Effects of selected chemical and physicochemical properties of humic acids from peat soils on their interaction mechanisms with copper ions at various pHs

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ABSTRACT

The main aims of this study were to investigate the interactions of copper ions (Cu(II)) at pH 5 and 7 with humic acids (HAs) isolated from four peat soils at different humification stages, as well as to determine the physico-chemical properties of HAs that may have an influence on the above interactions. For this purpose, a number of parameters were determined for the qualitative and quantitative analysis of peat soils, HAs, and HA-Cu(II) interactions. Processes in the HA-Cu(II) systems were studied using fluorescence spectroscopy (for chemical complexation) and atomic absorption spectrometry with carbon measurements (for coagulation processes). The influence of HA properties on their interactions with Cu(II) ions was evaluated using matrices of correlation coefficients between some HA properties and parameters describing the HA-Cu(II) interactions. Results showed that the complexation capacity was higher at pH 7 than at pH 5. The coagulation mechanism at pH 7 appeared to be based on the precipitation of Cu(II)-humates following the neutralization of HA functional groups by Cu(II) ions, while coagulation at pH 5 appeared to be controlled by the ionic strength. The complexation capacity increased significantly with increasing content of carboxylic and phenolic groups, O/H atomic ratio, degree of internal oxidation and surface negative charge of HAs. The binding of Cu(II) ions was stronger for HAs characterized by a higher aromaticity and humification degree (expressed respectively as absorbance at 280 nm and E4/E6 ratio). The stability constants were higher at pH 7 than at pH 5 and in most cases they did not correlate significantly with HA properties.

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1. Introduction

Humic acids (HAs) are among the most important components of soil in terms of determining its agricultural and environmental properties (Senesi and Loffredo, 1999). In particular, HAs show a high sorption capacity for several cations relevant to agriculture and the environment (Senesi, 1992; Senesi and Loffredo, 2008). Thus, HAs are among the main factors responsible for the concentration, form, mobility and toxicity of metal cations in soils. However, HAs possess a limited sorption capacity, i.e., an excessive dose of metal sorbed on HAs can cause the saturation of HA negative surface charges thus causing their coagulation. As a consequence, the risk arises of metal accumulation in soil precipitated as HA insoluble salts, which may lead to soil pollution and/or micronutrient deficiency for plants.

Copper (Cu) is one of the micronutrients necessary for plant growth, but in too high amounts it becomes a toxic metal. The presence of this metal is especially important in organic soils, where the amount of

HAs is high. Thus together with the low pH typical of these soils, the HA content and state can markedly influence the behavior of Cu (Boguta and Sokołowska, 2013a, 2013b). Furthermore, the ascertained significant variability of physicochemical properties of organic soils is known to affect the properties of their HAs (Boguta and Sokołowska, 2014), which, in turn, is expected to affect the mechanisms of interaction between HAs and Cu(II) ions.

Although the interactions between soil HAs and Cu(II) ions have been studied in the past (Fuentes et al., 2013; Garcia-Mina, 2006; Jerzykiewicz et al., 2002; Senesi, 1992), there are still several contradictory opinions concerning the interaction mechanisms and the influence of HA properties on these interactions. In general, it has been reported that the carboxylic groups of humic acids are mainly responsible for the binding processes with metal ions (Jeong et al., 2007; Rahman et al., 2010). However, other results also indicate both the carboxylic and phenolic functional groups of humic acids are capable of forming complexes (Christl and Kretzschmar, 2007; Christl, 2012; Erdogan et al., 2007; Pehlivan and Arslan, 2006); as well as the possibility of binding metal ions to HA particles by other donor atoms, e.g., nitrogen (Jimoh, 2011; Silva and Oliveira, 2002). Few researchers have studied copper

ion interactions taking into account the complex physicochemical nature of HAs, i.e., their variable composition of carbon, oxygen, hydrogen, nitrogen and other elements as well as their varying degree of structural transformation (i.e., their humification degree, aliphatic and aromatic structures, large variety of functional groups etc.). Moreover, most of the previous studies were based rather on the assumption of only a theoretical, model 1:1 ratio between metal ions and functional groups of humic acids (the simplest model; see Stevenson, 1994; Tipping, 2002). It should also be stressed that there is not much information in the literature about attempts of relating the mechanisms between humic acids and metals to the simple parameters describing humic acids only. Knowledge of such correlations would be helpful to establish a reasonable dosage of copper in soils rich in organic matter.

Taking into account the above arguments, the main aims of this paper are to study the interactions of selected HAs isolated from peat soils at different humification stages with Cu(II) ions at various concentrations (typical in the environment) at pH 5 and 7, and to discover the physicochemical properties of HAs that may have a statistically significant influence on the mechanisms of HA-Cu(II) interactions. A wide range of metal concentrations was chosen, which afforded a comprehensive approach to the problem of the various copper interactions with humic acids, starting from soluble complex formation to the processes of coagulation and flocculation.

2. Materials and methods

2.1. Soils

Four peaty-muck soils (S1 to S4) were collected from various locations at Polesie Lubelskie and the valley of the Biebrza River (East Poland). According to the Okruszko classification (Okruszko, 1976), sample S1 is a peaty muck (Z_1) and the other three soils (S2, S3, S4) are proper mucks (Z_3). Soil S1 at the early mucking stage (MtI) was sampled at depth of 5–10 cm and soils S2, S3 and S4 at a medium mucking stage (MtII) at a depth of 5–20 cm. As the mucking processes of these soils took place in the same climatic zone and under similar humidity and land-forming conditions, the studied samples can be assumed to be distinguished mostly by secondary transformations (Szajdak and Styla, 2010). These can be evaluated by the water absorption index (W_1) determined according to the method of Gawlik (1992), and the stage of the humification processes evaluated by the Springer humification number (H_z). Determination of the H_z index is based on spectrophotometric measurement of humic substance content in an extract obtained after boiling a soil sample in a mixture of 0.5% sodium hydroxide with 0.5% sodium oxalate; absorbance of the extract is recorded at 530 nm, then the H_z degree is calculated using calibration based on HAs standards (Schlichting et al., 1995).

