Synthesis and Use of Synthetic Humic-like Acid (SHLA) for The Remediation of Metal-Contaminated Water and Soil

Ting Yang
PhD

University of York
Environment and Geography

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Abstract

Humic acid (HA) can effectively bind several hazardous metals. The interaction between HA and metal ions plays an important role in metal mobility and bioavailability in the environment. Abiotic humification processes can provide a potential and promising way to synthesize humic acids with better metal binding ability than their naturally occurring counterparts. The aim of the PhD project is to investigate the synthesis and application of synthetic humic-like acid (SHLA) for water/wastewater treatment and soil remediation.

This thesis showed the log K and complexation efficiency of SHLA were higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration, and the effects of the above environmental factors on the complexation reaction are similar to natural HAs.

Eleven SHLAs were prepared via a range of abiotic humification conditions. The results showed that SHLAs with a higher content of carboxylic groups, more aromatic structures and a higher degree of humification, would have a better Cu complexation ability. The optimal abiotic humification conditions to synthesize SHLA with better copper complexation ability were also determined.

When applied as a soil washing agent, SHLA was more effective than some common washing agents, like Na_2EDTA, citric acid, commercial HA and tartaric acid, and reduced the bioavailability and environmental/human health risk of metals. When applied as an adsorbent, the adsorption process between SHLA and Cu^{2+} was favourable, spontaneous and endothermic, and was described by the pseudo-second-order kinetic model, Langmuir and Freundlich models. The Cu^{2+} adsorption capacity of SHLA was much higher than many of the humic acid-based materials reported in the literature and the SHLA can be regenerated by HCl. Besides, SHLA was able to remove co-contaminant metals from solution. When applied as a soil amendment, SHLA lowered the bioavailability of Cu, Zn and Ni, but increased the bioavailability of As. Besides, the addition of SHLA could increase TOC and WSC of soil but decrease the soil pH. This thesis has shown that it is possible to synthesize SHLAs that show great promise for use in remediation of contaminated water sources and soils.

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Author's declaration

As a PhD student at the Department of Environment and Geography, University of York under the supervision of Professor Mark Hodson (2015.10 - 2018.10), I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

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The data reported in Chapter 3 was presented at the "Early Careers Researcher Conference 2017". (April 2017, Lancaster University, United Kingdom)

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Chapter 3, 4, 5 and 6 have been written as articles for international peer-reviewed journals and all of them have been published, as shown in Table 0.1. This thesis is presented by publication format.

Signature:

Time:

Table 0.1 Status of publications for chapters presented in this thesis

Title	Authors	Journal	Status	Chapter
The copper complexation ability of a synthetic humic-like acid formed by an abiotic humification process and the effect of experimental factors on its copper complexation ability.	Ting Yang & Mark E. Hodson	*Environmental Science and Pollution Research*	Published DOI: 10.1007/s11356-018-1836-2	3
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	Ting Yang & Mark E. Hodson	*Ecotoxicology and Environmental Safety*	Published DOI: 10.1016/j.ecoenv.2018.09.057	4
Investigating the use of synthetic humic-like acid as a soil washing treatment for metal contaminated soil	Ting Yang & Mark E. Hodson	*Science of The Total Environment*	Published DOI: 10.1016/j.scitotenv.2018.07.457	5
Investigating the potential of synthetic humic-like acid to remove metal ions from contaminated water	Ting Yang & Mark E. Hodson	*Science of The Total Environment*	Published DOI: 10.1016/j.scitotenv.2018.04.176	6

“Man can do what he wants, but he cannot will what he wants.”

—Arthur Schopenhauer

CHAPTER 1

Introduction

Chapter 1 Introduction

1.1 General introduction

Soil and water contamination caused by hazardous metals (e.g., copper, cadmium, lead, zinc, chromium, and nickel) is a serious environmental and health issue worldwide (Ashraf et al., 2017; Gupta et al., 2016; Lesmana et al., 2009; Li et al., 2014; Machado et al., 2017; Saha et al., 2017). Hazardous metals are the most frequent contaminants (34.8%) affecting soil and groundwater in Europe and 82.8% of cases of soil contamination are caused by inorganic contaminants (mainly hazardous metals) in China (EEA, 2018; MEE, 2014). Since the 1970s, hazardous metal pollution has been recognized as one of the key water pollution problems in the seven major rivers of China (Qu and Fan, 2010). Elevated concentrations of metals in soils and water originate from many sources, including atmospheric deposition, sewage irrigation, waste disposal, mining activities, metal smelting, sewage sludge, electroplating, paper industry, batteries, pesticides and fertilizers (Al-Qodah and Al-Shannag, 2017; Fu and Wang, 2011; Li et al., 2014; Lwin et al., 2018; Mahar et al., 2016; Shi et al., 2009; Su et al., 2014). Hazardous metals are non-biodegradable and can accumulate in the human body by food chain, posing a great threat to both human health and the environment (Lesmana et al., 2009; Rezania et al., 2016; Suanon et al., 2016; Zou et al., 2016). Thus, it is essential to remove excessive concentrations of metals from soil and water/wastewater for human health and ecological stability.

Humic acid (HA) is a natural biopolymer and major component of humic substances (Stevenson, 1994). Due to the abundant oxygen-containing functional groups, especially the carboxylic and phenolic-OH groups present in HA, humic acid can effectively bind several metals (Perminova and Hatfield, 2005; Sparks, 1995). The use of humic acids has been considered in both soil remediation and water/wastewater treatment (Arslan et al., 2007; Clemente and Bernal, 2006; Jin et al., 2016; Khan et al., 2017; Kulikowska et al., 2015; Li et al., 2010; Meng et al., 2017; Wang and Mulligan, 2009; Yang et al., 2015). The majority of humic acids used in current studies are natural humic acids extracted from soil, lignite or sediment (Ashraf et al., 2017; Fuentes et al., 2013; He et al., 2016; Plaza et al., 2005; Xu et al., 2016; Yang et al., 2015; Zherebtsov et al., 2015). In addition to occurring naturally, humic acid can be synthesized by abiotic humification processes, that is the transformation of humic precursors (e.g., polyphenol, amino acids, and reducing sugars) to humic substances catalyzed by materials containing metallic oxide (e.g., pure MnO_2 and soil minerals); humic acid synthesized by abiotic humification is called synthetic humic-like acid (SHLA) (Fukushima et al., 2009; Hardie et al., 2009; Nishimoto et al., 2013; Qi et al., 2012a; Qi et al., 2012b; Wang and Huang, 2005; Zhang et al., 2015; Zhang et al., 2018). Compared with natural humic acid, the advantage of synthetic humic-like acid is that the structural characteristics of SHLA can be manipulated by changing the reaction conditions of the abiotic humification process, such as the species of precursors, pH, temperature and the ratio of catalyst (Fukuchi et al., 2012; Jokic et al., 2004; Yang and Hodson, 2018; Zhang et al., 2017; Zhang et al., 2015). Therefore, abiotic humification processes can provide a potential and promising way to synthesize humic acids with better metal binding ability than their naturally occurring counterparts.

1.2 Thesis aims, objectives and structures

The main aim of the PhD project is to investigate the synthesis and application of synthetic humic-like acid (SHLA) for water/wastewater treatment and soil remediation. Specifically, the project includes three objectives. Firstly, as the studies on remediation of metal contamination by SHLA are still rare, the metal binding ability of SHLA (Cu^{2+} is chosen as representative metal) and the influence of environmental factors are investigated (Chapter 3). Secondly, based on the first part, 11 SHLAs are prepared via a range of abiotic humification conditions and characterized to answer under what abiotic humification conditions SHLA with better metal binding ability can be synthesized and what structural characteristics of SHLA will affect its metal binding ability. Then the optimal humification conditions to produce SHLA with a high binding ability are achieved. Thirdly, a SHLA is synthesized under optimal conditions from the second part and applied in three ways: as a soil washing agent (in aqueous form, Chapter 5); as an adsorbent for water/wastewater treatment (in solid form, Chapter 6); as a soil amendment (in solid form, Chapter 7). The technology roadmap of this PhD thesis is shown as Figure 1.1.

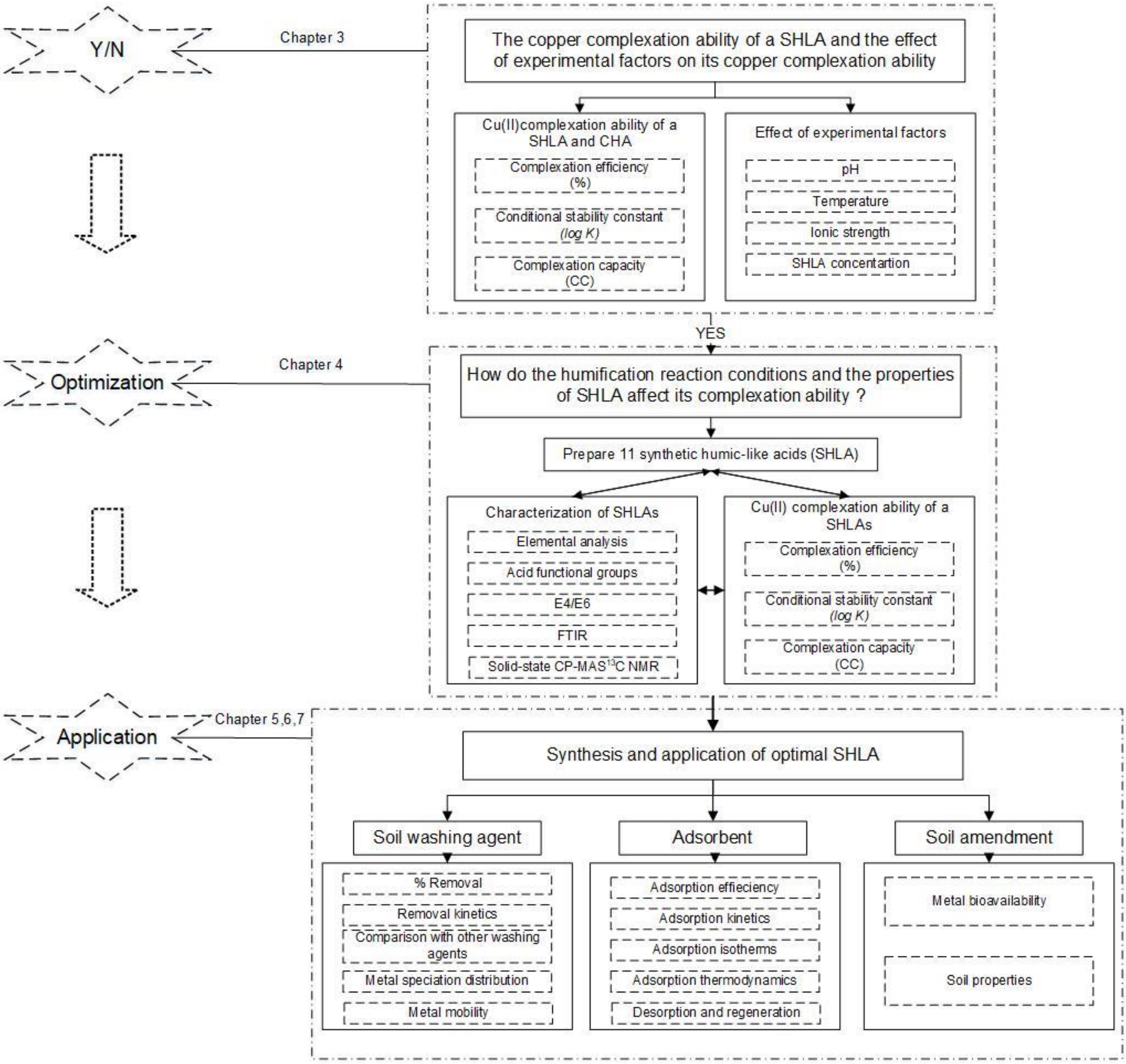

Figure 1.1 The technology roadmap of this PhD thesis

The objectives for each chapter in details are as follows.

In Chapter 3, a synthetic humic-like acid (SHLA) is synthesized under the environmental conditions based on the previous research. Then the complexation ability of SHLA for Cu^{2+} and the influence of environmental factors on the complexation reaction (ionic strength, pH, temperature and humic acid concentration) are investigated. Chapter 3 demonstrates that SHLA can be used for binding metals.

In Chapter 4, the aim is to achieve the optimal conditions to produce optimal SHLA with better metal binding ability. Eleven SHLAs are prepared via a range of abiotic humification conditions. The effect of the varying humification conditions on the complexation ability of the SHLA for Cu^{2+} is investigated together with the relationships between Cu complexation ability and the structural characteristics of the SHLAs. Chapter 4 demonstrates that the SHLA, which has a higher content of carboxylic groups, more aromatic structures and a higher degree of humification, will have a better Cu complexation ability. The optimal abiotic humification conditions to synthesize SHLA with better copper complexation ability are determined.

In Chapter 5, the optimal SHLA in liquid form is used as a soil washing agent to remove metals (Cu, Zn, Ni, Pb, As) from two contaminated agricultural soils. The effects of washing conditions on % metal removal are investigated, and optimal extraction conditions are achieved. Chapter 5 demonstrates that SHLA shows great potential as a soil washing agent.

In Chapter 6, the optimal SHLA in solid form is used as an adsorbent to remove metal ions from contaminated water. The adsorption behaviours of aqueous Cu^{2+} onto the SHLA including adsorption efficiency, adsorption kinetics, isotherm fits and thermodynamics are investigated together with the desorption and reuse potential of the SHLA and the potential adsorption of co-contaminant metals. Chapter 6 demonstrates that SHLA has great potential for use as an adsorbent for metal-contaminated waters treatment.

In Chapter 7, the optimal SHLA in solid form is used as a soil amendment to immobilize toxic metals in a metal-contaminated agricultural soil. The effects of SHLA amendment on metal bioavailability and soil properties are investigated. Chapter 7 demonstrates that SHLA is a promising soil amendment for immobilizing metals. However, due to the time limitation, the study about soil amendment is a preliminary study and deeper studies should be continued in the next step.

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CHAPTER 2

Literature Review

Chapter 2 Literature Review

In this literature review, the definition and methods used for the characterization of humic substances (HS) are summarized. Additionally, details concerning both the production of synthetic humic-like acid (SHLA) by abiotic humification processes and the methods used to determine metal and HS/HA interaction along with the current application of HA/HS in soil/water remediation are reviewed. The information outlined above can help to facilitate experimental design and explain/contextualize that constitute other parts of the present thesis. As a result of the review, three important knowledge gaps and subsequent research questions have been identified: 1) Can SHLA bind metals like natural humic acid (HA) and what is the metal binding performance of SHLA? 2) Can we use abiotic humification processes to synthesize SHLA with stronger metal binding ability? If yes, how can such processes be controlled to give optimal results? 3) Can we apply SHLA in environmental remediation and what types of remediation applications can SHLA be used in?

2.1 What are humic substances?

Humic substances (HS) are brown or black multifunctional organic compounds that naturally occur in soils, waters and sediments, and with major agricultural and environmental roles (Güngör and Bekbölet, 2010; Pehlivan and Arslan, 2006). For example, in terms of agricultural function, HS can dramatically benefit plant growth (Nardi et al., 2002). As for the environmental role, HS can interact with both metal ions and organic compounds within a natural environment (Perminova and Hatfield, 2005). HS are complex aromatic macromolecules with various linkages between the aromatic groups which include amino acids, amino sugars, peptides, aliphatic acids and other aliphatic compounds (Perminova and Hatfield, 2005; Stevenson, 1994). There are abundant functional groups in humic substances, including carboxylic, phenolic-OH, amine, enol and carbonyl groups, amongst others (Nardi et al., 2002; Perminova and Hatfield, 2005).

According to solubility, humic substances are commonly classified as: 1) humic acid (HA, soluble in base, 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). Because of the poor solubility of humin, current research has been mainly focused on HA and FA. Compared with HA, FA has a lower molecular weight, a lower C content and a higher O content (Gonzalez, 2002; Stevenson, 1994). In addition, the content of carboxylic, ketone and hydroxyl groups in FA is higher than that in HA (Stevenson, 1994).

However, because of the complicated structural characteristics, it has not been possible to describe the exact structure of HS macromolecules until now. This complexity is reflected in Figure 2.1, which shows the molecular structure model of HA and FA.

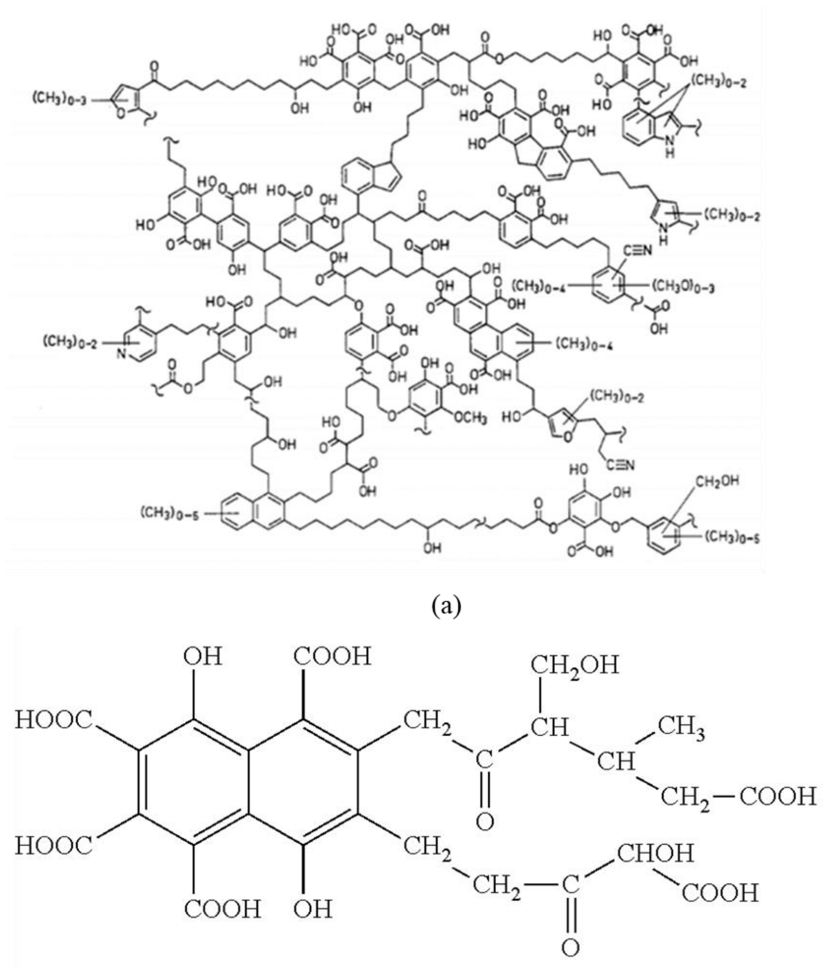


Figure 2.1 The molecular structure model of HA (a) and FA (b) (Buffle, 1977; Schulten and Schnitzer, 1993).

2.2 Methods for characterizing the structural characteristics of humic substances

Due to the complicated structure of HS, a number of analytical methods are used to characterize HS (Table 2.1). These include chemical/physical methods, spectroscopy, mass spectroscopy and chromatography. Chemical/physical methods determine elemental composition and the concentration of oxygen-containing and sulfur-containing groups quantitatively. Spectroscopic methods provide information about functional groups and molecular structure. Chromatography gives information about the molecular size and weight of HS.

2.2.1 Chemical/physical methods

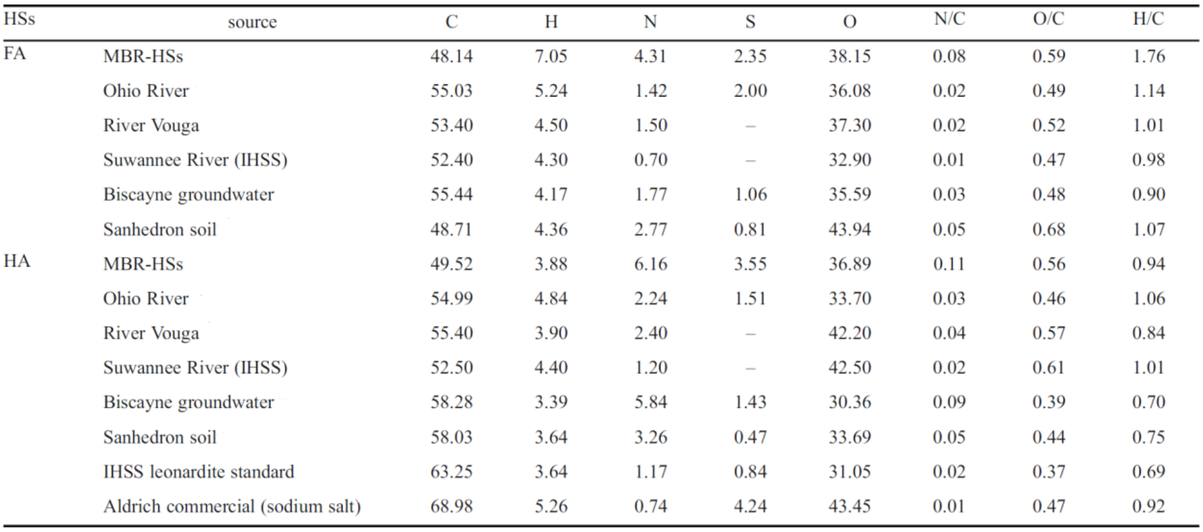
2.2.1.1 Elemental composition

HS consist of C, H, O, N, P and S (Stevenson, 1994). The H/C ratio is an indicator of the amount of saturation of C atoms and/or branched structures within HS (Rodrigues, 2010). A lower H/C ratio indicates more aromatic structures (Qi et al., 2012a). The O/C ratio is used to infer the carbohydrate content and degree of oxidation. The N content and N/C ratio give information about the quantity of nitrogen functional groups and humification degree. Generally, a higher N content and greater N/C ratio indicate a higher degree of humification (Mahieu et al., 2000; Qi et al., 2012a; Tu et al., 2017; Yang and Hodson, 2018). Table 2.2 shows an example of the elemental composition of humic acid and fulvic acid from different sources (Qi et al., 2012c).

Table 2.1 Analytical methods for the characterization of humic substances.

Method		Information
Chemical/physical (Abbt-Braun et al., 2004; Stevenson, 1994)	Elemental composition analysis	Elemental composition (C, H, N, O, P and S) and element ratio
	Titration	Functional groups (e.g. COOH content, phenolic-OH group ad carbonyl group); proton capacity
	Microscopy	Size and shape
	Viscometry	Size and shape
Spectroscopy (Abbt-Braun et al., 2004; Chen et al., 2010; Fukuchi et al., 2010; Hardie et al., 2009; He et al., 2016; Qi et al., 2012a; Qi et al., 2012b; Qi et al., 2012c; Thomsen et al., 2002; Zalba et al., 2016)	UV-VIS	E_{600}, A_{400nm}, A_{600}, E_4/E_6; degree of condensation of the aromatic carbon network, degree of humification and the molecular weight
	IR	Functional groups (qualitative)
	Fluorescence	Fluorescent groups (qualitative)
	NMR (^{1}H, 2-D, ^{13}C, ^{15}N, ^{31}P)	Functional groups (quantitative), monomeric units and substitution pattern (qualitative/quantitative), structural elements (qualitative);
	Raman	Functional groups (qualitative)
	ESR	Organic radicals and paramagnetic transition metals (quantitative)
Mass spectroscopy (Abbt-Braun et al., 2004; De la Rosa et al., 2011; Fukushima et al., 2009a; Okabe et al., 2011)	GC/MS Pyrolysis-GC/MS, Hydrolysis-GC/MS, Oxidation-GC/MS	Structures of organic molecules
Chromatography (Fukushima et al., 2009b; Woelki et al., 1997)	Size exclusion chromatography	Molecular size and weight
	HPLC	Fractions of different structural features

Table 2.2 Elemental composition of HA and FA extracted from different sources (Qi et al., 2012c).



HSs	source	C	H	N	S	O	N/C	O/C	H/C
FA	MBR-HSs	48.14	7.05	4.31	2.35	38.15	0.08	0.59	1.76
	Ohio River	55.03	5.24	1.42	2.00	36.08	0.02	0.49	1.14
	River Vouga	53.40	4.50	1.50	–	37.30	0.02	0.52	1.01
	Suwannee River (IHSS)	52.40	4.30	0.70	–	32.90	0.01	0.47	0.98
	Biscayne groundwater	55.44	4.17	1.77	1.06	35.59	0.03	0.48	0.90
	Sanhedron soil	48.71	4.36	2.77	0.81	43.94	0.05	0.68	1.07
HA	MBR-HSs	49.52	3.88	6.16	3.55	36.89	0.11	0.56	0.94
	Ohio River	54.99	4.84	2.24	1.51	33.70	0.03	0.46	1.06
	River Vouga	55.40	3.90	2.40	–	42.20	0.04	0.57	0.84
	Suwannee River (IHSS)	52.50	4.40	1.20	–	42.50	0.02	0.61	1.01
	Biscayne groundwater	58.28	3.39	5.84	1.43	30.36	0.09	0.39	0.70
	Sanhedron soil	58.03	3.64	3.26	0.47	33.69	0.05	0.44	0.75
	IHSS leonardite standard	63.25	3.64	1.17	0.84	31.05	0.02	0.37	0.69
	Aldrich commercial (sodium salt)	68.98	5.26	0.74	4.24	43.45	0.01	0.47	0.92

2.2.1.2 Titration

Titration is used to quantitatively determine the content of oxygen-containing functional groups. The advantages of titration method are with high degree of accuracy and precision, easy to operate, fast and cheap (Harris, 2010). The specific titration methods for different oxygen-containing functional groups of HS are listed in Table 2.3.

Table 2.3 Titration methods used to determine the content of oxygen-containing functional groups (Schnitzer and Kahn, 1972; Stevenson, 1994; Watanabe et al., 1994).

Group content	Titration method
Total acidity	Barium hydroxide method
Carboxylic group	Calcium acetate method
Total hydroxyl group	Acetylation method
Carbonyl group	Hydroxylamine method
Phenolic-OH	=Total acidity-COOH
Alcoholic-OH	=Total OH-phenolic-OH

2.2.2 Ultraviolet and visible absorption spectroscopy (UV-VIS)

Ultraviolet and visible absorption spectroscopy (UV-VIS) is employed for quantitative analysis and is widely used throughout chemical disciplines. Ultraviolet and visible absorption occurs due to transition of valence electrons (Christian et al., 2013). This method is attractive owing to the fact that it is highly sensitive and only requires a small quantity of sample. Additionally, samples being used for UV-VIS analysis do not require any special separation. Light absorbance of HS in the ultraviolet and visible range is a typical characteristic (from λ = 200 nm up to 800 nm). Generally, the light absorbance decreases gradually as the wavelength increases (Abbt-Braun et al., 2004). Figure 2.2 shows the basic schematic diagram of a typical UV-VIS spectrophotometer.

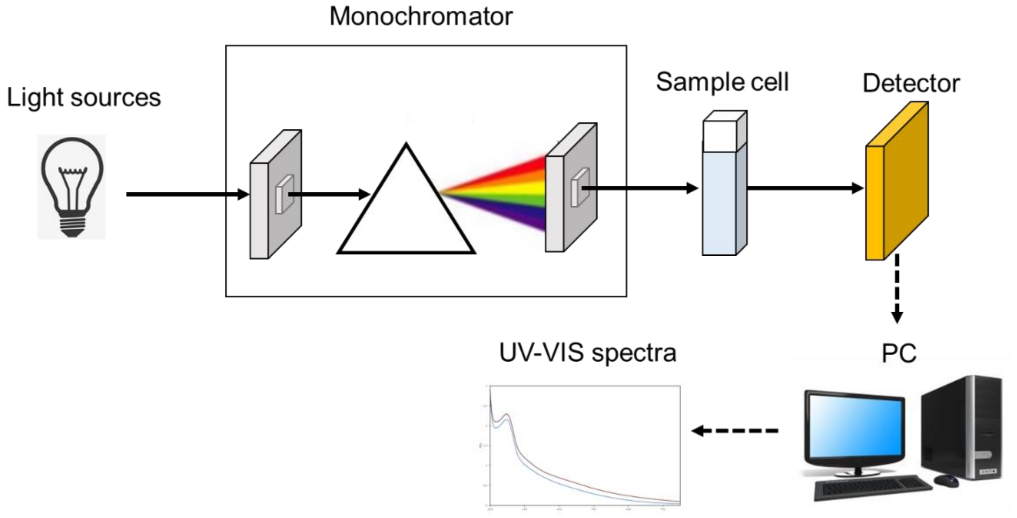


Figure 2.2 Basic schematic diagram of UV-VIS spectrophotometer

UV-VIS spectroscopy allows three characteristic parameters: E_4/E_6, E_{600} and finally A_{400} and A_{600} to be measured. Each will now be discussed in brief.

1) E_4/E_6

E_4/E_6 is the ratio of the absorbances at 465 and 665 nm, which is related to the degree of condensation of the aromatic carbon network, the degree of humification and the molecular weight of the humic acid (Chen et al., 1977; He et al., 2016; Yang and Hodson, 2018; Zalba et al., 2016). A lower value of E_4/E_6 indicates a more aromatic structure, a higher degree of humification and a larger molecular weight (Chen et al, 1977; He et al, 2016; Yang and Hodson, 2018; Zalba et al, 2016).

2) The extinction coefficient at a wavelength of 600 nm (E_{600})

E_{600}, a parameter derived from UV-VIS analysis, is defined as the extinction coefficient at a wavelength of 600 nm (Qi et al., 2012b; Zhang et al., 2018). E_{600} is used to monitor the kinetics of darkening and a semi-quantitative index of humification (Qi et al., 2012b; Zhang et al., 2017; Zhang et al., 2018). The calculation of E_{600} is shown in Equation (1).

$$E_{600} = \frac{A_{600}}{TOC \times L} \quad (1)$$

Where A_{600} is the absorbance at wavelength of 600 nm; TOC is the total organic carbon (g/L) and L is the length of the light path.

In general, E_{600} increases as the extent of humification increases (Fukuchi et al., 2010; Qi et al., 2012b; Zhang et al., 2017; Zhang et al., 2018). Figure 2.3 shows the change of E_{600} over time in five humification systems.

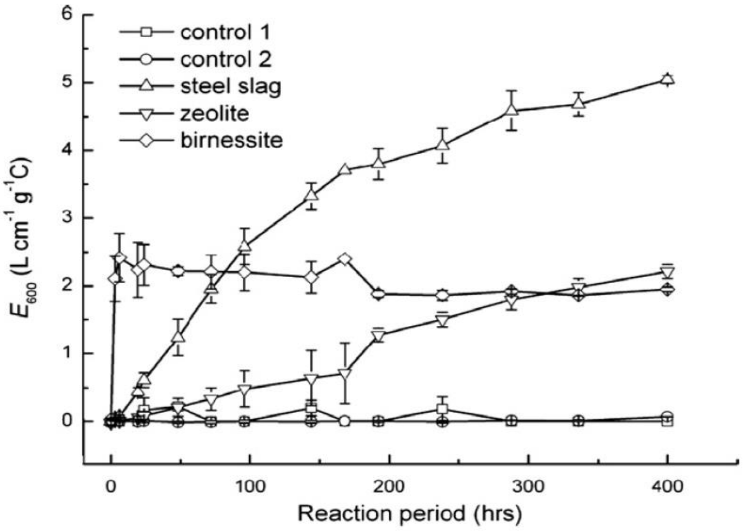


Figure 2.3 The change of E_{600} over time in five humification systems (Qi et al., 2012b)

3) A_{400} and A_{600}

Absorbances at 400 nm and 600 nm indicate the degree of humification in forming coloured substances (Hardie et al., 2009; Zhang et al., 2015).

2.2.3 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy is a fast and non-destructive analytical method. The principle of infrared spectrum method is predicated on the fact that when a substance is irradiated by infrared light, molecular vibration of functional groups within the analyte will occur. According to the relationship between molecular vibration and light absorption, it is possible to determine the functional groups within the sample under investigation (Christian et al., 2013). FTIR can be used for the determination of functional groups, but is primarily utilized as a qualitative technique. Figure 2.4 shows the basic scheme of an FTIR spectrophotometer. For HS samples, wavenumbers are usually recorded from 4000 to 400 cm^{-1}. The main FTIR absorption bands for HS are listed in Table 2.4 and Figure 2.5 shows the FTIR spectra of HA and FA from various sources. If HS are from different sources, the wavenumber and absorption intensity of the characteristic absorption bands may change (Chen et al., 2010; Qi et al., 2012a).

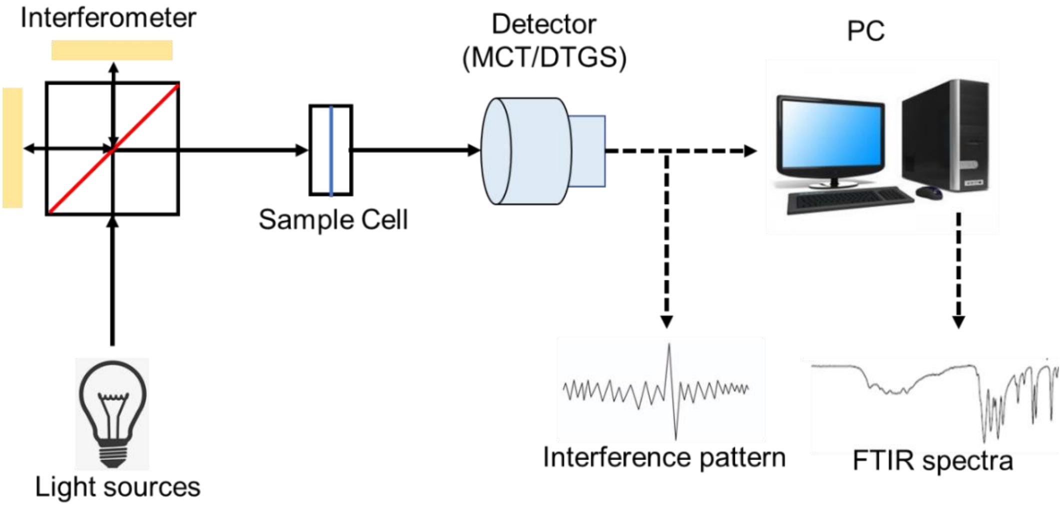


Figure 2.4 Basic schematic diagram of an FTIR spectrophotometer

Table 2.4 Assignment of wavenumbers to structural groups of HS (Fukushima et al., 2009b; Hardie et al., 2009; Jokic et al., 2004; Qi et al., 2012a; Rodrigues et al., 2009; Shiotsuka et al., 2015; Stevenson, 1994).

