the soil organic matter and minerals (secondary deficiency). According to Terelak et al. [5], 96.5% of arable soil in Poland shows natural copper concentrations ($15\text{-}40 \text{ mg}\cdot\text{kg}^{-1}$) [6]. Its limit content in these soils in Poland, according to the Decree of the Minister of the Environment [7], is $150 \text{ mg}\cdot\text{kg}^{-1}$. Copper contamination is most often local and situated near emission sources [1, 8, 9].

Cu accumulate in soils in various geochemical forms: water-soluble and exchangeable fractions are considered to be bioavailable; oxide-, carbonate-, and organic-matter-bound fractions may be potentially bioavailable; while the mineral fraction is mainly not available to either plants or microorganisms [1, 10].

Sequential extraction of elements from soil is a widely applied technique in geochemical exploration and environmental geochemistry. Fractionation gives information about fraction – not species of metals in soils. Sequential extraction procedure provides important information on mobile and stable heavy metal fractions in uncontaminated cultivated soils, as well as the actual and potential transport among different chemical forms. This method allows for the assessment of bioavailability and potential toxicity of these elements for organisms; it also helps to forecast the possible introduction of these elements into biological circulation under changing soil conditions (in particular pH, red-ox potential, conversions of organic matter). Metals from anthropogenic sources tend to be more mobile than pedogenic or lithogenic ones [1, 11-13].

A variety of protocols have been applied using different reagents, different sequencing of reagents, and different experimental conditions. Sequential fractionation procedure of metal proposed by the European Union's Standards, Measurements and Testing program (SM&T, formerly BCR) was introduced in order to standardize experimental conditions and to achieve comparable results in environmental studies (of soil) [10, 14, 15]. In the BCR procedure, acid-extractable, reducible, oxidable, and residual Cu were partitioned.

The aim of our study was to evaluate the content of total copper and its amounts in labile and stable fractions sequentially extracted with the BCR method in an environment of arable soils, as well as to determine and to compare their

various distribution in the particular genetic horizons of Cambisol and Luvisol profiles depending on soil properties.

Material and Methods

The studies were carried out on arable Eutric Cambisols (sites 1, 2, 3, 4), Haplic Luvisols (sites 5, 6, 7) and Stagnic Luvisols (sites 8, 9, 10, 11) in the Siedlce upland region (central-eastern Mazowieckie Province) located in Szaruty ($52^{\circ}21'N$, $22^{\circ}3'E$), Ruchenka ($52^{\circ}22'N$, $22^{\circ}5'E$), Wólka Leśna ($52^{\circ}12'N$, $22^{\circ}25'E$), Klimonty ($52^{\circ}10'N$, $22^{\circ}31'E$), Mordy ($52^{\circ}12'N$, $22^{\circ}32'E$), Pruszyń ($52^{\circ}10'N$, $22^{\circ}23'E$), Krześlin ($52^{\circ}13'N$, $22^{\circ}21'E$), and Jartypany ($52^{\circ}25'N$, $22^{\circ}7'E$) (Fig. 1). Luvisols and Cambisols are dominant in the structure of agricultural soils (Luvisols especially) of the Siedlce Upland and are the basis of agricultural activity in this region.

The soil samples from particular genetic horizons of 11 soil profiles were air dried and sieved through a 2 mm mesh, and then the following properties were determined: soil texture (according to the Polish Soil Science Society [16] – by the areometric method), pH in $1 \text{ mol KCl}\cdot\text{dm}^{-3}$ – potentiometrically, the amount of organic carbon (C_{org}) – by the oxidation-titration method [17]. The soil cation exchangeable capacity (CEC) values were calculated on the basis of hydrolytic acidity (Hh) determined by Kappen's method and exchangeable cations (Ca^{2+} , Mg^{2+} , K^{+} , Na^{+}) determined in $1 \text{ mol CH}_3\text{COONH}_4\cdot\text{dm}^{-3}$.

The total content of copper (Cu , pseudo-total content properly) were assessed by the ICP-AES method (Optima 3200 RL, Perkin-Elmer) after homogenization of soil samples in an agate mill and mineralization in a mixture of concentrated HCl and HNO_3 (3:1) in a microwave system (Multiwave, Anton Paar).

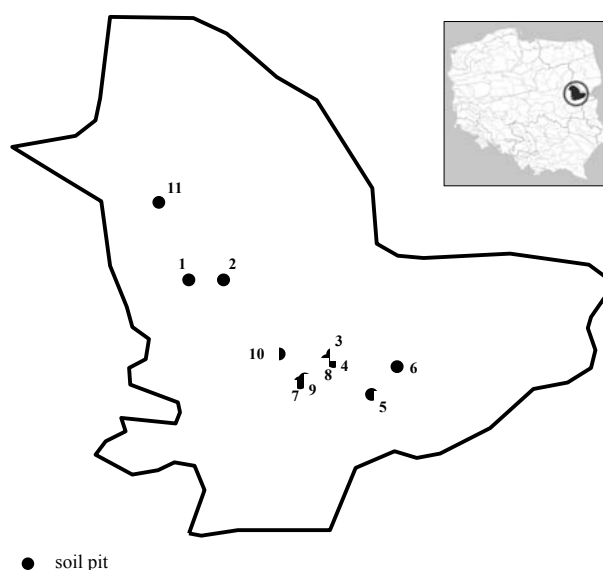


Fig. 1. Location of investigated soil profiles on the Siedlce upland (Eutric Cambisols: 1 – Szaruty, 2 – Ruchenka, 3 and 4 – Wólka Leśna; Haplic Luvisols: 5 – Klimonty, 6 – Mordy, 7 – Pruszyń; Stagnic Luvisols: 8 – Wólka Leśna, 9 – Pruszyń, 10 – Krześlin, 11 – Jartypany).

