**The impact of varying abiotic humification conditions and the resultant structural characteristics on the copper complexation ability of synthetic humic-like acids in aquatic environments**

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**Abstract**

Humic acid (HA) has a high complexation ability with metal ions due to its functional groups. In this study, 11 synthetic humic-like acids (SHLAs) were prepared under varying abiotic humification conditions: precursor species (glycine-catechol and glycine-catechol-glucose), precursor concentrations (from 0.25M:0.25M to 1M:1M), pH (6 - 8), temperature (25 °C - 45 °C) and mass of MnO2 catalyst (1.3-2.5% w/v ). The effect of the varying humification conditions on the complexation ability of the SHLA for Cu2+ were investigated together with the relationships between Cu complexation ability and the structure of the SHLAs (elemental composition, type and content of functional groups, AL/AR, E4/E6). Conditional stability constants (log *K*) of the SHLAs ranged from 6.00 to 6.42 and complexation capacities ranged from 1.76 mmol/g to 2.61 mmol/g. SHLAs synthesized at lower temperature (25 ℃), pH 8, low precursor concentrations (glycine:catechol= 0.25M:0.25M) and a larger proportion of catalyst (2.5% w/v) had a larger copper complexation ability. Log *K* values of SHLAs had significant positive correlations with carboxylic carbon (r =0.671, p <0.05), carboxylic group content (r =0.890, p<0.01) and O/C ratio (r =0.618, p <0.05), and significant negative correlations with aliphatic carbon (r = -0.616, p <0.05), total C (r = -0.685, p <0.05) and total H contents (r = -0.654, p<0.05). Complexation capacities had a significant positive correlation with total N (r = 0.826, p <0.01) and a significant negative correlation with C/N ratio (r = -0.823, p <0.01).

**Keywords:** Abiotic humification; synthetic humic-like acids; metals; copper complexation ability; structural characteristics

1. **Introduction**

Hazardous metal pollution is one of the most serious environmental issues throughout the world (Li et al., 2014; Nagajyoti et al., 2010; Zhang et al., 2017). Hazardous metals are of great concern because, unlike organic pollutants, they are non-biodegradable and can accumulate in living organisms (Lesmana et al., 2009; Mosayebi and Azizian, 2016). Copper is a potentially hazardous metal (Qing et al., 2016). Although copper is an essential metal to living organisms, long-term exposure to excess copper ions can cause gastrointestinal problems, kidney damage, cramps, hair loss, convulsions, anemia, hypoglycemia, and even death for animals and humans (Fu and Wang, 2011; Perera et al., 2014; Tang et al., 2014; Yin et al., 2016).

Humic substances (HS) are natural organic compounds (Güngör and Bekbölet, 2010; Pehlivan and Arslan, 2006) that are commonly classified as: 1) humic acid (HA, soluble in alkali but insoluble in acid); 2) fulvic acid (FA, soluble at all pH values) and; 3) humin (insoluble at any pH value) (Santosa et al., 2007; Yabuta et al., 2008). HA has a high complexation ability with metal ions due to its functional groups, especially carboxylic and phenolic-OH groups (Güngör and Bekbölet, 2010). The interaction between HA and metal ions plays an important role in metal mobility and bioavailability in the environment. Humic acids have been considered as complexation agents for use in both metal-contaminated soil remediation and wastewater treatment. (Fu and Wang, 2011; Perminova and Hatfield, 2005). Many studies have demonstrated that humic acids can form stable complexes with Cu2+, with stability constants ranging from 4.58 to 5.48 (Cao et al., 1995; Fuentes et al., 2013; He et al., 2016; Paradelo et al., 2012; Plaza et al., 2005; Plaza et al., 2005; Vidali et al., 2011; Xu et al., 2016).

HSs are known to be formed via the polycondensation of humification precursors derived from plants and microbial biopolymers, such as phenols, quinones, reducing sugars and amino acids (Okabe et al., 2011). Humification reactions between humic precursors can be enhanced by materials containing a metallic oxide (e.g., Mn-, Fe-, and Al-oxides), which is abiotic humification (Fukuchi et al., 2012; Fukushima et al., 2009a, Fukushima et al., 2009b; Nishimoto et al., 2013; Qi et al., 2012a; Qi et al., 2012b; Wang and Huang, 2000). To a certain extent, the structural characteristics and composition of the humic acid can be controlled by varying the abiotic humification reaction conditions, such as pH, temperature, the species and ratio of precursors, duration of the reaction and the amount of catalyst (Jokic et al., 2004; Yang and Hodson, 2018a; Zhang et al,2015). Abiotic humification reactions can provide a promising approach to synthesize a humic acid with a stronger copper complexation ability.