Wavenumber/cm^{-1}	Assignment
3450-3300	O–H stretching of phenol and/or alcohols; N-H stretching
2950-2900	C-H stretching of aliphatic structures
1725-1710	C=O stretching, mainly carboxylic groups
1630-1660	C=O stretching of amide groups (amide I band), quinone C=O and/or C=O of H-bonded conjugated ketones
1620-1570	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)
1480-1375	O-H deformation of phenols, symmetrical stretch of COO-, C–H deformation, CH_3 symmetric and asymmetric stretching
1280-1170	C–O stretching and C–O–H deformation of alcohols, phenols and ethers
975-775	Out-of-plane bending of aromatic C-H

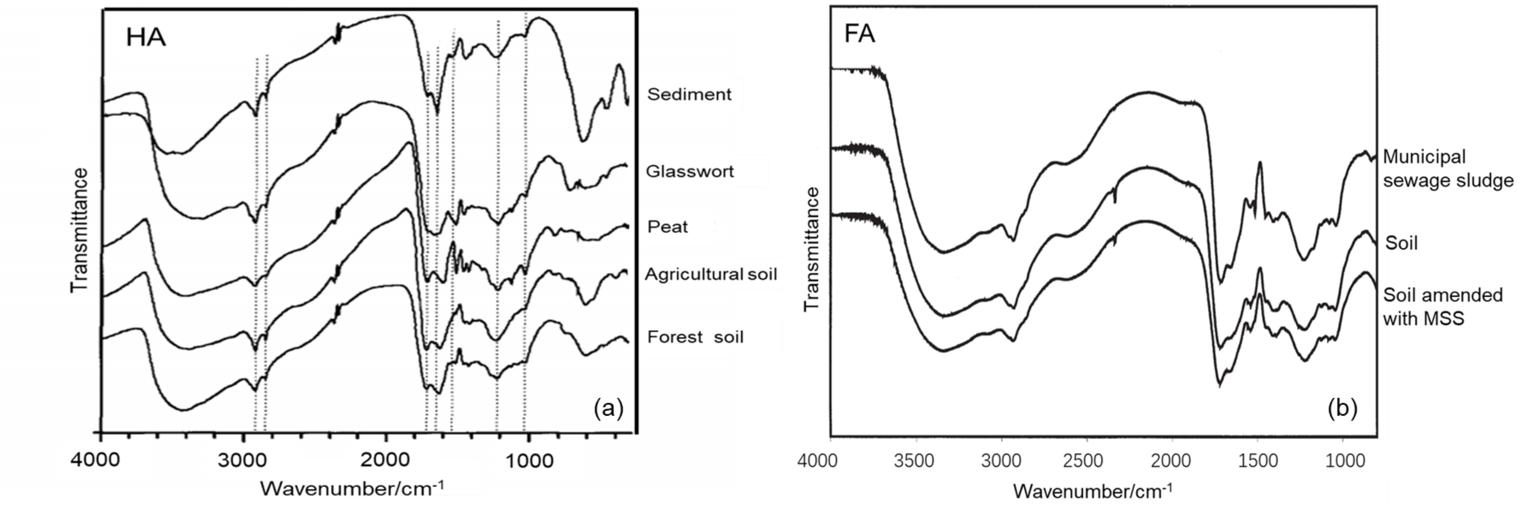


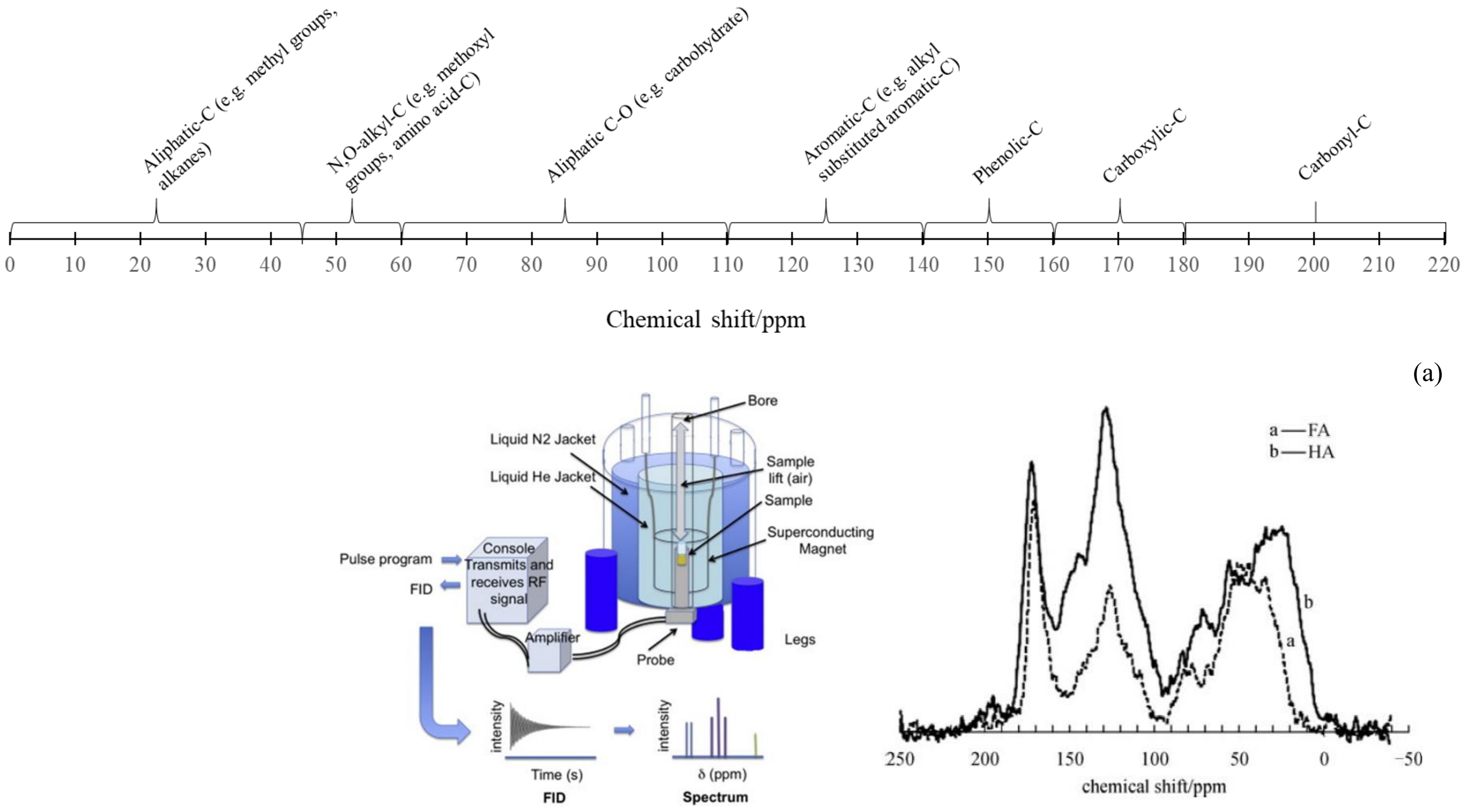
Figure 2.5 FTIR spectra of HA (a) and FA (b) from various sources (Fooken and Liebezeit, 2003; Gigliotti et al., 2001).

2.2.4 Nuclear magnetic resonance (NMR) spectroscopy

The NMR technique is based on the interaction of electromagnetic radiation with nuclear, atomic or molecular species (Abbt-Braun et al., 2004; Stevenson, 1994). Currently, the NMR techniques applied to characterize HS include ^{13}C-NMR, ^{1}H-NMR, ^{15}N-NMR and ^{31}P-NMR, which offer information about chemical bonding modes of C, H, N and P (Abbt-Braun et al., 2004). Among them, solid-state CP-MAS ^{13}C NMR spectroscopy is most widely used. The diagram of an NMR spectrometer, assignments of ^{13}C chemical shifts to structural groups and an example of CP-MAS ^{13}C NMR spectra of HA and FA are shown in Figure 2.6.

2.2.5 Size exclusion chromatography

To determine the molecular weight and size of HS, several approaches can be used, including size exclusion chromatography, ultracentrifugation, X-ray scattering, viscometry, cryoscopy, electron microscopy, vapor pressure osmometry, electrophoresis, and field flow fractionation (Abbt-Braun et al., 2004; Balch and Guéguen, 2015; Stevenson, 1994). Among them, size exclusion chromatography (SEC) is the most commonly used. SEC separates the sample based on the molecular size when it is passed through a column. Small molecules enter the pores of the column stationary phase while large molecules pass by the pores directly due to their prohibitively large size. Thus, larger molecules flow through the column faster than smaller molecules which accordingly, have longer retention time (Christian et al., 2013). The molecular weights of HSs range widely from 500 Da to 1000 kDa due to the different original sources and analytical methods (Balch and Guéguen, 2015; Perminova et al., 2003; Stevenson, 1994; Tipping, 2002).



(b)

(c)

Figure 2.6 The assignments of ^{13}C chemical shifts to structural groups (a), schematic diagram of an NMR spectrometer (b), and an example of CP-MAS ^{13}C NMR spectra of HA and FA (c) (Abbt-Braun et al., 2004; Fernández-Gómez et al., 2015; Nagasawa et al., 2016; Qi et al., 2012c; Rankin et al., 2014; Stevenson, 1994).

2.3 Synthesizing humic acid by abiotic humification pathway

2.3.1 Introduction to the humification process and abiotic humification

Humification is a process in which organic carbon molecules are transformed to humic substances. Humification results in the stabilization of organic matter and plays an important role in carbon sequestration (Jastrow et al., 2007; Song et al., 2014). Specifically, by mineralization and the humification process, organic matters ultimately achieve stabilization. Via mineralization, organic matters are transformed to CO_2, while organic matters are fixed in the soil by humification.

Humification typically includes two stages. Firstly, organic biomacromolecules (e.g. proteins, polysaccharides, lignins and polyphenols) are hydrolyzed to low-molecular-weight organic compounds (e.g. polyphenols, reducing sugars, amino acids and fatty acids) through processes which are generally driven by microorganisms and their extracellular enzymes in the natural environment (Jastrow et al., 2007; Nishimoto et al., 2013). Then, with the small organic molecules released from the first stage serving as precursors, condensation and polymerization reactions that create new larger molecules occur (Jastrow et al., 2007; Nishimoto et al., 2013). Currently, there are four hypotheses (Figure 2.7) that comprise the major pathways of humification: (a) sugar-amine pathway, (b) polyphenol-quinone pathway, (c) lignin-quinone pathway, and (d) modified lignin pathway (Stevenson, 1994).

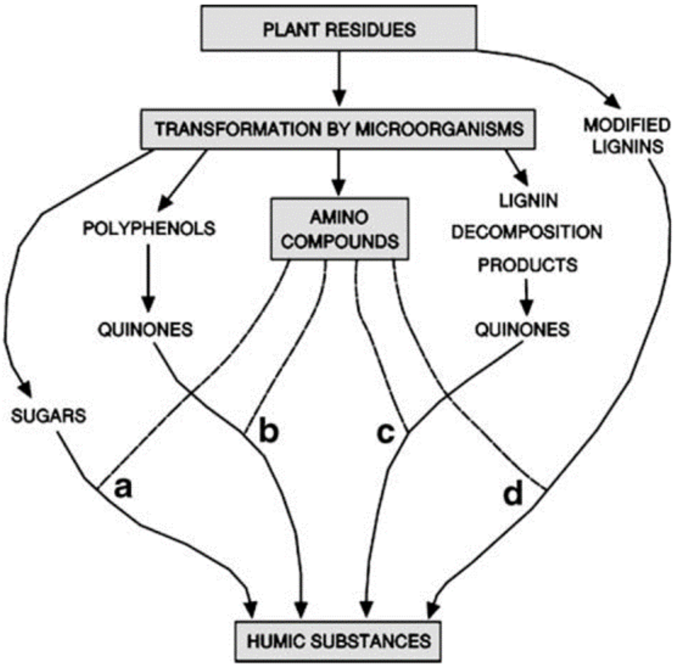


Figure 2.7 Major pathways for humification ((a) sugar-amine, (b) polyphenol-quinone, (c) lignin-quinone, and (d) modified lignin) (Stevenson, 1994).

Depending on whether the second stage is dominated by a microorganism, humification can be classified as either biotic humification or abiotic humification (Naidja et al., 1998). In terms of biotic humification, for example, polyphenol oxidase (i.e. tyrosinase) could enhance the polycondensation of small organic molecules (Martin and Haider, 1969). In terms of abiotic humification, it has been reported that materials containing metallic oxides (e.g., MnO_2, Fe_2O_3, Al_2O_3 and soil minerals) are able to catalyze and enhance the transformation of humic precursors (e.g., amino acids, sugars, and quinones) to humic substances; this process is called abiotic humification (Chen et al., 2006; Fukushima et al., 2009a; Hardie et al., 2009; Huang, 2000; Jokic et al., 2004; Okabe et al., 2011; Qi et al., 2012a; Qi et al., 2012b; Shindo and Huang, 1984; Wang, 1991; Wang and Huang, 2000; Wang and Huang, 2003; Zhang et al., 2015; Zhang et al., 2018). For example, soil minerals such as kaolinite, nontronite and zeolite can enhance the formation of humic substances from amino acids and polyphenol (Qi et al., 2012a; Wang and Huang, 1989; Wang and Huang, 2003). Pure oxides of Si, Mn, Al and Fe can accelerate the darkening of reaction mixtures that contain humic precursors (Chen et al., 2010; Wang and Huang, 2000; Zhang et al., 2017; Zhang et al., 2018). One of the possible catalytic mechanism of soil minerals and metallic oxides is that they can serve as Lewis acids (i.e. substances which can accept electrons) in the abiotic humification pathway (Chen et al., 2010; Huang, 2000). For example, using catechin as a precursor, the catalytic mechanism of MnO_2 in an abiotic humification system is shown in Figure 2.8. Humic and fulvic acids synthesized by abiotic humification are called synthetic humic-like acid (SHLA) and synthetic fulvic-like acid (SFLA), respectively.

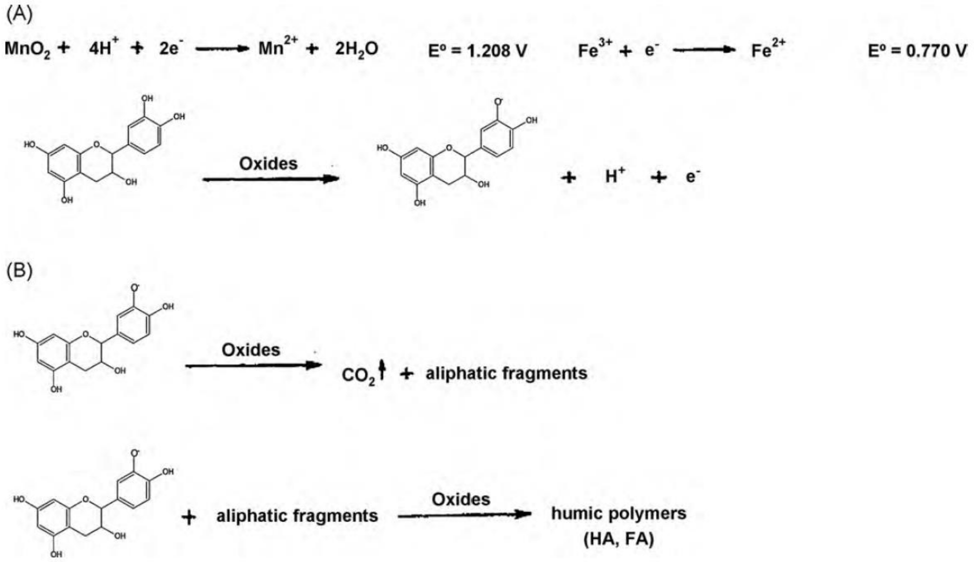


Figure 2.8 Proposed mechanisms for (A) the generation of protons caused by oxidative transformation of catechin as catalyzed by oxides and (B) the subsequent formation of HS (Chen et al., 2010).

2.3.2 Humic precursors used in abiotic humification studies

Following the sugar-amine pathway and polyphenol-quinone pathway hypotheses, most current studies concerning abiotic humification use low-molecular-weight phenolic compounds, reducing sugars, amino acids and mixtures of the aforementioned compounds. Specifically, catechin (Chen et al., 2010; Chen et al., 2006), catechol (Naidja et al., 1998; Wang and Huang, 2000) and pyrogallol (Wang and Huang, 2000) can all serve as individual humic precursors. The mixed precursors systems, such as the catechol-glycine system (Hardie et al., 2009; Jokic et al., 2004; Wang and Huang, 1989), hydroquinone-glycine system (Wang and Huang, 1989), pyrogallol-glycine system

(Wang and Huang, 1989; Wang and Huang, 2003), catechol-glucose system (Hardie et al., 2009), glucose-glycine system (Jokic et al., 2004) and catechol-glycine-glucose system (Jokic et al., 2004; Qi et al., 2012a, Zhang et al., 2015; Zhang et al., 2018) can also form humic substances via polycondensation.

2.3.3 The effect of reaction conditions on abiotic humification

A large number of studies investigating the influence of reaction conditions on abiotic humification have been carried out in the preceding four decades. A wide range of precursors, catalysts and environmental conditions have been used and the products have been characterized in a number of different ways. In particular, the difference in characterization methods can make comparisons of the HS products challenging.

Table 2.5 summarizes several abiotic humification processes under different reaction conditions and some properties of the resulting HS products. The materials used to catalyze abiotic humification process can be classified as either: 1) pure metallic oxides (e.g. MnO_2 and Fe_2O_3); 2) natural minerals (e.g. zeolite, kaolin and nontronite); and 3) waste residuals (e.g. steel slag and incineration residues). Many studies have used δ-MnO_2 as a catalyst because it is more effective in the humification process when compared to other metal oxides with respect to the abiotic humification among mineral materials (Hardie et al., 2009). Under comparable conditions, the sequence of the oxides that increase the yield of HA and the degree of humification is found to be: Mn (IV)-oxide>Fe (III)-oxide>Al-oxide>Si-oxide (Chen et al., 2010). A variety of soil minerals have also been used in the abiotic synthesis of HS, such as nontronite, bentonite, kaolinite, and zeolite (Fukuchi et al., 2012; Fukuchi et al., 2010; Wang and Huang, 1989). Under the same conditions, the sequence of soil minerals that increase the yield of HA is: Ca-nontronite > Ca- kaolinite > Ca- bentonite and can be attributed to the different specific surface areas and metals on the edge (Wang and Huang, 1989). Recently, researchers have also used waste residues to enhance abiotic humification. By using catechol, glycine and glucose as humic precursors, steel slag enhanced the polycondensation reactions of humic precursors (Qi et al., 2012a). The Fe (III) oxides on the surface of steel slag (i.e. hematite or magnetite) acted as catalytic sites for accelerating the formation of humic-like substances from humic precursors (Nishimoto et al., 2013). Kim et al. (2004) found that incineration residuals could also enhance abiotic humification (Kim and Osako, 2004).

Several factors can influence a humification reaction, such as precursors concentration, pH, temperature and catalyst type. The pH is an important factor that effects abiotic humification reactions. Low pH will inhibit formation of free radicals and autooxidation of phenol compounds, subsequently inhibiting the humification rate and formation of HS (Wang et al., 1980). Under alkaline conditions, some side reactions might occur which will have an adverse effect on abiotic humification. For example, in the catechol-glucose-glycine system, catechol will dissociate under alkaline condition and then might react with aldehyde group of glucose (Wang et al., 2010). Thus, current investigations into abiotic humification tend to focus on neutral pH conditions (pH = 6 to 7) and few experiments are carried out under acid or alkaline conditions (Table 2.5). Temperature also has a great effect on abiotic humification. Typically, the rate and degree of polycondensation increase dramatically with increasing temperature (Wang and Huang, 2003). However, the majority of studies have been conducted at a specific temperature and pH and the impact of variation of these two variables on abiotic humification has not been systematically investigated. In addition, increasing the reactant concentration can increase the yield and molecular weight of HA (Okabe et al., 2011; Zhang et al., 2015). Under the same conditions, the catalytic effect of Mn (IV) oxide is more pronounced than soil minerals and other metallic oxides (Chen et al., 2010; Qi et al., 2012a).

Table 2.5 Reaction conditions and characterization of HS products for several abiotic humification processes.

Catalyst Category	Catalyst and addition amount (w/w, %)		Precursors and concentration (mmol/L)	T /°C	pH	Time /h	UV-VIS analysis				Yield and ratio				MW (Da)
							A_{400}	A_{600}	A_{472}	A_{664}	HA /mg	HA /%	FA /mg	FA /%	
Pure metallic oxide (Chen et al., 2010; Hardie et al., 2009; Jokic et al., 2004; Qi et al., 2012a; Wang and Huang, 1992; Zhang et al., 2015)	δ-MnO_2	0.4	Catechin: 2.7	25	6	90	-	-	3.83	0.67	112.6	68.1	52.8	31.9	-
	δ-MnO_2	2.5	Catechol: 500	45	7	360	10.29	6.97	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glycine: 500 Catechol: 500	45	7	360	55.92	31.58	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glycine: 500 Catechol: 500 Glucose: 500	45	7	360	100.59	44.21	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Catechol: 500 Glucose: 500	45	7	360	15.65	10.63	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 500	45	7	360	0.07	0.04	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 50 Glycine: 50	45	7	360	14.7	1.5	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 50 Glycine: 50 Catechol: 50	45	7	360	73.7	24.7	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 50 Catechol: 50	45	7	360	242	115	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 50 Glycine: 50	25	7	1440	0.367	0.067	-	-	-	-	-	-	-
	δ-MnO_2	2.5	Glucose: 50 Glycine: 50 Catechol: 50	25	7	1440	90.4	37.6	-	-	-	-	-	-	-
	δ-MnO_2	0.3	Pyrogallol: 16.7	25	6	90	-	-	2.91	0.25	126	66.1	64.7	33.9	-

Table 2.5 continued

Catalyst Category	Catalyst and addition amount (w/w, %)		Precursors and concentration (mmol/L)	T /°C	pH	Time /h	UV-VIS analysis				Yield and ratio				
							A_{400}	A_{600}	A_{472}	A_{664}	HA /mg	HA /%	FA /mg	FA /%	MW /Da
Pure metallic oxide	δ-MnO_2	1.3	Glycine: 10 Catechol: 10 Glucose: 10	30	6	400	-	-	-	-	-	95.7	-	4.3	10660
	MnO_2	1.2	Glycine: 60 Catechol: 30-240 Glucose: 60	35	8	360	0.6-0.8	-	-	-	655.1-4534.4	-	-	-	-
	Fe-oxide	0.4	Catechin: 2.7	25	6	90	-	-	1.12	0.11	30.1	29.9	70.3	70.1	-
	Al-oxide	0.4	Catechin: 2.7	25	6	90	-	-	0.07	0.06	5.9	5.0	112.8	95.0	-
Natural mineral (Fukuchi et al., 2010; Qi et al., 2012a; Wang, 1991; Wang and Huang, 1989; Wang and Huang, 2003)	Mollisol	3.3	Pyrogallol: 166.7 Glycine: 333.3	25	6	90	-	-	-	-	25.8	37.7	42.7	62.3	-
	Mollisol	4	Pyrogallol: 200	25	6	90	-	-	-	-	14.5	28.8	35.8	71.2	-
	Oxisol	3.3	Pyrogallol: 166.7 Glycine: 333.3	25	6	90	-	-	-	-	15.6	20.4	60.8	79.6	-
	Oxisol	4	Pyrogallol: 200	25	6	90	-	-	-	-	13.4	21.6	48.5	78.4	-
	Nontronite	3.3	Pyrogallol: 166.7 Glycine: 333.3	25	6	90	-	-	7.83	4.01	16.3	16.8	80.6	83.2	-
	Nontronite	3.3	Catechol: 166.7 Glycine: 333.3	25	6	90	-	-	11.4	4.09	7.5	18.8	32.3	81.2	-
	Nontronite	3.3	Hydroquinone: 166.7 Glycine: 333.3	25	6	90	-	-	14.7	3.67	7.3	19.8	29.6	80.2	-
	Nontronite	4	Glycine: 400	25	6	90	-	-	0	0	-	-	-	-	-
	Nontronite	3.3	Pyrogallol: 166.7	25	6	90	-	-	6.58	3.42	8.1	10.6	68.5	89.4	-

Table 2.5 continued

Catalyst Category	Catalyst and addition amount (w/w, %)		Precursors and concentration (mmol/L)	T /°C	pH	Time /h	UV-VIS analysis				Yield and ratio				
							A_{400}	A_{600}	A_{472}	A_{664}	HA /mg	HA /%	FA /mg	FA /%	MW (Da)
Natural mineral (Fukushima et al., 2009a; Miura et al., 2011; Okabe et al., 2011)	Bentonite	3.3	Pyrogallol: 166.7	25	6	90	-	-	0.59	0.08	2.6	10.6	21.9	89.4	-
	Kaolinite	3.3	Pyrogallol: 166.7	25	6	90	-	-	1.34	0.25	3.8	10.6	32.2	89.4	-
	Zeolite	1.3	Glycine: 10 Catechol: 10 Glucose: 10	30	6	400	-	-	-	-	-	62.4	-	37.6	2670
	Zeolite	1.3	Glycine: 50 Catechol: 50 Glucose: 50	30	7	504	-	-	-	-	-	21.6	-	78.4	4200
	Allophanic soil	1.3	Catechol: 5 Glycine: 5	30	7	336	-	-	-	-	-	-	-	-	646
	Allophanic soil	1.3	Catechol: 10 Glycine: 10	30	7	336									2315
	Weathered pumice	0.6	Catechol: 50 Tryptophan: 50	30	7	336						18			1428
Waste residue (Nishimoto et al., 2013; Qi et al., 2012a)	Steel slag	1.3	Glycine: 10 Catechol: 10 Glucose: 10	30	6	400	-	-	-	-	-	94.8	-	5.2	2980
	Steel slag	1.1	Glycine: 5 Catechol: 5 Glucose: 5	25	6	506									3169

-: not available

2.4 Humic substances and metals

2.4.1 Interaction between HS and environmental contaminants

Due to the complicated structural characteristics of HS they can take part in a broad range of chemical interactions. HS can be involved in complexation, ion exchange, proton transfer and donor-acceptor interactions; can be oxidized by strong oxidants and function as reducing agents; and can also take part in hydrogen bonding and van-der-Waals interactions (Perminova and Hatfield, 2005; Stevenson, 1994). HS can react with both hazardous metals and organic contaminants.

HS play an important role in the distribution, toxicity, and bioavailability of hazardous metals in both aquatic and soil environments. The reactions between HS and hazardous metals mainly include two types of mechanisms (Perminova and Hatfield, 2005). Firstly, humic substances can react with metals by complexation and ion exchange and form HS-metal complexes. The carboxylic and phenolic groups of HS are two major functional groups for binding metals (Alvarez-Puebla et al., 2004; Baken et al., 2011; Meng et al., 2017; Perminova and Hatfield, 2005; Sparks, 1995; Zherebtsov et al., 2015). Some studies indicate that carboxylic groups dominate metal ion binding to humic acid because the dissociation ability of carboxylic group is much stronger than that of the phenolic group (Gondar et al., 2006; Xu et al., 2016; Yang et al., 2015). For example, Xu et al. studied the fraction of copper bound speciation on three humic acids using the NICA-Donnan model. The results showed that 76.5-97.0% of copper was bound to carboxylic groups compared to only the 3.2-23.5% bound to phenolic groups (Xu et al., 2016). However, some other researchers have suggested that the sites of phenolic group play a more significant role in metal ion binding than the carboxylic group (Borges et al., 2005; Cabaniss et al., 2007). It is important to note that the group which dominates the complexation process also depends on the type of metal, pH and temperature (Bosire et al., 2016; Fuentes et al., 2013). For instance, the complexation of Fe^{3+} is mainly dominated by carboxylic groups distributed within aliphatic domains, while Cu^{2+} complexation involves phenolic and O-alkyl groups (Fuentes et al., 2013). Secondly, redox reactions can occur between HS and metals. For example, previous studies have demonstrated that soil HA, peat HS and leonardite HS could reduce Cr^{6+} to Cr^{3+} (Fukushima et al., 1997; Wittbrodt and Palmer, 2005; Zhilin et al., 2004).

In addition to inorganic contaminants, HS can also react with organic pollutants via physical absorption, hydrogen bonding, charge transfer, and covalent bonding (Perminova et al., 2005; Stevenson, 1994). Several studies have shown that humic acid and humic acid-based materials can be used as adsorbents to remove dyes (e.g. methylene blue, rhodamine B, Midlon Black), pesticides (e.g. lindane, atrazine, diuron and thiram), phenanthrene, ethylene dibromide and 3,5-dichlorophenol from contaminated water (Chiou et al., 2000; Filipe et al., 2009; Janoš et al., 2005; Shenvi et al., 2015; Wang et al., 2017a; Wen et al., 2007). HS can also serve as a soil washing solution to remove polycyclic aromatic hydrocarbons, thiophenes and petroleum hydrocarbons from soil (Conte et al., 2005; García-Díaz et al., 2015).

2.4.2 Methods used to determine the interaction between HS and metals

Because the bonding mechanisms and influential factors occurring between HS and metals are complicated, a series of methods rather than only one should be used in combination in order to determine the interaction between HS and metals. These methods can be divided into spectroscopic, chromatography and finally electrochemical methods.

2.4.2.1 Spectroscopic methods

By analysing spectral characteristics of HS before and after a reaction, the bonding mechanism and stability constant (log K) can be determined. Currently, the most widely utilized spectroscopic methods include fluorescence, ultraviolet-visible, infrared, X-ray absorption fine structure and electron paramagnetic resonance techniques.

1) Fluorescence spectroscopy

Fluorescence spectroscopy is a fast, simple and inexpensive method based on evaluation of the fluorescent properties of a substance. The fluorescence spectroscopic method encompasses both two-dimensional fluorescence spectrum and three-dimensional fluorescence spectrum. Figure 2.9 shows the basic schematic diagram of a fluorescence spectrophotometer.

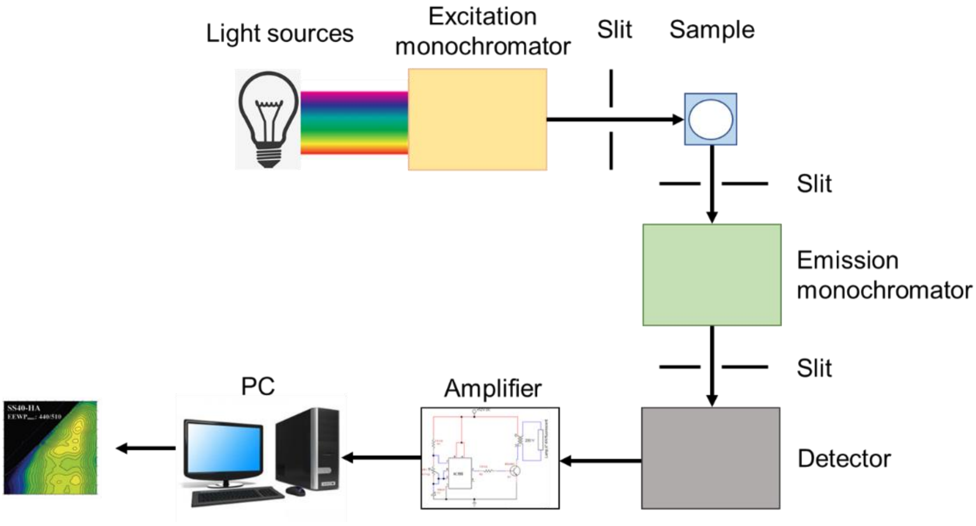


Figure 2.9 Basic schematic diagram of a fluorescence spectrophotometer.

During the two-dimensional fluorescence spectrum analysis, the excitation wavelength is fixed and the emission wavelength within a certain range is scanned. The result after scanning is a two-dimensional curve. The fluorescence quenching of HS can be calculated by using several fitting models and then stability constant between HS and metal can be achieved. The fitting models which are applied widely include NICA-Donnan and Stern-Volmer models (Kinniburgh et al., 1996; Puchalski et al., 1992). The NICA-Donnan model is a combination of the NICA and Donnan models. According to the two models, the addition of metal leads to the decrease of fluorescence intensity of HS. According to the fluorescence spectrum method, the stability constant (log *K*) of Fe (III) and Cu (II) are 4.19-5.33 and 4.67-5.10, respectively and the complexation capacity (*CC*) of Fe (III) and Cu (II) are 8.7-12 mmol/g HA and 0.21-0.98 mmol/g HA, respectively (Fuentes et al., 2013).

Three-dimensional fluorescence spectroscopy (EEM) is mainly used to study the reaction between mixed organic solution and metals. During the three-dimensional fluorescence spectrum analysis, a scanned area and steps of excitation/ emission wavelength are fixed on the basis of scan results of three-dimensional fluorescence spectrum. Natural organic matters can be divided into five regions. Region I and II include aromatic protein; region III includes fulvic acid-like substances (Ex < 250 nm, Em > 350 nm); region IV includes soluble microbial by-product-like substances (250 nm <Ex < 250 nm, Em < 380 nm); and region V includes humic acid-like substances (Ex > 250 nm, Em > 380 nm) (Chen et al., 2003). Parallel factor methods (PARAFAC) are often used in EEM analysis (Goldman et al., 2012; Sheng et al., 2013; Yu et al., 2010; Yu et al., 2011). It should be noted that, in general, EEM is not used to determine the reaction between single organic matter and metal, such as pure HA or FA.

2) Ultraviolet spectroscopy

Generally, ultraviolet spectroscopy is based on all-band ultraviolet scanning. For a specific ultraviolet band, the absorbance intensity of HS will change after complexing metals with a greater change in absorbance resulting from increasing metal concentration. By using the NICA-Donnan model or Ryan-Weber equation, the stability constant can be calculated (Bai et al., 2008; Yan et al., 2013a; Yan et al., 2013b).

However, overlapped spectra exists in traditional one-dimensional ultraviolet spectrum analysis. Recently, researchers have therefore combined both two-dimensional fluorescence and ultraviolet spectrum methods to circumvent the problems caused by overlapped spectra (Hur and Lee, 2011; Xu et al., 2013). For example, in order to study the interaction between three types of HS and Cu, Bai et al. used both two-dimensional fluorescence spectrum and ultraviolet spectrum, and then by employing the Ryan-Weber equation, determined the *CC* and log *K* values of the systems (Bai et al., 2008).

3) FTIR spectroscopy

The principle of FTIR spectroscopy is outlined previously in Section 2.2.3. Before and after the reaction between HS and metals, the functional groups of HS can undergo various chemical transformations which can be determined via FTIR spectroscopy (Jerzykiewicz, 2013; Lin et al., 2012; Reza et al., 2012). For example, the bands assigned to the stretching vibrations of C=O in the carboxylic groups (1720 cm^{-1}) and to the carboxylates (1650 cm^{-1}) experienced a reduction in their intensity when HA reacted with Hg (Jerzykiewicz, 2013).

The disadvantage of FTIR spectroscopy is its low sensitivity. When the concentration of metal added to experiment is low, an FTIR spectrum will not exhibit many (if any) distinct changes and thus, is not always applicable for quantitative research.

4) X-ray absorption fine structure spectroscopy

X-ray absorption fine structure (XAFS) spectroscopy is a physical measurement. When the energy of incident X-ray photons is equal to the ionization energy of certain inner electron which is included by the irradiate sample, the energy of incident X-ray photons will be absorbed largely, which leads to absorption edge. The fine structures are some distinguishable peaks and waviness near absorption edge (Penner-Hahn, 2004). XAFS includes extended x-ray absorption fine structure (EXAFS) and X-ray absorption near edge structure (XANES). When utilized to study the interaction between HS and metals, XAFS can judge what kind of functional group bind the target metal and whether bidentate ligand or polydentate ligand exists or not (Karlsson et al., 2005; Manceau and Matynia, 2010; Nebbioso and Piccolo, 2009; Xiong et al., 2013). The disadvantage of XAFS is that it is a semi-quantitative method rather than a quantitative method.

XANES is used to determine the coordination characteristic, bonding characteristics, valence state and spin states of target atom. For example, to study the valence state of Cr (VI) after reacting with HA, XANES was used in Jiang et al's research. The result showed Cr (VI) was reduced to Cr (III) when binding to HA (Jiang et al., 2014). EXAFS is employed to determine ligancy and change of bond length. For instance, Xiong et al. (2013) used EXAFS to determine the interaction between FA and Cr (III). The results showed a predominance of monomeric chromium (III)-FA complexes at low pH (<5), in which only Cr···C and Cr−O−C interactions were observed in the second coordination shell. At pH > 5 there were polynuclear chromium (III)-FA complexes with Cr···Cr interactions at 3.57 Å (Xiong et al., 2013).

5) Electron paramagnetic resonance spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy is used to detect paramagnetic substances including unpaired electrons and also to calculate the concentration of free radicals (Weil and Bolton, 2007). X-band, K-band and Q-band are used in EPR, and the most frequently-used microwave frequency is the X-band. EPR is a qualitative rather than a quantitative method and its sensitivity is low. The g-factor is an important parameter in EPR. After HS bind metals, by analysing EPR spectrum and g-factor, the changes in the types and concentration of free radicals can be evaluated. The results of such analyses can reflect the breakage and formation of old and new covalent bonds respectively that occur during the binding process between HS and metals (Hoffmann et al., 2013). For example, when HA bound with Cu^{2+}, Pb^{2+} and Sn^{2+}, g-factors would change significantly, indicating covalent bonds were formed between HA and the above three metals (Christoforidis et al., 2010). On the contrary, only electrostatic interaction existed for alkaline-earth metals (Christoforidis et al., 2010).

2.4.2.2 Chromatographic methods

HS is a kind of complicated macromolecular organic compounds. High-performance size exclusion chromatography (HPSEC) is widely used to determine the molecular weight of HS and works on the principle that substances of high molecular weight elute with a mobile phase earlier than those with low molecular weight. By using the linear relation between molecular weight and retention time, molecular weight of HS can be achieved (Liu et al., 2011; Trenfield et al., 2011; Trubetskoj et al., 2010). Specifically, HPSEC can determine the molecular weight of HS before and after binding metal, allowing for the binding process between HS with different molecular weight and metals to be studied (Laborda et al., 2008; Neubauer et al., 2013; Reiller et al., 2011). For instance, HPSEC was used to investigate the interaction between HA and Eu (III). The results showed that only 10% of HA fractions had stronger affinity for Eu (III) and they represented the lighter fractions which were retarded in the structure of the HPSEC immobile phase (Reiller et al., 2011). Additionally, HPSEC can be combined with other techniques, such as combined HPSEC-UV-ICP-MS method (Laborda et al., 2008; Liu and Cai, 2013).