The fractions of copper were determined by the optimized BCR sequential extraction procedure proposed by The European Union's Standards, Measurements, and Testing program [15], in which four fractions were separated: F1 – exchangeable, acid extractable ($40 \text{ cm}^3 \text{ } 0.11 \text{ mol CH}_3\text{COOH}\cdot\text{dm}^{-3}$; pH = 3; shake for 16 h at 20°C); F2 – reducible, bound to iron and manganese oxyhydroxides ($40 \text{ cm}^3 \text{ } 0.5 \text{ mol NH}_2\text{OH}\cdot\text{HCl}\cdot\text{dm}^{-3}$; pH = 2; shake for 16 h at 20°C); F3 – oxidizable, bound to organic matter and sulfides (digest with $10 \text{ cm}^3 \text{ } 8.8 \text{ mol H}_2\text{O}_2\cdot\text{dm}^{-3}$ (pH = 2-3); 1 h at 20°C , and 1 h at 85°C , reduced volume and followed extracted with $50 \text{ cm}^3 \text{ } 1 \text{ mol CH}_3\text{COONH}_4\cdot\text{dm}^{-3}$ (pH = 2), and shake for 16 h at 20°C); F4 – residual, strongly associated to crystalline structures of mineral, post-extraction remaining (calculated as the difference between the total content of a particular heavy metals and the sum of their fraction: F1, F2, and F3, extracted during the previous steps). Sequential extractions were carried out with 1 g of soil sample (homogenized in an agate mill additionally) weighed in the 50 ml polypropylene centrifuge tube. Suspensions from each extraction step were centrifuged at $5,000\cdot\text{g}$ for 15 min. Aliquots from the supernatants were removed with a pipette, stored in polyethylene bottles, and used for analysis.

The content of copper in particular fractions was determined by the ICP-AES method. The percentage contribution of separated four fractions of Cu in relation to its content in aqua regia solution were calculated.

The research was conducted in triplicates. The accuracy of the measurements was determined with the addition of a standard into each analyzed sample and with reference material WEPAL Soil Reference Material BCR 142R, Perkin Elmer. The analyses included control samples consisting of chemical reagents (corresponding to fractions). The interrelation between copper content in sequenced fractions and selected properties of analyzed soils was determined with linear correlation and a single-factor variance analysis (ANOVA) was conducted between separate genetic levels in a profile distribution using Statistica 9.1. software (StatSoft. Inc., Tulsa, USA).

Results and Discussion

The selected physical, physicochemical, and chemical characteristics of the analyzed soils were typical of Cambisols and Luvisols formed on the substratum composed of glacial deposits from the middle-Polish glaciation (Table 1). These soils were distinguished by a strong acid reaction, an acidic and weak acid reaction (surface horizons), a neutral and alkaline reaction (parent material horizons), a significantly smaller cation exchangeable capacity of the surface horizons than deeper layers, and accumulation of organic carbon compounds in humus horizons and particle size distribution characteristic of boulder loam (sandy loam, sandy clay loam, loam, clay loam, often sandy in the surface layers of the profile). Acidification of the surface soil horizons mainly is due to the fact that they were

composed of more sandy soil material with low sorption capacity, and more susceptible to the leaching process of a basic component. These cations in agricultural soils also can be downloaded by cultivated plants. The high pH of deeper horizons deposited in the soil profile, especially parent material, were primarily related to the presence of carbonates [11].

The different content of copper was measured in the analyzed soils between individual soil profiles and within each profile, and the average copper content was $7.35 \text{ mg}\cdot\text{kg}^{-1}$ in Eutric Cambisols, $7.20 \text{ mg}\cdot\text{kg}^{-1}$ in Stagnic Luvisols, and $6.00 \text{ mg}\cdot\text{kg}^{-1}$ in Haplic Luvisols. The highest content of this metal regardless of soil subtype was obtained in the enrichment horizons B: $5.91\text{-}10.7 \text{ mg}\cdot\text{kg}^{-1}$, whereas the lowest content was found in the surface layers Ap: $3.53\text{-}6.09 \text{ mg}\cdot\text{kg}^{-1}$ (Table 1). In the analyzed soils the copper content did not exceed the geochemical values for boulder loam ($2.5\text{-}19.8 \text{ mg}\cdot\text{kg}^{-1}$), and was within the natural content range [6, 18]. Low total Cu content in the soils resulted mainly from a small abundance of the parent material and low cation exchangeable capacity and acidity of surface horizons. Under such conditions, copper compounds are easily soluble and may migrate along soil solution inside the soil profiles, and also taken faster up by plants [11]. Total copper contents increased with the duration of agricultural use and the applied Cu persists in the upper soils because it seems to be strongly fixed by organic matter, clay minerals, and oxides of Fe and Mn [13].