Previously Yang and Hodson (Yang and Hodson, 2018b) demonstrated that a SHLA had better Cu complexation properties than a commercially available humic acid (CAS:1415-93-6 from Sigma- Aldrich). However, which structural characteristics in SHLAs result in a good complexation ability is not well defined in the literature. Thus to extend our previous study, here we aim to determine the impact of varying conditions during the humification reactions on the structural characteristics of SHLAs and their Cu complexation ability. To achieve these aims, 11 synthetic humic-like acids (SHLAs) were prepared via a range of abiotic humification conditions: varying precursor species (glycine-catechol system and glycine-catechol-glucose system), precursor concentrations (from 0.25M:0.25M to 1M:1M), pH (6 - 8), temperature (25 °C - 45 °C) and mass of MnO2 catalyst (1.3-2.5% w/v ). The conditional stability constant (log K), complexation capacity (CC) and chemical characteristics (element composition, acid functional groups, E4/E6, FTIR and 13C NMR) of the 11 SHLAs were determined.

1. **Materials and Methods**
	1. **Materials**

All chemicals were analytical reagent grade (for detail please see Yang and Hodson, 2018b).

* 1. **Preparation of** **11 synthetic humic-like acids by abiotic humification**

Sterile conditions were maintained throughout the experiments to guarantee the humification process was abiotic. Prior to use, all glassware and solutions were autoclaved at 121 °C under 0.12 MPa for 27 minutes (Model MLS-3751, SANYO, Japan). To synthesize the SHLAs, a 1-litre aliquot of phosphate buffer (autoclaved, pH 6, 7 or 8, 0.2 M Na2HPO4/NaH2PO4) containing 0.2 g (0.02% w/v) thimerosal was placed in a 3L beaker, and then either 13.33 g or 25.00 g of MnO2 was added inside. Combinations of powdered catechol, glycine and glucose were added to the suspensions to give a range of concentrations for each of the chemicals. The mixture was stirred in the dark (IKA C-MAG hotplate stirrers, Germany) at 25°C, 35°C or 45 °C for 240 h. The abiotic humification reaction conditions and concentrations of the precursors for the 11 SHLAs are listed in Table S1.

After incubation SHLAs were extracted and purified using the standard method of the International Humic Substances Society (IHSS) (Swift, 1996); details are given in Yang and Hodson (2018a) and Yang and Hoson (2018b).

* 1. **Characterization of synthetic humic-like acids**

The elemental composition (C, H, N, O), total acidity, carboxylic group content, phenolic-OH content, E4/E6 ratio, FTIR spectra and solid-state CP-MAS 13C NMR spectra of the SHLAs were determined. Details of characterization method (e.g. instrument model, operation procedures and conditions, quality control, software used, etc) are given in Yang and Hoson (2018b). The aromaticity and the ratio of alkyl to aromatic carbons (AL/AR) were calculated according to the following equations based on 13C NMR data (Qi et al., 2012a; Yang and Hodson, 2018b).



where AAlk are alkyl-C peak integration values and AAr are aromatic-C peak integration values.

**2.4 Determination of conditional stability constants (log *K*), complexing capacities (*CC*) and complexation efficiency**

For the complexation experiments, 100 mg SHLA was dissolved in 50 ml of 1 M NaOH, and then 50 ml of 1 M HNO3 was added. The SHLA solution was then diluted with ultrapure water to a volume of 1L. 50 ml of the SHLA solution and 50 ml of Cu(NO3)2 (2 μM, 10 μM, 20 μM, 100 μM, 200 μM, 250 μM and 300 μM) in 0.2 M NaNO3 were mixed. This produced solutions at seven Cu2+ concentrations (1 μM, 5 μM, 10 μM, 50 μM, 100 μM, 125 μM and 150 μM) each with an SHLA concentration of 50 mg/L in a background electrolyte of 0.1 M NaNO3 (Yang and Hodson, 2018b). All experiments were conducted at 25 °C and pH of 6.