2.4.2.3 Electrochemical methods

1) Stripping voltammetry method

The stripping voltammetry method is an electrochemical and quantitative method used for the determination of specific ionic species (Abate and Masini, 2002). It includes anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV). For example, by using CSV and the Scatchard equation, the log K of Pb and Cd were within the same order of magnitude, and binding of Pb^{2+} to HA was stronger than for Cd^{2+} (Abate and Masini, 2002).

2) Ion-selective electrode method

An ion-selective electrode (ISE) is a transducer that converts the activity of a specific ion into an electrical potential which can subsequently be measured by a voltmeter or pH meter (Tipping, 2002). Based on the Nernst equation, the voltage is theoretically dependent on the logarithm of the ionic activity (Voets et al., 2004). By using the ISE method, Liu et al. studied the complexation capacity of humic acid for Pb, Cu and Cd, and the binding strength of the three metals was found to follow the sequence of Pb>Cu>Cd (Liu and Gonzalez, 2000).

2.5 Application of humic substances in different remediation approaches

In terms of environmental remediation, HS have been applied as adsorbents, soil amendments, soil washing agents and also in other approaches (e.g. electrokinetic remediation and phytoremediation).

2.5.1 Application of HS as the adsorbents

Many approaches have been investigated to remove toxic metals from water, such as adsorption, chemical precipitation, ion exchange, membrane filtration, electrodialysis, photocatalysis and flotation (Mahmud et al., 2016; Rezania et al., 2016). Amongst them, adsorption is regarded as one of the most simple, cost-effective and efficient methods (Gupta et al., 2016; Gupta and Saleh, 2013) with the adsorbent playing a pivotal role in such an approach. Recently, humic acid-based materials have been considered as promising adsorbents and have attracted increasing attention (Ashraf et al., 2017; He et al., 2016; Lin et al., 2011; Yang et al., 2015; Yu et al., 2018).

Table 2.6 summarizes several humic acid-based adsorbents, related parameters and best-fit adsorption models. Current applied HA-based materials can be classified into four types: pure HA (e.g. soil HA, lignite HA and commercial HA), HA-based soil minerals (e.g. HA modified bentonite and HA modified montmorillonite); HA-based carbon materials (e.g. HA modified activated carbon) and HA-based metallic oxides (e.g. HA modified Fe_3O_4 and HA modified TiO_2). The studied metals include Cu^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Hg^{2+}, $Cr^{3+/6+}$, and $As^{3+/5+}$. For different HA-based materials, the adsorption capacity for same metal ion appears to vary greatly, which is caused by the different nature of HA-based materials and reaction conditions. For example, the maximum adsorption capacity ranges from 2.0 to 112.9 mg/g according to Table 2.6. For the same HA-based material, the adsorption capacity for different metals is widely varying due to differing affinities of metals towards an adsorbent (Irving and Williams, 1948). For example, Li et al. (2010) used a commercial HA to adsorb Cu^{2+} and Zn^{2+} and found that the adsorption capacity of Cu^{2+} was 23.0 mg/g while the value of Zn^{2+} was only 6.12 mg/g under the same reaction conditions (Li et al., 2010). The pH is the most studied environmental factor affecting the adsorption process of HA-based materials adsorption. Generally, the metal adsorption efficiency of HA-based materials decreases as pH decreases on account of increased H^+ competition for binding sites and electrostatic repulsion at low pH (Hamdaoui, 2017; Vidali et al., 2011; Zhang et al., 2016). Concerning kinetics, the pseudo-second-order model demonstrates the best fit for the majority of humic acid-based materials, indicating that chemisorption is the most probable rate-limiting step for adsorption of metal ions by many humic acid-based materials (Inyang et al., 2016; Xu et al., 2017). In terms of isotherm studies, the Langmuir model provides the best fit for most of the humic acid-based materials, indicating that the adsorption mechanism involves monolayer adsorption (Zhang et al., 2016).

Table 2.6 The related parameters and best-fit models of several humic-based adsorbents. (PFO and PSO are pseudo-first-order and pseudo-second-order kinetics respectively; L, F, L-F, bi-L, D-R are the Langmuir, Freundlich, Langmuir-Freundlich, bi-Langmuir, Dubinin-Radushkevich models respectively.)

Sample	Metal	Time (h)	pH	T (°C)	HA dose (g/L)	q_m (mg/g)	Kinetics	Isotherms	Thermodynamics	References
HA from soil	Co^{2+}, Cu^{2+}, Mn^{2+}	48	3.8-8.2	22	2	Co: 11.8-24.7 Cu: 26.4-38.1 Mn: 9.7-20.6	-	L	-	(Bibak, 1994)
HA from soil	Cu^{2+}, Ni^{2+}	6	2-3.7	25-45	1	Cu: 10.3-61.4 Ni: 13.7-29.2	-	L; F	-	(El-Eswed and Khalili, 2006)
HA from soil	V^{5+}	0-24	2-10	25	0.36-7.27	19.2	PFO; PSO	L	-	(Yu et al., 2018)
HA from lignite	Cu^{2+}, Ni^{2+}	2	2-5	20-65	1-4	Cu: 16.7-26.2 Ni: 12.9-21.2	-	L	$-\Delta S^0$, $-\Delta H^0$, $-\Delta G^0$	(Arslan et al., 2007)
HA (commercial)	Cu^{2+}	-	1-4	25	-	2.0	-	L; D-R	-	(Gezici et al., 2007)
HA (commercial)	Cu^{2+}, Zn^{2+}	0-3	3-8	10-30	1	Cu:23.0 Zn: 6.12	PSO	L	-	(Li et al., 2010)
HA from sediment	Cu^{2+}	24	2-4	25	0.5-5	7.55-85.1	-	L; F; bi-L	-	(Yang et al., 2015)
HA from sediment	Cu^{2+}	3	3-6	25	0.25	39.0-107.9	-	bi-L	-	(He et al., 2016)
HA modified starch hydrogel beads	Pb^{2+}	0-24	0.9-5.9	25-45	1	90.9-111.1	PSO	L	$+\Delta S^0$, $-\Delta H^0$, $-\Delta G^0$	(Chen et al., 2015)

Table 2.6 continued

Sample	Metal	Time (h)	pH	T (°C)	HA dose (g/L)	q_m (mg/g)	Kinetics	Isotherms	Thermodynamics	References
HA modified polymer/bentonite composite	Cu^{2+}	0-4h	2-6.5	20-50	2	101.3-112.9	PSO	L	$-\Delta S^0$, $+\Delta H^0$, $+\Delta G^0$	(Anirudhan and Suchithra, 2010)
HA modified Ca-montmorillonite	Cr^{3+}, Cu^{2+}, Cd^{2+}	0-5	5	25	20	Cr^{3+}: 15.7 Cu^{2+}: 15.3 Cd^{2+}: 14.1	PSO	F	-	(Wu et al., 2011)
HA modified zeolite	Cu^{2+}	0-6	3-7	25-45	0.4-2	19.8-21.5	PSO	L; F; D-R	$+\Delta S^0$, $+\Delta H^0$, $-\Delta G^0$	(Lin et al., 2011)
HA modified bentonite	Cu^{2+}	0-1.5	6.5	30	2	22.4	-	-	-	(Jin et al., 2016)
HA and goethite modified kaolinite clay	Cu^{2+}; Cd^{2+}; Zn^{2+}; Pb^{2+}, Ni^{2+}	0-6	3-9	30-60	5-100	Cu^{2+}: 1.7 Cd^{2+}: 15.5 Zn^{2+}: 0.93 Pb^{2+}: 6.1 Ni^{2+}: 13.88	-	L-F	$+\Delta S^0$, $+\Delta H^0$, $-\Delta G^0$	(Unuabonah et al., 2016)
HA modified HAP nanoparticles	Cu^{2+}	0-24	2.5-6.5	10-40	2	48.7-58.4	PSO	S	$+\Delta S^0$, $+\Delta H^0$, $-\Delta G^0$	(Yang et al., 2016)
HA modified activated carbon	Cu^{2+}	0-2	2-6	20	0.5	6.0	-	L	-	(Liu et al., 2014)
HA modified activated carbon	Cd^{2+}	0-3	1-8	20,25	2	12.3-26.3	PSO	L; F	-	(Jin et al., 2018)

Table 2.6 continued

Sample	Metal	Time (h)	pH	T (°C)	HA dose (g/L)	q_m (mg/g)	Kinetics	Isotherms	Thermodynamics	References
HA modified Fe_3O_4 nanoparticles	Cu^{2+}; Cd^{2+}; Hg^{2+}; Pb^{2+}	0.5	3-9	20	0.1	Cu^{2+}: 46.3 Cd^{2+}: 50.4 Hg^{2+}: 97.7 Pb^{2+}: 92.4	-	L	-	(Liu et al., 2008)
HA modified Fe_3O_4	Cu^{2+}	48	-	22	4	0.4-15.6	-	L, L-F	-	(Janoš et al., 2013)
HA modified Fe_3O_4 nanoparticles	Cr^{6+}	0-4	4-10	-	0.8; 10	3.37	PSO	L	-	(Jiang et al., 2014)
HA modified Fe_3O_4 nanoparticles	As^{3}, As^{5+}	0-3	3-8	25	0.2	As^{3+}: 12.2 As^{5+}: 12.6	PSO	F	-	(Rashid et al., 2018)
HA modified TiO_2 nanoparticles	Cd^{2+}, Cu^{2+}, Ni^{2+}	0-24	3-8	20	1	Cd: 9.9 Cu: 5.3 Ni: 24.2	PSO	L: Cd, Cu; F: Ni	-	(Mahdavi, 2016)

2.5.2 Application of HS as soil amendments

Soil amendment is regarded as a nondisruptive and cost-effective technique that can be used to remediate metal-contaminated soil (Houben et al., 2012). Soil amendments can not only reduce mobility and bioavailability of toxic metals, but also improve soil quality (e.g. soil organic matter content, enzyme activity, and nutrient status) (Janoš et al., 2010; Kumpiene et al., 2008; Pardo et al., 2014; Pérez-Esteban et al., 2014; Wu et al., 2017).

Humic acid can effectively complex with several metals and form stable metal–humic complexes, thereby reducing their mobility and bioavailability (Perminova and Hatfield, 2005; Stevenson, 1994). In addition, humic acid is also known to be a good plant-growth and mineral-nutrition promoter (Eyheraguibel et al., 2008; Lulakis and Petsas, 1995). Therefore, humic acid-based materials have been regarded as promising soil amendments (Clemente and Bernal, 2006; Janoš et al., 2010; Khan et al., 2017; Wang et al., 2017b; Wang and Mulligan, 2009; Yu et al., 2017).

Table 2.7 summarizes several humic acid-based soil amendments and their metal immobilization behaviour under different experimental conditions. Generally, HA can be applied in three different forms when applied as soil amendments: 1) pure humic acid, 2) materials rich in humic acid (e.g. compost from various sources), 3) humic acid-based materials and other soil amendments (e.g. fly ash, biochar) working synergistically.

The metal immobilization performance of amendments is affected by both the properties of the soil and characteristics of the humic acid-based materials. Soil pH is an important factor affecting metal mobility and bioavailability. For example, Khan et al. (2017) applied a commercial HA amendment in three soils and found that HA could reduce the bioavailability of Cd in acidic and neutral soil but increase it in alkaline soil. Additionally, the different affinities of metals (based on stability constants) for humic acid will affect the immobilization performance with the affinity generally following the Irving-William series. For instance, Chotpantarat et al. (2015) used a commercial HA amendment to remediate gold mine tailings and their results demonstrated that HA could dramatically decrease the mobility of Cu and Pb but slightly decrease that of Co and Zn, which was consistent with the Irving-William series (metal affinities decreasing in the order Pb > Cu > Zn >Co). In addition, it should be noted that the metal speciation distribution can also affect the metal immobilization behaviour of humic-based materials. Gusiatin and Kulikowska (2016) used a sewage sludge compost as a soil amendment and reported that it decreased the mobility of Cd, Ni and Zn but increased that of Cu and Pb, which was not consistent with the Irving-William series. One of the possible reasons was that Cd, Ni and Zn were primarily distributed within the exchangeable and acid soluble fractions (weakly bound) while Cu and Pb were predominately in the reducible fraction (strongly bound).

Table 2.7 Several humic acid-based soil amendments and their metal immobilization performance under different experimental conditions (all at lab scale).

Application type	Source of HA	Metals	Soil pH	Soil OM	Mix ratio (w/w, %) [a]	T/°C	Time/d	Metal mobility and bioavailability	Reference
Pure HA	HA from compost	Cu, Zn, Pb, Fe	3.4-4.7	0.7-0.8% OM	1.87%	26	196	pH=7.7: - pH=3.4: Cu↑, Zn↓, Pb↓, Fe↑	(Clemente and Bernal, 2006)
	HA from peat							pH=7.7: - pH=3.4: Cu↑, Zn↓, Pb↓, Fe↑	
	HA (commercial)	Cu, Pb, Cd	5.8-7.1	2.22-5.74%	1%	-	42	Cu: ↓; Cd, Pb: ↑	(Varrault and Bermond, 2011)
	HA (commercial)	Cu, Pb, Ni, Cd	7.71	3.82	0.1%	-	60	Cu, Pb, Ni, Cd: ↓	(Park et al., 2013)
	HA (commercial)	Cu, Zn, Pb, Ni, Cr, Co, Mn	8.25	1.27% OM	5-10%	-	30	↓: Cu, Zn, Pb, Cr, Co; ↑: Mn, Ni	(Chotpantarat et al., 2015)
	HA (commercial)	Cd	4.76-7.70	2- 19% OM	0.4%	-	14	pH=4.76: ↓ pH=6.91: ↓ pH=7.70: ↑	(Khan et al., 2017)
	HA salt from peat	Cd	4.94	4.82% OM	1%	20-25	42	Cd：↓（21 day） -（42 day）	(Yu et al., 2017)

Table 2.7 continued

Application type	Source of HA	Metals	Soil pH	Soil OM[a]	Mix ratio (w/w, %) [b]	T/°C	Time/d	Metal mobility and bioavailability[c]	Reference
Materials rich in HA	Lignite	Zn, Pb, Cd	5.05	0.67% OC	0.25%	-	365	Zn, Pb, Cd: ↓	(Kwiatkowska, 2006)
	Lignite	Cd	4.8-6.8	2.0-13.3% OC	1-10%		29	Cd: ↓	(Simmler et al., 2013)
	Lignite	Cu, As	7.3	0.43% OC	5-10%	20	270	Cu: ↓ As: ↑	(Tsang et al., 2013b)
	Lignite derived material	Zn, Pb, Cd	5.05	0.67% OC	0.32%	-	-	Zn, Pb, Cd: ↓	(Kwiatkowska-Malina, 2018)
	Compost from waste	Cu	7.0	3% LOI	1-2%	25	14	Cu: ↓	(Jones et al., 2016)
	Compost from sewage sludge	Cd, Cu, Ni, Pb, Zn	5.9	3.3% OC	20%	20	1050	Cd, Ni, Zn: ↓ Pb, Cu: ↑	(Gusiatin and Kulikowska, 2016)
HA based-material and other soil amendments working together	Peat & coal fly ash	Pb, Cu	4.1	0.03% OC	5% peat; 5% fly ash	-	500	Pb, Cu: ↓	(Kumpiene et al., 2007)
	Compost from waste & biochar	Cu	7.0	3% LOI	1-3%	25	14	Cu: ↓	(Jones et al., 2016)
	Compost from waste & red mud	Cu, Zn, Cd, Pb	8.3	1% OC	2.5% peat 2.5% red mud	25	60	Cu, Zn, Cd, Pb: ↓	(Zhou et al., 2017)

[a] OM: Organic matter, OC: Organic carbon, LOI: Loss on ignition;

[b] Mix ratio: the ratio of humic acid-based material and soil

[c]: Mobility decreased: ↓, Mobility increased: ↑, Mobility unchanged: –

2.5.3 Application of HS as soil washing agents

Soil washing is also regarded as one of the most efficient, rapid and cost-effective techniques for metal-contaminated soil remediation (Wuana and Okieimen, 2011). Humic substances are important components of soil organic matter and any residual HS left in the soil after soil washing can contribute to soil organic matter. Thus, the use of HS has the potential to be environmentally friendly compared with compounds such as EDTA.

Table 2.8 summarizes several HS/HA soil washing agents and their respective performances concerning metal removal under various experimental conditions. It can be seen that the % metal removal varies under different washing conditions such as initial washing agent pH and concentration, liquid/solid ratio (L/S), contact time and washing times. Typically, higher L/S ratio, higher HS concentration and more washing times lead to higher % metal removal as more metal binding sites are available. However, higher HS concentration will result in higher costs, higher L/S will increase the amount of wastewater generated by soil washing and greater washing times will lead to both. As such, it is important to select an optimal L/S ratio and HS concentration for economical and efficient practical applications. The effect of pH on % metal removal is not trivial. Generally, the % metal removal is low at low pH due to the competition from H^+ with metal ions for COOH binding sites (Kulikowska et al., 2015b). Additionally, at low pH, HS is less negatively charged and electrostatic attraction between HS and soil minerals (e.g. kaolinite, montmorillonite and hematite) increases (Chen et al., 2017; Hartley et al., 2014; Shaker and Albishri, 2014). As the HS is more readily adsorbed onto the soil, less is available for removal via complexation with the HS in solution (Hartley et al., 2014; Kulikowska et al., 2015b). However, this does not always appear to be the case. For example, Kulikowska et al (2015b) used HS from compost to wash three soils and the results showed that the highest % Cd removal was achieved using a pH of 3 and also that the % Cd removal decreased as pH increased.

Table 2.8 The metal removal performance and related experimental conditions of several HS/HA soil washing agents.

Washing agent source	Metals	Soil properties	pH	T (°C)	HS/HA concentration (mg/L)	Time (h)	L/S rario (mL/g)	Max % metal removal	Optimal condition	Reference
HS from cow slurry	Cu, Ni, Pb	pH: 5.3 OM:1.2 % OC Cu: 1150 mg/kg Ni: 90 mg/kg Pb: 58mg/kg	6	-	300 (C base)	1680	5:1	Cu: 39 % Ni: 13% Pb: 35 %	-	(Borggaard et al., 2009)
HS from forest litter								Cu: 38 % Ni: 11 % Pb: 37 %		
HS from forest litter								Cu: 34 % Ni: 9 % Pb: 34 %		
HS from cow slurry	Cd, Cu, Pb	pH: 7.5 OM: 1.4% OC Cd: 15.6 mg/kg Cu: 1000 mg/kg Pb: 500 mg/kg	6	-	300-1200 (C base)	24	5:1-100:1	Cd: 43.6 % Cu: 53.0 % Pb: 3.9 %	HS concentration: 1200 mg/L; L/S: 100:1	(Soleimani et al., 2010)
HA from leonardite	Cu, Cr, As	pH: 7.3 OM: 0.43% OC Cu: 2100 mg/kg As: 3300 mg/kg Cr:2800 mg/kg	4,8	20	20, 100	0-48	80:1	Cu: 37.7% As: 8.7% Cr: 1.6%	-	(Hartley et al., 2014; Tsang and Hartley, 2014; Tsang et al., 2013a)
HA (commercial)								Cu: 37.4% As: 9.5% Cr: 1.6%		
HS from lignite								Cu: 35.9% As: 8.8% Cr: 0.9%		

Table 2.8 continued

Washing agent source	Metals	Soil properties	pH	T (℃)	HS/HA concentration (mg/L)	Time (h)	L/S rario (mL/g)	Max % metal removal	Optimal condition	Reference
HS from compost	Cd, Cu, Ni, Pb, Zn	pH: 5.8-6.3 OM: 10.6-14.0% OM Cd: 46-47 mg/kg Cu: 981-1020 mg/kg Ni: 498-534 mg/kg Pb: 3924-4133 mg/kg Zn: 1952-2110 mg/kg	3-13	20	2170 (C base)	0-24	40:1	Cd: 73% Cu: 72% Ni: 36% Pb: 43% Zn: 34%	Cd: pH=3; Cu: pH=13; Ni: pH=3; Pb: pH=7; Zn: pH=3	(Kulikowska et al., 2015a)
HS from compost	Cd, Cu	pH: 6.1-7.1 OM: 1.6-10.3% OM Cd: 49.8-55.2 mg/kg Cu: 1984.1-2042.2 mg/kg	3-13	22-24	100-4000 (C base)	0-24	40:1	Cd: 53.2-80.7%; Cu: 36.5-69.1%	Cd: pH=5 or 7; Cu: pH=5 or 7; HS=3000 mg/L	(Kulikowska et al., 2015b)
HS from leonardite	Cd	pH: 5.51-6.57 OM:1.73-2.16% OC Cd: 1.33-6.57 mg/kg	3-9	20	157.5-4725 (C base)	0-12	5:1-30:1	88%-95%	pH: 6 HS concentration: 3150 mg/L; L/S: 5:1; washing time: 2h	(Meng et al., 2017)

2.5.4 Other application of HS

Electrokinetic remediation has been increasingly used in the remediation of metal-contaminated soils. Sawada et al. applied humic acid as a surfactant in an electrokinetic remediation system and the results showed that the amount of $Cu(OX)_2$ removed from the clay soils in the presence of HA was three times larger than those in the absence of HA (Sawada et al., 2003). In another example, Giannis et al. successfully used humic acid for electrokinetic remediation of cadmium-contaminated soil (Giannis et al., 2007).

Phytoremediation is an environmentally friendly approach for cleaning up soils contaminated by metals. HS can reduce readily soluble and exchangeable forms of metals in the contaminated soil whilst increasing the amount of plant-available forms (Park et al., 2013). HS can also increase the accumulation of metals in the shoots and roots of selected plants (Evangelou et al., 2004). The target contaminants include Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb and Zn (Vamerali et al., 2010).

2.6 Scientific gaps

Based on the above literature review, HS/HA have great potential for application in the remediation of soil and water contaminated by toxic metals. However, there are three remaining scientific gaps that must be addressed.

Most of the currently used HS/HA for soil/water remediation are derived from natural sources (e.g. soil, peat and lignite) or compost. Due to the variation in such sources, the structural characteristics of the applied HS/HA are generally diverse, resulting in disparate metal removal/immobilization behaviour. This is a great limitation which prevents the increased application and improved understanding of HS/HA utilization for soil/water remediation. As mentioned previously in Section 2.3, humic acid can also be synthesized by abiotic humification processes. Compared to natural humic acid, the advantage of synthetic humic-like acid (SHLA) is that the structural characteristics of SHLA can be manipulated to some extent by changing the reaction conditions of the abiotic humification process (e.g. the species and ratio of catalyst and precursors, pH and temperature) (Fukuchi et al., 2012; Jokic et al., 2004; Zhang et al., 2015). For example, the aliphatic structure content of SHLA could be increased by using Fe-loaded zeolite as a catalyst in place of an unloaded-zeolite (Fukuchi et al., 2012). If adding more catechol as a precursor, the N content of SHLA could be reduced (Zhang et al., 2015). However, current research concerning abiotic humification mainly focuses on the synthesis and characterization of SHLA products. Currently, studies that investigate the interaction between SHLA and metals and also the application of SHLA in metal-contaminated soil/water remediation are still rare. As such, three important questions can be formulated:

1. Can SHLA bind metals like natural HA and what is the metal binding performance of SHLA?
2. Can we use abiotic humification processes to synthesize SHLA with stronger metal binding ability? If yes, how can such processes be controlled to give optimal results?
3. Can we apply SHLA in environmental remediation and what types of remediation applications can SHLA be used in?

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CHAPTER 3

The copper complexation ability of a synthetic humic-like acid formed by an abiotic humification process and the effect of experimental factors on its copper complexation ability

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Chapter 3 The copper complexation ability of a synthetic humic-like acid formed by an abiotic humification process and the effect of experimental factors on its copper complexation ability

Ting Yang[a*], Mark E Hodson[a]

[a] Environment Department, University of York, Heslington, Wentworth Way, York YO10 5NG, United Kingdom

*Corresponding author. Tel.: +44 (0)7421 236832. Fax: +44 (0)1904 322998.

E-mail address: ty672@york.ac.uk

Abstract

Humic acids have an important impact on the distribution, toxicity and bioavailability of hazardous metals in the environment. In this study, a synthetic humic-like acid (SHLA) was prepared by an abiotic humification process using catechol and glycine as humic precursors and a MnO_2 catalyst. The effect of physico-chemical conditions (ionic strength from 0.01M to 0.5M $NaNO_3$, pH from 4 to 8, temperature from 25 °C to 45 °C and humic acid concentration from 5 to 100mg/L) on the complexation ability of SHLA for Cu^{2+} were investigated. A commercial humic acid (CHA, CAS: 1415-93-6) from Sigma-Aldrich was also studied for comparison. The results showed that for pH 4 to 8, the conditional stability constants (log *K*) of SHLA and CHA were in the range 5.63-8.62 and 4.87-6.23, respectively and complexation capacities (*CC*) were 1.34-2.61 mmol/g and 1.42-2.31 mmol/g, respectively. The Cu complexation ability of SHLA was higher than that of the CHA due to its higher number of acidic functional groups (SHLA: 19.19 mmol/g; CHA: 3.87 mmol/g), extent of humification and aromaticity (AL/AR: 0.333 (SHLA); 1.554 (CHA)), and O-alkyl functional groups (SHLA:15.56%; CHA:3.45%). The log *K* and complexation efficiency (fraction of metal bound to SHLA) of SHLA were higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration. Overall, SHLA was a good and promising complexation agent for copper in both soil washing of copper contaminated soil and the treatment of copper-containing wastewater .

Keywords: Abiotic humification; synthetic humic-like acid; copper; complexation

3.1 Introduction

Hazardous metal pollution is currently a serious environmental issue worldwide (Lesmana et al., 2009; Li et al., 2014). Human activities have introduced numerous potential hazardous trace elements into the environment. Elevated concentrations of metals in soils and water originate from many sources, including atmospheric deposition, sewage irrigation, intensive use of sewage sludge and compost, tanneries, mining activities, paper industries and use of pesticides and fertilizers (Fu and Wang, 2011; Li et al., 2014; Shi et al., 2009; Su, 2014). Unlike most organic contaminants, metals are of great concern because they are non-biodegradable and can accumulate in living organisms, posing a great threat to both human health and the environment (Lesmana et al., 2009). Hazardous metals of great concern in the treatment of water and soil include zinc, copper, cadmium, nickel, chromium and lead (Li et al., 2014; Tang et al., 2014). Among them, copper does essential work in animal metabolism. However, copper is very toxic at high concentrations, and can cause gastrointestinal problems, kidney damage, cramps, hair loss, convulsions, anemia, hypoglycemia, and even death (Fu and Wang, 2011; Tang et al., 2014). Permitted levels of Cu in soils and water vary across regulators, with land and water use, with soil and water chemistry and with protection targets (e.g. EPA, 2002, 2007; NRM, 2017; Recatalá et al., 2012) such that it is not meaningful to cite a single value for an acceptable soil or water Cu concentration. However, in all legislative regions across the globe examples can be found where Cu concentrations in soils and waters are above permitted levels and need to be reduced.

Although synthetic chelating agents such as DTPA and EDTA have been proposed as remedial agents given their ability to complex metals, these compounds can have adverse environmental and health effects (e.g. Peng et al., 2009). Humic substances (HS) are organic compounds that occur naturally in soils, waters and sediments. Humic acid (HA) is a major component of humic substances (Stevenson, 1994). HA has long been recognized to strongly complex metal ions and thus have an important impact on the distribution, toxicity and bioavailability of hazardous metals in the environment (He et al., 2016; Perminova and Hatfield, 2005; Sposito and Weber, 1986; Tang et al., 2014; Yang et al., 2016). The strong complexation ability with metal ions is usually attributed to the large number of oxygen-containing functional groups present in HA, including carboxyl, phenol, hydroxyl, enol and carbonyl structures of various types (Perminova and Hatfield, 2005). Among them, carboxylic group and phenolic-OH group are of particular importance for metal ion binding (Boguta et al., 2016; He et al., 2016; Vidali et al., 2011; Xu et al., 2016). The use of humic acids as complexation agents has been considered in soil remediation and water treatment; in polymer enhanced ultrafiltration (PEUF) metals are complexed and the resulting solutions filtered through membranes which have a pore size too small to permit passage of the complexed metals resulting in a cleaned solution (Perminova and Hatfield, 2005; Fu and Wang, 2011). The potential of PEUF methods has led to a large number of studies that show that humic acid can be used to form stable complexes with Cu^{2+}, Zn^{2+},Cr^{2+}, Cd^{2+}, Fe^{3+}, and Pb^{2+} (Dudare and Klavins, 2013; Fang et al., 2015; Fuentes et al., 2013; Gustafsson et al., 2014; Hernández et al., 2006; Pandey et al., 2000; Plaza et al., 2005a). Among these metals, the stability constant between copper and humic acid is highest (Pandey et al., 2000).

HAs used in most of the reported studies are natural humic acids extracted from soil, peat or leonardite, and have poorly defined structural characteristics (Chen et al., 2015; Fuentes et al., 2013; He et al., 2016; Plaza et al., 2005a, 2005b; Xu et al., 2016; Zherebtsov et al., 2015). It has been reported that materials containing metallic oxide (e.g., MnO_2, Fe_2O_3, and Al_2O_3) can catalyze and enhance the transformation of humic precursors (e.g. amino acids, sugars, and quinones) to humic substances; this process is called abiotic humification (Fukuchi et al., 2012; Fukushima et al., 2009a; Huang, 1995, 2000; Jokic et al., 2004; Nishimoto et al., 2013; Qi et al., 2012a, 2012b; Wang et al., 2000). To some extent the composition of humic acid can be controlled in abiotic humification reactions. Changing the species of humic precursors or catalyst used can alter the aromaticity and functional groups of the HA produced (Chen et al., 2010; Fukuchi et al., 2012; Hardie et al., 2009; Jokic et al., 2004). For example, the aliphatic structure content of SHLA can be increased when using Fe-loaded zeolite as the catalyst, compared with an unloaded-zeolite catalyst (Fukuchi et al., 2012) and by adding more catechol as a precursor, the N content can be reduced (Zhang et al., 2015). Controlling the humification processes raises the possibility of manipulating the composition of HAs to increase their complexation capacity for use in remediation. Recently, it has been reported that a humic-like substance associated with bentonite and synthesized by abiotic humification can be used to adsorb pentachlorophenol (Fukushima et al., 2014). However, studies on how to use synthetic HAs as metal complexing agents are still rare.

In this study, a synthetic humic-like acid (SHLA) was prepared via abiotic humification using catechol and glycine as humic precursors in the presence of a MnO_2 catalyst. These were chosen because they have been used in previous studies on the abiotic synthesis of humic acid (Hardie et al., 2009; Jokic et al., 2004; Zhang et al., 2015). The Cu^{2+} was chosen as the target metal. The complexation efficiency, conditional stability constant, complexation capacity and chemical characteristics of the SHLA were determined, and compared to a commercial humic acid available from Sigma-Aldrich. The effects of pH (4 - 8), ionic strength (0.01 - 0.5M $NaNO_3$), temperature (25 - 45 °C), and the concentration (5 - 100 mg/L) of SHLA on complexation between Cu^{2+} and the SHLA were investigated.

3.2 Materials and Methods

3.2.1 Materials

Catechol ($C_6H_6O_2$, >99% purity), glycine ($C_2H_5NO_2$, >99% purity), glucose ($C_6H_{12}O_6$, >99% purity), thimerosal ($C_9H_9HgNaO_2S$, >97% purity), manganese oxide (MnO_2, >99% purity), commercial humic acid (CHA, technical grade, CAS: 1415-93-6) and copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$, >99% purity) were purchased from Sigma-Aldrich. Sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$, >99% purity), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot H_2O$, >99% purity), sodium nitrate ($NaNO_3$, >99% purity) and barium hydroxide octahydrate ($Ba(OH)_2 \cdot 8H_2O$, >98% purity) were purchased from ACROS Organics. Calcium acetate monohydrate ($C_4H_6CaO_4 \cdot H_2O$, >99% purity) was purchased from Alfa Aesar. Sodium hydrogen carbonate ($NaHCO_3$, >99% purity) was purchased from Fisher Chemical. Ultra-pure water was prepared by PURELAB Classic ultra-pure system (ELGA, UK) from deionized water.

3.2.2 Preparation of a synthetic humic-like acid by abiotic humification

To ensure that the humification was abiotic, sterile conditions were maintained throughout the experiments. Pure water and glassware were autoclaved (121 °C for 27 minutes, 0.12 MPa). 1L autoclaved phosphate buffer (pH 7, 0.2 M Na_2HPO_4 / NaH_2PO_4 autoclaved at 121 °C for 27 minutes, 0.12 MPa) containing thimerosal (0.02% w/v) was prepared in a 3L beaker, to which 25 g of MnO_2 was added. Then catechol and glycine were added to give concentrations of 1 M for each of the two chemicals and the mixture stirred at 45 °C for 240 h in the dark on a heated magnetic stir plate (IKA, Germany). After incubation, SHLA was extracted from the dark brown solution and purified using the standard method recommended by the International Humic Substances Society (IHSS) (Swift., 1996). Details of the extraction and purification process of SHLA are given in the Supplementary materials.

3.2.3 Characterization of synthetic humic-like acid (SLHA) and CHA

3.2.3.1 Elemental analysis

The elemental composition (C, H, N) of the SHLA and CHA were determined using a vario MICRO cube type elemental analyzer (Elementar, Germany). The percentage of oxygen was calculated by subtracting the sum of C, H, and N from 100. Peat (NJV942, from LGC company) and organic sediment (B2150, from Elemental Microanalysis, National Institute of Standards and Technology (N.I.S.T.), U.S. Department of Commerce) standards were used as reference materials and to calculate analytical accuracy. The accuracy for C, N and H analysis was 86%-94%, 99%-103% and 86%, respectively. Sulphanilic acid (from Merck Company) was used as a drift corrector and to calculate precision. The precision, based on the standard deviation of 7 analyses carried out over the course of the analytical run for C, N and H were 0.16%, 0.54% and 0.22%, respectively. Before analyses, powdered SHLA and CHA were dehydrated under -0.1Mpa for 24 h at room temperature.

3.2.3.2 FTIR

FTIR spectra of the SHLA and CHA were obtained using an Alpha FT-IR Spectrometer (Bruker, Germany). Spectra were recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

3.2.3.3 Acid functional groups

Total acidity and carboxylic group content were measured using the barium hydroxide and calcium acetate methods. The phenolic-OH content was calculated as the difference between total acidity and carboxylic group content (Schnitzer and Kahn, 1972).

3.2.3.4 Ratio of the absorbances at 465 and 665 nm (E_4/E_6)

The UV spectra of the SHLA and CHA were determined using a Lambda 25 UV/Vis Spectrophotometer (PerkinElmer, USA). Absorbance at 465 and 665 nm (E_4 and E_6) was recorded on solutions of 20 mg of each HA dissolved in 100 ml of 0.05 M $NaHCO_3$, with the pH adjusted to 8 with NaOH and HCl. The E_4/E_6 ratio gives an indication of the degree of humification and the molecular weight of the humic acid was calculated as the ratio of the absorbances at 465 and 665 nm (Chen et al., 1977; He et al., 2016; Zalba et al, 2016).

3.2.3.5 Solid-state CP-MAS^{13} C NMR

Solid-state CP-MAS 13C NMR spectra of the SHLA and CHA were measured using an HD 400 spectrometer (Bruker, Germany) using the following conditions: spectrometer frequency, 100.64 MHz; contact time, 2 ms; pulse delay, 2.0 s; line broadening, 300 Hz; scans numbers, 800. The spectra were integrated into 6 chemical-shift regions: 0–45 ppm, 45–110 ppm, 110–140 ppm, 140–160 ppm, 160–185 ppm, and 185-215 ppm. The integrated areas were determined using Topspin 3.5 software. The aromaticity and AL/AR ratios (i.e. the ratio of alkyl to aromatic carbons) were calculated using equations (1) and (2) (Qi et al., 2012a):

$$\text{Aromaticity}=\frac{A_{Ar}(110\text{-}160\ \text{mm})}{A_{Ar}(110\text{-}160\ \text{mm})+A_{Alk}(0\text{-}110\ \text{mm})} \qquad (1)$$

$$\text{AL/AR}=\frac{A_{Alk}(0\text{-}110\ \text{mm})}{A_{Ar}(110\text{-}160\ \text{mm})} \qquad (2)$$

where A_{Alk} and A_{Ar} represent the peak integration values for alkyl and aromatic carbons, respectively.