A similar total content and diversity profile distribution of copper in cultivated Cambisols and Luvisols was reported by Kalembasa and Majchrowska-Safaryan [19], Dąbkowska-Naskręt and Róžański [20], and Wójcikowska-Kapusta and Niemczuk [21]. Kabata-Pendias and Pendias [1], Kalembasa and Pakuła [22], and Kobierski et al. [23] emphasize that the presence of copper in soil is related to its content in the parent material, and the distribution of this element in Cambisol and Luvisol profiles of different functionality indicates the significant impact of natural (soil-forming processes) and human-dependent (agrotechnology) factors.

The sequential chemical analysis demonstrated diverse copper distribution in its total content, in sequenced fractions and in separate genetic profiles of investigated soils (Table 2). The average percentage share of Cu fraction in its total content was arranged in the following decreasing orders: for Eutric Cambisols: F4 (69.6) > F3 (20.5) > F2 (7.90) > F1 (2.00); for Haplic Luvisols: F4 (49.1) > F2 (25.7) > F3 (23.0) > F1 (2.20); and for Stagnic Luvisols: F4 (54.5) > F2 (26.7) > F3 (16.4) > F1 (2.40). A similar order in soils formed with boulder loam material was reported by Wójcikowska-Kapusta and Niemczuk [21], Kalembasa and Pakuła [22], and Zemberyova et al. [24]. The distribution of copper to different fractions is the result of various soil processes and the equilibrium of these processes, which depend on soil physical-chemical conditions and crop cultivation [1, 2].

The lowest percentage share of copper in the analyzed soils occurred in the exchangeable fraction (F1): 0.61-3.02% in Eutric Cambisols; 0.41-4.60% in Haplic Luvisols;

Table 1. Some properties (mean[#] and ranges[#]) of investigated soils in the Siedlce Upland.

Genetic horizon (Mean depth in cm)		Clay fraction ø < 0.002 mm %	pH _{KCl}	CEC** mmol(+):kg ⁻¹	C _{org} g:kg ⁻¹	Cu _t *** mg:kg ⁻¹
SgP* (1989)	SgP (2011)					
Eutric Cambisols**** (4 profiles)						
Ap (0-30)	Ap	11.5 [#] 11-12 [#]	5.56 5.11-6.34	66.4 43.9-80.0	8.71 7.79-10.3	5.39 4.00-5.97
Bbr(tfe) (30-55)	Bw1	36.3 33-41	5.36 4.78-5.97	167.8 114-199	3.31 2.45-4.25	8.76 6.74-10.7
Bbr (55-100)	Bw2	31.0 24-35	5.76 4.90-6.39	172.3 127-211	2.00 1.38-3.17	8.68 7.62-10.2
Cca (100-150)	Ck	23.2 21-27	7.34 7.02-7.75	196.9 176-247	0.88 0.70-1.33	6.57 5.21-7.76
Mean for subtype of soil		25.5	5.65	150.9	3.73	7.35
Haplic Luvisols**** (3 profiles)						
Ap (0-30)	Ap	8.0 7-9	5.10 4.58-5.68	70.1 57.6-79.4	9.01 7.40-10.4	4.29 3.53-4.83
EB (30-45)	EB	20.0 18-23	5.44 4.60-6.20	89.6 65.4-119	2.93 1.70-3.19	5.01 4.22-6.18
Bt (45-95)	Bt	27.0 26-28	5.78 4.88-6.52	165.1 156-173	1.49 0.90-2.20	8.85 5.91-10.2
Cca (95-150)	Ck	21.4 19-23	7.44 7.08-7.96	180.8 175-187	0.99 0.70-1.30	5.85 3.93-7.49
Mean for subtype of soil		19.1	5.46	126.4	3.61	6.00
Stagnic Luvisols**** (4 profiles)						
Ap (0-25)	Ap	9.0 7-10	4.55 4.11-5.19	56.0 32.1-77.0	9.68 6.05-16.6	5.64 5.03-6.09
EB (25-40)	EB	13.8 11-16	4.60 4.55-5.43	87.5 50.7-137	2.00 1.30-2.75	6.62 5.39-7.72
Btg (40-100)	Btg	22.8 20-28	5.20 4.59-6.31	147.1 136-151	1.37 0.70-1.60	9.42 8.25-10.0
Cca (100-150)	Ck	18.9 15-24	7.15 6.51-7.60	151.6 125-192	0.86 0.50-1.12	7.12 6.20-8.13
Mean for subtype of soil		16.1	4.80	110.6	3.48	7.20

*Polish Soil Classification System [31, 32]; **cation exchangeable capacity; ***Cu_t – total content of copper; ****IUSS Working Group WRB [33]; mean pH values were calculated after conversion of pH on hydrogen ion concentration

and 0.30-5.94% in Stagnic Luvisols. The highest Cu concentrations in F1 fraction was measured in the Ap horizon (2.97-5.94%), with the lowest cation exchangeable capacity CEC (32.1-80.0 mmol(+):kg⁻¹), whereas the lowest was detected in the parent material horizon (0.30-1.26%), where CEC reached the maximal values for the analyzed soil profiles (175-247 mmol(+):kg⁻¹) (Tables 1 and 2). The percentage share of Cu in F1 fraction decreased considerably with

an increase in soil pH and clay mineral fraction (Ø < 0.002 mm). The mobility of copper in soil is strongly pH-dependent [1, 2, 25]. The acid-extractable fractions were most important in controlling Cu mobility and availability for plant uptake in soil, and the soluble organic matter could increase Cu availability [26]. Maderova et al. [12] reported that the mobility of exchangeable Cu decreased with increasing C_{org} content.