All solutions were shaken orbitally at 150 rpm for 24 hours and then free Cu2+ concentrations were measured using an Orion 9629BNWP Ion-Selective Electrode (ISE, Thermo Scientific, USA). All the measurements were performed in triplicate. Details of the calibration of the Cu-ISE are given in Yang and Hodson (2018b).

A 1:1 stoichiometric model was used to describe the reaction between humic acid and Cu (equation 3, Ružić, 1982; Tipping, 2002).



The conditional stability constant (*K*) is calculated as:



And the apparent complexation capacity (*Ct*, Plaza et al., 2005) is calculated as:



[*CuHA*] is then calculated as:



where *Cu*0 is initial concentration of Cu2+; [*Cu*] is concentration of free Cu2+ after 24h.

Combining and rearranging equations 4-6, the Equation 7 is given as :



Linear regression analysis was performed for 1/(*Cu*0-[*Cu*]) and 1/[*Cu*] in order to calculate *K* and *Ct* (Ružić, 1982).

**2.5 Statistical analysis**

The log *K* and *CC* values of SHLAs produced under various reaction conditions were analyzed using Kruskal-Wallis and Mann-Whitney tests. The significance level was set to p < 0.05 (SPSS 23.0, IBM, USA). Pearson (all but two properties) and Spearman rank (for aromatic C content and AL/AR) correlations were used to investigate the relationship between structural properties and Cu complexation ability (SPSS 23.0, IBM, USA).

1. **Results**
	1. **Characterization of 11 synthetic humic-like acids (SHLA)**
2. Elemental composition

The C, N, H and O contents of the 11 SHLAs were 53.48-56.58%, 3.26-4.59%, 2.83-3.16% and 36.60-39.36%, respectively (Table 1) similar to previous studies of synthetic humic-like acids produced by abiotic humification (Okabe et al., 2011; Qi et al., 2012a).

Compared with the 10 SHLAs synthesised from the catechol-glycine system, SHLA 2 which included glucose as a precursor had a higher C/N ratio and H content and lower N content which generally indicates a lower degree of humification (Qi et al., 2012a; Tu et al., 2017; Yang and Hodson, 2018b). The N content and N/C ratio increased with increasing temperature of the humification process and did not change with changing precursor concentration and relative ratio. The C/H of SHLA increased with decreasing temperature and increasing pH during humification, which indicates more aromatic structures formed under lower temperature and higher pH reaction conditions (Qi et al., 2012a).

Table 1. Elemental composition (N, C, H and O), acidic functional groups contents and E4/E6 of 11 SHLAs

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | %N | %C | %H | %O | C/N | C/H | O/C | COOH (mmol/g) | Phenolic-OH (mmol/g) | Total acidity (mmol/g) | E4/E6 |
| SHLA 1 | 4.23  | 54.62  | 2.98  | 38.17  | 12.91  | 18.35  | 0.699  | 4.10  | 8.61  | 12.72  | 2.52  |
| SHLA 2 | 3.26  | 56.58  | 3.16  | 37.00  | 17.36  | 17.90  | 0.654  | 3.64 | 12.26 | 15.90  | 2.81  |
| SHLA 3 | 4.30  | 53.48  | 2.90  | 39.33  | 12.44  | 18.47  | 0.735  | 4.61  | 6.72 | 11.32 | 3.15  |
| SHLA 4 | 4.56  | 54.44  | 2.89  | 38.11  | 11.94  | 18.81  | 0.700  | 4.83  | 7.41 | 12.24 | 3.39  |
| SHLA 5 | 4.30  | 56.06  | 3.04  | 36.60  | 13.04  | 18.42  | 0.653  | 4.57 | 9.53 | 14.10 | 2.96  |
| SHLA 6 | 4.50  | 55.57  | 2.97  | 36.97  | 12.35  | 18.74  | 0.665  | 4.62  | 8.92 | 13.54 | 3.12  |
| SHLA 7 | 3.72  | 54.44  | 2.83  | 39.01  | 14.63  | 19.24  | 0.717  | 4.81  | 7.86 | 12.67 | 2.55  |
| SHLA 8 | 3.88  | 56.22  | 2.93  | 36.97  | 14.49  | 19.21  | 0.658  | 4.31 | 8.54 | 12.85 | 2.59  |
| SHLA 9 | 3.64  | 54.02  | 2.98  | 39.36  | 14.84  | 18.13  | 0.729  | 4.33 | 9.29 | 13.62 | 2.64  |
| SHLA 10 | 3.85  | 54.79  | 2.93  | 38.43  | 14.23  | 18.71  | 0.701  | 4.66 | 11.55 | 16.21 | 3.29  |
| SHLA 11 | 4.59  | 53.78  | 3.02  | 38.61  | 11.72  | 17.81  | 0.718  | 4.59 | 14.61 | 19.19 | 3.18  |