Soil samples were analyzed for a number of properties including: pH, measured in H_2O and 1 M KCl water solution using a digital pH-meter (Radiometer, Copenhagen); total porosity, measured by mercury porosimetry (Autopore IV 9500); bulk density, determined by the pycnometric method; and ash content, calculated by weighing the residue after combustion at 550 °C in a muffle furnace (FCF 12 SP, Czylok). Hydrophobic/hydrophilic properties were evaluated by measuring the contact angles (Θ) (Dang-Vu et al., 2009) using a goniometer apparatus (DSA1, Kruss). The total soil carbon (TC) and organic carbon (TOC) were determined using a C/N analyzer (TOC MULTI N/C 2000, HT 1300, Analytik Jena).

2.2. Humic acids

The extraction of humic acids (HAs) from soil samples was carried out according to a slight modification of the procedure recommended by the International Humic Substances Society (Swift, 1996). Briefly, each air-dried and 2-mm-sieved soil sample was equilibrated at pH 1.0 with 0.1 M HCl at a ratio of 10 cm³ HCl/1 g soil. The suspension

was shaken for 1 h and then the supernatant was separated from the residue by centrifugation at 8000 g for 20 min and discarded. The soil residue was then extracted overnight with 0.1 M NaOH under an N_2 atmosphere and mechanical stirring at a soil:extractant ratio of 1:10. The supernatant was then separated from the residue by centrifugation at 8000 g for 20 min and acidified to pH 1.0 with 6 M HCl. After 12 h the precipitated HAs were centrifuged at 8000 g for 20 min and redissolved by adding a minimum volume of 0.1 M KOH under N_2. Solid KCl was added to attain a concentration of 0.3 M, and then the suspension was centrifuged at 8000 g for 20 min to remove the suspended solids. The HAs were reprecipitated at pH 1.0 by adding 6 M HCl and the suspension was centrifuged at 8000 g for 20 min after 12 h. The HAs were then purified with a 0.1 M HCl/0.3 M HF solution by mechanical shaking overnight. The procedure was repeated until the ash content was set below 1%. The HAs were then washed with distilled water until a negative Cl⁻ test was obtained, and finally lyophilised.

The ash content was determined as the residue obtained after heating a HA sample for 4 h at 550 °C. The elemental (C, H, N) composition was measured by a Perkin-Elmer CHN 2400 analyzer. The oxygen content was calculated by difference: O% = 100% − (H% + C% + N%). The atomic ratios H/C, O/H, O/C, C/N were calculated, as well as the degree of internal oxidation, by Zdanow's formula: $\omega = [(2O + 3N) - H] / C$ (Dębska et al., 2012). Carboxylic groups (COOH), and the sum of carboxylic and phenolic hydroxyl groups (COOH + OH) were determined according to Dragunowa and Kucharenko (Kononowa, 1968). The E_4/E_6 ratio was calculated as the ratio of absorbances at 465 and 665 nm of HA solutions (40 mg dm⁻¹, pH = 8) (Chen et al., 1977). The Kumada parameter (Δlogk) was calculated as the difference of the decimal logarithm of absorbances at 400 and 600 nm: Δlogk = $logA_{400} - logA_{600}$ (Kumada, 1987). Absorbance at λ = 280 nm was measured according to Traina et al. (1990). The surface charges at pH 5 and 7 (Q5 and Q7) were calculated on the basis of potentiometric titration curves recorded by an automatic titrator (Titrino 702 SM, Metrohm AG) (Matyka-Sarzyńska et al., 2000).

2.3. Interactions of humic acids with copper(II) ions

For complexation studies, three-dimensional fluorescence spectra in the form of excitation-emission matrices (EEM) were recorded for HA solutions to which Cu(II) ions were added at concentrations ranging from 0 to 10 mg dm⁻³ and adjusted to pH 5 and pH 7 with HCl or NaOH solutions. A PerkinElmer (Norwalk, CT) LS 55 luminescence spectrometer was used, setting emission and excitation slits at a 5 nm bandwidth and selecting a scan speed of 1200 nm min⁻¹ for the emission monochromator. The wavelength emission was scanned from 300 to 600 nm, while the excitation wavelength was increased sequentially by 5 nm steps from 250 to 500 nm. The excitation/emission matrices (EEM plots) were generated as contour maps from fluorescence data using the Surfer 8.01 software (Golden Software Inc., Golden, CO) and fluorescence spectra were electronically corrected for instrumental response. The sensitivity and stability of the instrument were previously measured using the Raman band intensity. Two fluorescence peaks (designed as α and β), which changed markedly as a function of Cu(II) concentration, were selected to determine the complexing capacity of HAs and the stability constants of HA-Cu(II) complexes by using the single-site fluorescence quenching model proposed by Ryan and Weber (1982). The relative fluorescence intensity (FI) values (arbitrary units) were also calculated for the two main peaks α and β recorded in EEM spectra of HAs and HA-Cu(II) complexes.