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

For the complexation experiments, 100 mg SHLA or CHA were dissolved in 50 ml of 1 M NaOH, and then 50 ml of 1 M HNO_3 was added. The HA solution was then diluted with ultrapure water to a volume of 1L. 50 ml of the CHA and SHLA HA solution and 50 ml of $Cu(NO_3)_2$ (2 µM, 10 µM, 20 µM, 60µM, 100 µM, 140 µM, 200 µM, 250 µM and 300 µM) in 0.2 M $NaNO_3$ were mixed. This produced solutions at nine Cu^{2+} concentrations (1 µM, 5 µM, 10 µM, 30µM, 50 µM, 70 µM, 100 µM, 125 µM and 150 µM) each with an HA concentration of 50 mg/L in a background electrolyte of 0.1 M $NaNO_3$. Experiments were performed at 25 °C and an initial pH of 4, 6, 7 and 8, obtained by addition of 0.1M NaOH or 0.1M HNO_3.

Complexation experiments at different ionic strengths, used 100 mL of solution of different concentrations of Cu^{2+} (1 µM, 5 µM, 10 µM, 50 µM, 100 µM, 125 µM and 150 µM) and 50 mg/L of humic acids at 25 °C and pH=6. The initial ionic strength in solution was set to 0.01M $NaNO_3$, 0.05 M $NaNO_3$, 0.1 M $NaNO_3$ and 0.5M $NaNO_3$.

Complexation experiments at different HA concentrations, used 100 mL of solutions of different concentrations of Cu^{2+} (1 µM, 5 µM, 10 µM, 50 µM, 100 µM, 125 µM and 150 µM) at 25 °C, 0.1 M $NaNO_3$ and pH=6. The initial humic acid concentration in solution was set to 5 mg/L, 50 mg/L and 100 mg/L. Complexation experiments at different temperatures, used 100 mL of solutions of different concentrations of Cu^{2+} (1 µM, 5 µM, 10 µM, 50 µM, 100 µM, 125 µM and 150 µM) and 50 mg/L of humic acids at 0.1 M $NaNO_3$ and pH=6. The initial temperature in solution was set to 25 °C, 35 °C and 45 °C.

All solutions were shaken orbitally in a water bath for 24 hours at 150 rpm and then free Cu^{2+} concentrations were measured using a Cu-ion selective electrode (Orion, model 9629 BNWP). The initial Cu^{2+} concentrations were determined on control, HA-free solutions. All the measurements were performed in triplicate. Prior to experiment, the electrode was calibrated using serially diluted Cu^{2+} stock solutions at the working ionic strength, following the procedures of the ISE manual (Thermo Scientific Orion, 2008). Calibration was repeated every hour, and direct electrode measurements were reproducible within ±4%.

The 1:1 stoichiometric model has been successfully applied to study the binding abilities between HA and Cu (Ružić, 1982; Tipping, 2002), and the stability constants (log *K*) and complexation capacities (*CC*) obtained represent the average properties of the ligands contained in the humic structures (Fuentes et al., 2013; Plaza et al., 2005; Ružić, 1982). Therefore, the reaction between HA and Cu (M) can be quantitatively described by equation (3).

$$M + HA \Leftrightarrow MHA \quad (3)$$

The conditional stability constant (*K*) which represents the average binding ability was calculated as:

$$K = \frac{[MHA]}{[M][HA]} \quad (4)$$

The mass balance equations for the ligand are

$$CC = [MHA] + [HA] \quad (5)$$

where CC is the apparent complexation capacity (Plaza et al., 2005a).

[MHA] is calculated by mass balance as:

$$[MHA] = M_0 - [M] \quad (6)$$

where M_0 is the initial Cu^{2+} concentration and M is free Cu^{2+} concentration after 24h. Combining equations (4), (5) and (6) and rearranged into a linear form gives equation (7):

$$\frac{1}{M_0 - [M]} = \frac{1}{K \cdot CC \cdot [M]} + \frac{1}{CC} \quad (7)$$

Linear regression analysis was performed for $1/(M_0-[M])$ and $1/[M]$ allowing K and C_t to be calculated (Ružić, 1982).

Complexation efficiency at different copper concentrations was calculated as:

$$\text{Complexation efficiency} = \frac{M_0 - M}{M_0} \times 100\% \quad (8)$$

where M_0 is the initial Cu^{2+} concentration and M is free Cu^{2+} concentration after 24h.

3.2.5 Thermodynamic study

Log *K* values at 25℃, 35℃ and 45℃ were used to determine the thermodynamic parameters. The thermodynamic parameters (free energy change (ΔG), the entropy change (ΔS) and enthalpy change (ΔH)) were calculated following the method of Garcia-Valls and Hatton (Garcia-Valls and Hatton, 2003).

$$\Delta G = -RT\ln(K) \quad (9)$$

$$\ln(K) = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \quad (10)$$

where *R* (8.314 J/(mol·K) is the ideal gas constant, *T* (K) is the absolute temperature, and *K* is the conditional stability constant.

3.2.6 Statistical analysis

The Shapiro-Wilk test was used to assess whether the data were subject to normal distribution. T-tests were used to determine whether there was a significant difference in complexation ability between SHLA and CHA. The non-parametric Friedman test was used to assess whether the log *K*, *CC* and complexation efficiency of SHLA changed significantly as a result of changes in pH, ionic strength, temperature and humic acid concentration. The level of significance between the data was set to $p < 0.05$. Data analysis was carried out using SPSS 23.0 (IBM, USA).

3.3 Results and Discussion

3.3.1 Characterization and Cu complexation ability of synthetic humic-like acid (SHLA) and commercial humic acid (CHA)

3.3.1.1 Characterization of synthetic humic-like acid and CHA

The SHLA and CHA were characterized by determining the elemental composition, acidic functional group content and E_4/E_6, and by recording FTIR and ^{13}C NMR spectra. Compared with CHA, SHLA had a higher concentration of acidic functional groups, and higher degrees of humification and aromatization.

(a) Elemental composition

The elemental composition and atomic ratios of SHLA and CHA are summarized in Table 3.1.

Table 3.1 Elemental composition, acidic functional group content and E_4/E_6 of the SHLA and CHA (n=3; mean value ± standard deviation).

Samples	N/%	C/%	H/%	O/%	N/C	H/C	O/C	COOH (mmol/g)	Phenolic-OH (mmol/g)	Total acidity (mmol/g)	E_4/E_6
SHLA	4.61±0.02	53.81±0.07	2.97±0.06	38.61±0.09	0.086±0.000	0.055±0.001	0.717±0.002	4.59±0.34	14.61±4.45	19.19±4.79	3.18±0.00
CHA	0.94±0.02	46.29±0.06	2.99±0.09	49.78±0.08	0.020±0.000	0.064±0.002	1.075±0.003	1.22±0.34	2.65±0.34	3.87±0.68	4.32±0.01

Compared with CHA, SHLA had larger C and N contents and a greater N/C ratio which generally indicates a higher degree of humification (Mahieu et al., 2000; Qi et al., 2012a; Tu et al., 2017). The O/C ratio of SHLA (0.717) was lower than that of CHA (1.075), suggesting that CHA had more oxygen-containing functional groups (Fukushima et al., 2014). The lower H/C ratio of SHLA (0.055) indicates that it has more aromatic structures than CHA (0.064) (Qi et al., 2012a).

(b) Acidic functional group content and E_4/E_6

Table 3.1 shows the acidic functional group content and E_4/E_6 ratio of the SHLA and CHA. SHLA contained 3.8 times as many carboxylic acids groups and 5.5 times as many phenolic hydroxyl groups as CHA. Thus, SHLA has a higher concentration of acidic functional groups than CHA, suggesting that SHLA has more hydrophilic properties than CHA (Nagasawa et al., 2016). These results, together with the higher O/C ratio of CHA indicate that the oxygen-containing functional groups in CHA may be based on ketone, ethers, aldehydes, quinones or esters (Fukushima et al., 2014).

The E_4/E_6 ratio is related to the degree of condensation of the aromatic carbon network, the degree of humification and the molecular weight of the humic acid (Chen et al., 1977; He et al., 2016; Zalba et al., 2016). SHLA had a lower value of E_4/E_6 value than CHA (3.18 vs 4.32) indicating a more aromatic structure, a higher degree of humification and a larger molecular weight (Chen et al., 1977; He et al., 2016; Zalba et al., 2016), consistent with the elemental analysis.

(d) FTIR

The FTIR spectra of SHLA and CHA are shown in Figure 3.S1. Several difference are apparent. Assignment of the absorption bands in Figure 3.S1 is given in Table 3.2. The absence of a peak at 1710 cm^{-1} for CHA is consistent with its lower carboxylic acid group content.

(f) Solid-state CP-MAS^{13} C NMR

The Solid-state CP-MAS ^{13}C NMR spectrums of SHLA and CHA are shown in Figure 3.S2 and show differences. Peak assignments for the spectra are given in Table 3.3. The percentages of both phenolic carbon (140-160 ppm) and carboxylic carbon (160-185 ppm) of SHLA were higher than those of CHA, consistent with the determination of acidic functional group contents. The alkyl carbon content of CHA (52.23%) was much higher than that of SHLA (6.67%), indicating that CHA has more aliphatic characteristics. The lower AL/AR ratio (AL/AR: 0.333 (SHLA); 1.554 (CHA)) and higher aromaticity (aromaticity: 0.750 (SHLA); 0.391 (CHA)) of SHLA relative to CHA indicate that SHLA has a lower degree of unsaturation (Qi et al., 2012a; Tu et al., 2017). The above results are consistent with the lower H/C ratio of SHLA determined by elemental analysis.

Table 3.2 Assignments of FTIR absorption bands present in the spectra for SHLA and CHA (Fukushima et al., 2009a, 2009b; Hardie et al., 2009; Rodrigues et al., 2009; Qi et al., 2012a)

SHLA		CHA	
Wavenumber/cm^{-1}	Assignment	Wavenumber/cm^{-1}	Assignment
2925	C-H stretching of aliphatic structures	2921	C-H stretching of aliphatic structures
1715	C=O stretching of carbonyl groups		
1592	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)	1572	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)
1439	O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and C-O stretch of COO-	1375	O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and C-O stretch of COO-
1175	C–O stretching and C–O–H deformation of alcohols, phenols and ethers		
		1008	C-O stretching vibration in alcohols, phenols and ethers

Table 3.3 Interpretation of the ^{13}C NMR spectra of SHLA and CHA (Fernández-Gómez et al., 2015; Nagasawa et al., 2016)

SHLA		CHA	
Chemical shift /ppm	Assignment	Chemical shift /ppm	Assignment
33	Methylene carbon in long aliphatic chains	29	Methylene carbon in long aliphatic chains
45	Methyl and methylene groups in aliphatic rings and chains		
		76	Aliphatic alcohols and esters from carbohydrates and flavonoids
118	Protonated aromatic carbon, and/or aromatic carbon ortho to oxygen-substituted aromatic carbon		
		130	Aromatic carbon bound to H
145	Phenolic carbon		
171 and 183	Carboxyl carbon	176	Carboxyl carbon

3.3.1.2 The Cu complexation ability of synthetic humic-like acid

Figure 3.1 shows the conditional stability constants (log *K*) and complexation capacity of SHLA and CHA at pH values of 4 – 8 for HA concentrations of 50 mg/L, a temperature of 25 °C and an ionic strength of 0.1M.

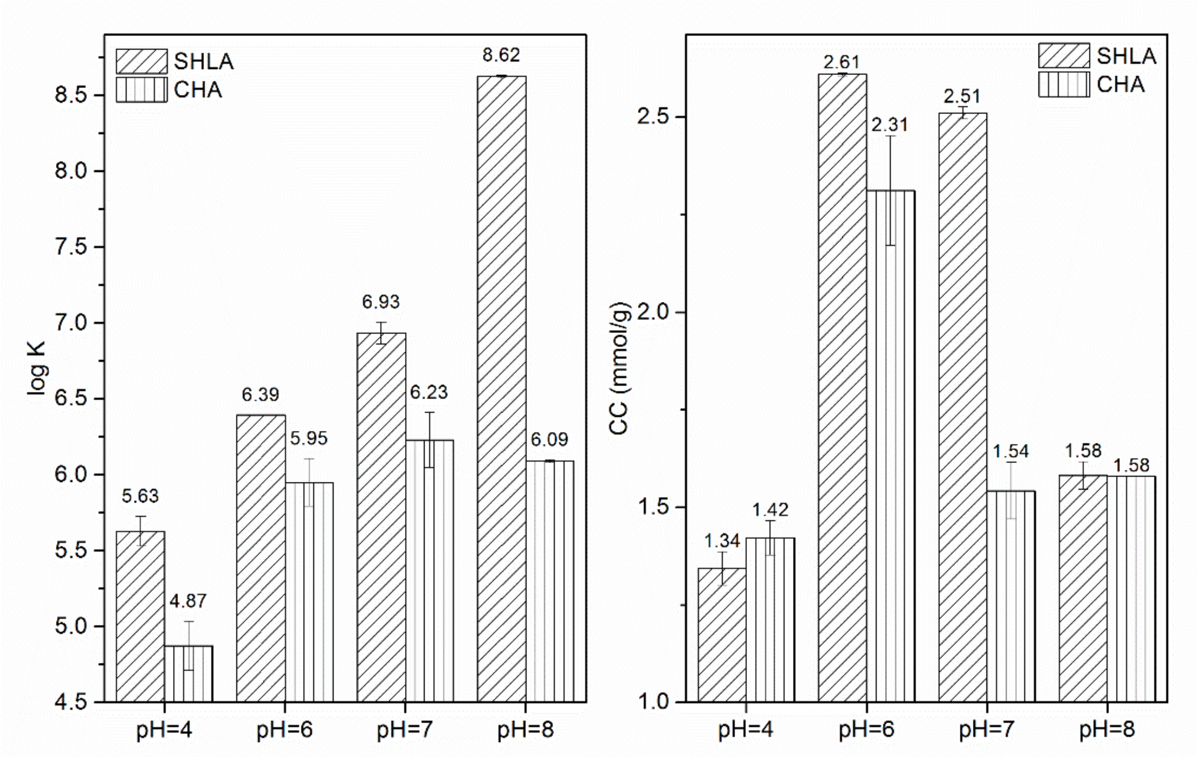

Figure 3.1 Mean conditional stability constant (log *K*) and complexation capacity (*CC*) of SHLA and CHA (mean values, n=3 ± standard deviation).

The log *K* values of SHLA were significantly higher than those of CHA ($p<0.05$). The complexation capacities of SHLA were higher at pH 6 and pH 7 ($p<0.05$) and similar at pH 4 and pH 8, compared with CHA. The stronger Cu^{2+} complexation ability of SHLA was due to its composition. Carboxylic and phenolic functional groups play a significant role in binding metals (Alvarez-Puebla et al., 2004; Baken et al., 2011; Kautenburger et al., 2014; Zherebtsov et al., 2015) as do acidic functional groups (Tipping, 2002; Baker and Khalili, 2005), and all are present at higher concentrations in SHLA than CHA (Table 3.1). Although both SHLA and CHA contain a higher concentration of carboxylic groups than phenolic groups (Table 3.1), our current data do not allow us to state which of these groups is more important in the complexation reaction. The larger log *K* values of SHLA are related to the higher aromaticity and higher degree of humification of the SHLA relative to the CHA. It has been demonstrated that extremely stable, Cu^{2+}-bearing, salicylate-like and catechol-like ring structures can be formed by an aromatic COOH and adjoining phenolic-OH or two adjoining aromatic COOH. (Fuentes et al., 2013; Hernández et al., 2006; Xu et al., 2016). Fuentes et al. report that the log K of Cu is positively correlated to numbers of O-alkyl groups (Fuentes et al., 2013). The NMR data indicated that the O-alkyl carbon content of SHLA was 12% higher than that of CHA.

Synthetic organic chelating agents (e.g. EDTA, DTPA, etc) and natural organic acids (e.g. oxalic acid, citric acid, and tartaric acid, etc) can also be used as metal chelate agents (Alkorta et al., 2004; Yuan et al., 2007). The sequence of log K_{Cu} is: DTPA (20.5-21.9) > EDTA (18.8-19.4) > SHLA (5.6-8.6) > citric acid (5.2-7.3) > oxalic acid (4.5-5.1) > tartaric acid (3.2-3.4) (Baumann, 1974; Buffle et al., 1977; Playle et al., 1993; Martell and Smith., 1974, Yuan et al., 2007).

Though synthetic chelate agents have much higher metal complexation efficiency than humic acids, synthetic chelate agents such as EDTA and DTPA can lead to adverse health and environmental effects, which restricts their use (Peng et al., 2009). Humic substances are important components of soil organic matter (Stevenson, 1994) and if SHLAs were used in soil washing, any residual SHLA left in the soil could contribute to soil organic matter. Thus the use of SHLAs has the potential to be environmentally friendly compared to using compounds such as DTPA or EDTA though clearly before their widespread use their application would need to be assessed for any possible toxic side effects. Previous research on natural humic acids from various sources (i.e. peat, soil, leonardite, etc) indicates that log K_{Cu} values ranged from 4.58 to 5.36 at similar reaction conditions to those considered here (Fuentes et al., 2013; Paradelo et al., 2012; Plaza et al., 2005a). The higher log K_{Cu} of the SHLA (5.63-8.62) suggests that it can be a reasonable substitute for natural humic acids as a metal chelating agent.

3.3.2 The effect of experimental factors on the complexation of Cu^{2+} on synthetic humic-like acids

The complexation ability of humic acid can be influenced by pH, ionic strength, temperature, and the concentration of humic acids (Tipping, 2002). In our tests the effect of these factors on the complexation of Cu^{2+} by SHLA was similar to the effects reported on natural humic acids.

3.3.2.1 The effect of pH

In this study, pH was set to 4, 6, 7 and 8 to investigate the effect of pH on complexation of Cu^{2+} by SHLA at an SHLA concentration of 50 mg/L, temperature of 25 ℃ and ionic strength of 0.1M (Figure 3.2)

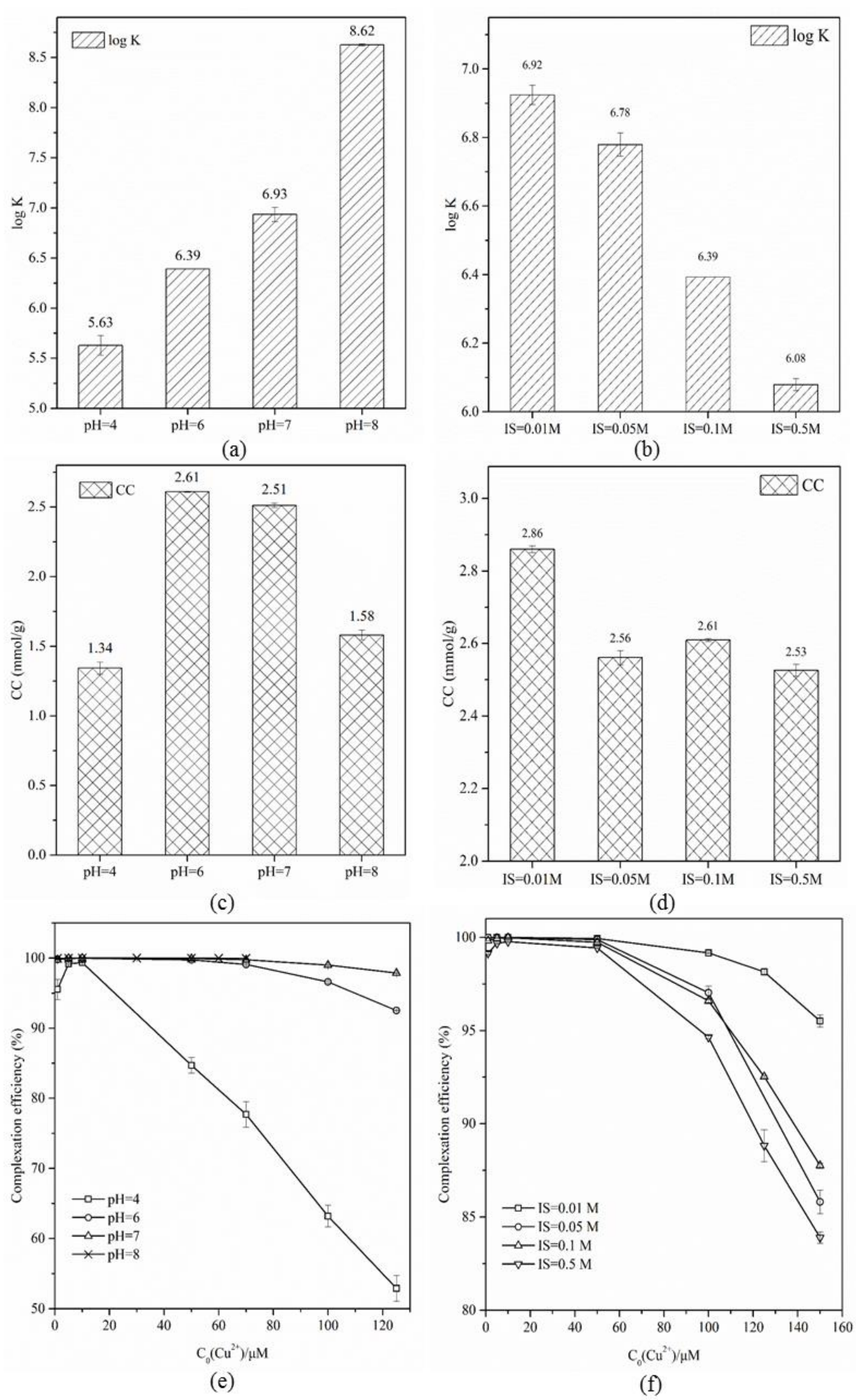

Figure 3.2 The effect of pH and ionic strength on complexation of Cu^{2+} and SHLA (a & b: log *K*; c & d: complexation capacity; e & f: complexation efficiency; SHLA concentration of 50 mg/L and temperature of 25 °C; mean values, n=3 ± standard deviation).

The complexation efficiency and log *K* increased with pH ($p<0.05$). This trend is consistent with studies of natural humic acid (Christl, 2012; Vidali et al., 2011; Xu et al., 2016). Proton concentration is higher at lower pH and protons will compete for binding sites with Cu^{2+} (Tipping, 2002; Christl, 2012). Also, at lower pH conditions the acidic functional groups of SHLA will be less deprotonated, providing fewer binding sites for Cu (Cao et al., 2004; Fang et al., 2015; Lippold et al., 2007). By increasing the pH, the negative charge of SHLA will increase as carboxylic groups and phenolic OH groups deprotonate; this enhances the electrostatic interaction between Cu^{2+} and the SHLA (Cao et al., 2004; Vidali et al., 2011). The complexation capacity increased from pH 4 to 6 ($p<0.05$), and decreased from pH 7 to 8 ($p<0.05$). The decrease at pH 8 might be caused by changes in the speciation of Cu due to increases in the concentration of OH^- (Liu et al., 2014).

3.3.2.2 The effect of ionic strength

Experiments were conducted at ionic strengths of 0.01 M, 0.05 M, 0.1 M and 0.5 M to investigate the effect of ionic strength on complexation of Cu^{2+} by SHLA at the SHLA concentration of 50 mg/L, temperature of 25 °C and pH of 6 (Figure 3.2).

When ionic strength increased from 0.01M to 0.5M, both the complexation efficiency and log *K* decreased sharply ($p<0.05$). Complexation capacity decreased ($p<0.05$) when ionic strength increased from 0.01M to 0.05 M and did not change much when ionic strength increased from 0.05 M to 0.5 M. The results are consistent with other studies on natural humic acids (Tipping, 2002; Xu et al., 2016). Ionic strength can affect the electric potential and spatial structure of SHLA thereby influencing the complexation ability of SHLA (Fang et al., 2015). As ionic strength increases, counter ion condensation in the HA's diffuse double layer increases as well, which attenuates the ability of binding sites to bind Cu^{2+} (Cao et al., 2004; Tipping, 2002; Vidali et al., 2011). At the same time the electrostatic repulsion between adjacent functional groups can be lowered causing the humic acid to fold up on itself to form a more compact shape (Chin and Gschwend, 1991). This reduces the diffusion of Cu^{2+} to binding sites (Fang et al., 2015).

3.3.2.3 The effect of temperature

Experiments were conducted at 25 °C, 35 °C and 45 °C to investigate the effect of temperature on complexation of Cu^{2+} by SHLA at an SHLA concentration of 50 mg/L, ionic strength of 0.1M and pH of 6 (Figure 3.3).

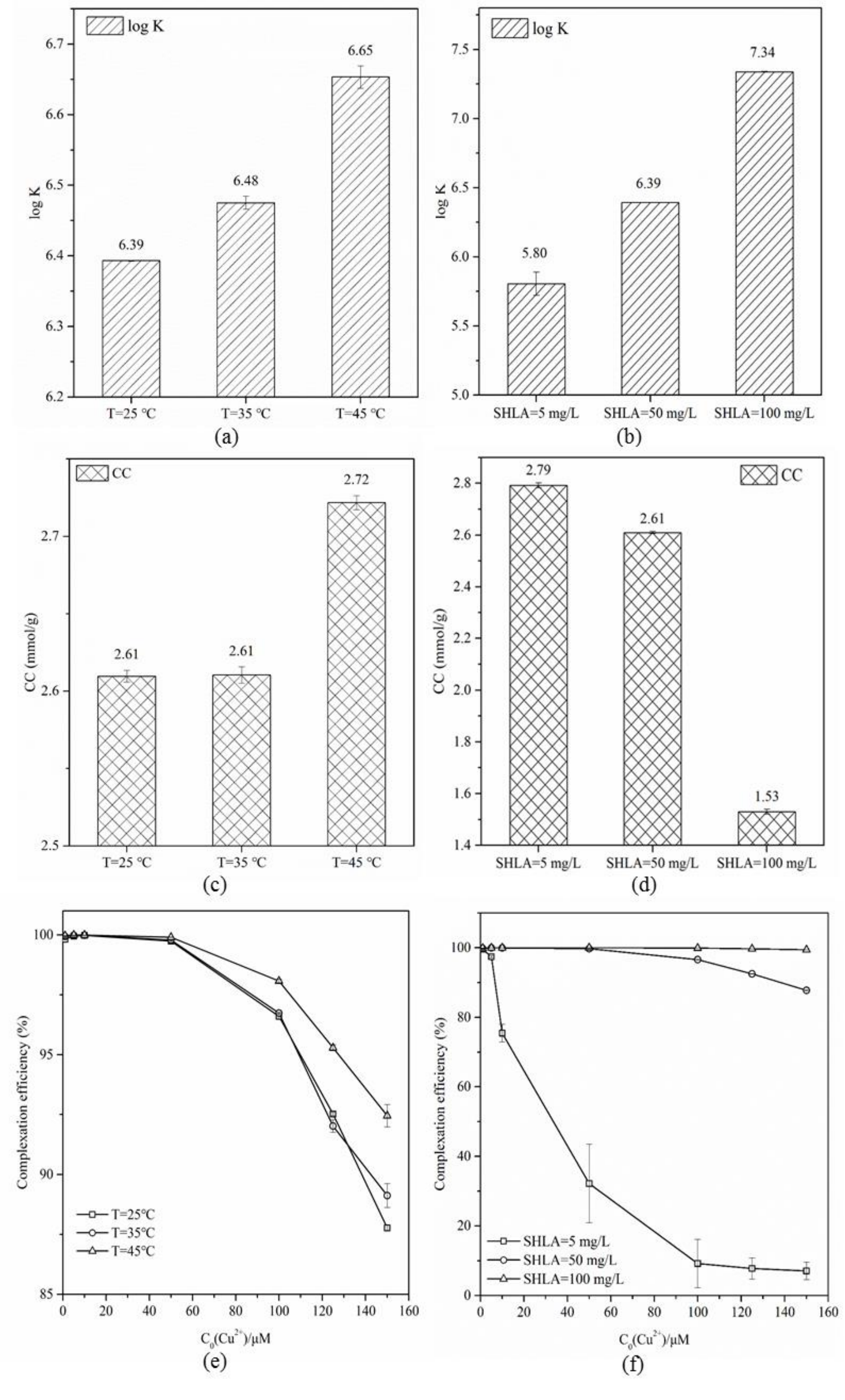

Figure 3.3 The effect of temperature and SHLA's concentration on complexation of Cu^{2+} and SHLA (a & b: log *K*; c & d: complexation capacity; e & f : complexation efficiency; ion strength of 0.1M and pH of 6; n=3 for each condition; error bar indicates the standard deviation).

The complexation efficiency (from 87.8% to 92.5%, $p<0.05$), log *K* (from 6.39 to 6.65, $p<0.05$) and complexation capacity (from 2.61 mmol/g to 2.72 mmol/g, $p = 0.097$) increased with increasing temperature.

Thermodynamic parameters for the Cu^{2+}-SHLA complexation reaction are shown in Table 3.4. The positive ΔH indicated that complexation reaction between Cu^{2+} and SHLA was endothermic, which would result in the trend seen in our experiments and was consistent with previous studies (Baker and Khalili, 2003, 2005). The negative ΔG suggested that the Cu^{2+}-SHLA complexation reaction was spontaneous. With increasing temperature from 25℃ to 45 ℃, ΔG^{o} decreased from -4.60 kJ/mol to -5.01 kJ/mol, indicating that higher temperatures favoured complexation.

Table 3.4 Thermodynamic parameters for the complexation reaction between Cu^{2+} and SHLA.

T (K)	ΔG (kJ/mol)	ΔS (kJ/(mol·K)	ΔH (kJ/mol)
298.15	-4.5976	0.0207	1.5670
308.15	-4.7876		
318.15	-5.0114		

3.3.2.4 The effect of concentration of synthetic humic-like acid

SHLA concentrations of 5 mg/L, 50 mg/L and 100 mg/L were used to investigate the effect of SHLA concentration on the complexation of Cu^{2+} by SHLA at 25 ℃, an ionic strength of 0.1M and pH of 6 (Figure 3.3).

The complexation efficiency and log *K* (from 5.80 to 7.34) increased with increasing SHLA concentration; the complexation capacity (from 2.79 mmol/g to 1.53 mmol/g) had the opposite trend. Increasing the concentration of SHLA increased the total number of binding sites available, thereby enhancing the complexation efficiency as more Cu^{2+} was able to be complexed. In the same fashion, the Cu^{2+} bound to SHLA per unit humic acid decreases due to the excess binding sites (Vidali et al., 2011).

3.4. Conclusions

In this study, a synthetic humic-like acid (SHLA) was prepared by an abiotic humification reaction between catechol and glycine in the presence of a MnO_2 catalyst. The complexation ability of SHLA for Cu^{2+} and the influence of solution physico-chemical factors on the complexation reaction (ionic strength, pH, temperature and humic acid concentration) were investigated. The Cu complexation ability of the SHLA was compared to that of a commercial humic acid (CHA) from Sigma-Aldrich. The results showed that the SHLA had a stronger complexation ability than CHA and most natural humic acids, which was attributed to the higher levels of acidic functional groups, degree of humification , aromaticity, and higher O-alkyl groups content of the SHLA. The log K and complexation efficiency of SHLA were higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration, and the effects of solution physico-chemical factors on the complexation reaction were similar to natural humic acids. In conclusion, SHLA was a good and promising complexing agent for copper in both soil washing of copper contaminated soil and polymer enhanced ultrafiltration of copper-containing wastewater.

The composition and structure of the SHLA could be further manipulated by changing factors during the synthesizing process, such as the species of precursors, the ratio of precursors, the duration of synthesizing process and temperature, etc (Jokic et al., 2004; Zhang et al., 2015). This provides a feasible way to produce an SHLA with a better copper complexation ability. Besides, to reduce the cost of raw materials, waste containing polyphenols, sugars, amino acids or metal oxides can be considered as precursors or catalyst for abiotic humification process in the future study. For example, Brunetti et al show that SHLA can be produced from olive oil mill wastewater catalyzed by MnO_2 (Brunetti et al., 2008). And some waste residue, like steel slag and municipal solid waste incineration residue, can be used as catalyst to enhance abiotic humification process (Kim and Osako, 2004; Nishimoto et al., 2013; Qi et al., 2012a)

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FTIR, ^{13}C-NMR (University of York) and for elemental analysis (University of Leeds).

Appendix 3. Supplementary data

Details of the HA extraction process and the FTIR spectra (Figure 3.S1) and solid-state CP-MAS ^{13}C NMR spectrums (Figure 3.S2) are shown in Appendix 3.

Appendix 3. Supplementary data

The method for extracting and purifying SHLA

The reaction mixture was centrifuged at 10,000 rpm for 10 minutes, and the supernatant filtered through a 0.45 μm polyethersulfone membrane by vacuum filtration. The filtrate was acidified to pH 1 with concentrated HCl and stirred for 24 h. The SHLA fraction precipitated. The slurry was centrifuged at 10,000 rpm for 15 minutes. The precipitate was transferred to a beaker, re-dissolved in 0.1 M NaOH and filtered through a 0.2 μm polyethersulfone membrane twice by vacuum filtration. The filtrate was acidified to pH 1 with concentrated HCl, stirred for 24 h and centrifuged at 10,000 rpm for 15 minutes. The resulting precipitate (SHLA) was dialyzed by a Spectra/Por 6 dialysis membrane (Spectrum Labs, USA, molecular weight cut-off (MWCO) 1000 Da) and freeze-dried.

The Mn concentration level of SHLA

The solution with SHLA concentration of 1000 mg/L was measured by ICP-OES and the concentration of Mn was below detection limitation (0.002 mg/L). Given that 1 g of SHLA was dissolved in 1000 mL of solution, this would give a maximum concentration of Mn in the SHLA of 2 mg/kg. Additionally, SHLA was extracted and purified using the standard method recommended by the International Humic Substances Society (IHSS). During this method, SHLA is acidified to pH 1 with concentrated HCl twice and thus SHLA is H^+ saturated. That makes it highly unlikely that manganese ions would be present adsorbed to the SHLA.