1. Acidic functional group contents and E4/E6

Table 1 shows the acidic functional group contents and the E4/E6 ratio of the SHLAs. SHLA 4 had the greatest carboxyl content (4.83 mmol/g), while SHLA 2 had the smallest (3.64 mmol/g). SHLA 11 had the highest phenolic-OH content (14.61 mmol/g) and total acidity (19.19 mmol/g), while SHLA 3 had the lowest values. The carboxyl content of SHLA increased with decreasing temperature of humification, decreasing the glycine and catechol concentration whilst maintaining their mass ratio or just decreasing the concentration of catechol. The values of total acidity, carboxyl content and phenolic-OH content all became lower at neutral conditions or by decreasing the mass of MnO2 catalyst used. Compared with SHLA 2 from the catechol-glycine-glucose system, SHLA1 from the catechol-glycine system had a higher carboxyl content, but a lower phenolic-OH content and total acidity.

The E4/E6 ratio is related to the degree of condensation of the aromatic carbon network, the humification degree and molecular weight of HA (Chen et al., 1977; He et al., 2016; Zalba et al., 2016). SHLA 1 had the lowest E4/E6 (2.52), while SHLA 4 had the greatest (3.39). Decreasing the mass of MnO2 catalyst, increasing the glycine and catechol concentration whilst maintaining their mass ratio or just increasing the concentration of catechol whilst maintaining a neutral pH all led to a lower E4/E6. In comparison, humification temperature had only a small influence on the E4/E6 of SHLAs. The difference between the E4/E6 ratio of SHLA 1 and SHLA 2 indicated the presence of glucose could increase the E4/E6 ratio.

(c) FTIR

The FTIR spectra of the SHLAs and initial precursors are shown in Figure S1. The common features in the FTIR spectra are summarized in Table 2.

Table 2. Assignments of common FTIR absorption bands present in the spectra for the SHLAs (El-Menyawy et al., 2014; Fukushima et al., 2009a; Hardie et al., 2009; Qi et al., 2012a; Rodrigues et al., 2009; Senesi et al., 2003; Shiotsuka et al., 2015; Yang and Hodson, 2018b).

|  |  |
| --- | --- |
| Wavenumber/cm-1 | Assignment |
| 2115-2122 | C☰C stretching and the presence of cyano group |
| 1715-1722 | C=O stretching, mainly carboxylic groups |
| 1590-1598 | C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band) |
| 1439-1480 | O-H deformation of phenols, symmetrical stretch of COO-, C–H deformation, CH3 symmetric and asymmetric stretching  |
| 1172-1180 | C–O stretching and C–O–H deformation of alcohols, phenols and ethers  |
| 749-850 | Out-of-plane bending of aromatic C-H |

The different features exhibited in the FTIR spectra were the intense and broad band between 2925-2950 cm-1 attributed to C-H stretching of aliphatic structures present in all the SHLAs except for SHLA 2 and the intense and broad band around 3055 cm-1 attributed to aromatic C-H stretching for SHLA 2 (Senesi et al., 2003). The shapes of the SHLA spectra are similar but the relative absorption intensity of each peak varied, indicating that the SHLAs contained the same functional groups but in relatively different ratios.