In order to analyze the stability of HA-Cu(II) systems, coagulation studies were conducted in a range of Cu(II) ion concentrations from 0 to 40 mg dm⁻³ at a constant HA concentration. A series of HA solutions containing increasing amounts of Cu(II) ions at pH 5 and 7 were stirred for 24 h. The solutions were then centrifuged at 8000 g for 20 min, and analyzed for their Cu and C contents by atomic absorption spectrometry (AAS, contrAA 300, Analytik, Jena) and C analyzer (Multi NC2000,

Table 1
Selected physicochemical properties of the peaty-muck soils studied. Standard deviations do not exceed 5% in all cases.

Soil	Kind of soil	Mt	pH (H$_2$O)	pH (1 M KCl)	W$_1$	H$_z$	TC (mg g^{-1} d.m.)	TOC (mg g^{-1} d.m.)	Ash (% d.m)	d (g cm^{-3})	Porosity (%)	Contact angle Θ
S1	Z1	Mt I	5.22	4.85	0.44	11	371.1	336.3	25.8	0.19	88.5	130.0
S2	Z3	Mt II	5.42	4.93	0.60	14	292.7	272.3	25.3	0.30	81.4	125.0
S3	Z3	Mt II	5.25	4.85	0.72	21	312.4	301.6	20.0	0.33	77.8	111.2
S4	Z3	Mt II	6.01	5.55	0.74	19	249.1	241.2	25.1	0.32	84.1	91.42

S1–S4: soils; Z$_1$, peaty muck; Z$_3$, proper muck; Mt, mucking degree; W$_1$, water absorption index; H$_z$, humification index; d, bulk density, d.m., dry mass.

Analytik, Jena, module for liquid samples, platinum catalyst, furnace temperature 800 °C), respectively. The analyses were replicated three times, and data obtained averaged. The C data were used for the determination of coagulation points (C$_{crI}$, beginning of coagulation, C$_{crII}$, end of coagulation, and ΔC$_{cr}$, difference between C$_{crI}$ and C$_{crII}$) according to the modified method of Khil'ko et al. (2001).

The influence of some properties of HAs on their interactions with Cu(II) ions was evaluated by statistical analysis (Student's t-test) by calculating the correlation coefficients between the properties of each HA sample and each quantitative parameter describing the HA-Cu(II) interactions. The assumption of a normal distribution for all data used in statistical calculations was verified by the Shapiro-Wilk test.

3. Results and discussion

3.1. Physicochemical properties of soils

Results of soil analyses are presented in Table 1. The pH data show that samples S1–S3 are strongly acidic soils, whereas sample S4 is weakly acidic. According to the Gawlik classification for water absorption index (W$_1$) (Gawlik, 1992), S1 was a soil in the initial stage of transformation, S2 a weakly secondary transformed soil, and S3 and S4 medium-secondary transformed soils. Sample S3 showed the highest humification index (H$_z$) and sample S1 the lowest. The highest TC content was shown by sample S1 and the smallest by sample S4, which correspond, respectively, to the lowest and highest transformation of peat into muck. The TOC contents were only a little lower than the corresponding TC contents. The higher organic C content confirmed the weakly advanced processes of C mineralization (Ilnicki, 2002). The ash content was similar for all samples studied. The difference in bulk density between sample S1 and the other samples may be reasonably due to their different secondary transformation degree. The lowest bulk density was shown by the peaty muck (Z$_1$, soil S1), which also showed the lowest index W$_1$ and the highest porosity, whereas higher densities are typical of proper mucks (Z$_3$, soils S2, S3 and S4) that showed higher W$_1$ indexes and lower porosity. The smallest contact angle was exhibited by sample S4 and the highest by sample S1. A higher value of the contact angle was evidence of higher surface hydrophobicity of soil particles.

3.2. Physicochemical properties of humic acids

The physicochemical properties of the HA samples are presented in Table 2. The elemental composition and H/C, O/H and O/C atomic ratios

do not differ significantly among the various HA samples studied, whereas HA1 shows a C/N ratio higher than that of the other HAs, which suggests a lower humification state of HA1 with respect to the other HAs.

The contents of COOH and COOH + OH functional groups, the value of the internal oxidation degree (ω) and the total surface charges (Q5 and Q7) of sample HA1 were much lower than those of the other HAs, which were similar to each other. These results suggest a sorption capacity of HA1 lower than that of the other HA samples. The charge measured at pH 5 (Q5) would originate mostly from negatively charged functional groups of stronger acids, i.e., COOH, whereas the charge at pH 7 (Q7) would result from the dissociation of both stronger and weaker, i.e., phenolic OH, acidic functional groups.

The values of the E$_4$/E$_6$ ratio were all below 6 and increase in the order: HA3 < HA2 < HA4 < HA1, which suggests a decreasing humification degree and molecular mass and the decreasing presence of condensed aromatic structures. The order above is almost confirmed by the Δlogk values, which would suggest that sample HA1 is the less humified HA and sample HA3 is the most humified one. The highest value of A$_{280}$ shown by sample HA3 confirms a relatively high content of aromatic units, whereas the lowest A$_{280}$ value of HA1 would suggest a higher contribution of aliphatic than aromatic structures (Uyguner and Bekbolet, 2005).

3.3. Humic acid-copper(II) ion systems

3.3.1. Fluorescence spectroscopy

Three-dimensional (EEM) fluorescence spectra of all HAs and HA-Cu(II) systems at pH 5 and 7 reveal that increasing doses of Cu(II) ions caused significant changes of two fluorescent sites (peaks) designed as α and β with respect to control samples of the studied HAs. Fig. 1 shows examples of EEM characteristics of samples HA1 and HA3 and their HA-Cu(II) complexes for selected metal concentrations. The changes of α and β sites are observed as decreases in fluorescence intensity with increase of Cu(II) doses. The locations of these peaks were similar for all HA samples studied with the wavelength maxima differing only a few nm. According to the literature, the fluorescence of β sites would originate from structurally simple organic compounds containing low-molecular-mass fluorophores with a weak degree of aromatic structure condensation and bonded with substituents like methoxyl or phenolic groups. The α sites would reflect the presence of highly coupled systems of quinones and phenols with a high degree of polycondensation, typical for HAs at an advanced stage of humification (Senesi et al., 1991).