Table 2. The percentage contribution of copper fractions (mean[#] and ranges[#]) in the investigated soils of the Siedlce Upland.

Genetic horizon (Mean depth in cm)		Fraction			
SgP* (1989)	SgP (2011)	F1	F2	F3	F4
Eutric Cambisols** (4 profiles)					
Ap (0-30)	Ap	3.00 [#] 2.97-3.02 [#]	10.6 6.57-14.3	35.7 31.1-40.8	50.7 45.8-59.3
Bbr(tfe) (30-55)	Bw1	2.20 1.67-2.82	9.80 5.13-13.5	24.1 21.3-29.1	63.8 58.1-70.5
Bbr (55-100)	Bw2	2.00 1.50-2.50	6.80 4.83-8.08	18.7 20.6-26.0	72.5 64.7-77.8
Cca (100-150)	Ck	0.70 0.61-0.88	4.40 2.51-6.50	3.70 2.10-5.57	91.2 88.5-94.3
Mean for subtype of soil		2.00	7.90	20.5	69.6
Haplic Luvisols** (3 profiles)					
Ap (0-30)	Ap	4.00 3.55-4.60	20.1 14.7-24.1	45.8 41.1-53.3	30.1 27.4-33.6
EB (30-45)	EB	2.70 1.90-3.50	24.3 20.0-33.6	24.2 21.9-25.8	48.8 43.2-51.3
Bt (45-95)	Bt	1.10 0.80-1.60	27.8 21.7-33.9	17.3 14.1-19.3	53.3 46.8-62.6
Cca (95-150)	Ck	0.80 0.41-1.19	30.7 29.5-32.0	4.80 3.43-6.65	63.8 59.4-66.2
Mean for subtype of soil		2.20	25.7	23.0	49.1
Stagnic Luvisols** (4 profiles)					
Ap (0-25)	Ap	5.00 3.77-5.94	18.6 16.9-22.3	31.4 28.6-35.4	45.0 37.7-49.7
EB (25-40)	EB	3.10 2.16-4.50	26.7 22.4-29.4	21.1 18.0-25.6	49.1 40.5-52.5
Btg (40-100)	Btg	1.10 0.47-1.77	30.7 24.9-36.4	10.3 7.23-12.5	57.9 53.7-67.4
Cca (100-150)	Ck	0.50 0.30-1.26	31.0 25.2-36.7	2.30 1.37-3.09	66.2 60.8-71.4
Mean for subtype of soil		2.40	26.7	16.4	54.5

*Polish Soil Classification System [31, 32]; **IUSS Working Group WRB [33]; Fraction: F1- exchangeable, F2 – reducible (bound to Fe-Mn oxides), F3 – oxidizable (bound to organic matter), F4 – residual

The high content of copper in the easily soluble and bioavailable F1 fraction in low pH soil may be toxic to plants and animals [1, 11, 12]. Several studies have shown a very low content of Cu in most labile fractions, and especially in unpolluted soils [22, 25], but Kabała and Singh [8], Guan et al. [26], and Hu et al. [27] report that with an escalation in environmental pollution, the content of copper in the exchangeable fraction may increase to 45-62% of its

total content. Yu et al. [28] report that water-soluble Cu added to soils is rapidly retained by soil colloids, as mobile fractions initially, and transformed into stable fractions in time. This transformation decreased with increasing Cu contamination levels.

The highest concentration of copper in the analyzed soils was detected in the residual fraction (F4): 45.8-94.3% in Eutric Cambisols, 37.7-71.4% in Stagnic Luvisols, and

27.4-66.2% in Haplic Luvisols, and it increased with depth in soil profiles. The highest percentage share of this metal in F4 fraction was determined in the enrichment horizons (Bbr, Bt): 46.8-77.8% and in the parent material horizon (Cca): 59.4-94.3% (Table 2), with the highest values of soil pH, CEC, and clay content. Zemberyova et al. [24] report that in the surface layers of cultivated soils, the residual fraction of this metal constituted from 71.8% (Cambisols) to 81.2% (Luvisols). Guan et al. [26] and Graf et al. [29] found in the soils to a depth of 0-20 cm, the residual fractions ranged from 7.8 to 69.0%. Schramel et al. [13] found most of the copper in the residual phase: 65-85% for the uncontaminated and 40-50% for the contaminated arable soils. Kabala and Singh [8] report that in subsurface horizons of unpolluted soils this fraction ranged from 88.0 to 97%, like a result of the high CEC and clay content. The residual fraction was not expected to be solubilized over a reasonable period of time under natural conditions. The copper in the minerals and in the stable organic complexes is unavailable for uptake by plants. It is for this reason that mineral soils with high contents of organic matter are the most prone to copper deficiency [2]. In the investigated soils, the concentrations of Cu in residual fraction, negatively associated with oxidizable ones, were higher in Cambisols ($r=-0.435$; $p < 0.01$) than in Luvisols ($r=-0.316$; $p < 0.01$).