(d) Solid-state CP-MAS13 C NMR

The peak assignments for 13C NMR spectra of the SHLAs are shown in Table S2 (Fernández-Gómez et al., 2015; Jokic et al., 2004; Nagasawa et al., 2016; Qi et al., 2012a). The compositions of different carbon species, AL/AR ratio and aromaticity for the SHLAs are summarized in Table 3. SHLA 1 had the highest aliphatic carbon (18.85%) and O-alkyl carbon (27.31%) content, and lowest aromatic carbon (30.08%) and phenolic carbon (17.20%) content, which indicated SHLA 1 had more aliphatic characteristics than the other SHLAs. SHLA 2 had the smallest carboxylic carbon content (4.00%) while SHLA 7 had the greatest (13.73%). The AL/AR ratio and aromaticity can be used to evaluate the degree of unsaturation (Qi et al., 2012a; Tu et al., 2017; Yang and Hodson, 2018b). SHLA 1 has the lowest aromaticity (0.506) and highest AL/AR ratio (0.976), indicating that SHLA 1 had the fewest aromatic structures and lower degree of saturation. In contrast, SHLA 7 had the highest aromaticity (0.223) and lowest AL/AR ratio (0.817).

Table 3. Compositions of different carbon species assessed by solid-state 13C NMR for 11 SHLAs.

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Chemical shift/ppm | AL/AR | Aromaticity |
| 0-45 | 45-110 | 110-140 | 140-160 | 160-185 | 185-215 |
| Aliphatic-C | O-alkyl-C | Aromatic-C | Phenolic-C | Carboxylic-C | Carbonyl-C |
| SHLA 1 | 18.85  | 27.31  | 30.08  | 17.20  | 6.09  | 0.48  | 0.976  | 0.506  |
| SHLA 2 | 11.32  | 17.32  | 42.07  | 23.29  | 4.00  | 2.01  | 0.438  | 0.695  |
| SHLA 3 | 11.99  | 18.37  | 40.64  | 21.75  | 6.74  | 0.51  | 0.487  | 0.673  |
| SHLA 4 | 6.75  | 15.49  | 40.68  | 24.83  | 10.30  | 1.94  | 0.340  | 0.747  |
| SHLA 5 | 10.41  | 9.71  | 41.83  | 27.59  | 8.88  | 1.59  | 0.290  | 0.775  |
| SHLA 6 | 7.90  | 13.88  | 39.33  | 25.08  | 10.76  | 3.04  | 0.338  | 0.747  |
| SHLA 7 | 8.93  | 6.30  | 36.42  | 31.73  | 13.73  | 2.89  | 0.223  | 0.817  |
| SHLA 8 | 9.19  | 20.05  | 38.87  | 23.16  | 7.81  | 0.90  | 0.471  | 0.680  |
| SHLA 9 | 10.80  | 16.74  | 39.38  | 24.22  | 7.25  | 1.59  | 0.433  | 0.698  |
| SHLA 10 | 9.17  | 18.45  | 38.52  | 24.06  | 8.82  | 0.98  | 0.441  | 0.694  |
| SHLA 11 | 6.67  | 15.60  | 40.48  | 26.36  | 9.41  | 1.48  | 0.333  | 0.750  |

**3.2 The effect of varying humification conditions on the complexation ability of synthetic humic-like acids**

Below, the effect of the different variables (humification precursor species and relative concentrations, pH, temperature, and relative amounts of catalyst) on the copper complexation ability of SHLAs are shown. Generally, SHLAs synthesized at lower temperature (25℃), pH 8, low precursor concentration (glycine:catechol = 0.25M:0.25M) and larger mass of catalyst (2.5% w/v) had a higher copper complexation ability and these conditions should be considered for use in future studies.

**3.2.1 The effect of precursor species**

Glycine-catechol (Jokic et al., 2004; Zhang et al., 2017) and glycine-catechol-glucose (Fukuchi et al., 2012; Qi et al., 2012a; Zhang et al., 2015) systems are used most commonly to synthesize SHLA. Log *K* and *CC* of SHLAs produced by these different precursor species (SHLA1 was produced from glycine-catechol-glucose, SHLA 2 from glycine-catechol) are shown in Figure S2.

The log *K* values of SHLA 1 and SHLA 2 are similar (p = 0.1) and the *CC* of SHLA 1 is a little higher (p<0.05) than that of SHLA 2, indicating that the addition of glucose did not have a large effect on the Cu complexation ability. Also, the SHLA yield of the glycine-catechol system was much higher than that of glycine-catechol-glucose system, consistent with other studies (Jokic et al., 2004). For these reasons the glycine-catechol system was chosen for further study.