Table 2
Physicochemical properties of HAs studied. Standard deviations do not exceed 4% in all cases.

HA	C	N	H	O	H/C	O/H	O/C	C/N	COOH (cmol × kg^{-1})	COOH + OH (cmol × kg^{-1})	ω	Q5 (cmol · kg^{-1})	Q7 (cmol · kg^{-1})	E$_4$/E$_6$	A$_{280}$	Δlogk
	Weight %				Atomic ratios											
HA1	47.9	3.4	5.2	43.6	1.3	0.5	0.7	16.6	306	489	0.25	308	348	5.7	1.55	0.91
HA2	47.9	3.9	4.9	43.3	1.2	0.5	0.7	14.5	360	564	0.33	439	465	5.1	1.67	0.86
HA3	46.2	3.8	4.4	45.6	1.1	0.6	0.7	14.1	401	642	0.54	475	509	3.4	2.01	0.70
HA4	47.3	3.9	4.8	44.0	1.2	0.6	0.7	14.2	422	576	0.39	439	479	5.3	1.83	0.83

HA1–HA4, humic acids; A$_{280}$, absorbance at 280 nm; Δlogk, Kumada's humification index; ω, internal oxidation degree; Q5 and Q7, surface charge at pH 5 and 7, respectively.
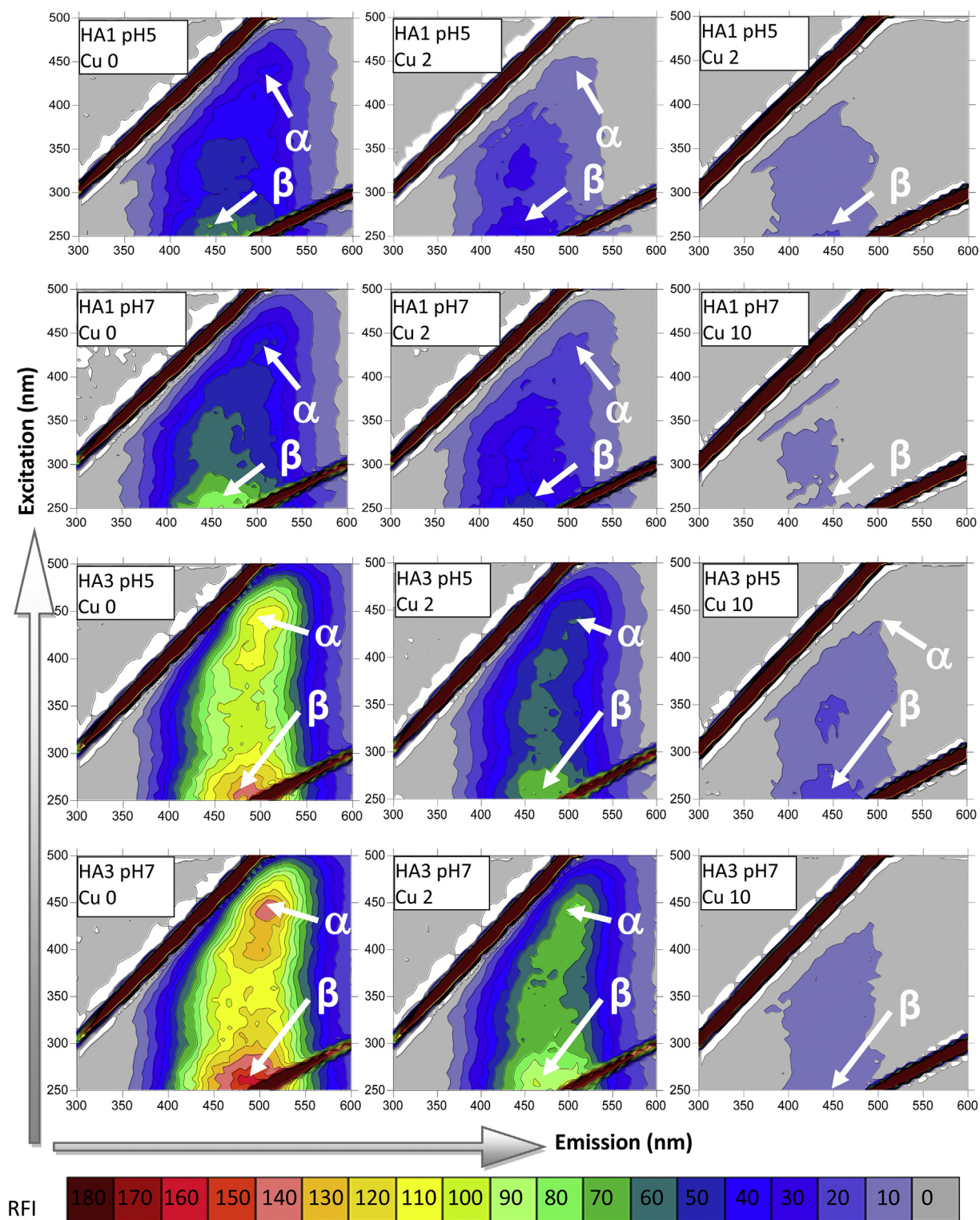
Fig. 1. Contour EEM maps of humic acids HA1 and HA3 with Cu(II) ions added at 0, 2 and 10 mg dm^{-3} at pH 5 and 7.