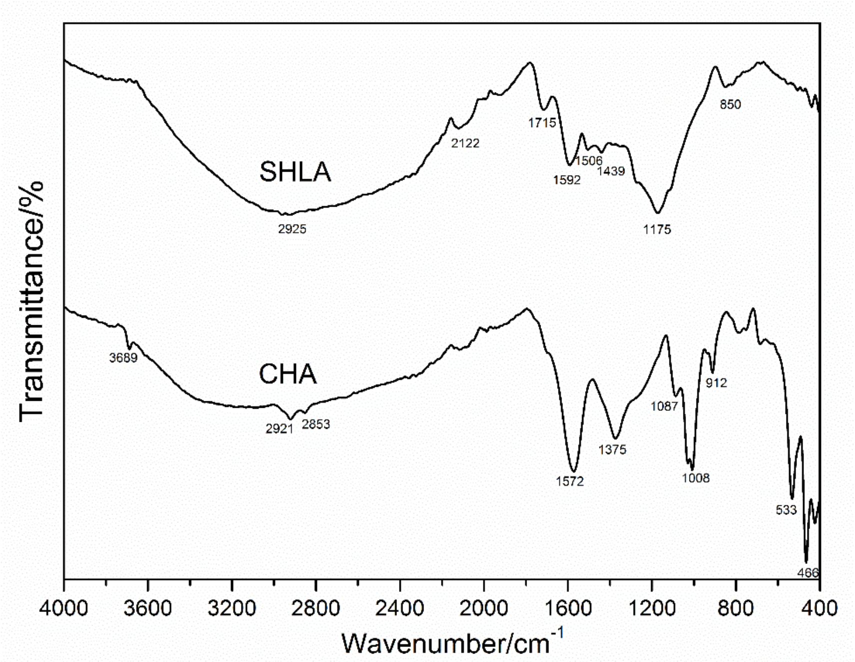
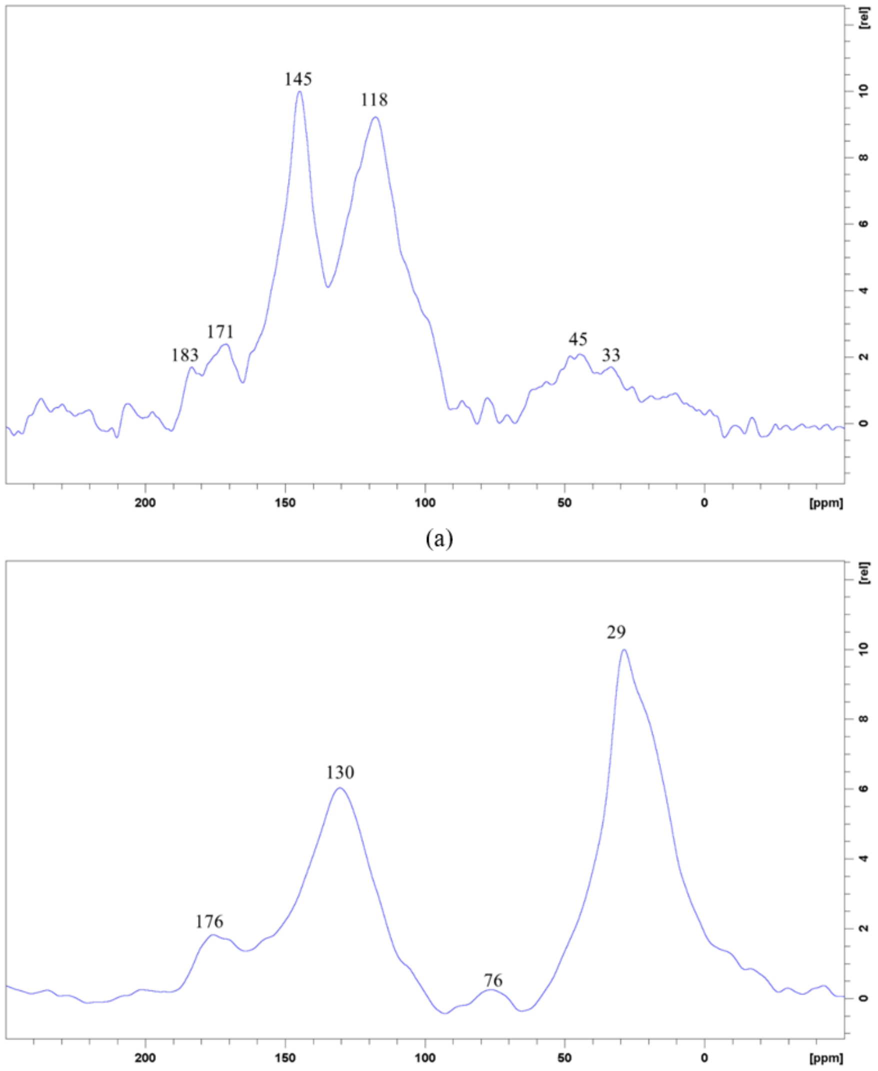


Figure 3.S1 FTIR spectra of SHLA and CHA.(n=1 for each sample; peaks at 2925 and 2921 cm^{-1} attributed to C-H stretching of aliphatic structures; at 1715 cm^{-1} attributed to C=O stretching of carbonyl groups; at 1572 and 1592 cm^{-1} attributed to aromatic C=C ring stretching, symmetric C–O stretch of COO- and N–H; at 1439 cm^{-1} attributed to O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and and C-O stretch of COO-; at 1375 cm^{-1} attributed to symmetric bending vibration of CH_3; at 1175 cm^{-1} attributed to C–O stretching and C–O–H deformation of alcohols, phenols and ethers; at 1008 cm^{-1} attributed to the C-O stretching vibration in alcohols, phenols and ethers)



(a)

(b)

Figure 3.S2 The Solid-state CP-MAS ^{13}C NMR spectrums of SHLA (a) and CHA (b).

(n=1 for each sample; peaks at 29 ppm assigned to methylene carbon in aliphatic long chains; at 45ppm assigned to methyl and methylene groups in aliphatic rings and chains; at 76 ppm attributed to aliphatic alcohols and esters from carbohydrates and flavonoids; at 118 ppm attributed to protonated aromatic carbon, and/or aromatic carbon ortho to oxygen-substituted aromatic carbon; at 130 ppm assigned to aromatic carbon bound to H; at 145 ppm attributed to phenolic carbon; at 171, 176 and 183 ppm ascribed to carboxyl carbon)

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CHAPTER 4

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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Chapter 4 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

Ting Yang[a*], Mark E Hodson[a]

[a] Department of Environment and Geography, University of York, Heslington, Wentworth Way, York YO10 5NG, United Kingdom

*Corresponding author. Tel.: +44 (0)7421 236832. Fax: +44 (0)7421 236832.

E-mail address: ty672@york.ac.uk

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 MnO_2 catalyst (1.3-2.5% w/v). The effect of the varying humification conditions on the complexation ability of the SHLA for Cu^{2+} 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, E_4/E_6). 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 °C), 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

4.1 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 Cu^{2+}, 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 MnO_2 catalyst (1.3-2.5% w/v). The conditional stability constant (log *K*), complexation capacity (CC) and chemical characteristics (element composition, acid functional groups, E_4/E_6, FTIR and ^{13}C NMR) of the 11 SHLAs were determined.

4.2 Materials and Methods

4.2.1 Materials

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

4.2.2 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 Na_2HPO_4/NaH_2PO_4) 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 MnO_2 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 4.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).

4.2.3 Characterization of synthetic humic-like acids

The elemental composition (C, H, N, O), total acidity, carboxylic group content, phenolic-OH content, E_4/E_6 ratio, FTIR spectra and solid-state CP-MAS ^{13}C 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 Hodson (2018b). The aromaticity and the ratio of alkyl to aromatic carbons (AL/AR) were calculated according to the following equations based on ^{13}C NMR data (Qi et al., 2012a; Yang and Hodson, 2018b).

$$\text{Aromaticity}=\frac{A_{Ar}(110\text{-}160\text{ mm})}{A_{Ar}(110\text{-}160\text{ mm})+A_{Alk}(0\text{-}110\text{ mm})} \quad (1)$$

$$\text{AL/AR}=\frac{A_{Alk}(0\text{-}110\text{ mm})}{A_{Ar}(110\text{-}160\text{ mm})} \quad (2)$$

where A_{Alk} are alkyl-C peak integration values and A_{Ar} are aromatic-C peak integration values.

4.2.4 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 HNO_3 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 $Cu(NO_3)_2$ (2 μM, 10 μM, 20 μM, 100 μM, 200 μM, 250 μM and 300 μM) in 0.2 M $NaNO_3$ were mixed. This produced solutions at seven Cu^{2+} 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 $NaNO_3$ (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 Cu^{2+} 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).

$$Cu + HA \Leftrightarrow CuHA \quad (3)$$

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

$$K = \frac{[CuHA]}{[Cu][HA]} \quad (4)$$

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

$$CC = [CuHA] + [HA] \quad (5)$$

$[CuHA]$ is then calculated as:

$$[CuHA] = Cu_0 - [Cu] \quad (6)$$

where Cu_0 is initial concentration of Cu^{2+}; $[Cu]$ is concentration of free Cu^{2+} after 24h. Combining and rearranging equations 4-6, the Equation 7 is given as :

$$\frac{1}{Cu_0 - [Cu]} = \frac{1}{K \cdot CC \cdot [Cu]} + \frac{1}{CC} \quad (7)$$

Linear regression analysis was performed for $1/(Cu_0-[Cu])$ and $1/[Cu]$ in order to calculate *K* and *CC* (Ružić, 1982).

4.2.5 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).

4.3 Results

4.3.1 Characterization of 11 synthetic humic-like acids (SHLA)

(a) 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 4.1 Elemental composition (N, C, H and O), acidic functional groups contents and E_4/E_6 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)	E_4/E_6
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

(b) Acidic functional group contents and E_4/E_6

Table 1 shows the acidic functional group contents and the E_4/E_6 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 MnO_2 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 E_4/E_6 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 E_4/E_6 (2.52), while SHLA 4 had the greatest (3.39). Decreasing the mass of MnO_2 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 E_4/E_6. In comparison, humification temperature had only a small influence on the E_4/E_6 of SHLAs. The difference between the E_4/E_6 ratio of SHLA 1 and SHLA 2 indicated the presence of glucose could increase the E_4/E_6 ratio.

(c) FTIR

The FTIR spectra of the SHLAs and initial precursors are shown in Figure 4.S1. The common features in the FTIR spectra are summarized in Table 2.

Table 4.2 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, CH_3 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-MAS^{13} C NMR

The peak assignments for ^{13}C NMR spectra of the SHLAs are shown in Table 4.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 4.3 Compositions of different carbon species assessed by solid-state ^{13}C 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

4.3.2 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°C), 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.

4.3.2.1 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 4.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.

4.3.2.2 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.

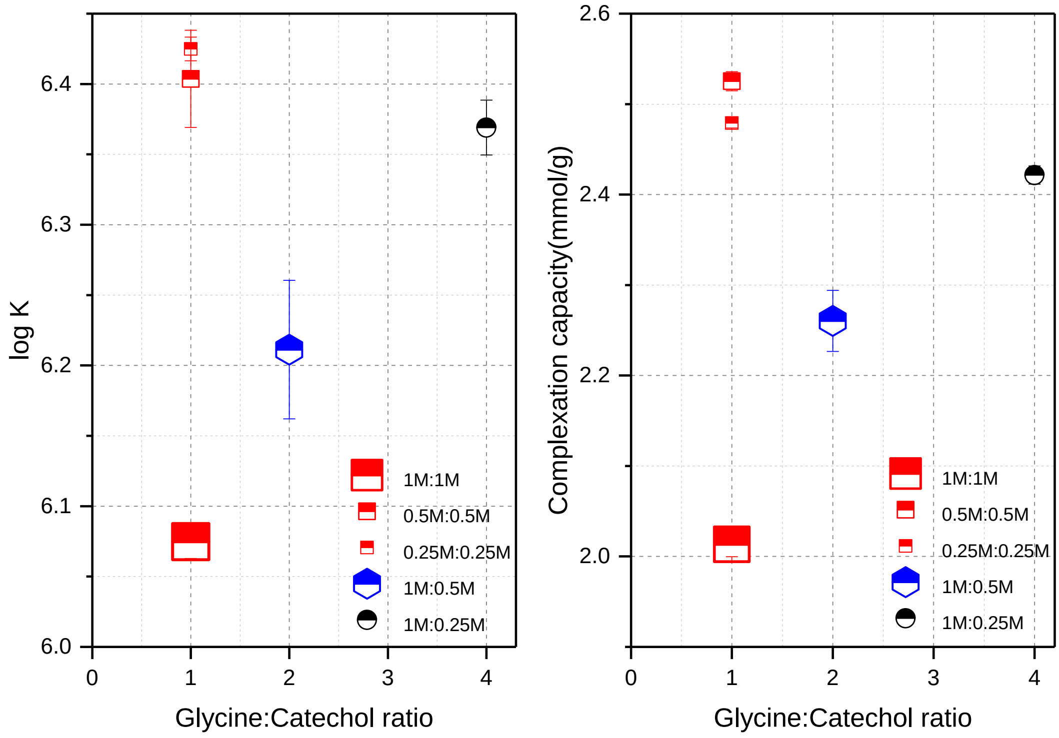

Figure 4.1 Log *K* and *CC* of SHLAs (SHLA 1, SHLA 3, SHLA 4, SHLA 5 and SHLA 6) produced by different precursors concentrations and ratios (mean values, n=3 ± standard deviation).

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.

4.3.2.3 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 °C), SHLA 7 (T=25 °C) and SHLA 8 (T=35 °C)) 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 °C to 45 °C ($p < 0.05$), while the *CC* increased from 25 °C to 35 °C ($p < 0.05$) and then remained unchanged (from 35 °C to 45 °C, $p = 0.275$). 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 MnO_2. 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°C.

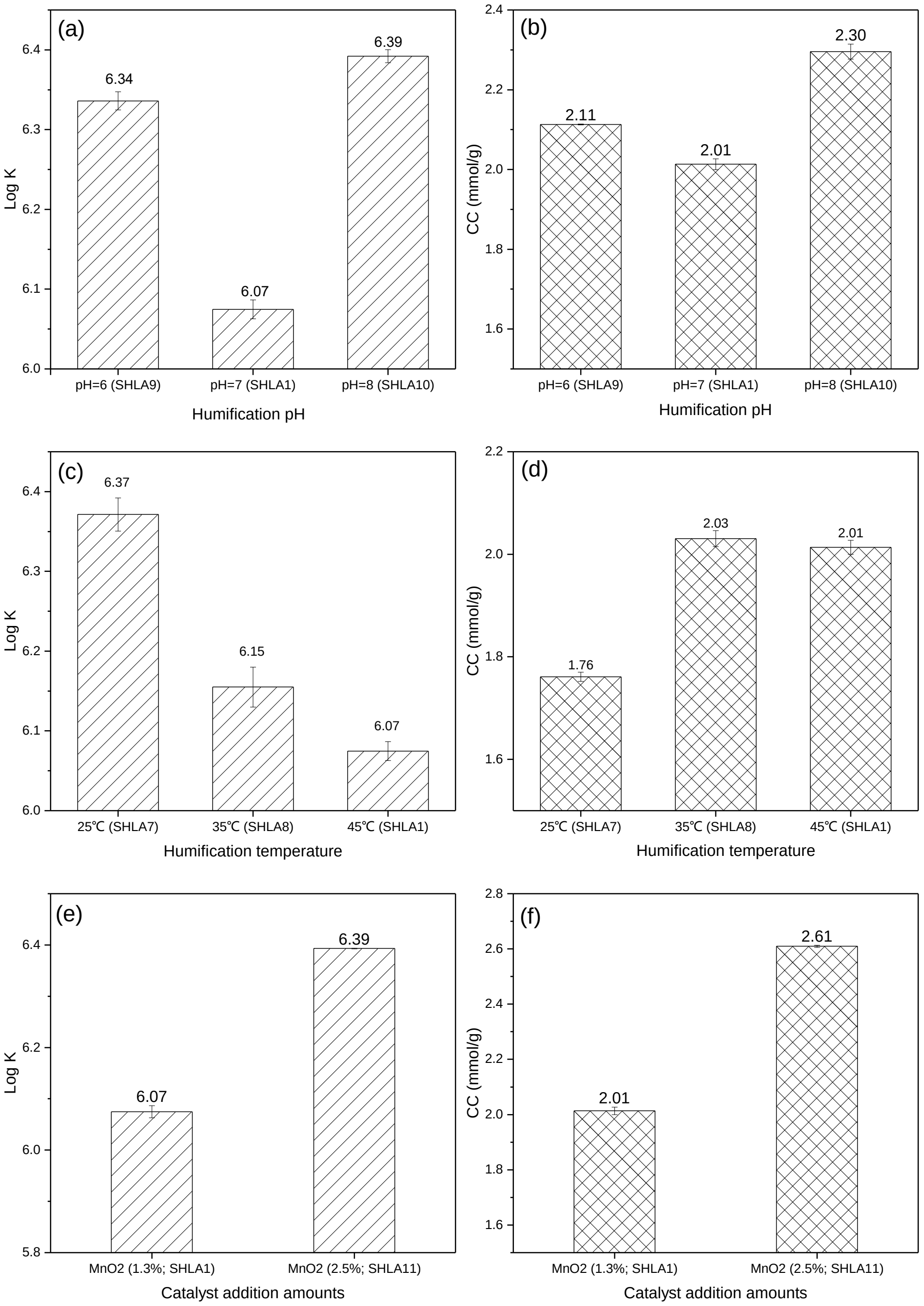

Figure 4.2 Log *K* (a,c,e) and *CC* (b,d,f) of SHLAs produced by different humification pH (SHLA 1, SHLA 9 and SHLA 10),temperatures (SHLA 1, SHLA 7 and SHLA 8) and different masses of catalyst (SHLA 1 and SHLA 11) (mean values, n=3 ± standard deviation).

4.4 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 K_{Cu} 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 4.4.

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 Cu^{2+} from contaminated water, the FTIR spectra of SHLA before and after adsorption of Cu^{2+} also demonstrated the importance of the carboxylic groups as binding sites for Cu^{2+}. 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 $NaNO_3$, 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 4.4 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	E_4/E_6	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
E_4/E_6														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).

4.5 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°C), 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.

Appendix 4. Supplementary Materials

Information about the abiotic humification reaction conditions and concentrations of the precursors for the 11 SHLAs (Table 4.S1), FTIR spectra of humification precursors and 11 SHLAs (Figure 4.S1), interpretation of the ^{13}C NMR spectra of the SHLAs (Table 4.S2), Log *K* and *CC* of SHLAs produced by different precursors systems (Figure 4.S2) are shown in supplementary materials.

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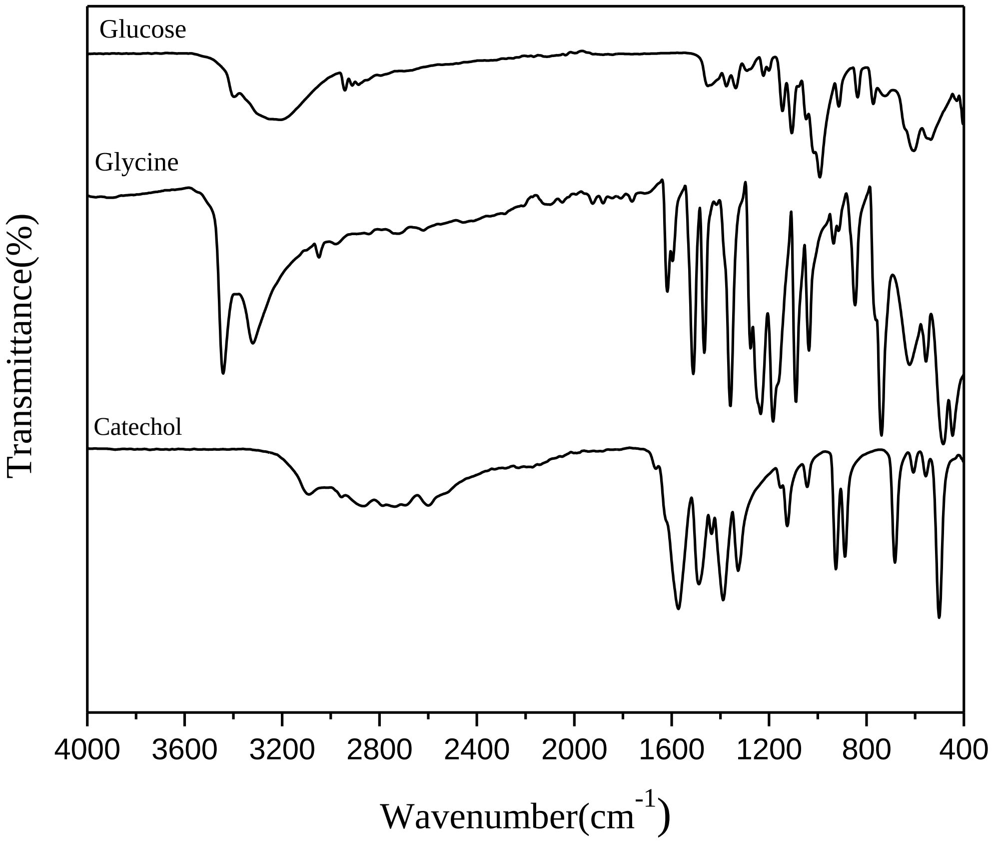
Competing interests statement

The authors declare that they have no competing interests.

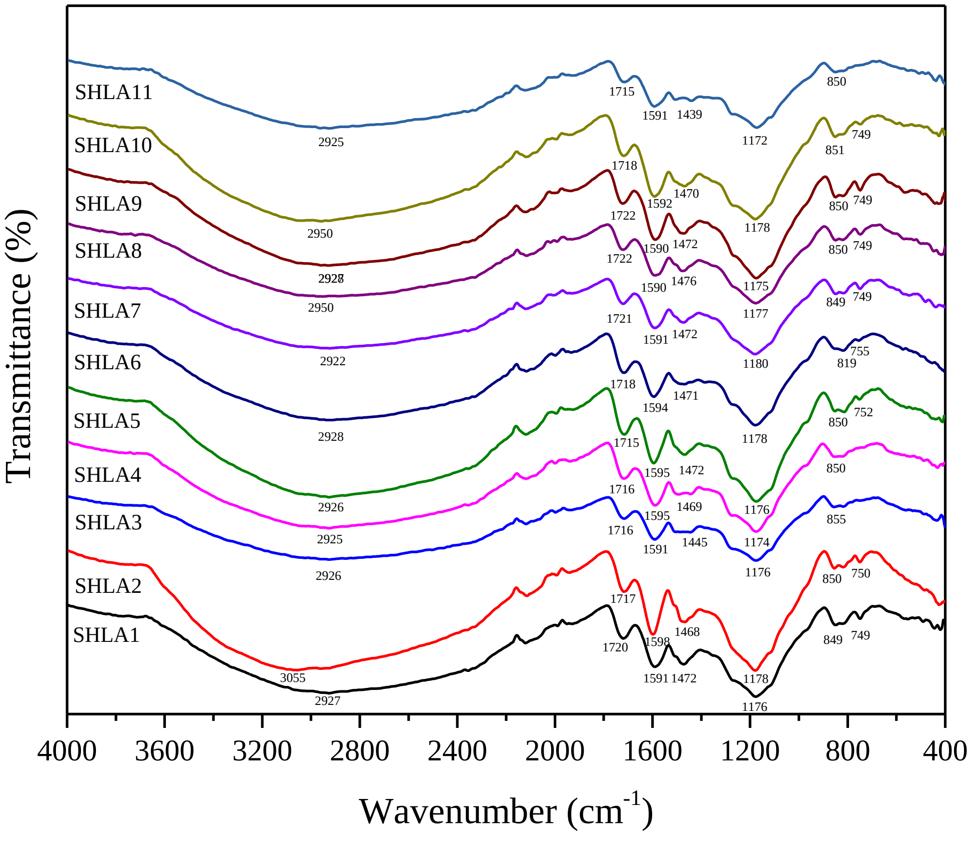
Appendix 4. Supplementary materials

Table 4.S1 Abiotic humification reaction conditions for the 11 SHLAs

HA Samples	Catechol /mol/L	Glycine /mol/L	Glucose /mol/L	Temperature /°C	pH	MnO_2 addition amount/g	Reaction time
SHLA 1	1	1	-	45	7	13.33	240 h
SHLA 2	1	1	1	45	7	13.33	
SHLA 3	0.5	0.5	-	45	7	13.33	
SHLA 4	0.25	025	-	45	7	13.33	
SHLA 5	0.5	1	-	45	7	13.33	
SHLA 6	0.25	1	-	45	7	13.33	
SHLA 7	1	1	-	25	7	13.33	
SHLA 8	1	1	-	35	7	13.33	
SHLA 9	1	1	-	45	6	13.33	
SHLA 10	1	1	-	45	8	13.33	
SHLA 11	1	1	-	45	7	25	


Glucose
Glycine
Catechol
Transmittance(%)
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber(cm^{-1})

(a)


SHLA11
SHLA10
SHLA9
SHLA8
SHLA7
SHLA6
SHLA5
SHLA4
SHLA3
SHLA2
SHLA1
Transmittance (%)
2925
1715
1591
1439
1172
850
2950
1718
1592
1470
1178
851
749
2927
2928
1722
1590
1472
1175
850
749
2950
1722
1590
1476
1177
850
749
2922
1721
1591
1472
1180
849
749
2928
1718
1594
1471
1178
819
755
2926
1715
1595
1472
1176
850
752
2925
1716
1595
1469
1174
850
2926
1716
1591
1445
1176
855
3055
1717
1598
1468
1178
850
750
2927
1720
1591
1472
1176
849
749
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm^{-1})

(b)

Figure 4.S1 FTIR spectra of humification precursors ((a); glycine, catechol and glucose) and 11 SHLAs (b). (n=1 for each sample; peaks at 3055 cm^{-1} attributed to aromatic C-H stretching; at 2925 -2950 cm^{-1} attributed to C-H stretching of aliphatic structures; at 1715-1722 cm^{-1} attributed to C=O stretching, mainly carboxylic groups; at 1572 and 1590-1598 cm^{-1} attributed to C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band); at 1439-1480 cm^{-1} attributed to O-H deformation of phenols, C–H deformation and CH3 symmetric and asymmetric stretching, symmetrical stretch of COO^{-}, at 1172-1180 cm^{-1} attributed to C–O stretching and C–O–H deformation of alcohols, phenols and ethers; at 749-850 cm^{-1} attributed to out-of-plane bending of aromatic C-H)

Table 4.S2 Interpretation of the ^{13}C NMR spectra of the SHLAs (Fernández-Gómez et al., 2015; Jokic et al., 2004; Nagasawa et al., 2016; Qi et al., 2012a)

Chemical shift /ppm	Assignment
45*	Methyl and methylene groups in aliphatic rings and chains
116-120	Protonated aromatic carbon, and/or aromatic carbon ortho to oxygen-substituted aromatic carbon
143-146	Phenolic or aromatic amine carbon
171-183	Carboxyl carbon

(*SHLA 2 did not have any peak around 45 ppm)

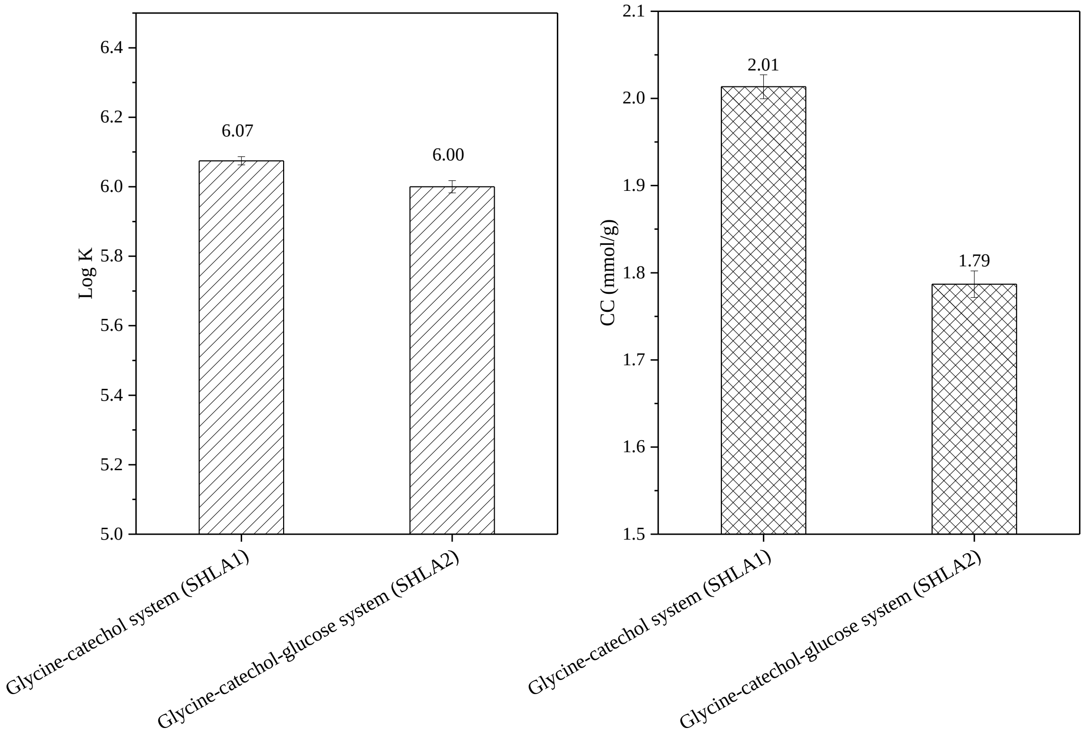


Figure 4.S2 Log *K* and *CC* of SHLAs (SHLA 1 and SHLA 2) produced by different precursors systems (mean values, n=3 ± standard deviation)

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CHAPTER 5

Investigating the use of synthetic humic-like acid as a soil washing treatment for metal contaminated soil

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Chapter 5 Investigating the use of synthetic humic-like acid as a soil washing treatment for metal contaminated soil

Ting Yang[a*], Mark E Hodson[a]

[a] Department of Environment and Geography, University of York, Heslington, Wentworth Way, York YO10 5NG, United Kingdom

*Corresponding author. Tel.: +44 (0)7421 236832. Fax: +44 (0)7421 236832.
E-mail address: ty672@york.ac.uk

Abstract

Humic acid can effectively bind several metals and is regarded as a promising soil washing agent. Previous studies indicate that carboxylic groups dominate metal binding to humic acid. In this study, a synthetic humic-like acid (SHLA) with high COOH content (5.03 mmol/g) was used as a washing agent to remove metals (Cu, Zn, Ni, Pb, As) from two contaminated agricultural soils (Soil 1 (pH: 6.17 ± 0.11; organic carbon: 5.91±0.40%; Cu: 302.86±3.97 mg/kg; Zn: 700.45±14.30 mg/kg; Pb 323.56±4.84 mg/kg; Ni: 140.16±1.59 mg/kg) and Soil 2 (pH: 9.83±0.01; organic carbon: 2.52%±0.25%; Cu: 242.81±10.66 mg/kg; Zn: 841.00±22.31 mg/kg, Pb 451.21±1,92 mg/kg, As: 242.23±5.24 mg/kg)). The effects of solution pH (4 to 11), liquid/solid ratio (L/S ratio, 5:1 (mL:g) to 80:1 (mL:g)), SHLA concentration (100 mg/L to 2000 mg/L), and contact time (0 to 1440 min) on % metal removal were investigated and optimum conditions identified: pH of 9, L/S ratio of 1:80, SHLA concentration of 1500 mg/L at 25 °C for 6 h. Under optimum conditions, a single washing removed 45.2% of Cu, 34.6% of Zn. 42.2% of Ni and 15.6 % of Pb from Soil 1, and 30.6% of Cu, 28.1% of Zn. 14.6% of As and 18.1% of Pb from Soil 2. A modified BCR extraction of the two soils before and after washing indicated that the SHLA mainly removed metals in the exchangeable and acid soluble fraction and reducible fraction, which could effectively reduce bioavailability and environmental risk of metals. On a molar basis, SHLA was a more effective washing agent than commercial humic acid, Na_2EDTA, citric acid and tartaric acid. Overall, SHLA shows great potential for use as a soil washing agent.

Keywords: Synthetic humic-like acid; toxic metals; soil washing; metal speciation distribution; reduced partition index; metal bioavailability factor

5.1 Introduction

Soil contamination by hazardous metals is currently a serious environmental issue worldwide. For example, hazardous metals are the most frequent contaminants (34.8%) affecting soil and groundwater in Europe and 82.8% of cases of soil contamination are caused by inorganic contaminants (mainly hazardous metals) in China (EEA, 2018; MEE, 2014). The original sources of excess hazardous metals in soils are various, including atmospheric deposition, sewage irrigation, waste disposal, mining activities, metal smelting, sewage sludge, pesticides and fertilizers (Lwin et al., 2018; Mahar et al., 2016; Su et al., 2014). Hazardous metals are of great concern because not only are they non-biodegradable, but they can enter the food chain and finally accumulate in the human body, posing a great threat to both human health and the environment (Lesmana et al., 2009; Suanon et al., 2016). The most common metal contaminants include Cd, Cr, Hg, Pb, Cu, Zn, and As (Ashraf et al., 2017; Kumpiene et al., 2008).

Various remedial approaches for metal-contaminated soil have been investigated in the past decades, including soil washing, solidification/stabilization, soil amendment, phytoremediation, electrokinetic processes, and bioremediation (Bolan et al., 2014; Leštan et al., 2008; Liu et al., 2017; Rosestolato et al., 2015; Wan et al., 2016; Xia et al., 2017). Of these, soil washing is regarded as one of the most efficient, rapid and cost-effective techniques (Wuana and Okieimen, 2011). Washing agents, which are the key to soil washing techniques, can be classified into four types: 1) inorganic acid (e.g. HCl, H_2SO_4, HNO_3); 2) synthetic organic chelating agents (e.g., EDTA, DTPA, EDDS); 3) surfactants (e.g. humic acid, saponin, rhamnolipid) and 4) low-molecular-weight organic acids (e.g., oxalic acid, citric acid, tartaric acid) (Ferraro et al., 2016; Gusiatin and Radziemska, 2018; Jiang et al., 2017; Kulikowska et al., 2015; Meng et al., 2017; Yoo et al., 2017). However, although inorganic acid and synthetic organic chelating agents have high metal removal efficiencies, inorganic acid will acidify soil, change soil structure and decrease soil fertility and microorganism activity; while synthetic chelating agents have low biodegradability and can have adverse effects on groundwater quality and soil microorganism health (Gusiatin and Klimiuk, 2012; Meng et al., 2017; Yang et al., 2017). Thus, using environmentally friendly and cost-effective washing agents is encouraged to reduce risks to ecosystems and human health.

Humic acid (HA) is a natural surfactant (Conte et al., 2005) that can not only effectively complex many different metals due to its abundant oxygen-containing functional groups especially carboxylic group and phenolic-OH group, but also improve soil properties

(Meng et al., 2017; Perminova and Hatfield, 2005; Sparks, 1995). Thus, humic acid has been regarded as a promising soil washing agent (Hartley et al., 2014; Kulikowska et al., 2015; Meng et al., 2017; Tsang and Hartley, 2014). In addition to occurring naturally, humic acid can be synthesized by abiotic humification processes, that is the transformation of humic precursors (e.g., polyphenol, amino acids, and reducing sugars) to humic substances catalyzed by materials containing metallic oxide (e.g., pure MnO_2, zeolite, and steel slag). Humic acid synthesized by abiotic humification is called synthetic humic-like acid (SHLA) (Fukushima et al., 2009; Hardie et al., 2009; Qi et al., 2012; Zhang et al., 2017; Zhang et al., 2015). Compared with natural humic acid, SHLA has the advantage that its structural characteristics can be controlled to some extent by changing the abiotic humification conditions, such as the species of precursors, pH, temperature and the ratio of catalyst (Fukuchi et al., 2012; Jokic et al., 2004; Yang and Hodson, 2018a, b; Zhang et al., 2017; Zhang et al., 2015). Recently, we have reported a SHLA which was optimized to complex Cu^{2+} and can be used as an adsorbent for removing several metal ions from metal-contaminated water (Yang and Hodson, 2018a; Yang and Hodson, 2018b). However, studies on how to apply SHLA as part of the soil washing process are still rare. The novelty of our study lies in the application of a SHLA that has been synthesized to optimize its metal removal efficiency for soil washing.

In this study, we examined the use of aqueous SHLA as a soil washing agent. Previous studies indicate that carboxylic groups dominate metal ion binding to humic acid (Gondar et al., 2006; Xu et al., 2016; Yang et al., 2015). An optimal SHLA with a high COOH content was produced using synthesis conditions informed by our previous experiments and characterized by elemental analysis, acidic functional group content, FTIR and UV-VIS absorbance. A series of batch removal experiments were carried out to determine the effect of initial SHLA concentration (100 -2000 mg/L), pH (4-9), liquid/soil ratio (L/S ratio, 5:1 to 80:1 (mL:g)) and reaction time (0-1440 min) on % metal removal in two agricultural soils (Soil 1 and Soil 2); optimum extraction conditions were determined. Using the BCR sequential extraction method (Zhang et al., 2017), I_R (reduced partition index) and M_F (metal bioavailability factor) parameters, metal speciation distribution before and after SHLA washing and the influence on metal stability and mobility in two soils were also investigated. Finally, a comparison of metal washing efficiency between SHLA and four other washing agents (CHA, Na_2EDTA, citric acid and tartaric acid) was conducted.

5.2 Materials and methods

5.2.1 Materials

Catechol ($C_6H_6O_2$, >99% purity), glycine ($C_2H_5NO_2$, >99% purity), glucose ($C_6H_{12}O_6$, >99% purity), thimerosal ($C_9H_9HgNaO_2S$, >97% purity), manganese oxide (MnO_2, >99% purity), ethylenediaminetetraacetic acid disodium salt dihydrate ($C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$, 98.5-101.5% purity), citric acid ($C_4H_8O_7$, >99% purity), tartaric acid ($C_4H_8O_7$, >99% purity) and commercial humic acid (CHA) were purchased from Sigma-Aldrich. Sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$, >99% purity), sodium phosphate monobasic dihydrate ($NaH_2PO_4{\cdot}H_2O$, >99% purity) and hydrogen peroxide (H_2O_2, 30% purity) were purchased from ACROS Organics. Calcium chloride ($CaCl_2$, >99% purity), acetic acid (CH_3COOH, >99% purity) and ammonium acetate (CH_3COONH_4, >99% purity) were purchased from Fisher Chemical. Hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, >99% purity) were purchased from Alfa Aesar. Ultra-pure water was prepared using a PURELAB Classic ultra-pure system (ELGA, UK) from deionized water.