The highest concentration of copper in the reducible fraction (F2) was detected in the humus horizon (average 10.6%) of Eutric Cambisols and it decreased with depth. In Luvisols, the highest content of F2 fraction was measured in the enrichment horizons (average 27.8-30.7% Cu) and in the parent material horizons (average 30.7-31.0% Cu) (Table 2). The profile distribution of copper in the F2 fraction resulted from specific soil-formation processes, soil texture, and changing red-ox conditions in the soil profile [1]. Copper associations with Fe and Mn oxides in the arable soils indicate the influence of agricultural activities on the soils [27]. The reducible fractions increased most rapidly with the increase of Cu addition to soil [26] and increased with progressing soil development [29]. Maderova et al. [12], Wójcikowska-Kapusta and Niemczuk [21], Kalembasa and Pakuła [22], and Graf et al. [29] reported a similar correlation in the cultivated soils. Zemberyova et al. [24] found in arable Cambisol and Luvisol 7-19% of total copper content in reducible fraction, and Lua et al. [9] found an average 5.4% in the A horizons of non-urban soils. The Fe-Mn oxides-bound fraction was unstable under low Eh conditions and appeared to control mobility and bioavailability of anthropogenic Cu, and had potential to impact plant growth [9, 28]. Quantities ranging from 15 to 30% could be extracted from the iron oxyhydroxides and oxides, showing slightly higher amounts for the uncontaminated soils [13].

The highest Cu percentage share in the oxidizable fraction (F3) was detected in Haplic Luvisols and the lowest in Stagnic Luvisols, and the highest – in the humus horizons (Ap) 28.6-53.3%, and the lowest – in the parent material horizons (Cca) 1.37-6.65%, which reflected the distribution of organic carbon compounds (C_{org}) and varied pH in the

profiles of analyzed soils (Table 2). Kaasalainen and Yli-Halla [30] report that the concentrations of Cu oxidizable fraction were substantially higher in the A horizons than in the deeper horizons, while the concentrations in Cambisols were less compared to Luvisols, irrespective of the loading and the concentration of organic matter. Guan et al. [26], Hu et al. [27], and Graf et al. [29] emphasize the major role of organic matter and very durable organic-mineral bounds and mineral bounds in the circulation of copper in the soil. Copper is more readily adsorbed and the most strongly complexed by organic matter. These authors reported that the content of copper in the oxidizable (organic) fraction in the cultivated soils ranges from 3.86 to 53.4% and from 22.8 to 97.0% in the residual fraction, whereas copper in the oxidizable fraction dominates in the humus horizons. This strong sorption of copper to associate with the crystalline structures of the minerals and the organic ligand by most soils results in very restricted mobility of the element [2, 13]. Alva et al. [11] reported the proportion of the organic fraction of Cu decreased along with an increase in that of the precipitate form in the higher pH soil. Fernandez-Calvino et al. [25] suggests that a pH rise in soil containing naturally occurring copper has a more marked effect on the stability of organically bound copper than does a pH fall.

The oxidizable fraction is a relatively stable fraction, which could be transferred to mobile fractions as a result of its dissolution, and the risk of extracted reagent (CH_3COOH) attacking the oxide bound in the first-step BCR sequential procedure because the $-COO$ functional group of the acetate anion has a strong affinity for Cu^{2+} [28]. In the investigated soils, the concentrations of Cu in oxidizable fraction, strongly positively associated with exchangeable ones, were higher in Cambisols ($r=0.912$; $p < 0.01$) than in Luvisols ($r=0.773$; $p < 0.01$), showing that this accumulated metal is found in this fraction as well. Wójcikowska-Kapusta and Niemczuk [21] report that the concentration copper in F3 fraction confirmed strong Cu affinity to form complexes with functional groups of soil organic matter, which effects the mobility and thus the availability of the element in the soil [1].

Based on the single-factor variance ANOVA analysis, statistically significant differences were found between the average copper content in the sequentially extracted fractions (exchangeable F1, reducible F2, oxidizable F3, residual F4) in the separate genetic horizons (Ap, EB, Bbr, Bt, Cca) in the analyzed Cambisols and Luvisols (Fig. 2).

The statistical calculations (Table 3) showed the significant impact of organic carbon compounds (C_{org}) on the content of copper in the exchangeable fraction F1 and the oxidizable fraction F3 (positive), and the residual fraction F4 (negative), strongly in Luvisols. A highly significant negative correlation was observed between the pH of soil and the F1, F2, and F3 fractions, whereas a positive correlation was found with F4 fraction of this metal, strongly in Cambisols. In the analyzed soils, the content of the F2 and F4 fractions was significantly positively correlated with total copper content (C_u) and the content of clay fraction ($\phi < 0.002$ mm), more strongly in Luvisols than Cambisols. The values of cation exchangeable capacity (CEC) were significantly cor-

Table 3. The coefficients of correlation between the copper fractions and some properties of investigated soils on the Siedlce Upland.