The effect of Cu(II) ions on fluorescence changes of HAs was described quantitatively by mathematical model of Ryan and Weber (1982), based on a non-linear regression analysis of the quenching profiles. Fig. 2 shows examples of model curves (solid lines) that fit very well to the experimental FI data (symbols) for peak α at pH 5, with r^2 values exceeding 0.98 in all cases, thus confirming the reliability and accuracy of the model used. Fluorescence is quenched in all cases with increasing Cu(II) ion concentration, which provides evidence of

complexation of Cu(II) ions by HAs (Hernandez et al., 2006; Plaza et al., 2005; Plaza et al., 2006).

The highest FI decrease occurred at low metal concentrations, while at higher Cu(II) doses the FI changes were observed less and less frequently, until the Cu(II) concentration obtained a value above which no further decrease of FI was measured (Fig. 2), thus suggesting that the maximum sorption of Cu(II) ions by HA ligands was attained (Ryan and Weber, 1982). Strong quenching of fluorescence signals at
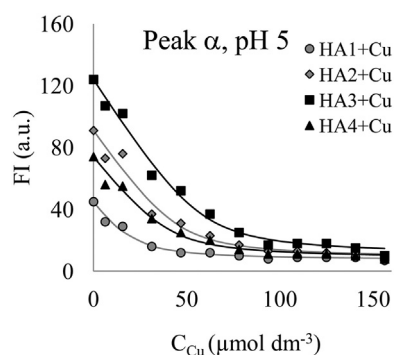
Fig. 2. Fluorescence intensity (FI) of peak α of HAs determined experimentally (symbols) compared with the theoretical curves calculated from the model (solid lines) as a function of the initial Cu(II) amount at pH 5.

Fig. 3. Ratio of the C concentration after 24 h ($Conc_{C1}$) to the C remaining in the liquid phase before Cu(II) addition ($Conc_{C0}$) as a function of added Cu(II) amount ($Conc_{Cu}$) for HA-Cu(II) systems at pH 5 and 7.

low Cu(II) concentrations is probably connected with the existence of a high amount of free, unsaturated sites on the HA surfaces to which metal ions have easy access and can be abundantly complexed. Higher concentrations of Cu(II) are more weakly bonded, mainly due to the fact that the number of reactive sites becomes lower and moreover, the reactivity of free binding sites is reduced due to steric effects by neighboring complexed functional groups. Sample HA1 featured the lowest total decrease and sample HA3 the highest total decrease of FI for α and β peaks when treated with Cu(II) ions at pH 5 and 7 (Table 3, FI_0/FI_{sat}).

For all HAs studied, the quenching of fluorescence at pH 7 appears stronger than that at pH 5 (Table 3), which would suggest a more extended HAs-Cu(II) complexation at higher pH values. This fact would result from a higher degree of dissociation at pH 7 of acidic functional groups responsible for complexation by HAs and in consequence better access of Cu cations to the negatively charged ligands.

Apparently, all the above interpretations are confirmed by the values of the complexation capacity and stability constants of HA-Cu(II) complexes calculated on the basis of the Ryan and Weber (1982) model (Table 3). The stability constants of HA-Cu(II) ion complexes are quite high and similar for the peaks α and β, which confirms the high stability of bindings formed between Cu(II) ions and HA functional groups. This parameter is slightly higher for the complexes at pH 7 that pH 5. This may be due to a more spatially expanded structure at pH 7, which facilitates access of copper to new functional groups forming more stable chemical bonds. The values of the complexation capacity (C_L) of HAs for Cu(II) ions were higher at pH 7 than at pH 5 and simultaneously slightly higher for the HA structures related to peak β than for those related to peak α. However, the complexation capacity of HAs at α fluorescent sites appears to be more affected by pH, showing a slightly higher increase of C_L values from pH 5 to 7, when compared to the difference measured for the peak β. This may indicate that α-area is related to structures more sensitive to pH than β-sites.

3.3.2. Coagulation experiments

Fig. 3 shows the trends of the C content remaining in solution at pH 5 and 7 with increasing Cu(II) concentrations. The data show that no precipitation of HAs occurs at Cu(II) concentrations up to ~8–10 mg dm^{-3}
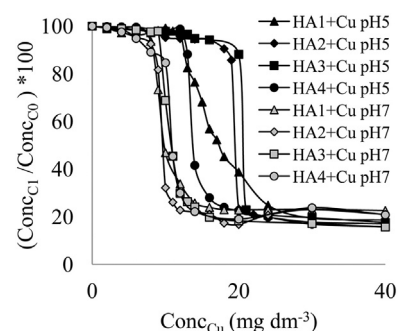
at pH 7, and up to ~12–20 mg dm^{-3} at pH 5, thus indicating that HA solutions added with Cu(II) ions are stable in this concentration range. These results may be ascribed to the formation of soluble HA-Cu(II) complexes.

Above these Cu(II) concentrations, coagulation of HAs is observed. The Cu(II) critical concentrations at which the coagulation of HAs takes place at pH 7 (defined by the coagulation points, C_{crI}) do not differ much, and follow the order: HA1 < HA2 < HA3 < HA4, whereas C_{crI} at pH 5 varies in the order: HA1 < HA4 < HA2 < HA3 and the difference between the minimum and maximum values of this parameter is higher in comparison to the above difference at pH 7 (Table 4). This Table also refers to the critical thresholds of Cu(II) concentrations at which coagulation is completed (C_{crII}), and to the difference between the starting and final points of this process (ΔC_{cr}). At pH 5, the lowest C_{crII} value was attained by HA4 and the other three HAs showed higher and similar values of C_{crII}, whereas the highest ΔC_{cr} was shown by HA1 and much lower and similar values were featured by the other three HAs. At pH 7 both C_{crII} and ΔC_{cr} were almost similar for the four HAs.