5.2.2 Soil sampling and characterization

Two agricultural soils with similar bulk metal concentrations above permitted levels for agricultural use (see below) but different pH and organic matter contents that could result in differing performances of the SHLA washing agent were sampled for this study. Soil 1 in this study was collected from a farm located on the Stoke Bardolph Estate (GR 52.97104, -1.05515; Nottingham, UK). Soil 1 has been treated with sewage sludge for several decades and is used for growing corn for the Stoke Bardolph Energy Crop Plant (Riddell-Black, 1994; Severn Trent Water, 2016). Soil 2 was obtained from an upland farm near Laurencekirk (GR 56.89450, -2.54512; Aberdeenshire, UK) and was previously treated with metal(loid) rich wood ash arising from waste wood combustion, applied in controlled experiments at 10% vol (Trakal et al., 2017; Mitchell et al., 2018). The soils were air dried ar room temperature and sieved to 2 mm. Soil pH was measured in a 1:2.5 (w/v) soil/ ultrapure water mixture (Rowell, 1994). Soil organic carbon was determined using a vario MACRO C/N analyzer (Elementary, Germany). Cation exchange capacity (CEC) of soil was measured following the method of Hendersot and Duquette (Hendershot and Duquette, 1986). Soil texture was determined using a Malvern Laser granulometer (Malvern Instruments, UK). Methodological details are provided in the Supplementary materials. Total metals in soil and the concentration of metals in the

BCR residual fraction (see below) were measured following the International Standard Organization (ISO)-method BS7755 (British, 1995).

5.2.3 Preparation and characterization of optimal synthetic humic-like acid

In preliminary experiments, 11 synthetic humic-like acids (SHLAs) were synthesized under varying abiotic humification conditions (different temperatures, pH, precursor species and initial concentrations, and masses of catalyst) to determine optimal synthesis conditions to produce a SHLA with a high COOH content (Yang and Hodson, 2018a). Subsequent to these preliminary investigations, the SHLA used in this study was synthesized using these conditions: a pH 8, 0.25M glycine: 0.25M catechol mixture at 25 °C with 2.5 wt% MnO_2 as a catalyst. In detail, all glassware and pure water were autoclaved (121 °C for 27 minutes, 0.12 MPa) prior to use to ensure that the humification was abiotic. An 1 L aliquot of autoclaved phosphate buffer (pH 8, 0.2 M Na_2HPO_4 / NaH_2PO_4 autoclaved at 121 °C for 27 minutes, 0.12 MPa) containing 0.02% (w/v) thimerosal (to ensure sterility) was placed in a 3L beaker, to which 25.00 g of MnO_2, 27.53 g of powdered catechol and 18.77g of glycine were added. The mixture was shaken in the dark at 25 °C for 240 h. After incubation, SHLA was extracted and purified using the standard method recommended by the International Humic Substances Society (IHSS) (Swift, 1996). The synthesis and purification of the SHLA are described in detail in Yang and Hodson (2018a). Importantly the purification process involves a dialysis step that separates the high molecular number HA from the low molecular mass precursors catechol and glycine and the thimerosal.

The elemental composition (C, H, N) of the SHLA was determined using a vario MICRO cube type elemental analyzer (Elementary, Germany). Total acidity and carboxylic group content were measured using the $Ba(OH)_2$ and $Ca(CH_3COO)_2$ titration methods, respectively (Schnitzer and Kahn, 1972). The phenolic-OH content was calculated by subtracting the carboxylic group content from total acidity. The E_4/E_6 ratio, an indicator of the degree of humification and the molecular weight of humic acid, was calculated as the ratio of the UV/Vis absorbances (Lambda 25 UV/Vis Spectrophotometer, PerkinElmer, USA) at 465 and 665 nm (Chen et al., 1977). An Alpha FT-IR Spectrometer (Bruker, Germany) was used to obtain the FTIR spectra of the SHLA. Spectra were recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. For each spectrum, 24 scans were averaged to reduce noise.

Humic acids contain a number of functional groups; the carboxylic and phenolic groups are the two major functional groups that are reported as binding metals (Boguta et al., 2016; He et al., 2016; Vidali et al., 2011). Several studies indicate that carboxylic groups dominate metal ion binding to humic acid (Gondar et al., 2006; Xu et al., 2016; Yang et al., 2015) and in our preliminary work we found that log K_{cu} for SHLAs was positively correlated with carboxylic group content ($r = 0.89$, $p < 0.01$), but not phenolic-OH content. For these reasons, we focus on the carboxylic and phenolic-OH functional groups in this study when considering sorption mechanisms.

5.2.4 The effect of washing conditions on % metal removal and kinetics of the SHLA washing process

To simulate the soil washing process, batch experiments were carried out at different SHLA concentrations, pH, liquid/soil ratio (L/S ratio, mL: g) and duration. For the SHLA concentration experiment, experiments were conducted at a pH of 6, a L/S ratio of 40:1 (mL:g) and a temperature of 25 °C for 24h. The initial SHLA concentration in the washing solution was set to 100 mg/L, 200 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L, and 2000 mg/L. For the pH experiment, experiments were conducted at a SHLA concentration of 1000 mg/L, a L/S ratio of 40:1 (mL:g) and a temperature of 25 °C for 24h. The initial pH in the washing solution was set to 4, 5, 6, 7, 8, and 9. For the L/S ratio (mL:g) experiment, experiments were conducted at a pH of 6, a SHLA concentration of 1000 mg/L and a temperature of 25 °C for 24h. The initial L/S ratio (mL:g) was set to 5:1, 10:1, 20:1, 40:1, and 80:1. For the washing duration experiment, experiments were conducted at a pH of 6, a SHLA concentration of 1000 mg/L, a L/S ratio of 40:1 (mL:g) and a temperature of 25 °C. The contact time was set to 5 min, 10 min, 30 min, 60 min, 120 min, 180 min, 240 min and 360 min.

All solutions were shaken orbitally at 250 rpm. Then, the mixtures were separated by centrifugation at 4500 rpm for 20 min. The supernatant was filtered using a 0.45μm nylon syringe filter (Gilson, UK). The metal concentrations in the supernatants were determined by ICP-OES (iCAP 7000, Thermo Scientific, USA). All the experiments were performed in triplicate. In the results, all calculated values (see below) are quoted as mean values ± standard deviations.

The % removal of metals from the soil by the SHLA solution was calculated as:

$$\% \text{ Removal} = \frac{M_0 - M}{M_0} \cdot 100\% \quad (1)$$

where M_0 (mg/kg) is the initial total metal concentration in the soil sample and M (mg/kg) is total metal concentration in the soil sample after soil washing.

For the kinetic study, the experimental data were fitted to first-order (equation 2), second-order (equation 3), power function (equation 4), Elovich (equation 5) and Parabolic function (6) models (Almaroai et al., 2013; Xu et al., 2008).

$$\ln Q_t = \ln(Q_0) - k_1 \mathrm{t} \quad (2)$$

$$\frac{1}{Q_t} = \frac{1}{Q_0} + k_2 t \quad (3)$$

$$\ln q_t = \ln(b) + k_f \ln(\mathrm{t}) \quad (4)$$

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \quad (5)$$

$$q_t = k_p t^{0.5} + C \quad (6)$$

where Q_t and Q_0 are the mole fraction of metals in soils at time t and t = 0, respectively; q_t is the mole fraction of metals desorbed by extractants time t; and k_1, k_2, k_f, b, α, β, k_p, and C are constants.

5.2.5 Metal speciation distribution study

The speciation analysis of metals before and after SHLA washing for the soil samples was carried out using a modified BCR sequential extraction method (Table 5.1; Zhang et al., 2017).

Table 5.1 BCR sequential extraction process for metals of soil samples.

Step	Fraction	Extraction agent	Reaction conditions
1	Exchangeable and acid-soluble fraction(F1)	0.11 M CH_3COOH	16h, 25 °C
2	Reducible fraction (F2)	0.1M $NH_4OH \cdot HCl$	16h, 25 °C
3	Oxidizable fraction (F3)	8.8M H_2O_2 1M CH_3COONH_4	85 °C for 1h and room temperature for 16 h.
4	Residual fraction (F4)	HNO_3: HCl =1:3	See Section 2.2

I_R (reduced partition index) and M_F (metal bioavailability factor) values before and after SHLA washing were calculated using the BCR results to evaluate metal binding ability and mobility (Gusiatin and Klimiuk, 2012; Tang et al., 2017; Wei et al., 2016), as follows.

$$I_R = \frac{\sum_{i=1}^{k} i^2 F_i}{k^2} \quad (7)$$

$$M_F = \frac{F_1'}{\sum_{i=1}^{k} F_i'} \times 100\% \quad (8)$$

where i is the index number of the extraction step, the value is from 1 (for the weakest) to the most aggressive extractant (for the BCR method, $k = 4$), F_i is the percentage of the considered metal in fraction i, and F_i' is actual concentration of the considered metal in fraction i.

5.2.6 Comparison of 4 washing agents and the change of metal species

In this study, commercial humic acid (CHA, as a representative of natural humic acid), Na_2EDTA, citric acid and tartaric acid were chosen as washing agents to compare against SHLA. The COOH content, phenolic-OH content and total acidity of the CHA were 1.22 mmol/g, 2.65 mmol/g and 3.87 mmol/g, respectively. In detail, 1500 mg/L of each washing agent was shaken with each of the two soils at a L/S ratio of 80:1 (mL:g) and at 25 °C. The pH of CHA and SHLA were adjusted to 9, while the pH of Na_2EDTA, citric acid and tartaric acid were not adjusted. The above washing conditions were used as they were optimal for the SHLA (Section 3.2). pH was not adjusted to 9 for Na_2EDTA, citric acid and tartaric acid, because they do not work well at high pH since metal hydrolysis and precipitation is favoured over complexation (Zhang et al., 2017; Elliott and Shastri, 1999; Zou et al., 2009). All solutions were shaken orbitally for 360 min at 250 rpm. Then, the mixtures were separated by centrifugation at 4500 rpm for 20 min. The supernatant was filtered using a 0.45μm nylon syringe filter (Gilson, UK). The metal concentrations in the supernatants were determined by ICP-OES. All the experiments were performed in triplicate. In the results, all calculated values (see below) are quoted as mean values ± standard deviations.

5.2.7 Statistical analysis and quality control

The % metal removal data were analyzed using a one-way ANOVA (Sigmaplot 12.5, USA). The post-hoc Turkey test was used to determine differences between pairs of treatments with significance set at $p \leq 0.05$. The actual concentrations of each fraction before and after extraction with the SHLA solution were compared using paired t-tests with significance set at $p \leq 0.05$ (Sigmaplot 12.5, USA). The I_R and M_F values before and after extraction with the SHLA solution were analyzed using paired t-tests (for individual metals) and one-way ANOVA (for comparison between metals) with significance set at $p \leq 0.05$ (Sigmaplot 12.5, USA).

The accuracy of the total metal analysis was assessed using a standard loamy sandy soil certified reference material (CRM036, Sigma-Aldrich, USA. The accuracies for different metals were 93%-94% for Cu, 99%-106% for Zn, 97%-98% for Ni, 87%-89% for Pb and 97%-98% for As. For the BCR sequential extraction, the sum of fractions F1 to F4 for both soils (plus the metals removed by soil washing for the SHLA treated soils) gave total metal recoveries of 86%-101% for Cu, 84%-112% for Zn, 83%-98% for Ni, 94%-120% for Pb and 80%-105% for As. The ICP-OES detection limits for the metals were 0.006-0.008 mg/L for Cu, 0.001-0.007 mg/L for Zn, 0.001-0.002 mg/L for Pb, 0.001 mg/L for Ni and 0.004 mg/L for As which were equivalent to c. 0.25-0.32 mg/kg for Cu, 0.03-0.29 mg/kg for Zn, 0.06-0.10 mg/kg for Pb, 0.04 mg/kg for Ni and 0.16 mg/kg for As.

5.3 Results and discussions

5.3.1 Characterization of optimal synthetic humic-like acid and two tested soils

Table 5.2 lists the elemental composition, acidic functional group content and E_4/E_6 of the SHLA and CHA. Compared with the CHA used in these experiments, the SHLA had larger C and N contents and a higher N/C ratio which generally indicates a higher degree of humification (Mahieu et al., 2000; Qi et al., 2012a; Tu et al., 2017). SHLA contained 4.1 times as many carboxylic acids groups and 2.5 times as many phenolic hydroxyl groups as CHA, indicating SHLA has a higher concentration of acidic functional groups and more hydrophilic properties than CHA (Nagasawa et al., 2016).

Table 5.2. Elemental composition, acidic functional groups content and E_4/E_6 of SHLA and CHA

Sample	N/%	C/%	H/%	O/%	N/C	C/H	O/C	COOH (mmol/g)	Phenolic-OH (mmol/g)	Total acidity (mmol/g)	E_4/E_6
SHLA	5.14	47.68	2.96	44.23	0.108	16.131	0.928	5.03	6.55	11.58	3.20
CHA	0.94	46.29	2.99	49.78	0.020	15.508	1.075	1.22	2.65	3.87	4.32

Figure 5.1 shows the FTIR spectra of SHLA which shows clear differences from those of the unpolymerized catechol and glycine and also the CHA. Peak assignments are given

in Table 5.S1 in the supplementary material.

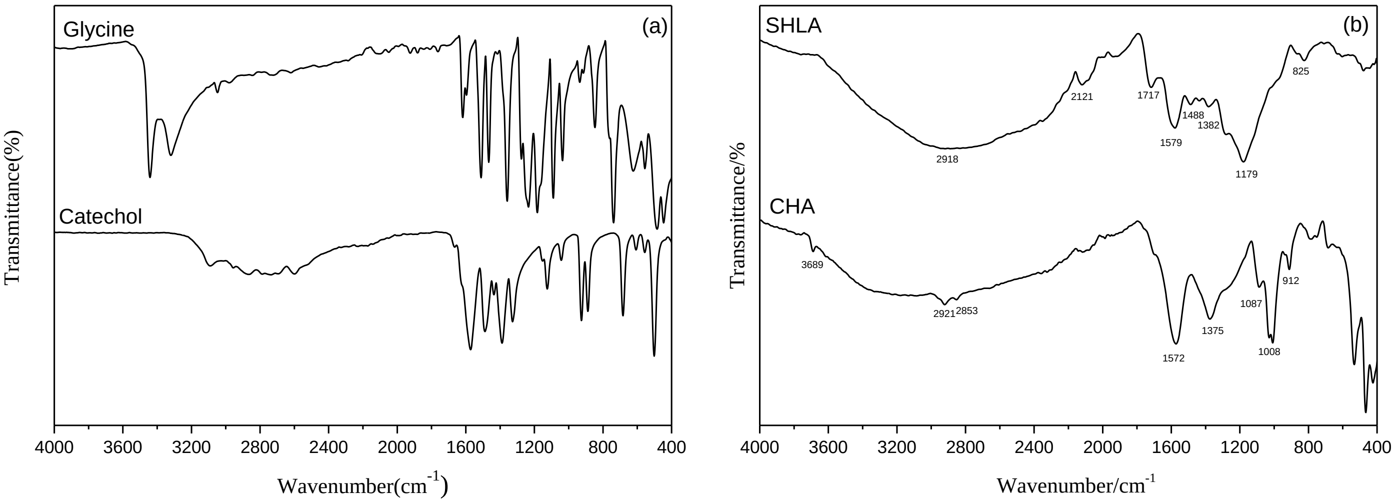

Figure 5.1 FTIR spectra of glycine (a), catechol (a), SHLA used in this study (b) and CHA (b).

The physico-chemical properties of the two soils are listed in Table 5.3. The two soils varied in pH and organic carbon content. The pH of Soil 1 was slightly acid while pH of Soil 2 was alkaline. Based on the EU standard for agricultural soil (EC, 1986), the metal concentrations of Cu, Zn, Ni, Pb of Soil 1, and Cu, Zn, As, Pb of Soil 2 are above permitted metal concentrations. The content of clay and organic matter (less than 10%-20%) indicated that the two soils are suitable to be remediated by soil washing (Mulligan et al., 2001).

Table 5.3 Physico-chemical properties of tested soil (n=3, mean values ± standard deviation)

Parameters	Soil 1	Soil 2	EU standard*
pH	6.17 ±0.11	9.83 ±0.01	
Organic carbon (%)	5.91 ±0.40	2.52 ±0.25	
Cation exchange capacity (cmol/kg)	9.48 ±0.13	8.76 ±0.5	
Composition (%)			
Sand	64.9	75.1	
Silt	33.4	24.0	
Clay	1.7	0.9	
Texture	Sandy loam	Loamy sand	
Metal (mg/kg)			
Total Cu	302.86 ±3.97	242.81 ±10.66	135 for pH 6-7; 200 for pH >6-7
F_1	18.73±0.84	14.84±0.07	
F_2	62.19±5.34	86.10±9.56	
F_3	173.77±7.70	78.48±2.99	
F_4	18.51±3.84	44.76±1.76	
Total Zn	700.45 ±14.30	841.00 ±22.31	300 for pH 6-7; 450 for pH >6-7
F_1	280.44±8.67	368.05±23.81	
F_2	214.28±9.26	215.11±10.67	
F_3	43.25±1.20	66.22±9.61	
F_4	127.13±18.38	134.81±18.01	
Total Pb	323.56 ±4.84	451.21 ±1.92	300
F_1	0.54±0.06	15.42±1.75	
F_2	195.48±11.29	235.58±13.03	
F_3	34.09±2.71	51.55±7.18	
F_4	79.59±15.54	122.51±13.78	
Total Ni	140.16 ±1.59	2.74 ±0.27	75 for pH 6-7; 110 for pH >6-7
F_1	53.15±1.61	0.64±0.04	
F_2	44.46±2.38	1.10±0.03	
F_3	13.21±0.29	0.69±0.02	
F_4	18.71±2.51	0.26±0.00	
Total As	24.59 ±0.93	242.23 ±5.24	50
F_1	4.52±0.23	22.61±1.29	
F_2	10.86±0.37	92.05±4.57	
F_3	5.37±0.09	21.74±2.38	
F_4	1.07±0.01	58.42±21.69	

(*EU standard for permitted metal concentrations in agricultural soil)

5.3.2 The effect of washing conditions on % metal removal

Figure 5.2 shows % metal removal of the SHLA and Figure 5.S1 shows metal concentration of soil after washing at different initial SHLA concentrations, pH, L/S ratios and contact time. The % metal removal varies for the different metals for a number of reasons. Firstly, metals have differing affinities (based on stability constants) for humic acid, with affinities of divalent metal ions decreasing in the order Pb > Cu > Ni >Zn (Irving and Williams, 1948). In this study, the % Cu removal in both two soils and % Ni removal in Soil 1 was higher than that of Zn, which was consistent with the Irving-William series (Irving and Williams, 1948; Pandey et al., 2000). Secondly, metal speciation also plays an important role in % metal removal. For example, though Pb has a higher affinity to SHLA than Zn (Irving and Williams, 1948), the % Pb removal is much lower than Zn in both two soils. This is because the exchangeable and acid-soluble fraction of Pb was much lower in both soils than that of Zn (see Table 5.3). Compared with the other four metals, As removal varied less with washing conditions. Under the solution conditions investigated here, As is present in anionic forms (e.g. AsO_4^{3-}, $HAsO_4^{2-}$, and AsO_3^{3-})and does not bond to the humic acid via exchange with the H^+ of the COOH groups. The result was similar to its interactions with other compounds such as Na_2EDTA (Qiu et al., 2010). Instead bonding via cation bridges and associative ligand exchange for the positively charged As centre of the As oxyanions with H present in the humic acid COOH and phenolic-OH functional groups may occur (Buschmann et al., 2006). In addition to the characteristics of the washing agent and the washing conditions, soil physico-chemical properties, like soil pH, soil texture, cation exchange capacity, buffering capacity, metal concentration and speciation, and organic matter content, can also affect metal removal performance (Dermont et al., 2008). Soil 1 and Soil 2 have different pH, soil texture and organic carbon content, which will contribute to the differences in metal removal. The content of clay (Soil 1:1.7%; Soil 2: 0.9%) and organic matter (Soil 1: 5.91%; Soil 2: 2.52%) were < 10-20%, indicating that both soils are suitable to be remediated by soil washing (Mulligan et al., 2001). However, the % metal removal was higher in Soil 1 than that in Soil 2, possibly due to the lower pH of Soil 1 (pH 6.17) relative to Soil 2 (pH 9.83). Metals in the lower pH soil will be more soluble and therefore more readily removed by washing (Lu et al., 2017; Sungur et al., 2015). Also, the higher concentrations of metals in F1 and F2 of Soil 2 might cause the lower % metal removal as the complexation capacity of the SHLA may be closer to saturation. The interaction of different soil physico-chemical properties in controlling the efficiency of soil washing is still hard to predict with chemical modelling and warrants further study.

5.3.2.1 SHLA concentration

In this study, SHLA concentrations were set to 100 mg/L, 200 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L and 2000 mg/L to investigate the effect of the SHLA concentration on metal % removal under conditions of a pH of 6, a L/S ratio of 40:1 (mL:g), a temperature of 25 °C and a duration of 24 h (Figure 5.2(a) and (b)). The % removal of the five metals under various SHLA concentrations followed a similar trend in both soils. It increased sharply with SHLA concentration up to 1000 mg/L or 1500 mg/L and then the rate of increase reduced. For Soil 1, the % removal of Ni and Pb increased with SHLA concentration ($p<0.001$), initially sharply as SHLA concentration increased from 100 mg/L to 1000 mg/L ($p<0.001$), and then more gradually by just 1.8% for Ni and 2.6% for Pb after 1000 mg/L ($p<0.001$). For Cu and Zn, the % removal increased gradually with SHLA concentration up to 1500 mg/L ($p<0.001$), and then the rate of increase reduced ($p<0.01$). For Soil 2, the % removal of Cu, Pb and As increased rapidly with increasing SHLA concentration from 100 mg/L to 1000 mg/L ($p<0.001$), then increased slightly after 1000 mg/L for Pb ($p<0.001$) and As ($p<0.01$) but remained stable for Cu ($p>0.05$). For Zn, the inflection point happened at 1500 mg/L of SHLA ($p<0.01$). High concentrations of SHLA provided more metal binding sites, resulting in the high % metal removal.

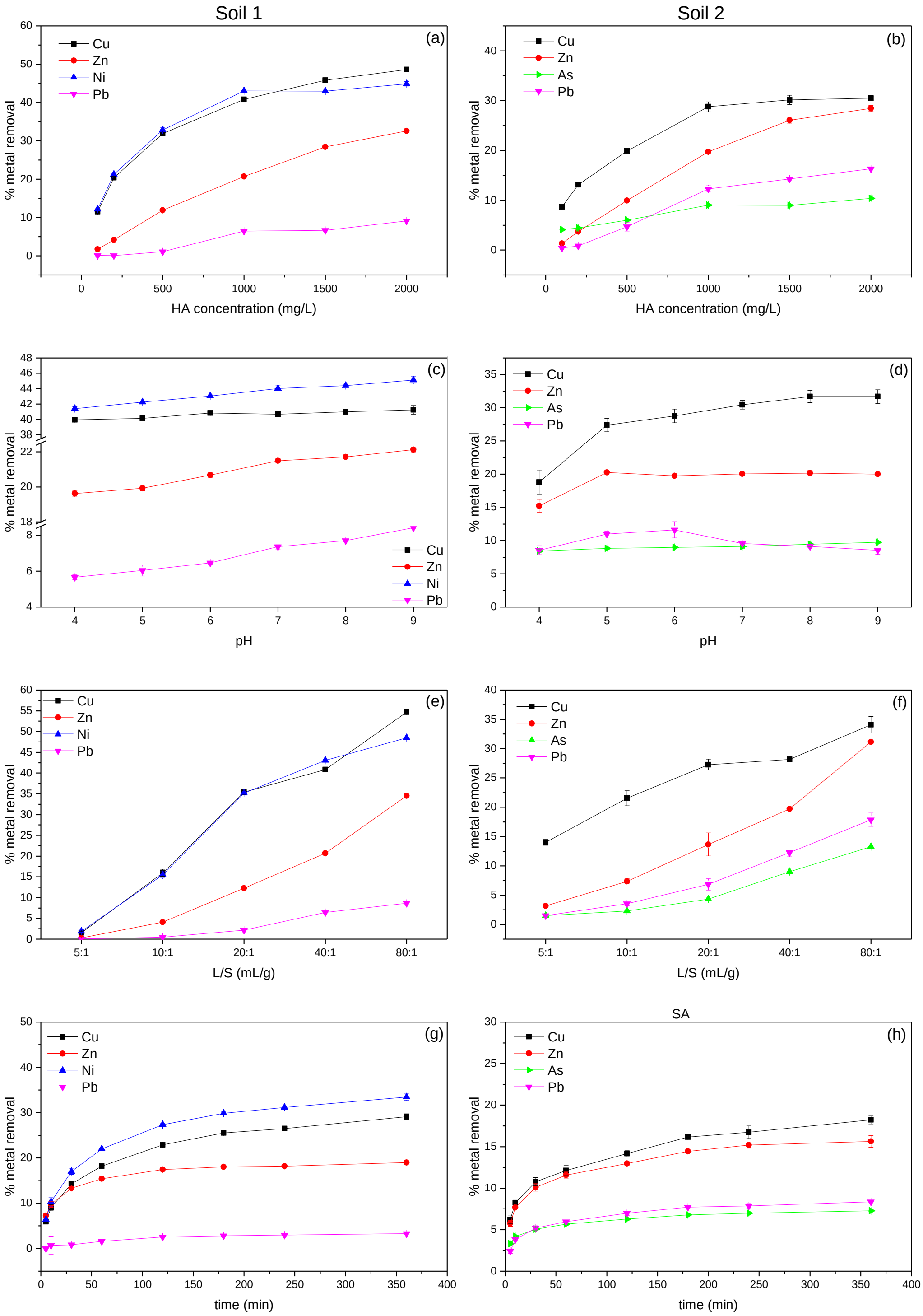

Figure 5.2 The effect of initial SHLA concentration (a,b), pH (c,d), L/S ratio (e,f) and contact time (g,h) on % metal removal (n=3, mean values ± standard deviation). The standard conditions were a SHLA concentration of 1000 mg/L, pH of 6, L/S ratio of 40:1 and contact time of 24h at 25°C; one of these variables was varied at a time.

5.3.2.2 pH

The pH was set to 4, 5, 6, 7, 8 and 9 to investigate the effect of pH on the % metal removal of SHLA for a SHLA concentration of 1000 mg/L, a L/S ratio of 40:1 (mL:g), a temperature of 25 °C and a washing duration of 24 h (Figure 5.2(c) and (d)). For Soil 1, the % removal of Zn, Ni and Pb increased gradually with increasing pH ($p<0.01$), while there is no apparent trend for Cu. For Soil 2, the % removal of Zn increased sharply from pH 4 to 5 ($p<0.001$), then remained stable around 20% from pH 5 to 9 ($p>0.05$). For Cu and As, the % removal increased with increasing pH ($p<0.001$), while the % removal of As just increased by 1.2% ($p<0.001$). The % removal of Pb increased from pH 4 to 6 ($p<0.05$), then decreased from pH 6 to 9 ($p<0.01$). The low % metal removal at low pH is due to the competition from H^+ with metal ions for COOH binding sites (Kulikowska et al., 2015). Also at low pH, SHLA is less negatively charged and electrostatic attraction between SHLA and soil minerals (e.g. kaolinite, montmorillonite and hematite) increases (Chen et al., 2017; Shaker et al., 2012). As the SHLA is more readily adsorbed onto the soil less is available for removal via complexation with the SHLA in solution (Hartley et al., 2014; Kulikowska et al., 2015). Typically in aqueous solutions, metal solubility decreases from acidic to neutral conditions, but for metals like Cu and Zn, begins to increase again as pH rises further (simulated by Visual MINTEQ 3.0 (KTH, Sweden)). It might, therefore, be expected that % metal removal would decrease from low pH to neutral conditions and then begin to increase again. However, the presence of a chelating agent has a significant impact because of the formation of organo-metal complexes in solution (e.g. Wang and Mulligan, 2009). The SHLA contains phenolic-OH groups and at higher pHs, these tend to dissociate (pKa: 8–11) and can react with metal cations, which contributes to the metal removal at high pH (Kulikowska et al., 2015; Vidali et al., 2011).

5.3.2.3 L/S ratio

The L/S ratio (mL:g) was set to 5:1, 10:1, 20:1, 40:1 and 80:1 to investigate the effect of the L/S ratio on % metal removal of the SHLA at a SHLA concentration of 1000 mg/L, a pH of 6 and a temperature of 25 °C for a wash duration of 24 h (Figure 5.2(e) and (f)). For both Soil 1 and Soil 2, % metal removal increased with an increasing L/S ratio ($p<0.01$), because more SHLA is available at the higher L/S ratios. However, when considering the practical applications of this potential technology, higher L/S ratios will lead to more residual metal-bearing solution for subsequent treatment.

5.3.2.4 Contact time and kinetics

Suspensions were shaken for 5 min, 10 min, 30 min ,60 min, 90 min, 120 min, 180 min, 240 min, 360 min and 1440 min to investigate the effect of reaction time on % metal removal by SHLA for an initial SHLA concentration of 1000 mg/L, a pH of 6, a L/S of 1:40 and a temperature of 25 °C (Figure 5.2(g) and (h)). In general, the % metal removal increased rapidly over the first 30-60 minutes ($p<0.001$) and then slowly increased ($p<0.05$). The % metal removal for each metal at 360 min was just 1.67-11.7 % lower that at 1440 min ($p<0.05$). Thus, despite this slight increase, considering the practical application of this technology, we suggest that 360 min is a better choice as a washing time.

Five commonly used kinetic models, the first order model, second-order model, Elovich model, power function model, and parabolic diffusion equations, were applied to the experiments and the results were evaluated by comparing the regression coefficient (R^2), SEE (standard errors of the estimate) and p-value (Table 5.4). The low R^2 values of the first-order (R^2: 0.5678-0.8305) and second-order (R^2: 0.5693-0.8454) models did not describe the experiments very well. However, for all metals in these two soils, the Elovich model and power function equation fit the experimental data well, indicating that the desorption process is a heterogeneous diffusion process (Zhang et al., 2017). The results are consistent with previous studies' findings that data are better fit by mass transfer and diffusion-based models than simple models like first order model for many soil chemical process (Sparks, 1995).

5.3.2.5 Summary

Considering both the % removal of metals and the potential costs of using increasing masses of SHLA in practical applications, we conclude that the optimal washing conditions are a pH of 9, a L/S ratio of 80:1 and a SHLA concentration of 1500 mg/L at 25 °C for 6 h.

Table 5.4 Kinetics parameters (R^2, SEE, p-value) for the first order, second order, Elovich, power function and parabolic function models.

		First order			Second order			Elovich			Power function			Parabolic function		
		R^2	SEE	p-value	R^2	SEE	p-value	R^2	SEE	p-value	R^2	SEE	p-value	R^2	SEE	p-value
Soil 1	Cu	0.7927	0.0588	<0.01	0.8263	0.0002	<0.001	0.9959	1.7828	<0.0001	0.9774	0.0929	<0.0001	0.9462	6.4749	<0.0001
	Zn	0.5784	0.0495	<0.05	0.5893	0.0001	<0.05	0.9825	4.3351	<0.0001	0.9455	0.0877	<0.0001	0.8457	12.8792	<0.01
	Ni	0.7747	0.0745	<0.01	0.8113	0.0006	<0.001	0.9978	0.7105	<0.0001	0.9630	0.1244	<0.0001	0.9242	4.2050	<0.001
	Pb	0.8305	0.0058	<0.001	0.8454	0.00001	<0.001	0.9610	0.8477	<0.0001	0.9457	0.1633	<0.001	0.9405	1.0470	<0.0001
Soil 2	Cu	0.7198	0.0366	<0.01	0.7456	0.0002	<0.01	0.9897	1.1307	<0.0001	0.9893	0.0420	<0.0001	0.9593	2.2523	<0.0001
	Zn	0.6511	0.0355	<0.01	0.6822	0.00004	<0.01	0.9966	1.9219	<0.0001	0.9778	0.0573	<0.0001	0.9219	9.1455	<0.001
	As	0.5678	0.0169	<0.05	0.5693	0.0001	<0.05	0.9969	0.2050	<0.0001	0.9799	0.0423	<0.0001	0.9152	1.0770	<0.001
	Pb	0.6385	0.0187	<0.01	0.6539	0.00004	<0.01	0.9898	1.0156	<0.0001	0.9418	0.1106	<0.0001	0.8829	3.4420	<0.001

5.3.3 Metal speciation before and after SHLA washing and the influence on metal stability and mobility in two soils

The BCR sequential extraction was applied to determine how metal speciation in the soil (F1: exchangeable and acid-soluble fraction; F2: reducible fraction; F3: oxidizable fraction; F4: residual fraction) changed after SHLA washing for metals identified as being above permitted EU concentrations in agricultural soils, i.e. Cu, Zn, Ni, Pb for Soil 1, and Cu, Zn, As, Pb for Soil 2 (Table 5.3, Figure 5.3 for distributions in terms of actual concentration, Figure 5.S1 for percentage distributions). The fate and behaviour of the four fractions are different. Generally, F1 and F2 are bioavailable, relatively soluble and easily extracted, resulting in greater environmental risk than F3 and F4 (Zhang et al., 2017). In both soils, SHLA mainly removed F1 ($p<0.05$) and F2 ($p<0.05$) for Cu, Zn, Ni and Pb. A small portion of F3 was removed as well for Cu, Zn, Ni and Pb ($p<0.05$ except $F3_{Zn}$ in Soil 2), but F4 was not be removed ($p>0.05$ except $F4_{Cu}$ in Soil 2). The results are consistent with previous studies (Zhang et al., 2017). For instance, SHLA significantly removed 93.4% Cu and 85.0% Ni of F1 ($p<0.05$), 79.6% Cu and 51.8% Ni of F2 ($p<0.05$), 43.3% Cu and 16.3% Ni of F3 ($p<0.05$), but F4 for both Cu and Ni did not change significantly ($p>0.05$) in Soil 1. In Soil 1, the percentage distributions of Zn and Ni were similar initially, but became different after soil washing, which might be because Ni has a higher affinity to SHLA than Zn which caused a much higher % removal from F1 of Ni (85%) than of Zn (48%) (Pandey et al., 2000). In Soil 2, the extraction behaviour for As was different from the removal of Cu, Zn and Pb. SHLA only removed 25.8% As from F2 ($p<0.05$) but could not remove As from F1, F3 and F4 ($p>0.05$). As mentioned above, under the experimental conditions, As would have been present in anionic forms and would not have bonded to the humic acid via exchange with the H^+ of the COOH groups. This led to the low % removal of As from all the fractions. However, humic acid can also act as an electron donor which might explain the relatively higher % removal of As from the F2 fraction (He et al., 2014).

I_R (reduced partition index) and M_F (metal bioavailability factor) before and after SHLA washing (Figure 5.4) were calculated to evaluate metal binding ability and mobility based on the data from the BCR extraction (Tang et al., 2017; Wei et al., 2016). Generally, high I_R and low M_F values indicate that metals in soils are stable with low mobility and bioavailability (Gusiatin and Klimiuk, 2012). The I_R values for all metals (except Pb) in both two soils increased ($p <0.05$) following SHLA extraction, indicating that the metals that remained in the soils were more stable and strongly bound to the soil, thus reducing their potential mobility and bioavailability. Specifically, after SHLA washing, in Soil 1 Cu had the largest I_R value ($p<0.001$) of all the metals and the I_R of Ni had the largest increase (by 0.2 units, $p<0.001$). Similarly in Soil 2, Cu had the largest I_R value ($p<0.001$) of all the metals and the largest increase in I_R value ($p<0.01$). In Soil 1 the M_F values for Cu ($p<0.001$), Zn ($p<0.01$) and Ni ($p<0.001$) decreased after washing, indicating that metal mobility and bioavailability were reduced. However, there were no significant changes in the M_F values of either Zn or As ($p \geqq 0.05$) in Soil 2, which might be caused by the low F1 extraction efficiency of both Zn and As.