**3.2.2 The effect of precursors’ concentration and ratio**

Log *K* and *CC* of SHLAs (SHLA 1 (glycine:catechol=1M:1M), SHLA 3 (glycine:catechol=0.5M:0.5M, SHLA 4 (glycine:catechol=0.25M:0.25M), SHLA 5 (glycine:catechol=1M:0.5M) and SHLA 6 (glycine:catechol=1M:0.25M)) produced by different precursors concentrations and ratios are shown in Figure 1.

 

Figure 1. Log *K* and *CC* of SHLAs (SHLA 1, SHLA 3, SHLA 4, SHLA 5 and SHLA 6) produced by different precursors concentrations and ratios (mean values, n=3 ± standard deviation).

The log *K* and *CC* of SHLAs increased significantly (p<0.05) with decreasing equimolar-concentrations of glycine and catechol from 1 M:1 M to 0.5 M:0.5 M but did not increase further when the concentrations of glycine and catechol were reduced to 0.25 M (p = 0.513 for log *K*, p = 0.18 for *CC*). When just the concentration of catechol decreased form 1 M to 0.25 M, the log *K* and *CC* increased significantly (p<0.05). In addition, comparing the complexation ability of SHLA 3 and SHLA 5, or SHLA 4 and SHLA 6, the results showed that decreasing the concentration of glycine enhanced complexation ability. In conclusion, SHLA, which was synthesized from low concentrations of glycine and catechol had a greater Cu complexation ability.

**3.2.3 The effect of pH, temperature and** **mass of catalyst**

The log *K* and *CC* of SHLAs produced at different pHs (SHLA 1 (pH 7), SHLA 9 (pH 6) and SHLA 10 (pH 8)),temperatures (SHLA 1 (T=45 ℃), SHLA 7 (T=25 ℃) and SHLA 8 (T=35 ℃)) and different masses of catalyst (SHLA 1 (1.3% w/v) and SHLA 11 (2.5% w/v)) are shown in Figure 2.

SHLA 1 produced at pH 7 had the lowest log *K* and *CC*, while SHLA 10 synthesized at pH 8 had the highest log *K* and *CC*. The log *K* of SHLA decreased with increasing reaction temperature from 25 ℃ to 45 ℃ (p <0.05), while the CC increased from 25 °C to 35 °C (p <0.05) and then remained unchanged (from 35 ℃ to 45 ℃, p=0.275). Both log *K* (from 6.07 to 6.39) and *CC* (from 2.01 to 2.61) increased significantly (p<0.05) with increasing masses of MnO2. In summary, both log *K* and *CC* of synthesized SHLA were low at neutral pH and lower masses of catalyst; for temperature, log *K* decreased as reaction temperature increased, while *CC* did not change above 35℃.



Figure 2. Log *K* (a,c,e) and *CC* (b,d,f) of SHLAs produced by different humification pH (SHLA 1, SHLA 9 and SHLA 10),temperatures (SHLA 1, SHLA 7 and SHLA 8) and different masses of catalyst (SHLA 1 and SHLA 11) (mean values, n=3 ± standard deviation).

**4. Discussion**

The log *K* values of the SHLAs ranged from 6.00 to 6.42 and complexation capacities ranged from 1.76 mmol/g to 2.61 mmol/g. At similar reaction conditions, other studies of natural humic acids report log *KCu* values in the range 4.58 - 5.48 and complexation capacity in the range of 0.07-1.33 mmol Cu/g HA, thus the SHLA had a better copper complexation ability than most natural humic acids (Abate and Masini, 2001; Cao et al., 1995; Carter et al., 1992; Fuentes et al., 2013; Fukushima et al., 1992; Paradelo et al., 2012; Taga et al., 1989).

The difference in Cu complexation ability among the SHLAs depended on their structural characteristics which were controlled by the humification conditions. Correlations between the structural characteristics of the SHLAs and their Cu complexation ability are presented in Table 4.