Fig. 4 shows the corresponding trends of the amount of Cu(II) ions remaining in solution (Cu1) with increasing concentration of Cu (II) added initially (designed as Cu0). At pH 7, the transfer of Cu(II) from the solution to the precipitate began at the same Cu(II) doses as in the case of the beginning of HA coagulation at this pH, and almost reached completeness (Fig. 4, grey symbols). At pH 5 only a slight removal of Cu(II) from solution was measured, and most of the Cu(II) remained in solution until the end of the experiment (Fig. 4, black symbols) despite the almost total coagulation of HAs (Fig. 3, black symbols).

The above results suggest that the coagulation process of HAs due to Cu(II) addition at pH 7 is governed by a mechanism different than that at pH 5. Coagulation at pH 7 is possibly caused by precipitation of HA-Cu(II) complexes, which is suggested by the simultaneous and almost total precipitation of Cu(II) and HAs, and by the fact that Cu(II) precipitated following the same order as HA coagulation: HA < HA2 < HA3 < HA4. In these interactions, Cu(II) ions gradually saturate negatively charged functional groups of the HAs by forming soluble complexes, but when the point of neutralization of the surface is obtained, coagulation can be initiated. The decline of Cu(II) concentration in the liquid phase at pH 7 is considerably higher than that at pH 5, which suggests a higher degree of Cu(II) binding

Table 3

Complexation capacity (C_L) of HAs expressed as mg dm^{-3}, stability constants (logK) of HA-Cu(II) complexes calculated for the two fluorescence peaks α and β at pH 5 and 7as well as fluorescence intensities before Cu treatment (FI_0) and after saturation of HA by Cu ions (FIsat).

HA-Cu(II) complex	$FI_0/FIsat(\alpha)$	$C_L(\alpha)$	logK(α)	$FI_0/FIsat(\beta)$	$C_L(\beta)$	logK(β)	$FI_0/FIsat(\alpha)$	$C_L(\alpha)$	logK(α)	$FI_0/FIsat(\beta)$	$C_L(\beta)$	logK(β)
	pH 5						pH 7					
HA1-Cu	45/7	1.31	5.28	78/28	2.26	5.20	54/5	3.24	5.46	101/11	3.40	5.41
HA2-Cu	91/8	2.90	5.36	117/20	3.36	5.40	115/7	4.47	5.77	140/12	4.45	5.61
HA3-Cu	124/10	3.71	5.38	153/26	3.37	5.37	153/9	5.05	5.77	186/16	5.12	5.57
HA4-Cu	74/8	2.59	5.33	83/20	2.97	5.23	74/7	4.44	5.54	112/11	4.33	5.55

Table 4
Values of HA coagulation points, C_{crI}, C_{crII}, ΔC_{cr}, expressed as concentration of Cu(II) ions ($mg\ dm^{-3}$) in the HA-Cu systems at pH 5 and 7.

|	pH 5			pH 7		
	C_{crI}	C_{crII}	ΔC_{cr}	C_{crI}	Cc_{rII}	ΔC_{cr}
HA1	12.2	20.7	8.5	7.9	11.0	3.1
HA2	19.3	20.0	0.7	8.6	10.4	1.8
HA3	20.3	21.2	0.9	9.1	12.0	2.9
HA4	12.8	14.5	1.7	10.0	11.7	1.7

at pH 7, but would also suggest the formation of small amounts of Cu(II) hydroxide that may act as a flocculant and lead to co-precipitation of HAs together with the formed HA-Cu(II) complexes.

The concentrations of organic C and Cu(II) in solution did not drop to zero in any studied case, which suggests the presence in solution of small amounts of soluble HA-Cu(II) complexes, dissolved HAs and free Cu(II) ions. At pH 5, the almost total coagulation of HAs (Fig. 3, black symbols) is accompanied by only a small decrease of Cu(II) (Fig. 4, black symbols). This result is probably related to the increase of the ionic strength of the solution after the addition of high amounts of Cu(II) ions, which make it difficult to form HA-Cu(II) complexes at pH 5. The majority of Cu(II) ions would thus remain not bonded in the liquid phase with small amounts of soluble HA-Cu(II) complexes, and not completely coagulate the HAs. The electrolyte thus appears to play a relevant effect on HA coagulation at pH 5, whereas coagulation at pH 7 would result from the neutralization of the HA surface charge by strong binding to Cu(II) ions.

3.3.3. Correlations between humic acid properties and complexation and coagulation parameters

The analysis of correlations existing between parameters describing HA-Cu(II) interactions and the physicochemical properties of HAs may reveal the existence of significant statistical relationships proving the influence of a given property of the HA on their interactions with Cu(II) ions.