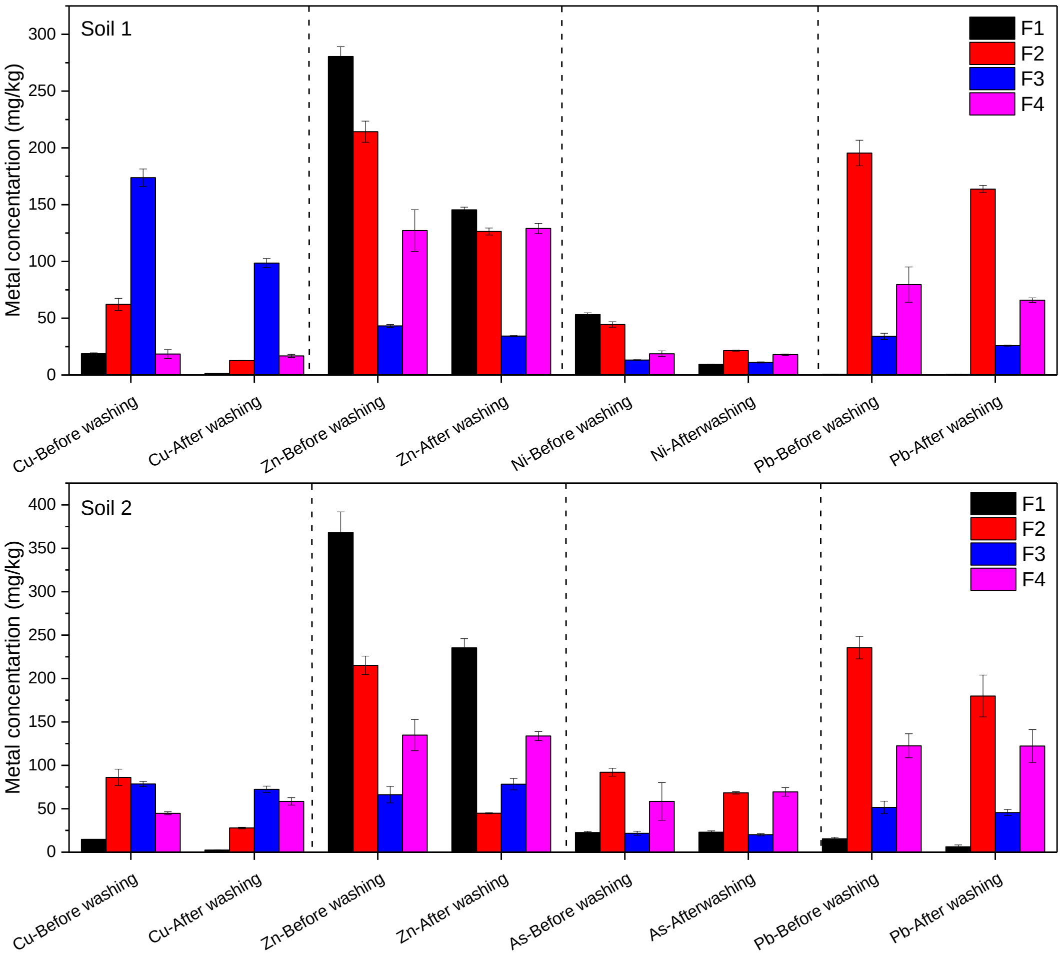

Figure 5.3 BCR-defined metal speciation distribution of Soil 1 (a,b) and Soil 2 (c,d) before and after SHLA washing in terms of actual concentration (n=3, mean values ± standard deviation). The percentage of different fractions are given in Figure 5.S2 (Supplementary Material).

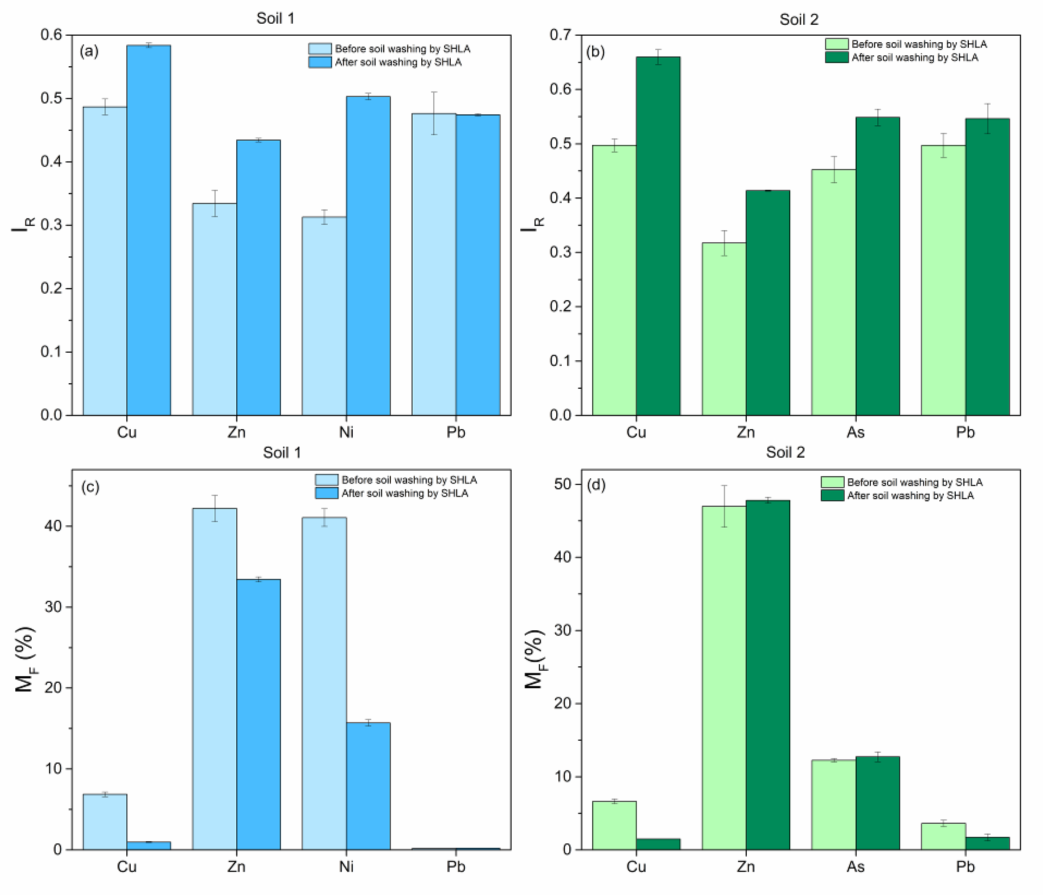

Figure 5.4 I_R (a,b) and M_F (c,d) values before and after SHLA washing of two soils (n=3, mean values ± standard deviation).

5.3.4 Comparison with CHA, Na$_2$EDTA, citric acid and tartaric acid

In addition to humic acid, synthetic organic chelating agents (e.g. EDTA, DTPA, and EDDS, etc.) and low-molecular-weight organic acids (e.g. oxalic acid, citric acid, and tartaric acid, etc.) are also studied and applied widely as soil washing agents (Begum et al., 2012). In this study, commercial humic acid (CHA, as a representative of natural humic acid), Na$_2$EDTA, citric acid and tartaric acid were used as washing agents with which to compare the performance of SHLA (Figure 5.5).

In both soils, % metal removal for all metals by SHLA was 2.2-15.2 times higher than that of CHA ($p<0.001$), most likely due to the higher COOH and phenolic-OH content of SHLA than CHA. In Soil 1, the % Cu removal ($p<0.001$) and % Pb removal ($p<0.001$) of SHLA was just below N$_2$EDTA. Na$_2$EDTA had the greatest % metal removal for Cu ($p<0.001$), Ni ($p<0.01$), and Pb ($p<0.05$), while citric acid had the better extraction ability for As ($p<0.01$). The overall metal extraction behaviour of SHLA seemed not as good as Na$_2$EDTA, citric acid and tartaric acid. However, if the concentrations of the washing agents are considered on a mole basis rather than on a mass basis (1500 mg/L), they were 4.46 mmol/L for Na_2EDTA, 9.99 mmol/L for citric acid, 7.81 mmol/Lfor tartaric acid, 0.375 mmol/L for CHA (the molecular weight of CHA is c. 4000 g/mol (Nghiem et al., 2008)) and 0.14-0.71 mmol/L for SHLA (the molecular weight of synthetic-humic like acid ranges from 2100 to 11000 g/mol (Fukuchi et al., 2012; Fukuchi et al., 2010; Nishimoto et al., 2013; Okabe et al., 2011; Qi et al., 2012)). Compared with Na_2EDTA, citric acid and tartaric acid, the molar concentration of SHLA was much lower, which caused the apparent low % metal removal. Accordingly, Figure 5.5(c) and 5(d) show metal removal efficiency in terms of mg metal removed /mmol washing agent; SHLA shows the highest removal efficiency in both Soil 1 and 2 ($p<0.05$). Besides, although Na_2EDTA had excellent metal extraction efficiency for most metals in this study, synthetic chelate agents can lead to adverse health and environmental effects, which restricts their use (Peng et al., 2009). Humic substances are important components of soil organic matter (Stevenson, 1994) and if SHLAs were used in soil washing, any residual SHLA left in the soil could contribute to soil organic matter. Thus the use of SHLAs has the potential to be environmentally friendly compared to using compounds such as EDTA. The toxicity of the reagents used to synthesize materials should be considered as well. However, although the SHLA were synthesized with catechol and thimerosal which can be toxic at high concentrations (Sigma-Aldrich, 2013; Sigma-Aldrich, 2017), the purification process of SHLA involves a dialysis step that separates the high molecular number HA from the low molecular catechol and thimerosal. Thus, catechol and thimerosal do not exist in SHLA product. However, clearly before their widespread use, their application would need to be assessed for any possible toxic side effects.

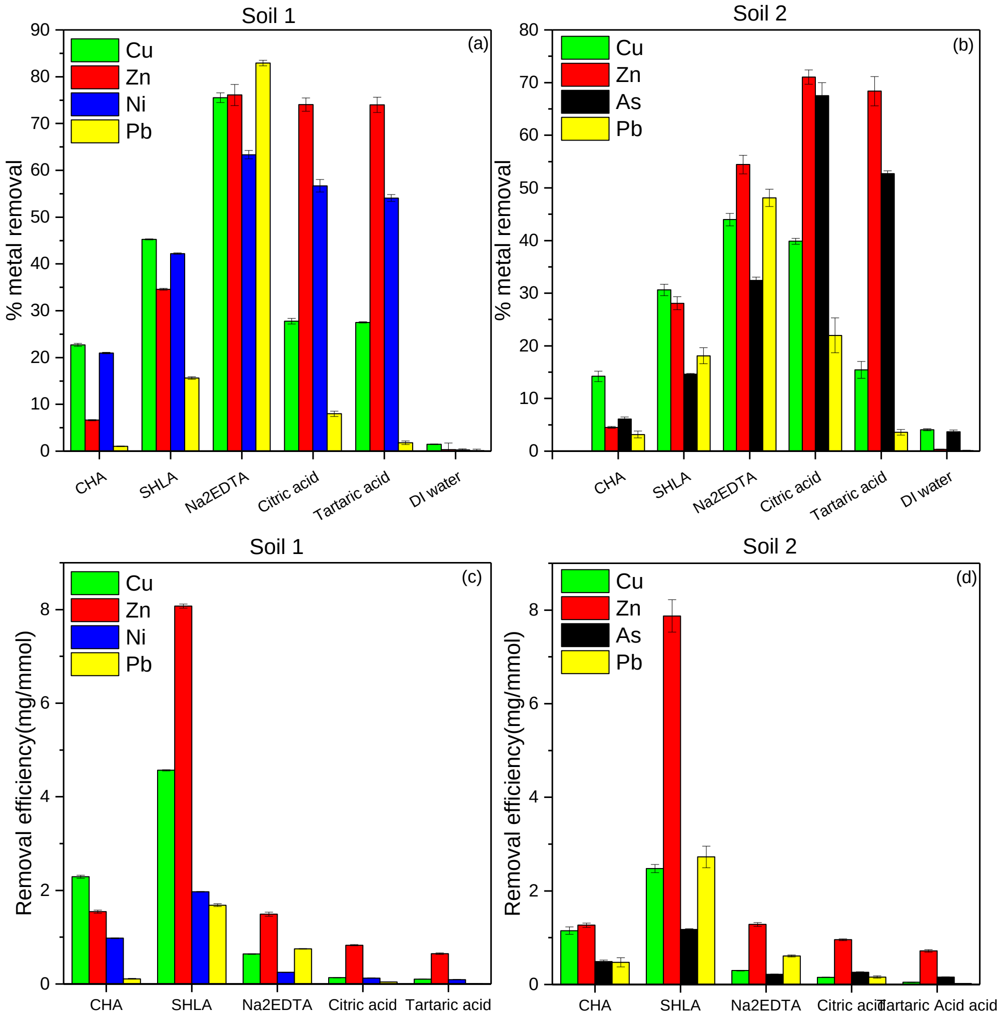

Figure 5.5 Comparison of % metal removal of SHLA, CHA, Na_2EDTA, citric acid and tartaric acid (a and b in terms of % metal removed, c and d in terms of mg metal removed /mmol washing agent; the molecular weight of SHLA and CHA was set to 4000 g/mol for calculation; n=3, mean values ± standard deviation).

5.4 Conclusions

In this study, a synthetic humic-like acid (SHLA) with high COOH content was applied as a washing agent to remove metals (Cu, Zn, Ni, Pb, As) from two contaminated agricultural soils (Soil 1 and Soil 2). The effects of solution pH (4 to 11), L/S ratio (5:1 (mL:g) to 80:1 (mL:g)), SHLA concentration (100 mg/L to 2000 mg/L), and contact time (0 to 1440 min) on % metal removal were investigated and optimal extraction conditions (pH=9, L/S ratio of 80:1 mL/g, initial SHLA concentration of 1500 mg/L, contact time of 360 min) were determined. Under these conditions, a single washing removed 45.2 % (136.98 mg/kg) of Cu, 34.6 % (242.21 mg/kg) of Zn, 42.2 % (59.16 mg/kg) of Ni and 15.6 % (50.52 mg/kg) of Pb from Soil 1, and 30.6% (74.37 mg/kg) of Cu, 28.1 % (236.24 mg/kg) of Zn. 14.6 % (35.35 mg/kg) of As and 18.1% (81.78 mg/kg) of Pb from Soil 2, respectively. The SHLA mainly removed metals in the BCR defined F1 (exchangeable and acid soluble) and F2 (reducible) fractions, which would effectively reduce bioavailability and environmental risk of the metals. In terms of removal efficiency on a molar basis, SHLA was more effective than commercial humic acid, Na_2EDTA, citric acid and tartaric acid. Economic studies are now required, taking into account costs of production, to determine whether larger scale trials of SHLA in soil washing are warranted. Overall, SHLA shows great potential as a soil washing agent.

Appendix 5. Supplementary Materials

Methodological details for measuring soil physico-chemical properties are provided in the Supplementary materials. FTIR peak assignments (Table 5.S1) are shown in supplementary materials. Figure 5.S1 shows metal concentrations in soils after SHLA washing at different washing conditions. Figure 5.S2 shows metal speciation distribution (%) of Soil 1and Soil 2 before and after SHLA washing in terms of percentage.

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Appendix 5. Supplementary materials

Soil characterization methods in details

pH: 10 g of air-dried soil was added to 25 mL deionised water in 50 mL centrifuge tube and shaken for 15 minutes. Thermo Scientific Orion Model 420 plus pH meter was calibrated with pH buffer solutions (pH 4.00, 7.00 and 10.00). Then pH electrode was placed in the soil suspension and the pH was recorded. (Rowell, 1994).

Organic carbon was measured using a vario MACRO C/N analyzer (Elementary, Germany).

Cation exchange capacity (CEC): 1) 2 g of air-dried soil was added to 20 mL 0.2 M $BaCl_2$ solution in 50 mL centrifuge tube and shaken for 2 hours. Then exchangeable cations in the supernatant were analysed by ICP-OES. 2) The soil was washed for 3 times with 20 mL 0.01 $BaCl_2$ and shaken for 1 hour every time. Then the supernatant was discarded. 3) 20 ml of 0.025 M $MgSO_4$ solution was added to soil and the mixture was shaken overnight. Then Mg concentrations in the supernatant were analysed by ICP-OES and CEC was calculated by the difference (Hendershot and Duquette, 1986).

Soil texture was determined using a Malvern Mastersizer Hydro2000 MU laser granulometer (Malvern Instruments, UK).

Table 5.S1 Assignments of FTIR absorption bands present in the spectra for SHLA and CHA (Stevenson, 1994; Jokic et al., 2004; Fukushima et al., 2009; Hardie et al., 2009; Shiotsuka et al., 2015; Yang and Hodson, 2018a; Yang and Hodson, 2018b).

SHLA		CHA	
Wavenumber/cm^{-1}	Assignment	Wavenumber/cm^{-1}	Assignment
2918	C-H stretching of aliphatic structures	2921	C-H stretching of aliphatic structures
2121	C≡C stretching and the presence of cyano group		
1717	C=O stretching of carbonyl groups		
1579 & 1488	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)	1572	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)
1382	O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and C-O stretch of COO-	1375	O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and C-O stretch of COO-
1179	C–O stretching and C–O–H deformation of alcohols, phenols and ethers		
		1008	C-O stretching vibration in alcohols, phenols and ethers

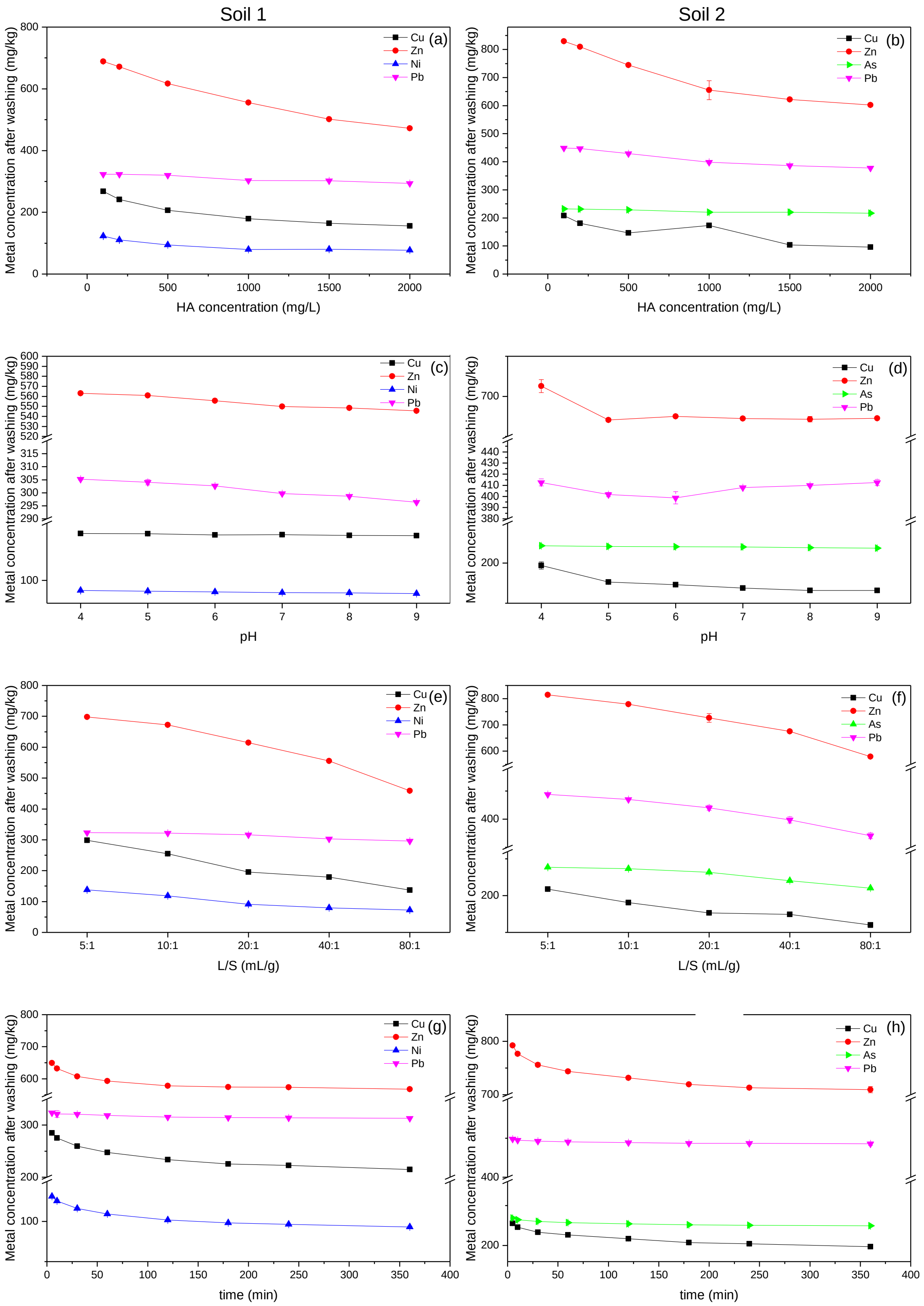
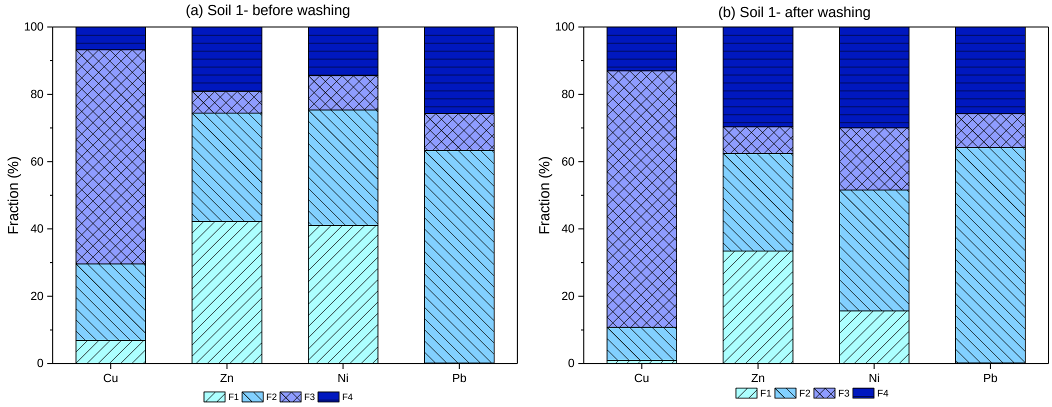
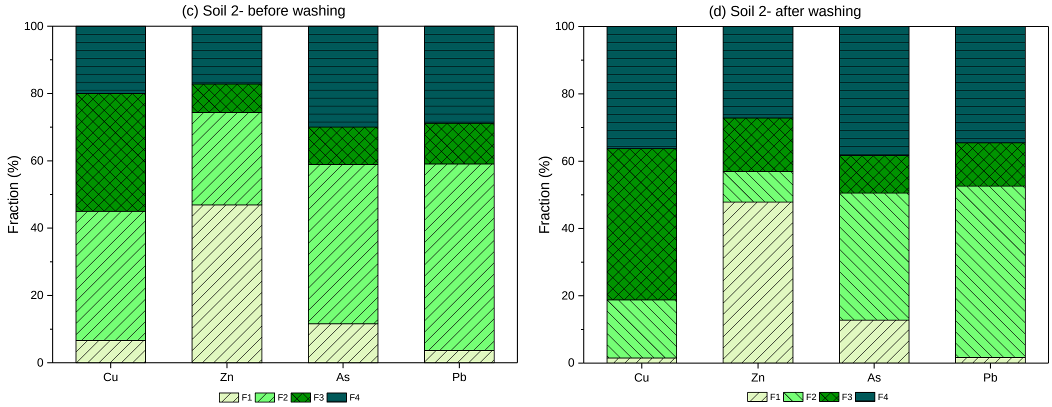


Figure 5.S1 Metal concentrations after SHLA washing at different initial SHLA concentrations (a,b), pH (c,d), L/S ratios (e,f) and contact time (g,h). (n=3, mean values ± standard deviation). For a & b, experiments were conducted at a pH of 6, a L/S ratio of 40:1 and a temperature of 25 °C for 24h. For c & d, experiments were conducted at a SHLA concentration of 1000 mg/L, a L/S ratio of 40:1 and a temperature of 25 °C for 24h. For e & f, experiments were conducted at a

pH of 6, a SHLA concentration of 1000 mg/L and a temperature of 25 °C for 24h. For g & h, experiments were conducted at a pH of 6, a SHLA concentration of 1000 mg/L, a L/S ratio of 40:1 and a temperature of 25 °C.


(a) Soil 1- before washing

(b) Soil 1- after washing


(c) Soil 2- before washing

(d) Soil 2- after washing

Figure 5.S2 BCR-defined metal speciation distribution (%) of Soil 1 (a,b) and Soil 2 (c,d) before and after SHLA washing.

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CHAPTER 6

Investigating the potential of synthetic humic-like acid to remove metal ions from contaminated water

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Chapter 6 Investigating the potential of synthetic humic-like acid to remove metal ions from contaminated water

Ting Yang[a*], Mark E Hodson[a]

[a] Environment Department, University of York, Heslington, Wentworth Way, York YO10 5NG, United Kingdom

*Corresponding author. Tel.: +44 (0)7421 236832. Fax: +44 (0)7421 236832.

E-mail address: ty672@york.ac.uk

Abstract

Humic acid can effectively bind metals and is a promising adsorbent for remediation technologies. Our studies initially focussed on Cu^{2+} as a common aqueous contaminant. Previous studies indicate that carboxylic groups dominate Cu^{2+} binding to humic acid. We prepared a synthetic humic-like acid (SHLA) with a high COOH content using catechol (0.25M) and glycine (0.25M) with a MnO_2 catalyst (2.5% w/v) at pH = 8 and 25 °C and investigated the adsorption behaviour of Cu^{2+} onto it. The SHLA exhibited a range of adsorption efficiencies (27% - 99%) for Cu^{2+} depending on reaction conditions. A pseudo-second-order kinetic model provided the best fit to the experimental data (R^2 = 0.9995-0.9999, $p \leq 0.0001$), indicating that chemisorption was most likely the rate-limiting step for adsorption. The equilibrium adsorption data showed good fits to both the Langmuir ($R^2 = 0.9928 – 0.9982$, $p \leq 0.0001$) and Freundlich ($R^2 = 0.9497 – 0.9667$, $p \leq 0.0001$) models. The maximum adsorption capacity (q_m) of SHLA increased from 46.44 mg/g to 58.78 mg/g with increasing temperature from 25 °C to 45 °C. Thermodynamic parameters (ΔG^0=-3.69 - -2.50 kJ/mol; ΔS^0=0.06 kJ/(mol·K); ΔH^0=15.23 kJ/mol) and values of R_L (0.0142-0.3711) and n (3.264-3.527) show that the adsorption of Cu^{2+} onto SHLA was favourable, spontaneous and endothermic in nature. Over six adsorption/desorption cycles using 0.5M HCl for the desorption phase, there was a 10% decrease of the adsorption capacity. A final experiment using a multi-metal solution indicated adsorption efficiencies of up to 84.3-98.3% for Cu, 86.6-98.8% for Pb, 30.4-82.9% for Cr, 13.8-77.4% for Ni, 9.2-62.3% for Cd, 8.6-51.9% for Zn and 4.6-42.1% for Co. Overall, SHLA shows great potential as an adsorbent to remove metals from water and wastewater.

***Keywords*:** Synthetic humic-like acid; Metal ions; Adsorption; Kinetics; Curves; Thermodynamics

6.1 Introduction

Water contamination caused by metals (e.g., copper, cadmium, lead, zinc, chromium, and nickel) is a serious environmental and health issue worldwide (Gupta et al., 2016; Kavcar et al., 2009; Machado et al., 2017; Saha et al., 2017; Zhang et al., 2017a). Metals can be of great concern because at elevated concentrations they can be extremely toxic, non-biodegradable and tend to accumulate in living organisms (Rezania et al., 2016; Zou et al., 2016). Thus, it is essential to remove excessive concentrations of metals from water and wastewater for human health and ecological stability. Significant industrial sources of copper release into the environment include metal cleaning, electroplating, smelting, mining, the paper industry, batteries and pesticides (Al-Qodah and Al-Shannag, 2017; Awual et al., 2013; Gupta, 1998). Excessive intake of copper can cause serious toxicological concerns, such as gastrointestinal problems, cystic fibrosis, kidney damage, neurotoxicity, cramps, hair loss, anaemia, hypoglycemia, and even death (Al-Qodah and Al-Shannag, 2017; Fu and Wang, 2011; Ihsanullah et al., 2016). The maximum permissible concentrations of Cu in drinking water as defined by the EPA, WHO and EEA are 1.3mg/L, 1.5mg/L and 2 mg/L, respectively (EPA, 2009; EU, 1998; WHO, 2004).

Various methods to remove metals from water have been investigated in the past decades, including chemical precipitation, adsorption, ion exchange, membrane filtration, phytoremediation, electrodialysis, photocatalysis and flotation (Mahmud et al., 2016; Rezania et al., 2016). Of these, adsorption is regarded as one of the most simple, cost-effective and efficient (Gupta et al., 2016; Gupta and Saleh, 2013). As the adsorbent is the key to adsorption techniques, many organic and inorganic adsorbents have been studied, such as active carbon, nanosized metal oxides, carbon nanotubes, clay minerals, biochar and polymers (e.g. chintin/chitosan based materials and humic acid based materials) (Adebisi et al., 2017; Ahmaruzzaman and Gupta, 2011; Anastopoulos et al., 2017; Côa et al., 2017; Gupta and Nayak, 2012; Ihsanullah et al., 2016; Inyang et al., 2016; Li et al., 2010; Mahdavi, 2016; Saleh and Gupta, 2012; Tang et al., 2014; Uddin, 2017; Zhang et al., 2016).

Humic acid (HA) is a natural biopolymer and major component of humic substances (Stevenson, 1994). Due to the abundant oxygen-containing functional groups present in HA (e.g., including carboxyl, phenol, hydroxyl, amine, and enol), humic acid can effectively bind several metals (Perminova and Hatfield, 2005). Recently, humic acid and humic acid-based materials have been considered as a promising adsorbent and have attracted increasing attention (Arslan et al., 2007; Jin et al., 2016; Lin et al., 2011; Yang et al., 2015). The majority of humic acids used in these studies are natural humic acids extracted from soil, lignite or sediment (Arslan et al., 2007; He et al., 2016; Unuabonah et al., 2016; Yang et al., 2015). However, humic acid can also be synthesized by abiotic humification processes, that is the transformation of humic precursors (e.g., amino acids, sugars, and quinones) to humic substances catalyzed by materials containing metallic oxide (e.g., MnO_2, Fe_2O_3, and Al_2O_3). Humic acid synthesized by abiotic humification is called synthetic humic-like acid (SHLA) (Fukushima et al., 2009; Hardie et al., 2009; Qi et al., 2012(a); Qi et al., 2012(b); Zhang et al., 2015; Zhang et al., 2017b). Compared with natural humic acid, the advantage of synthetic humic-like acid is that the structural characteristics of SHLA can be manipulated by changing the reaction conditions of the abiotic humification process, such as the species of catalyst and the ratio of precursors (Fukuchi et al., 2012; Jokic et al., 2004; Zhang et al., 2015). Therefore, abiotic humification processes can provide a potential and promising way to synthesize humic acids with better metal sorption ability than their naturally occurring counterparts and the novelty of our study lies in its investigation of a SHLA that has been synthesized to optimize its adsorption capacity.

The aims of the experiments reported here were to determine the adsorption efficiency and adsorption kinetics, together with isotherm fits and thermodynamics for adsorption of Cu^{2+} by an optimized SHLA under a range of conditions, to determine how re-usable the material was via a series of adsorption/desorption cycles and to investigate whether a SHLA optimized for Cu^{2+} would also efficiently remove metals from solutions containing a mixture of metals.

6.2 Materials and methods

6.2.1 Materials

Catechol ($C_6H_6O_2$, >99% purity), glycine ($C_2H_5NO_2$, >99% purity), glucose ($C_6H_{12}O_6$, >99% purity), thimerosal ($C_9H_9HgNaO_2S$, >97% purity), manganese oxide (MnO_2, >99% purity), and copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$, >99% purity) were purchased from Sigma-Aldrich. Sodium phosphate dibasic dihydrate ($Na_2HPO_4 \cdot 2H_2O$, >99% purity), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot H_2O$, >99% purity) and sodium nitrate ($NaNO_3$, >99% purity) were purchased from ACROS Organics. Calcium chloride ($CaCl_2$, >99% purity) was purchased from Fisher Chemical. Multi-element standard solution IV (1000 mg/L) was purchased from MERCK. Ultra-pure water was prepared using a PURELAB Classic ultra-pure system (ELGA, UK) from deionized water.

6.2.2 Preparation of optimal synthetic humic-like acid

Previous studies indicate that carboxylic groups dominate Cu^{2+} binding to humic acid (Gondar et al., 2006; Xu et al., 2016; Yang et al., 2015). In preliminary work for this study, SHLAs were synthesized at different temperatures (25 °C - 45 °C), pH (6 - 8), and with varying precursor species (glycine, catechol and glucose), initial precursor concentrations (0.25M:0.25M - 1M:1M) and mass of MnO_2 catalyst (1.3% 2.5%) (Table 6.S1). Based on these initial experiments we synthesized a SHLA (SHLA 12 in Table 6.S1) with an optimised high COOH content from a pH 8, 0.25M glycine: 0.25M catechol mixture at 25 °C with 2.5 wt% MnO_2 as a catalyst.

To ensure that the humification was abiotic, sterile conditions were maintained throughout the experiments. All glassware and pure water were autoclaved (121 °C for 27 minutes, 0.12 MPa) prior to use. An 1000-mL aliquot of autoclaved phosphate buffer (pH 8, 0.2 M Na_2HPO_4 / NaH_2PO_4 autoclaved at 121 °C for 27 minutes, 0.12 MPa) containing 0.02% (w/v) thimerosal (to ensure sterility) was placed in a 3L beaker, to which 25.00 g of MnO_2 was added. 27.53 g of powdered catechol and 18.77g of glycine to give 0.25M concentrations of each were added to the suspensions. The mixture was shaken in the dark at 25 °C for 240 h. After incubation, SHLA was extracted and purified using the standard method recommended by the International Humic Substances Society (IHSS) (Swift, 1996). Details of the extraction and purification process are given in the Supplementary materials. When synthesizing materials for environmental deployment, the toxicity of the reagents and products should be considered. The extraction process used to isolate the SHLA results in the isolation of pure SHLA; an attraction of the use of SHLAs in environmental applications is that humic acids and SHLAs occur naturally in the environment and therefore represent less of a risk to the environment than some other materials such as nanoparticles. The reagents used to synthesize the SHLA have low toxicities (see Material safety data sheets such as: (Sigma-Aldrich, 2014; Sigma-Aldrich, 2015; Sigma-Aldrich, 2017(a); Sigma-Aldrich, 2017(b)). Whilst thimerosal is highly toxic in concentrated form (Sigma-Aldrich, 2013), the dose used in this study was just 0.02% (w/v), concentrations higher than this (0.001 - 0.1%) are routinely used in human medical applications (National, 2001; Veen and Joost, 1994). Thimerosal was used in this study to maintain sterile conditions as we wished to be certain that our SHLA was produced by abiotic processes. However, to reduce environmental risks we would anticipate that in the commercial production of SHLA for use as a remedial agent, thimerosal would not be used; sterility would be established by autoclaving.

6.2.3 Characterization of the synthetic humic-like acid

The elemental composition (C, H, N) of the SHLAs was determined using a vario MICRO cube type elemental analyzer (Elementary, Germany). Total acidity and carboxylic group content were measured using the $Ba(OH)_2$ and $Ca(CH_3COO)_2$ titration methods, respectively (Stevenson, 1994). The phenolic-OH content was calculated by subtracting the carboxylic group content from total acidity. The UV spectra of the SHLAs were determined using a Lambda 25 UV/Vis Spectrophotometer (PerkinElmer, USA). Absorbance at 465 and 665 nm was recorded on solutions of 20 mg of each HA dissolved in 100 mL of 0.05 M $NaHCO_3$, with the pH adjusted to 8 with NaOH and HCl. The E_4/E_6 ratio was calculated as the ratio of the absorbances at 465 and 665 nm (Chen et al., 1977). FTIR spectra of the SHLAs were obtained using an Alpha FT-IR Spectrometer (Bruker, Germany). Spectra were recorded from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. For each spectrum, 24 scans were averaged to reduce noise.