Log *K* values of SHLAs had significant positive correlations with carboxylic carbon (r =0.671, p <0.05), carboxylic group content (r =0.890, p<0.01) and O/C ratio (r =0.618, p <0.05), and had significant negative correlations with aliphatic carbon (r = -0.616, p <0.05), total C (r = -0.685, p <0.05) and total H contents (r = -0.654, p<0.05). Complexation capacities had a significant positive correlation with total N (r = 0.826, p <0.01) and a significant negative correlation with C/N ratio (r = -0.823, p <0.01). There was no significant relation between phenolic-OH group content and either log *K* and *CC*. The carboxylic group and phenolic group are two major functional groups for HA binding to metals (Alvarez-Puebla et al., 2004; Baken et al., 2011; Zherebtsov et al., 2015). From our data it appears that the carboxylic groups are dissociated more strongly and have stronger conditional affinity compared with phenolic-OH and hence dominate copper binding to the SHLA. Similar results have been reported by others. For example, Xu et al. (2016) and Gondar et al. (2006) studied the complexation behaviour between soil/peat humic acids and copper using the NICA-Donnan model, and the results showed that 76.5-97.0% of Cu was bound to COOH groups while 2.9-23.5% of Cu was bound to phenolic-OH. In another study (Yang and Hodson, 2018a) in which we used SHLA as an adsorbent to remove Cu2+ from contaminated water, the FTIR spectra of SHLA before and after adsorption of Cu2+ also demonstrated the importance of the carboxylic groups as binding sites for Cu2+. In addition, phenolic-OH groups (pKa: 8–11) tend to dissociate at high pH while the pH was set at 6 in this study, which might explain the not significant effect of phenolic-OH (Vidali et al., 2011). Additionally complexation ability is stronger when HA has more aromatic structures and a higher degree of humification, because an aromatic carboxylic group and adjacent phenolic OH group, or two adjacent aromatic carboxylic groups can form highly-stable salicylate-like and catechol-like ring structures with metal ions (Fuentes et al., 2013; Hernández et al., 2006; Xu et al., 2016). SHLAs with a lower aliphatic carbon content have a higher aromatic structure content, and higher N contents and N/C ratios indicate a higher degree of humification. Thus, our findings are consistent with previous research that log *K* has a significant negative relation with aliphatic carbon content and *CC* has a significant positive correlation with N content and N/C ratio (Hernández et al., 2006; Fuentes et al., 2013; Xu et al., 2016). To achieve a better Cu complexation ability, the SHLA should have a higher content of carboxylic groups, more aromatic structures and a higher degree of humification.

When using SHLA in practical applications, in addition to the structural characteristics of SHLA, the effect of environmental factors, such as the temperature, pH and ionic strength, on copper complexation ability of SHLA should also be considered. For example, in another study (Yang and Hodson, 2018b) we investigated various environmental factors (ionic strength: 0.01-0.5M NaNO3, pH: 4-8, temperature: 25-45 °C) and showed that the copper complexation ability of SHLA is higher at higher pH, lower ionic strength and higher temperature, and the effects of environmental factors on the complexation reaction were similar to natural humic acids.