Parameter	Fraction				Cu _t
	F1	F2	F3	F4	
Cambisols					
Cu _t	0.539*	0.625**	0.277	0.813**	-
pH _{KCl}	-0.659**	-0.673**	-0.882**	0.572**	0.034
CEC	-0.332	-0.238	0.556**	0.770**	0.466*
C _{org}	0.612**	0.285	0.580**	-0.757**	-0.413*
ø < 0.002 mm	0.293	0.483*	0.172	0.562**	0.707**
Luvisols					
Cu _t	0.413*	0.878**	-0.012	0.890**	1
pH _{KCl}	-0.634**	-0.617**	-0.733**	0.445**	-0.032
CEC	-0.742**	0.642**	0.617**	0.629**	0.504**
C _{org}	0.696**	-0.287	0.613**	-0.576**	-0.416*
ø < 0.002 mm	-0.254	0.655**	-0.318	0.649**	0.606**

Significant at: * $p < 0.05$ and ** $p < 0.01$;

Fraction: F1 – exchangeable, F2 – reducible (bound to Fe-Mn oxide), F3 – oxidizable (bound to organic matter); F4 – residual, Cu_t – total content of copper, CEC – cation exchangeable capacity, ø < 0.002 mm – clay fraction

related with the F3 and F4 copper fraction and F1 and F2 in Luvisols only. In the investigated soils we found the positive impact of CEC and clay fraction, also C_{org} content (but negatively) on the copper content in aqua regia extract. This is consistent with the findings of Alva et al. [11] and Graf et al. [29], and underlines the importance of soil pH and soil organic matter for the sorption of Cu in soils.

A correlation analysis revealed the statistically significant (at $p < 0.01$) impact of C_{org} on F3 fraction ($r=0.810$) in the humus horizons (Ap); the impact of clay fraction on Cu content in F2 fraction in Ap horizons ($r=0.639$), and the parent material Cca ($r=0.579$) and in F4 fraction in Cca ($r=0.692$). In addition, a significant correlation (at $p < 0.05$) was detected between CEC value and the content of copper in F4 fraction in the Bt enrichment horizons ($r=0.550$) and the parent material ($r=0.652$), as well as between soil pH and the content of this metal in the F1 fraction ($r=-0.746$) and the F2 fraction ($r=-0.724$) in EB horizons, and in the F4 fraction ($r=0.703$) in the Bbr enrichment horizons of the analyzed soils.

Schramel et al. [13], Wójcikowska-Kapusta and Niemczuk [21], Kalembasa and Pakuła [22], Fernandez-Calvino et al. [25] report the significant impact of total copper content, organic carbon compounds, soil pH, cation exchangeable capacity, and clay fraction on copper fractions in the soil.

Alva et al. [11] found in cultivated soils correlation between the exchangeable (negatively) and residual (positively) copper forms and soil pH. Maderova et al. [12] observed in sludge-amended soils no impact of soil pH on Cu mobility, significant availability of Cu related to total

soil copper, and reducible form of this metal with soil organic matter. Lua et al. [9] reported that in non-urban soils significant positive correlation between C_{org} and Cu concentrations suggests that this metal has a strong affinity with organic matter and the contents of this metal are inversely correlated with clay content. These probably result in different distributions of Cu in particle size fractions. Total Cu concentration in cultivated soils was negatively correlated with the residual fraction and positively correlated with F2 and F3 fractions, and the organic carbon compounds and clay fraction was positively correlated with the F3 and F4 fractions [9, 30].

Conclusions

1. In the examined Cambisols and Luvisols in the Siedlce upland region, the varied natural, not contaminated levels of total copper was determined and the distribution of this metal in the soil profile was influenced by pedogenesis and soil properties.
2. The low total content of copper, the highest concentration of this metal in the stable residual fraction, and the lowest – in the exchangeable, bioavailable forms indicated deficit of this element for plants. Taking into account normal growth of crops, it is advisable to supplement copper deficiency by fertilization.
3. A statistical analysis revealed the significant role of selected soil properties (soil pH, C_{org}, CEC, the content of Cu and clay fraction) on relations between copper mobility and stability fractions in examined soils.