Results of this analysis, which are displayed in the form of a correlation coefficient matrix in Table 5, showed that in many cases the HA complexation capacity, C_L, correlated significantly with properties related to the sorption capacity of the HAs. In particular, highly positive and statistically significant correlation coefficients ($r > 0.9$) existed at both pHs between the C_L values and the total amount of functional groups, COOH + OH, which confirms the marked participation of these groups in the processes of Cu(II) complexation by HAs. This relationship can result from the fact that COOH and OH functional groups demonstrate good chemical properties as ligands for metal binding as well as from the high content of such groups in the structure of humic acids. Slightly higher correlation coefficients were calculated for the above
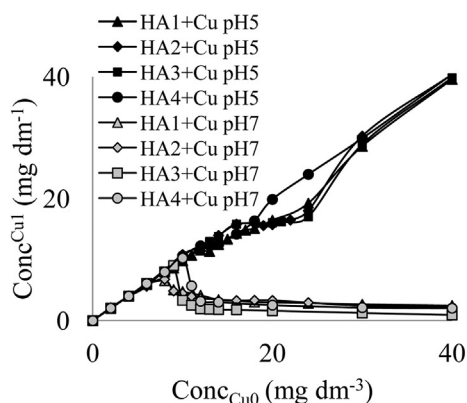
Fig. 4. Content of Cu(II) remaining in the liquid phase ($Conc_{Cu1}$) as a function of added Cu(II) amount ($Conc_{Cu0}$) for HA-Cu systems at pH 5 and 7.

relationships related to pH 7, which can be connected with better dissociation of OH groups and almost total dissociation of COOH groups. On the other hand, an interesting finding was the significant participation of COOH + OH groups in Cu binding at pH 5. A portion of the COOH groups may already be dissociated under such conditions; however OH groups are considered to be dissociated only under alkaline conditions, so their participation in metal binding should be hampered. However, there are some reports indicating possibility of OH group interactions at lower pHs with selected metals, including copper ions (Benedetti et al., 1995; Martyniuk and Więckowska, 2003). Our results confirm that OH groups together with COOH have significant influence on Cu(II) binding even at pH 5, however it should be emphasized that this relationship was improved at higher pH, probably due to lower competition with protons. The influence of oxygen-containing functional groups on Cu(II) binding was also confirmed by the high, positive correlations of C_L with the surface charge of HAs measured at both pH 5 and 7 (Q5 and Q7). In general, negative charges are generated on humic acid surfaces during the dissociation of acidic functional groups (Boguta and Sokołowska, 2013b), so the values of Q5 and Q7 parameters reflect the amount of all deprotonated functional groups at pH 5 and 7, respectively, that are allowed to bond with Cu(II) ions. The C_L parameter was also correlated significantly and positively with the degree of internal oxidation (ω). The influence of this parameter on C_L can result from the fact that the ω index describes the content of O-containing structures in HAs, but also includes in its calculation nitrogen – a second electron donor atom that can belong to functional groups complexing Cu(II) ions.

High but not statistically significant correlation coefficients were also shown between C_L and O/H and O/C ratios and content of COOH groups. The positive signs of all the above relationships still show the importance of oxygen in complexation, however, as shown above, binding of Cu(II) is determined not only by COOH concentration but also by the total content of COOH + OH groups. Statistically significant negative correlations existed between C_L and H/C and C/N atomic ratios of HAs, and high but not statistically significant negative correlations existed between C_L and E_4/E_6 ratios. These results suggest the increasing binding of Cu(II) to HAs with high molecular mass and highly condensed aromatic units in structures, which was also confirmed by the high positive and, in some cases, statistically significant correlation coefficients between C_L and A_{280}. The relationship of C_L with the C/N ratio shows that Cu(II) binding could be also dependent on nitrogen content. Despite the fact that this element occurs in small amounts in the structure of humic acids (about 3%), it can play an important role in interactions with metals, because, according to Pearson theory, complexes of metals with N-containing structures should be more stable than with O-containing structures (Boguta and Sokołowska, 2013b). In general, significant relationships of C_L with HA properties can be found at both pH 5 and 7 for both fluorescence peaks α and β. However, at pH 5 the number of significant correlations for peak α was higher than that for peak β, whereas this number was similar at pH 7. These effects may be ascribed to the different influence of fluorescing functional groups on Cu(II) complexation by HAs.

In the majority of cases, the stability constants of HA-Cu(II) complexes (LogK) were not significantly correlated with HA properties. However, statistically significant positive relationships existed at pH 5 between logK (α) and the surface charges (Q5 and Q7) as well as total number of COOH and OH groups. Activity of fluorophores designed as α-areas is related to the presence of coupled systems of quinones and phenols with a high degree of polycondensation and humification (Senesi et al., 1991). As a consequence, the increase of stability of complexes at the α-areas with increase of content of oxygen-containing structures may indicate formation of highly stable, chelation complexes between copper, highly condensed (typical for α-sites) ring structures, and their functional side groups like COOH or OH. Such complexes can demonstrate higher stability than simple, monodentate complexes.

In almost all cases the threshold concentrations of Cu(II) needed for starting (C_{crI}) and for completing (C_{crII}) HA coagulation did not exhibit

Table 5
Coefficients of linear Person's correlation calculated between selected physicochemical properties of HAs and the complexation capacity of HAs (C_L), the stability constants of HA-Cu(II) complexes (logK) and the coagulation points (C_{crI}, initial; C_{crII}, end; ΔC_{cr}, difference between C_{crI} and C_{crII}) at pH 5 and 7 for the fluorescence peaks α and β. Bold lettering refers to statistically significant relationships (α = 0.1).