Table 4 General correlation matrix for the measured parameters.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | log *K* | *CC* | Aliphatic-C | O-alkyl-C | Aromatic-C | Phenolic-C | Carboxylic-C | Carbonyl-C | AL/AR | Aromaticity | Phenolic-OH | COOH | Total acidity | E4/E6 | N | C | H | O | C/N | C/H | O/C |
| log *K* | 1 | .652\* | -.616\* | -.420 | .082 | .444 | .671\* | .223 | -.227 | .519 | -.150 | .890\*\* | -.019 | .090 | .489 | -.685\* | -.654\* | .555 | -.583 | .240 | .618\* |
| *CC* |  | 1 | -.390 | .043 | .327 | -.079 | .144 | -.183 | -.036 | .124 | .087 | .547 | .174 | .482 | .826\*\* | -.470 | -.144 | .146 | -.823\*\* | -.201 | .285 |
| Aliphatic-C |  |  | 1 | .637\* | -.009 | -.704\* | -.577 | -.539 | .664\*\* | -.847\*\* | -.232 | -.547 | -.324 | .279 | -.204 | .000 | .187 | .071 | .164 | -.208 | .043 |
| O-alkyl-C |  |  |  | 1 | -.300 | -.963\*\* | -.702\* | -.743\*\* | .945\*\* | -.947\*\* | .029 | -.510 | -.048 | .345 | .009 | -.061 | .187 | .043 | -.020 | -.256 | .052 |
| Aromatic-C |  |  |  |  | 1 | .145 | -.164 | .273 | -.218 | .237 | .209 | -.118 | .164 | .036 | .164 | .105 | .420 | -.251 | -.064 | -.391 | -.227 |
| Phenolic-C |  |  |  |  |  | 1 | .761\*\* | .708\* | -.991\*\* | .947\*\* | .095 | .531 | .180 | -.372 | -.051 | .023 | -.243 | .019 | .047 | .294 | .001 |
| Carboxylic-C |  |  |  |  |  |  | 1 | .607\* | -.782\*\* | .682\* | -.210 | .840\*\* | -.088 | -.283 | .358 | -.240 | -.678\* | .153 | -.403 | .604\* | .187 |
| Carbonyl-C |  |  |  |  |  |  |  | 1 | -.747\* | .714\* | .015 | .226 | .051 | -.678\* | -.059 | .228 | -.026 | -.207 | .123 | .203 | -.224 |
| AL/AR |  |  |  |  |  |  |  |  | 1 | -.998\*\* | -.227 | -.445 | -.236 | .445 | -.200 | .036 | -.027 | .114 | .091 | -.118 | .082 |
| Aromaticity |  |  |  |  |  |  |  |  |  | 1 | .094 | .544 | .180 | -.339 | .051 | .056 | -.171 | -.064 | -.024 | .231 | -.062 |
| Phenolic-OH |  |  |  |  |  |  |  |  |  |  | 1 | -.312 | .989\*\* | .150 | -.122 | .129 | .641\* | -.138 | .185 | -.646\* | -.136 |
| COOH |  |  |  |  |  |  |  |  |  |  |  | 1 | -.170 | .114 | .597 | -.504 | -.772\*\* | .332 | -.675\* | .507 | .407 |
| Total acidity |  |  |  |  |  |  |  |  |  |  |  |  | 1 | .173 | -.035 | .057 | .546 | -.093 | .089 | -.592 | -.079 |
| E4/E6 |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | .380 | -.338 | .036 | .183 | -.402 | -.303 | .253 |
| N |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | -.370 | -.273 | -.021 | -.979\*\* | .025 | .140 |
| C |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | .527 | -.919\*\* | .520 | .136 | -.971\*\* |
| H |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | -.514 | .406 | -.770\*\* | -.525 |
| O |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | -.154 | -.083 | .986\*\* |
| C/N |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | -.067 | -.308 |
| C/H |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | -.112 |
| O/C |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |
| \*. Correlation is significant at the 0.05 level (2-tailed). |
| \*\*. Correlation is significant at the 0.01 level (2-tailed). |

**5. Conclusions**

In this study, 11 synthetic humic-like acids (SHLAs) were prepared by abiotic humification under different reaction conditions. The log K of the SHLAs ranged from 6.00 to 6.42 and complexation capacities ranged from 1.76 mmol/g to 2.61 mmol/g. SHLAs synthesized at lower temperature (25℃), pH 8, low precursors concentration (glycine:catechol= 0.25M:0.25M) using a larger mass of catalyst ( 2.5% w/v) had better copper complexation abilities. Log *K* values of SHLAs had significant positive correlations with carboxylic carbon (r =0.671, p <0.05), carboxylic group content (r =0.890, p<0.01) and O/C ratio (r =0.618, p <0.05), and had significant negative correlations with aliphatic carbon (r = -0.616, p <0.05), total C (r = -0.685, p <0.05) and total H contents (r = -0.654, p<0.05). Complexation capacities had a significant positive correlation with total N (r = 0.826, p <0.01) and a significant negative correlation with C/N ratio (r = -0.823, p <0.01). Thus, to achieve a better Cu complexation ability, the SHLA should have a higher content of carboxylic groups, more aromatic structures and a higher degree of humification. Future studies should consider the application of SHLA for the remediation of contaminated water and soil.

**Supplementary Materials**

Information about the abiotic humification reaction conditions and concentrations of the precursors for the 11 SHLAs (Table S1), FTIR spectra of humification precursors and 11 SHLAs (Figure S1), interpretation of the 13C NMR spectra of the SHLAs (Table S2), Log *K* and *CC* of SHLAs produced by different precursors systems (Figure S2) are shown in supplementary materials.

**Acknowledgements**

This work is part of Ting Yang’s PhD work funded by the China Scholarship Council (No. 201506210102), University of York Overseas Student Scholarship and the Department of Environment and Geography, University of York (D017).

**Competing interests statement**

The authors declare that they have no competing interests.

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