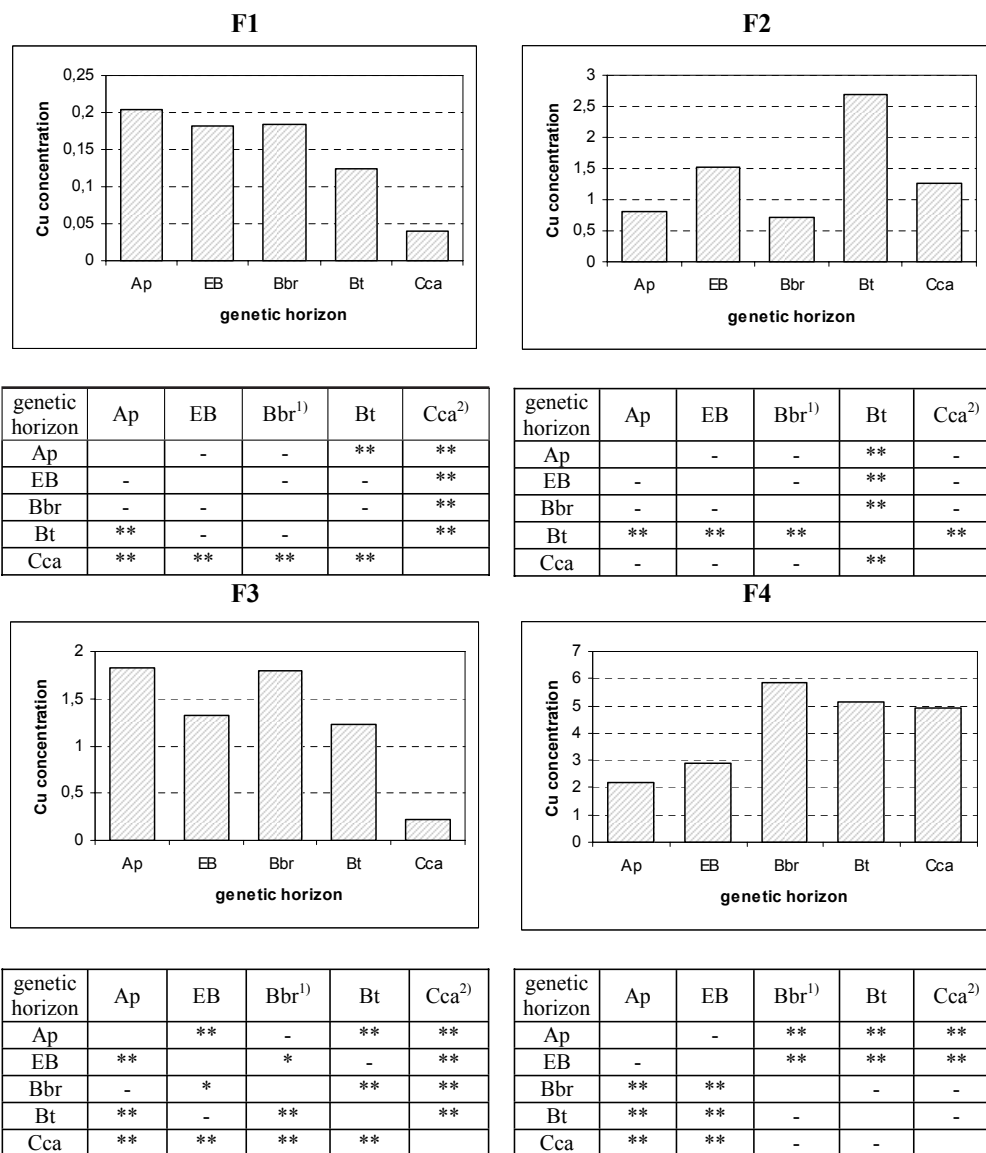


Fig. 2. The statistical significance of differences between the average contents of Cu in the fractions separated in different genetic horizons of the investigated soils.

**significant at 0.01 probability level; *significant at 0.05 probability level; - insignificant; Fraction: F1 – exchangeable, F2 – reducible (bound to Fe-Mn oxide), F3 – oxidizable (bound to organic matter), F4 – residual; ¹⁾Bbr = Bw; ²⁾Cca = Ck; Cu concentration in mg·kg⁻¹

4. The highest copper concentration in the F1 and F3 fractions was detected in the humus horizons of the examined soils, whereas it in the F4 fraction in the enrichment horizons and the parent material. The highest Cu percentage share in the F2 fraction was measured in the humus horizon of Cambisols and in the enrichment horizons and in the parent material horizons of Luvisols.

References

- KABATA-PENDIAS A., PENDIAS H. Biogeochemistry of trace elements. PWN, Warszawa, pp 398, 1999 [In Polish].
- ADRIANO D.C. Trace Elements in Terrestrial Environments. Springer-Verlag, New York/Berlin/Heidelberg, pp. 867, 2001.
- SMOLDERS E., OORTS K., LOMBI E., SCHOETERS I., MA Y., ZRNA S., MCLAUGHLIN M. J. The Availability of Copper in Soils Historically Amended with Sewage Sludge, Manure, and Compost. *J. Environ. Qual.*, **41**, 506, 2012.
- SPIAK Z. Rules for determining the needs of micronutrient fertilization of crops in sustainable agriculture. [In:] Kalembasa S. (Eds), *Diagnosis of soil and plants in sustainable agriculture*, Wyd. Akademii Podlaskiej w Siedlcach, **54**, 82, 2004 [In Polish].
- TERELAK H., STUCZYŃSKI T., PIOTROWSKA M. Heavy metals in agricultural soils in Poland. *Polish J. Soil Sci.*, **30**, 2, 35, 1997.
- KABATA-PENDIAS A., PIOTROWSKA M., MOTOWICKA-TERELAK H., MALISZEWSKA-KORDYBACH B., FILIPEK K., KRAKOWIAK A., PIETRZAK C. Basis of chemical evaluation of soil contamination. *Bibliot. Monit. Środ.*, Wyd. IOŚ Warszawa, pp. 41, 1995 [In Polish].