	pH 5		pH 7		pH 5		pH 7		pH 5			pH 7		
	C_L (α)	C_L (β)	C_L (α)	C_L (β)	logK (α)	logK (β)	logK (α)	logK (β)	C_{crI}	C_{crII}	ΔC_{cr}	C_{crI}	C_{crII}	ΔC_{cr}
E_4/E_6	−0.87	−0.69	−0.81	−0.88	−0.82	−0.64	−0.73	−0.50	−0.78	−0.37	0.58	−0.27	−0.60	**−1.00**
A_{280}	0.88	0.70	**0.90**	**0.91**	0.79	0.43	0.56	0.58	0.53	−0.12	−0.70	0.69	0.81	0.84
O/C	0.73	0.47	0.71	0.77	0.62	0.32	0.44	0.28	0.51	0.13	−0.47	0.45	0.85	**0.92**
H/C	**−0.96**	−0.81	**−0.95**	**−0.97**	−0.89	−0.61	−0.72	−0.69	−0.73	−0.05	0.79	−0.57	−0.64	−0.98
C/N	**−0.91**	**−0.91**	**−0.96**	**−0.90**	−0.88	−0.62	−0.70	**−0.92**	−0.60	0.35	**0.98**	−0.80	−0.38	−0.67
O/H	0.87	0.66	0.85	**0.90**	0.78	0.48	0.60	0.50	0.63	0.15	−0.60	0.47	0.77	**0.96**
ω	**0.90**	0.72	**0.90**	**0.93**	0.82	0.51	0.63	0.57	0.63	0.03	−0.69	0.58	0.76	**0.94**
COOH	0.74	0.67	0.83	0.76	0.66	0.26	0.37	0.68	0.27	−0.60	−0.81	**0.96**	0.64	0.11
COOH + OH	**0.97**	0.86	**0.98**	**0.99**	**0.92**	0.65	0.75	0.75	0.73	−0.02	−0.84	0.62	0.61	**0.98**
Q5	**0.96**	**0.95**	**0.99**	**0.96**	**0.95**	0.71	0.79	**0.92**	0.71	−0.20	**−0.98**	0.71	0.39	**1.00**
Q7	**0.95**	**0.92**	**0.99**	**0.96**	**0.92**	0.65	0.74	0.89	0.67	−0.24	**−0.96**	0.75	0.46	**0.92**

any significant correlation with HA properties. However at pH 5, ΔC_{cr} showed a significant positive correlation with the C/N ratio and a significant negative correlation with the surface charges (Q5 and Q7), whereas at pH 7, ΔC_{cr} showed significant positive correlations with O/C and O/H ratios, ω, COOH + OH group contents and Q5 and Q7, as well as a highly significant negative correlation with the E_4/E_6 ratio. Among the above results, there are interesting differences observed in the sign of the correlation coefficients at pH 5 and 7 between ΔC_{cr} and properties of HAs related to oxygen content (O/C, O/H, ω, COOH + OH group, Q5 and Q7). Negative relationships at pH 5 and positive ones at pH 7 provide evidence for the different mechanisms of coagulation under various pHs. As our experiments showed, HA coagulation at pH 5 might be caused mostly by the electrolyte (Cu(II) ions) and ionic strength, whereas coagulation at pH 7 may be due to the neutralization of negative surface charges of HAs by strongly bound Cu(II) ions. As a consequence, the significant positive correlations observed at pH 7 may be explained by the fact that amount of Cu(II) ions (in this case expressed as ΔC_{cr}) needed for the completion of coagulation increases with the increase of oxidation degree and content of COOH + OH groups, generating negative charge. These HA properties do not have such importance when coagulation is governed by ionic strength.

4. Conclusions

Knowledge of the interactions between soil HAs and Cu(II) ions may allow the elucidation of the possible consequences of Cu(II) inputs into the soil environment. Further, statistical analysis of the correlations between HA properties and their interaction parameters with Cu(II) ions may help in predicting HA behavior after Cu(II) addition. Finally, the results of this study may help in the optimal adaptation to agricultural purposes of special soils such as peaty-mucks.

The main conclusions of this work can be itemized as it follows:

1. The pH has a significant influence on Cu(II) binding by HAs. Values of complexation capacity of the HA samples are higher at pH 7 than at pH 5. This effect is related to the increase of the negative charge on HA surfaces resulting from the consecutive functional group dissociation with increasing pH. The stability constants are higher at pH 7 than at pH 5. Greater values of these constants may be attributed to the higher involvement of multi-chelate coordinate structures.

2. Coagulation of HAs and Cu(II) is almost total at pH 7, whereas at pH 5, only HAs precipitate almost totally, while Cu(II) precipitates only very minimally. Further, Cu(II) ions precipitate simultaneously with HAs, which possibly consists of the precipitation of Cu(II) humates and/or, at pH 7, in a co-precipitation of small amount of Cu(II) hydroxide. The differences between the above processes at pH 5 and 7 probably result from different mechanisms of coagulation: a) one governed by ionic strength at pH 5 and b) one based on HA surface charge neutralization by Cu(II) ions at pH 7.

3. Some HA properties have a high influence on Cu(II) binding. In particular, the amount of bound Cu(II) ions increases with increasing content of carboxylic and phenolic functional groups, which indicates a significant participation of these groups in complexation mechanism.

4. The influence of oxygen-containing HA structures on the binding of Cu(II) ions is additionally confirmed by the highly positive correlations between the HA complexation capacity and O/H atomic ratio and degree of HA internal oxidation.

5. The parameters related to the HA humification degree and aromaticity, i.e., the H/C and E_4/E_6 ratio, as well as absorbance at 280 nm, also influence Cu(II) binding by HAs, particularly at pH 7. The binding of Cu(II) ions appears to be more extended for HAs characterized by a higher humification degree and by a higher aromaticity.

6. In general, the physicochemical properties of HAs are not correlated significantly with the stability constants. However, a significant increase in this parameter with increasing HA surface charge and content of HA acidic functional groups is found in some cases.

7. The threshold concentrations of Cu(II) ions needed to start and complete HA coagulation (designated as ΔC_{cr}) show several significant, positive correlations at pH 7 with some HA chemical properties related to oxygen-containing functional groups and show negative relationships with surface charges of HAs at pH 5. The above differences are explained by different mechanisms of coagulation.

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