7. THE DECREE OF THE MINISTER OF THE ENVIRONMENT of 9 September 2002 on Soil Quality Standards, Journal of Laws of the Republic of Poland, No. 165, Item 1359, **2002**.
8. KABAŁA C., SINGH B.R. Fractionation and mobility of copper, lead and zinc in soil profiles in the vicinity of a copper smelter. *J. Environ. Qual.*, **30**, 485, **2001**.
9. LUA Y., GONGA Z., ZHANGA G., BURGHARDT W. Concentrations and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China. *Geoderma*, **115**, 101, **2003**.
10. KARCZEWSKA A., BOGDAA., GAŁKA B., KRAJEWSKI J. Assessment of environmental hazards in the vicinity of polymetallic ore deposits Żeleźniak (Wojcieszów-Kaczawskie Mts, SW Poland). *Wyd. Akademii Rolniczej we Wrocławiu*, pp. 262, **2005** [In Polish].
11. ALVA A. K., HUANG B., PARAMASIVAM S. Soil pH effects copper fractionation and phytotoxicity. *Soil Sci. Soc. Am. J.*, **64**, 955, **2000**.
12. MADEROVA L., WATSON M., PATON G.I. Bioavailability and toxicity of copper in soils: Integrating chemical approaches with responses of microbial biosensors. *Soil Biol. Biochem.*, **43**, 6, 1162, **2011**.
13. SCHRAMEL O., MICHALKE B., KETTRUP A. Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. *Sci. Total Environ.*, **263**, 11, **2000**.
14. URE A. M., QUEVAUVULLER PH., MUNTAU H., GRIEPINK B. Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under auspices of the BCR of Commission of The European Communities. *Intern. J. Environ. Anal. Chem.*, **51**, 135, **1993**.
15. RAURET G., LÓPEZ-SÁNCHEZ J.F., SAHUQUILLO A., RUGIO R., DAVIDSON C., URE A., QUEVAUILLER PH. Improvement of the BCR three step sequential extraction procedure priori to the certification of new sediment and soil reference materials. *J. Environ. Monit.*, **1**, 57, **1999**.
16. POLISH SOCIETY OF SOIL SCIENCE. Particle size distribution and textural classes of soils and mineral materials - classification of Polish Society of Soil Sciences 2008. *Rocz. Glebozn.*, **60**, 2, 5, **2009** [In Polish].
17. KALEMBASA S., KALEMBASA D. The quick method for the determination of C:N ratio in mineral soils. *Polish J. Soil Sci.* **25**, 1, 41, **1992**.
18. CZARNOWSKA K. The total content of heavy metals in parent rocks as geochemical background of soil. *Rocz. Glebozn.*, **47**, supl., 43, **1996** [In Polish].
19. KALEMBASA D., MAJCHROWSKA-SAFARYAN A. Soil degradation on the morainic slope of Siedlce high plain. *Zesz. Probl. Post. Nauk Rol.*, **520**, 83, **2007** [In Polish].
20. DĄBKOWSKA-NASKRĘT H., RÓŻAŃSKI S. Total content and fitoavailability of Zn, Cu, Mn and Fe in selected arable soils of Gniezno lake district. *Zesz. Probl. Post. Nauk Rol.*, **541**, 97, **2009** [In Polish].
21. WÓJCIKOWSKA-KAPUSTA A., NIEMCZUK B. Copper speciation in different-type soil profiles. *J. Elementol.*, **14**, 4, 815, **2009**.
22. KALEMBASA D., PAKUŁA K. Heavy metals fractions in soils fertilized with sewage sludge. *Environment Protection Engineering*, **35**, 2, 157, **2009**.
23. KOBIERSKI M., KOBIERSKA H., DĄBKOWSKA-NASKRĘT H. Distribution of Zn and Cu in profiles of arable Luvisols of The Chełmno-Dobrzyn Lake district. *Ochr. Środ. i Zasob. Natur.*, **31**, 33, **2007**.
24. ZEMBERYOVA M., BARTEKOVA J., HAGAROVA I. The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. *Talanta*, **70**, 973, **2006**.
25. FERNANDEZ-CALVINO D., PEREZ-NOVO C., NOVOA-MUNOZ J.C., ARIAS-ESTEVEZ M. Copper fractionation and release from soils devoted to different crops. *J. Hazard. Mater.*, **167**, 797, **2009**.
26. GUAN T.X., HE H.B., ZHANG X.D., BAI Z. Cu fractions, mobility and bioavailability in soil-wheat system after Cu-enriched livestock manure applications. *Chemosphere*, **82**, 215, **2011**.
27. HU N., LI Z., HUANG P., TAO C. Distribution and mobility of metals in agricultural soils near a copper smelter in South China. *Environ. Geochem. Health*, **28**, 19, **2006**.
28. YU S., HE Z.L., HUANG C.Y., CHEN G.C., CALVERT D.V. Copper fraction and extractability in two contaminated variable charge soils. *Geoderma*, **123**, 163, **2004**.
29. GRAF M., LAIR G.J., ZEHETNER F., GERZABEK M.H. Geochemical fractions of copper in soil chronosequences of selected European floodplains. *Environ. Pollut.*, **148**, 788, **2007**.
30. KAASALAINEN M., YLI-HALLA M. Use of sequential extraction to assess metal partitioning in soils. *Environ. Pollut.*, **126**, 225, **2003**.
31. POLISH SOIL CLASSIFICATION SYSTEM. *Rocz. Glebozn.*, **62**, (3), 193, **2011** [In Polish].
32. POLISH SOIL CLASSIFICATION SYSTEM. *Rocz. Glebozn.*, **42**, (3-4), 150, **1989** [In Polish].
33. IUSS WORKING GROUP WRB. World reference base for soil resources. 2nd edition. *World Soil Resources Reports*, No. 103, FAO, Rome, pp. 132, **2006**.