6.2.4 Adsorption kinetics and removal efficiency

The effects of temperature, pH, initial Cu^{2+} concentration and SHLA dose on Cu^{2+} adsorption kinetics and removal efficiency were investigated via a series of adsorption experiments. All the experiments used 200 mL of $Cu(NO_3)_2$ solution in a background electrolyte of 0.1 M $NaNO_3$. Initial solution pH was adjusted by additions of either NaOH or HNO_3. The experiments at different pH were conducted at 25 °C and used initial concentrations of 10 mg/L Cu^{2+} and 1 g/L SHLA. The initial pH in solution was set to 1, 2, 3, 4, 5, 6, 7 and 8. The experiments at different temperatures were conducted at a pH of 5 and initial concentrations of Cu^{2+} of 50 mg/L and 1 g/L of SHLA. The initial temperature of the solutions was set to 25 °C, 35 °C and 45 °C. The experiments at different initial Cu^{2+} concentrations, were conducted at pH 5 and had initial Cu^{2+} concentrations of 10, 20, 50, 100 and 150 mg/L and 1 g/L of SHLA at 25 °C. The experiments that investigated SHLA doses used concentrations of SHLA of 0.5 g/L, 1 g/L and 2 g/L at an initial Cu^{2+} concentration of 50 mg/L at pH 5 and a temperature of 25 °C. The dissolution of SHLA was assessed by measuring the dissolved organic carbon (DOC) after 24 hours and back-calculating the mass of dissolved SHLA on the basis of the determined SHLA C content. Dissolution ranged from 1.65% to 1.93% in this study, indicating that the SHLA is stable in aqueous solution. Visual MINTEQ 3.0 (KTH, Sweden) was used to confirm that Cu^{2+} would not precipitate under these experimental conditions.

For each experiment, a sample of 2 mL was taken by pipette at 1, 3, 5, 10, 15, 20, 30, 60, 90, 120, 180, 240 and 1440 (24h, for removal efficiency calculation) minutes, filtered using a 0.45μM nylon syringe filter (Gilson, UK) and diluted in 5% HNO_3. The Cu^{2+} concentration was determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES) (iCAP 7000, Thermo Scientific, USA). Copper recovery after filtration with the nylon syringe filter ranged from 98.5% to 101.9%, and thus Cu adsorption by the nylon syringes filter can be ignored. All the experiments were performed in triplicate. In the results, all calculated values (see below) are quoted as mean values ± standard deviations. Equilibrium time for each adsorption experiment was determined as the time when consecutive concentration measurements showed no significant difference and the time when the concentration showed no significant difference to that at 24 hours by Kruskal-Wallis test using SPSS 23.0 (IBM, USA).

The kinetic study data were fitted to pseudo-first-order (equation 1), pseudo-second-order (equation 2), intraparticle diffusion (equation 3) and Elovich (equation 4) models.

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (1)$$

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e} \quad (2)$$

$$q_t = k_{id} t^{0.5} + C \quad (3)$$

$$q_t = \frac{1}{b}\ln(ab) + \frac{1}{b}\ln(t) \quad (4)$$

where q_e and q_t are the concentrations of Cu^{2+} adsorbed (mg/g) at equilibrium and time t. k_1, k_2 are the rate constants of pseudo-first-order and pseudo-second-order adsorption. k_{id} (mg/(g·min$^{0.5}$)) is the intra-particle diffusion rate constant and C (mg/g) is the constant which is proportional to the boundary layer thickness. a (mg/(g·min)) is the initial sorption rate and b (g/mg) is the desorption constant.

The removal efficiency of Cu^{2+} was calculated as:

$$\%\text{Removal} = \frac{C_0 - C_e}{C_0} \cdot 100 \quad (5)$$

where C_0 and C_e (mg/L) are the initial and equilibrium concentrations of Cu^{2+} in solution.

The adsorption capacity of Cu^{2+} was calculated as:

$$q_e = \frac{(C_0 - C_e)V}{M} \quad (6)$$

where q_e (mg/g) is the equilibrium adsorption capacity; V(L) is the volume of aqueous solution containing Cu^{2+}; and M(g) is the weight of the adsorbent.

6.2.5 Adsorption curves study

Adsorption curves were studied at three different temperatures. In detail, 30.0 mg of SHLA was shaken at pH 5 for 24 h with 30.0 mL of $Cu(NO_3)_2$ solution at 8 different initial concentrations (5, 10, 20, 50, 75, 100, 125 and 150 mg/L) at 25 °C, 35 °C and 45 °C. The background electrolyte was 0.1 M $NaNO_3$. Solution compositions were modelled using Visual MINTEQ 3.0 (KTH, Sweden) to confirm that Cu^{2+} would not precipitate from solution.

All solutions were shaken orbitally for 24 hours at 250 rpm. Then, the mixtures were separated by centrifugation at 4500 rpm for 20 min. The supernatant was filtered using a 0.45μM nylon syringe filter (Gilson, UK) and diluted in 5% HNO_3. The copper concentrations in the supernatants were determined by ICP-OES. All the experiments were performed in triplicate. In the results, all calculated values (see below) are quoted as mean values ± standard deviations.

The data were fitted to the Langmuir (equation 7), Freundlich (equation 8) and bi-Langmuir (equation 9) models:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad (7)$$

where q_m (mg/g) is the maximum adsorption capacity when an adsorbent is fully covered, and K_L (L/mg) is the Langmuir adsorption constant which relates to the adsorption energy.

$$\ln(q_e) = \ln(K_f) + \frac{1}{n}\ln(C_e) \quad (8)$$

where K_f (mg/g(L/mg)$^{1/n}$) is the Freundlich isotherm constant and n (dimensionless) is the heterogeneity factor.

$$q_e = \frac{q_{m,1}k_{L,1}C_e}{1+k_{L,1}C_e} + \frac{q_{m,2}k_{L,2}C_e}{1+k_{L,2}C_e} \quad (9)$$

where $q_{m,1}$ (mg/g) and $q_{m,2}$ (mg/g) are the adsorption capacity of Cu^{2+} on sorption site 1 and site 2, respectively; $k_{L,1}$ (L/mg) and $k_{L,2}$ (L/mg) are the adsorption affinity constants on sorption site 1 and site 2.

6.2.6 Adsorption thermodynamic study

Data from the adsorption studies conducted for 24 hours at 25℃, 35℃ and 45℃ with initial Cu^{2+} and SHLA concentrations of 50 mg/L and 1g/L and a pH of 5 were used to determine the thermodynamics of adsorption. The thermodynamic parameters (free energy change (ΔG°), the entropy change (ΔS°) and enthalpy change (ΔH°) were calculated following the method of (Li et al., 2015).

$$K_c = \frac{q_e}{C_e} \quad (10)$$

$$\Delta G^\circ = -RT\ln(K_c) \quad (11)$$

$$\ln(K_c) = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (12)$$

where R (8.314 J/(mol·K) is the ideal gas constant, T (K) is the absolute temperature, and K_c is the thermodynamic equilibrium constant.

6.2.7 Desorption and reusability study

The use of sorbents to remove metals from solution is likely to be more economically feasible if the sorbent can be re-used (Venkateswarlu and Yoon, 2015; Zhang et al., 2016). Therefore, we investigated the desorption of Cu^{2+} from our SHLA and its reuse. In initial experiments, desorption of Cu^{2+} in solutions of HCl (0.1 M, 0.5M), HNO_3 (0.1 M), Na_2EDTA (0.1 M) and CH_3COONH_4 (0.1 M) were investigated. On the basis of these results (Figure 6.S1), 0.5M HCl was selected for further investigation as a desorbing agent. 35.0 mg of SHLA was shaken at pH 5 for 4 h with 35.0 mL of 50 mg/L $Cu(NO_3)_2$ solution at 25 °C and background electrolyte of 0.1M $NaNO_3$. The Cu loaded SHLA was centrifuged for 20 minutes at 4500 rpm and the supernatant decanted, filtered and analysed for Cu by ICP-OES. Adsorption of Cu^{2+} to the SHLA was calculated. Then 35 mL of 0.5 M HCl solution was added to the centrifuge tube which was shaken on an orbital shaker at 25 °C for 4 h. The suspension was again centrifuged for 20 minutes at 4500 rpm and the supernatant decanted for Cu analysis by ICP-OES. Desorption of Cu^{2+} from the Cu^{2+} loaded SHLA was calculated. 35 mL of fresh 50 mg/L $Cu(NO_3)_2$ was added and the adsorption-desorption process was repeated five times.

6.2.8 Adsorption of metals from a mixed metal solution

Multi-metal solutions containing Cu, Pb, Cr, Ni, Cd, Zn and Co, were prepared at 3 different initial concentrations (1 mg/L, 10 mg/L, and 50 mg/L) by diluting a multi-element standard solution (MERCK ICP multi-element standard solution IV, USA; the concentration of each metal is 1000 mg/L). 30.0 mg of SHLA was shaken at pH 5 for 24 h with 30.0 mL of the multi-metal solution at 25 °C. The mixtures were centrifuged at 4500 rpm for 20 minutes; the supernatant was filtered and diluted in 5% HNO_3 and analysed for metals by ICP-OES. All the experiments were performed in triplicate.

6.3 Results and discussions

6.3.1 Preparation and characterization of optimal synthetic humic-like acid

Following the preliminary optimization process, a SHLA (SHLA 12) was synthesized as described above. Table 6.1 lists elemental composition, acidic functional group content and E_4/E_6 of SHLA 12. SHLA 12 had a higher COOH content (5.03 mmol/g) than the SHLAs synthesized in the preliminary work (3.64 - 4.81 mmol/g, see Table 6.S2 in the supplementary material). The yield of SHLA 12 was 73.3%.

Table 6.1 Elemental composition, acidic functional groups content and E_4/E_6 of SHLA 12

Sample	N/%	C/%	H/%	O/%	C/N	C/H	O/C	COOH (mmol/g)	Phenolic-OH (mmol/g)	Total acidity (mmol/g)	E_4/E_6
SHLA 12	5.14	47.68	2.96	44.23	9.28	16.13	0.928	5.03	6.55	11.58	3.20

Figure 6.1 shows the FTIR spectra of glycine, catechol and SHLA 12 before and after adsorption of Cu^{2+}(at initial Cu^{2+} of 100 mg/L, HA dose =1 g/L, pH=5, T=25 °C and $NaNO_3$=0.1M). The FTIR spectra of SHLA 12 shows clear differences from those of the unpolymerized catechol and glycine. Peak assignments are given in Table 6.S3 in the supplementary material. The SHLA spectra are very similar to previously reported FTIR spectra of SHLA (Chen et al., 2010; Jokic et al., 2004). After reacting with the Cu^{2+}, the 1717 cm^{-1} peak present in the SHLA 12 spectra almost disappeared due to the formation of a coordinate bond between COOH and Cu^{2+}, indicating the importance of the carboxylic groups as binding sites for Cu^{2+} (Boyd et al., 1981).

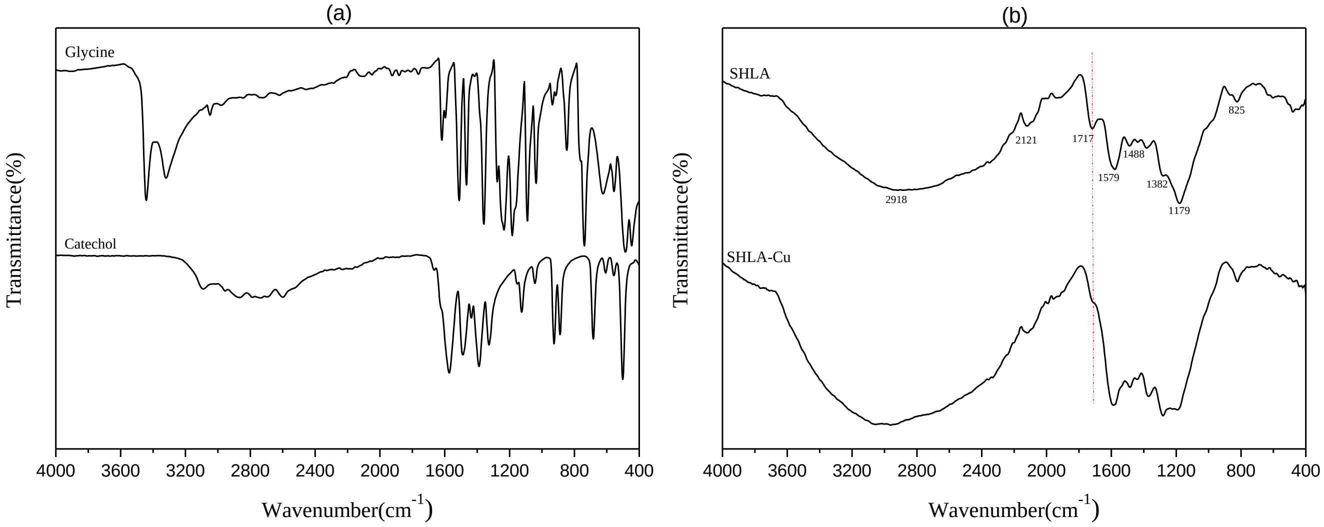

Figure 6.1 FTIR spectra of glycine (a), catechol (a), SHLA 12 before (SHLA, (b)) and after (SHLA-Cu, (b)) adsorption of Cu^{2+}. The red dotted line shows the change of peak at 1717 cm^{-1} due to the formation of a coordinate bond between COOH and Cu^{2+}.

6.3.2 Adsorption kinetics and efficiency

Figure 6.2 shows adsorption kinetics and adsorption efficiency of Cu^{2+} on SHLA at different pH, temperatures, initial Cu^{2+} concentrations and SHLA doses. In general, the adsorption of Cu^{2+} increased rapidly over the first 30 minutes and then slowly increased towards an equilibrium concentration. The equilibrium time ranged from 90 to 180 minutes for the various adsorption conditions.

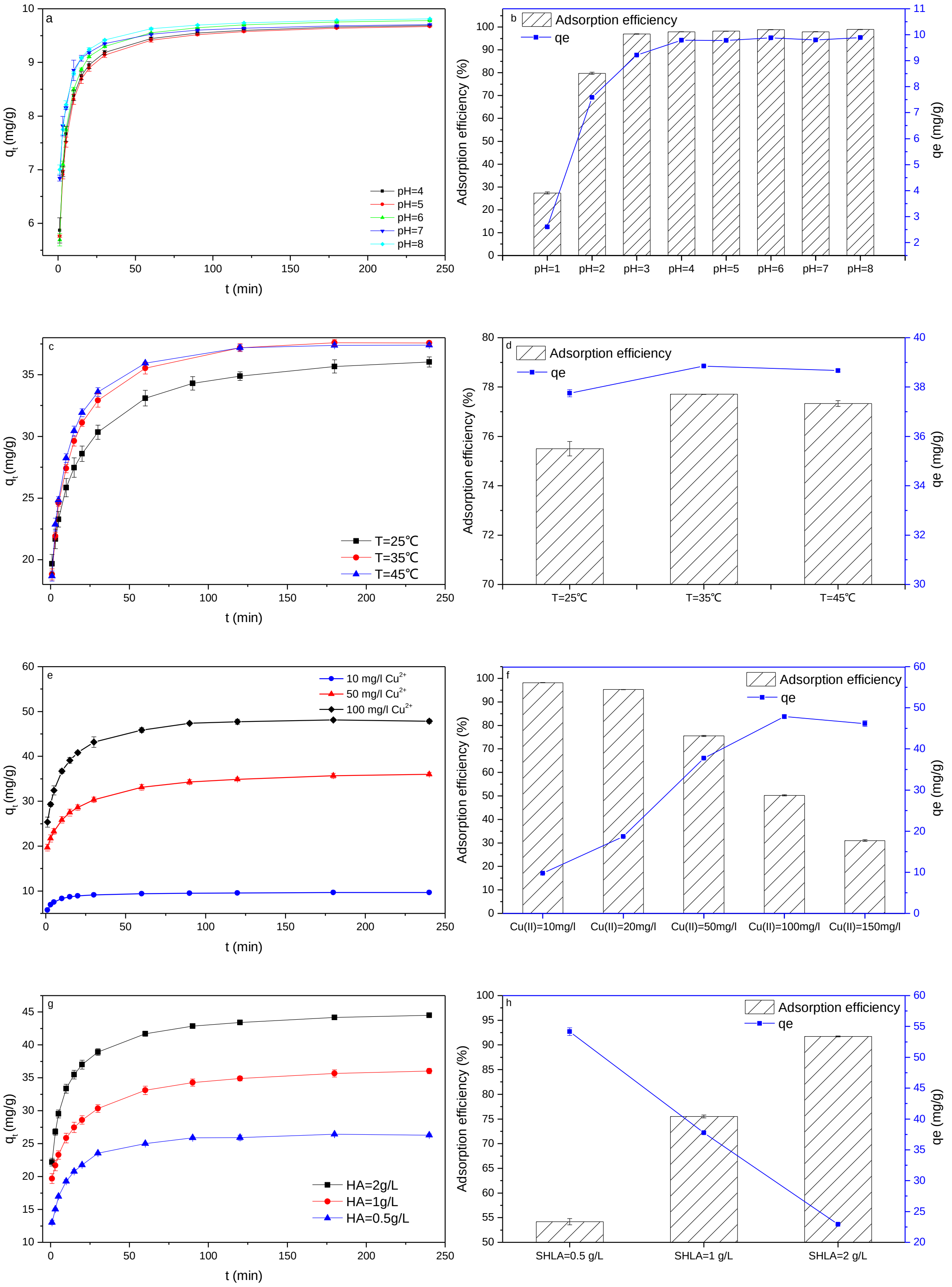

Figure 6.2 Adsorption kinetics and adsorption efficiency of Cu^{2+} on SHLA at different pH (a, b), temperatures (c, d), initial Cu^{2+} concentrations (e, f) and SHLA doses (g, h) (mean values, n=3 ± standard deviation; where error bars appear to be missing they are too small to see).

Adsorption kinetics can be used to calculate adsorption rates and investigate both the mechanism of adsorption and rate-limiting steps (Adebisi et al., 2017; Hu et al., 2017; Xu et al., 2017). Adsorption kinetics can also be helpful for selecting optimum reaction conditions for removing metals from solution (Zhang et al., 2016). In this study, four of the most widely used models (pseudo-first-order model, pseudo-second-order model, intraparticle diffusion model and Elovich model) were used to fit the experimental data. In the pseudo-first-order model, diffusion and mass transfer of adsorbate from solution to adsorption sites is the rate-limiting step. In the pseudo-second-order model, adsorption is controlled by chemical adsorption. The Intraparticle diffusion model fits adsorption data for which intraparticle diffusion is the rate-controlling step (Macías-García et al., 2017; Xu et al., 2017; Yang et al., 2016). The Elovich model describes adsorption which does not involve displacement of one moiety by another at the exchange site, i.e. no associated desorption and is considered suitable for some chemisorption processes (Adebisi et al., 2017).

Adsorption kinetics parameters for fits of the four models to our data are presented in Table 6.2. The correlation coefficients (R^2) of the intraparticle model (0.56 – 0.86) were lower than those for the fits to the other models (0.87 – 1.00) suggesting that intraparticle diffusion was not the only rate-limiting step in the adsorption process. The kinetic data are consistent with the adsorption of Cu^{2+} on SHLA involving three individual stages (Figure 6.S2, Table 6.S4): 1) rapid adsorption due to external surface adsorption and boundary layer diffusion; 2) a gradual adsorption due to intraparticle diffusion; 3) a final equilibrium stage (Li et al., 2010; Lin et al., 2011; Macías-García et al., 2017; Yang et al., 2016). For the pseudo-first-order model, the calculated values of q_e were much lower than those measured after 24 hours in all the experiments, whereas there was good agreement between calculated and measured values for q_e for fits to the pseudo-second-order model. Also, the R^2 values of fits to the pseudo-second-order model were all higher than those for the pseudo-first-order model fits. Thus, the pseudo-second-order model appears to be more appropriate for modelling the adsorption processes, indicating that chemisorption was the most probable rate-limiting step for adsorption of Cu^{2+} on SHLA (Inyang et al., 2016; Venkateswarlu and Yoon, 2015; Xu et al., 2017). The chemical bonding might be mainly coordinate bond between COOH and Cu^{2+} through surface complexation, and salicylate-like ring structures might be formed by an aromatic COOH and adjoining phenolic-OH or two adjoining aromatic COOH (Yang et al., 2016; Yang and Hodson, 2018)

The impacts of pH, temperature, initial Cu^{2+} concentration and SHLA dose on adsorption efficiency, q_e and k_2, the rate constant for pseudo-second order adsorption are detailed below. Adsorption efficiency and q_e increased rapidly from pH 1 to 3, and then gradually from pH 3 to 8 (Figure 6.2b). At lower pH, some functional groups like carboxylic groups (pKa: 3-5) in SHLA may be protonated which would lead to a positive charge on the SHLA and electrostatic repulsion between the Cu^{2+} and SHLA (Hamdaoui, 2017; Vidali et al., 2011; Zhang et al., 2016). Also, H^+ concentration is higher at lower pH and will compete for binding sites with Cu^{2+} (Tan et al., 2015). pH impacted adsorption efficiency and kinetics in the same way. The fitted values of k_2 also increased with pH, from 6.84×10^{-2} g/(mg·min) to $8.72.\times10^{-2}$ g/(mg·min) The decrease at high pH may be because of competition between OH^- and SHLA for Cu^{2+} (Lan et al., 2013).

Table 6.2 Adsorption kinetics parameters for pseudo-first-order, pseudo-second-order, intraparticle diffusion and Elovich models (modelled values ± standard deviation).

Adsorption conditions		**$Q_{e,exp}$ (mg/g)**	**Pseudo-first-order**			**Pseudo-second-order**			**Elovich model**			**Intraparticle diffusion**		
			$Q_{e,cal}$ (mg/g)	k_1 (1/min)	R^2	$Q_{e,cal}$ (mg/g)	k_2 (g/(mg·min))	R^2	a (mg/(g·min))	b (g/mg)	R^2	K_{id} (mg/(g·min$^{0.5}$))	C (mg/g)	R^2
pH	4	9.79 ±0.00	1.78 ±0.30	2.51×10^{-2} $\pm0.25\times10^{-2}$	0.9188	9.74 ±0.01	6.84×10^{-2} $\pm0.57\times10^{-2}$	0.9999	9.85×10^{3} $\pm3.62\times10^{3}$	1.48 ±0.14	0.9064	0.202 ±0.049	7.31 ±0.39	0.6298
	5	9.78 ±0.00	1.85 ±0.31	2.48×10^{-2} $\pm0.24\times10^{-2}$	0.9192	9.72 ±0.01	6.48×10^{-2} $\pm0.55\times10^{-2}$	0.9999	6.65×10^{3} $\pm2.22\times10^{3}$	1.44 ±0.13	0.9120	0.208 ±0.049	7.21 ±0.40	0.6386
	6	9.88 ±0.00	1.77 ±0.31	2.58×10^{-2} $\pm0.26\times10^{-2}$	0.9151	9.83 ±0.02	6.99×10^{-2} $\pm1.28\times10^{-2}$	0.9999	7.51×10^{3} $\pm2.81\times10^{3}$	1.43 ±0.15	0.8878	0.207 ±0.054	7.37 ±0.43	0.6008
	7	9.79 ±0.01	1.25 ±0.22	2.45×10^{-2} $\pm0.27\times10^{-2}$	0.9074	9.74 ±0.01	9.73×10^{-2} $\pm0.93\times10^{-2}$	0.9999	2.02×10^{6} $\pm1.40\times10^{6}$	2.06 ±0.24	0.8726	0.137 ±0.041	8.10 ±0.34	0.5631
	8	9.89 ±0.00	1.34 ±0.23	2.39×10^{-2} $\pm0.25\times10^{-2}$	0.9081	9.83 ±0.03	8.72×10^{-2} $\pm1.66\times10^{-2}$	0.9999	1.08×10^{6} $\pm0.63\times10^{6}$	1.97 ±0.17	0.9161	0.153 ±0.036	8.01 ±0.29	0.6466
Temperature (℃)	25	37.75 ±0.15	12.68 ±0.86	2.05×10^{-2} $\pm0.10\times10^{-2}$	0.9810	36.40 ±0.26	6.73×10^{-3} $\pm0.89\times10^{-3}$	0.9995	9.49×10^{2} $\pm0.34\times10^{2}$	0.30 ±0.01	0.9879	1.108 ±0.139	21.94 ±1.11	0.8644
	35	38.86 ±0.00	14.60 ±1.05	3.20×10^{-2} $\pm0.14\times10^{-2}$	0.9846	38.08 ±0.24	7.32×10^{-3} $\pm1.02\times10^{-3}$	0.9998	5.85×10^{2} $\pm0.12\times10^{2}$	0.27 ±0.01	0.9763	1.210 ±0.198	22.95 ±1.59	0.7892
	45	38.67 ±0.06	14.85 ±1.32	3.96×10^{-2} $\pm0.13\times10^{-2}$	0.9916	37.88 ±0.13	9.29×10^{-3} $\pm0.97\times10^{-3}$	0.9999	8.44×10^{2} $\pm0.47\times10^{2}$	0.28 ±0.02	0.9628	1.122 ±0.222	23.68 ±1.75	0.7400
Initial Cu^{2+} concentration (mg/L)	10	9.78 ±0.00	1.85 ±0.31	2.48×10^{-2} $\pm0.24\times10^{-2}$	0.9192	9.72 ±0.01	6.48×10^{-2} $\pm0.55\times10^{-2}$	0.9999	6.65×10^{3} $\pm2.22\times10^{3}$	1.44 ±0.13	0.9120	0.208 ±0.049	7.21 ±0.40	0.6386
	50	37.75 ±0.15	12.68 ±0.86	2.05×10^{-2} $\pm0.10\times10^{-2}$	0.9810	36.40 ±0.26	6.73×10^{-3} $\pm0.89\times10^{-3}$	0.9995	9.49×10^{2} $\pm0.34\times10^{2}$	0.30 ±0.01	0.9879	1.108 ±0.139	21.94 ±1.11	0.8644
	100	47.83 ±0.45	19.09 ±1.33	4.26×10^{-2} $\pm0.13\times10^{-2}$	0.9919	48.54 ±0.20	7.57×10^{-3} $\pm0.89\times10^{-3}$	0.9998	1.44×10^{3} $\pm0.09\times10^{3}$	0.22 ±0.01	0.9663	1.430 ±0.255	30.90 ±2.05	0.7583
SHLA dose (g/L)	0.5	54.17 ±0.62	19.55 ±2.29	3.13×10^{-2} $\pm0.24\times10^{-2}$	0.9544	53.39 ±0.23	5.75×10^{-3} $\pm0.62\times10^{-3}$	0.9998	7.14×10^{2} $\pm0.11\times10^{2}$	0.19 ±0.01	0.9685	1.716 ±0.295	32.01 ±2.37	0.7723
	1	37.75 ±0.15	12.68 ±0.86	2.05×10^{-2} $\pm0.10\times10^{-2}$	0.9810	36.40 ±0.26	6.73×10^{-3} $\pm0.89\times10^{-3}$	0.9995	9.49×10^{2} $\pm0.34\times10^{2}$	0.30 ±0.01	0.9879	1.108 ±0.139	21.94 ±1.11	0.8644
	2	22.93 ±0.03	7.27 ±0.71	2.24×10^{-2} $\pm0.14\times10^{-2}$	0.9661	22.47 ±0.10	1.31×10^{-2} $\pm0.14\times10^{-2}$	0.9998	4.81×10^{2} $\pm0.17\times10^{2}$	0.47 ±0.02	0.9791	0.682 ±0.114	13.84 ±0.91	0.7822

(*p values are less than 0.01)

Adsorption efficiency and q_e increased slightly from 25℃ to 45 ℃ (Figure 6.2d). This may be due to increased diffusion rates of the Cu^{2+} at the higher temperatures enhancing the transport of Cu^{2+} from the bulk solution to adsorption sites (Li et al., 2010) The impact of temperature on k_2 is similar to adsorption efficiency. The fitted values of k_2 also increased with temperature, from 6.73×10^{-3} g/(mg·min) to 9.29×10^{-3} g/(mg·min).

The q_e increased with increasing initial Cu^{2+} concentration, while adsorption efficiency and k_2 values decreased (Figure 6.2f, Table 6.2). The decrease in adsorption efficiency is due to the saturation of adsorption sites (Hamdaoui, 2017).

The q_e decreased from 54.17 ± 0.62 mg/g to 22.93 ± 0.03 mg/g when the SHLA dose was increased from 0.5 g/L to 2 g/L, but adsorption efficiency and k_2 values increased. This is because increasing the dose of the adsorbent increases the available adsorption sites, which results in higher metal removal efficiency but at the same time reduces the adsorption per unit mass of adsorbent (Li et al., 2015).

6.3.3 Adsorption curves

The adsorption curves of $Cu^{2+}$and SHLA at 25 °C, 35 °C and 45 °C resemble classic L-shaped isotherms indicating an excess of adsorption sites at low solution concentrations such that adsorption can be limited by the transfer of Cu^{2+} from the bulk solution to the surface of the SHLA and increasing saturation of adsorption sites at higher solution concentrations (Figure 6.3) (Chen et al., 2015; Giles et al., 1974(a); Giles et al., 1974(b)).

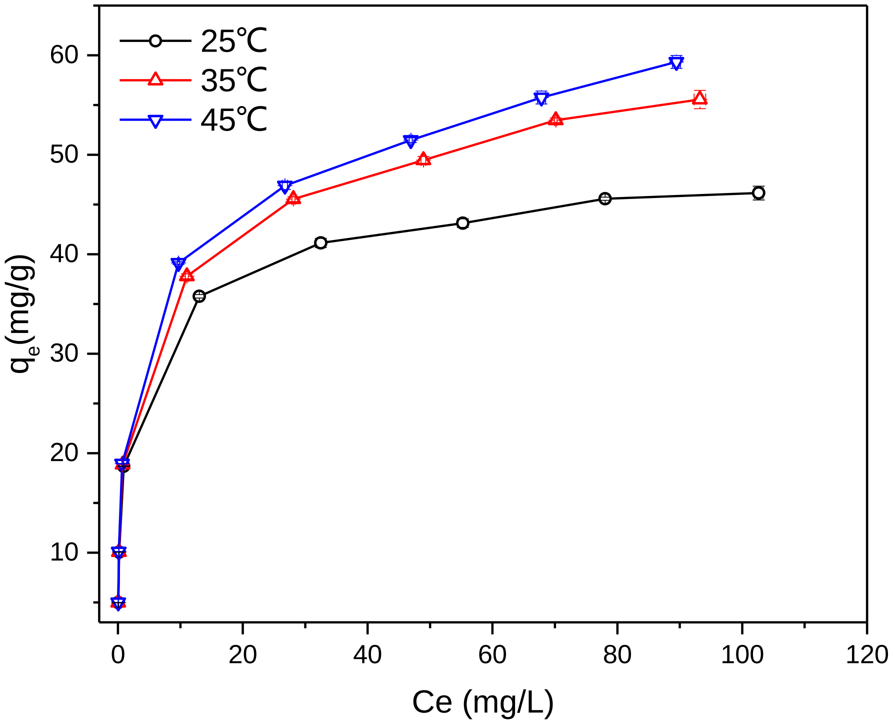

Figure 6.3 The adsorption curves of Cu^{2+} onto SHLA at three temperatures (mean values, n=3 ± standard deviation; where error bars appear to be missing they are too small to see).

The adsorption data were fitted to the Langmuir and Freundlich models (Table 6.3). Fits to the Langmuir model suggest that the maximum adsorption capacity (q_m) increased from 46.45 ± 0.79 mg/g to 58.79 ± 1.98 mg/g with increasing temperature. The fits to the Langmuir model were used to calculate separation factors, R_L (Adebisi et al., 2017) as:

$$R_L = \frac{1}{1+K_L C_0} \quad (13)$$

where C_0 (mg/L) is the initial Cu^{2+} concentration and K_L (L/mg) is the Langmuir adsorption constant.

R_L values indicate whether adsorption is favourable ($0<R_L<1$), linear ($R_L=1$), unfavorable ($R_L>1$) or irreversible ($R_L=0$). All the calculated R_L values at the three temperatures were in the range from 0 to 1 (Table 6.3), indicating that the Cu^{2+} adsorption process onto SHLA was favourable. The heterogeneity factor, *n*, in the Freundlich model can also be used to assess the favourability of adsorption (Xiao and Hu, 2017). The values of n ($1<n<10$) indicate that the adsorption of Cu^{2+} onto SHLA was favourable (Xu et al., 2017), consistent with the R_L values. K_F values increased from 14.49 ± 1.21 to 16.71 ± 1.19 with increasing temperature from 25°C to 45°C, indicating that the adsorption process was endothermic in nature (Gupta et al., 2017).

Table 6.3 Adsorption isotherms parameters at 25 °C, 35 °C and 45 °C for Langmuir and Freundlich models (modelled values ± standard deviation).

Temperature /℃	Langmuir					Freundlich			
	q_m (mg/g)	K_L (L/mg)	R^2	p	R_L	K_f (mg/g(L/mg)$^{1/n}$)	n	R^2	p
25	46.45 ±0.79	0.466 ±0.129	0.9982	<0.0001	0.0142-0.2985	14.488 ±1.206	3.527 ±0.303	0.9497	<0.0001
35	55.62 ±1.58	0.344 ±0.102	0.9949	<0.0001	0.0192-0.3656	15.704 ±1.204	3.264 ±0.245	0.9616	<0.0001
45	58.79 ±1.98	0.336 ±0.106	0.9928	<0.0001	0.0196-0.3711	16.711 ±1.191	3.276 ±0.231	0.9667	<0.0001

Both Langmuir and Freundlich models showed good fits to the experimental data with R^2 values of 0.9928-0.9982 for the Langmuir model and 0.9497-0.9669 for the Freundlich model.

To obtain a deeper insight into the adsorption mechanism, the bi-Langmuir model was applied to the experimental data (Table 6.S5). The bi-Langmuir model assumes that there are two types of adsorption site, one related to ion exchange and the other to surface complexation (He et al., 2016; Yang et al., 2015). The bi-Langmuir model fits the data well. The results show that the surface complexation sites had a smaller sorption capacity ($q_{m,1}$) but higher sorption affinity ($k_{L,1}$) for Cu^{2+}, than the ion-exchange sites ($q_{m,2}$ and $k_{L,2}$ respectively) (Gezici et al., 2007; Yang et al., 2015). The higher $k_{L,1}$ values suggest that the surface complexation sites had a stronger attraction and higher sorption energy than the ion exchange sites, and the lower $q_{m,1}$ values were due to fewer available adsorption sites for surface complexation (Yang et al., 2015). Generally, carboxylic and phenolic-OH groups are the two most significant functional groups present in humic acid that are active in binding metals (Alvarez-Puebla et al., 2004; Baken et al., 2011; Kautenburger et al., 2014; Zherebtsov et al., 2015). However, phenolic-OH (pKa: 8-11) would not be dissociated at pH 5 (Vidali et al., 2011), suggesting that carboxylic groups might be the predominant functional groups for SHLA to adsorb Cu^{2+}. Carboxylic groups could bind with Cu^{2+} through surface complexation but also by electrostatic attraction. This result indicates that increasing the COOH content of SHLA is a feasible way to increase the ability of SHLA to adsorb Cu^{2+}.

6.3.4 Thermodynamic analysis

Thermodynamic parameters for the adsorption of Cu^{2+} onto SHLA were calculated following the method of Li et al (2015) (Table 6.4).

Table 6.4 Thermodynamic parameters of Cu^{2+} onto SHLA.

T (K)	ΔG° (kJ/mol)	ΔS° (kJ/(mol·K))	ΔH° (kJ/mol)	R^2	p
298.15	-2.4992	0.0595	15.2315	0.9951	<0.05
308.15	-3.1534				
318.15	-3.6873				

The parameters indicate that the adsorption of the Cu^{2+} onto SHLA was endothermic (positive ΔH°) and occurred spontaneously (negative ΔG°). With increasing temperature from 25℃ to 45 ℃, ΔG° decreased from -2.50 kJ/mol to -3.69 kJ/mol, indicating that higher temperatures favoured adsorption (Hu et al., 2017). The positive value of $\Delta S°$ is similar to that reported in Yang et al. (2016) for Cu^{2+} adsorption to humic acid-modified HAP nanoparticles which was attributed to both the affinity of the adsorbent to Cu^{2+} and also an increase in the degree of randomness in the system following Cu adsorption.

6.3.5 Comparison with other humic acid-based adsorbents

The maximum adsorption capacity, K_L and k_2 values of SHLA and other humic acid-based adsorbents are summarized in Table 6.5. The maximum Cu^{2+} adsorption capacity of SHLA was much higher than most of the pure humic acid adsorbents, most likely due to the higher COOH content of SHLA. The adsorption capacity was also greater than many materials comprising humic acid and an inorganic component, e.g. humic acid modified Fe_3O_4 (Janoš et al., 2013) but not as good as the humic acid-immobilized polymer/bentonite composite (Anirudhan and Suchithra, 2010) or the humic acid modified carbon nanotubes (Côa et al., 2017). However, our studies suggest that the performance of these composite materials could be improved further if a SHLA was used rather than a natural humic acid. Also, in terms of humic acid modified nanomaterials, potential adverse impacts to the environment should be of great concern calling into question their practical use. For example, carbon nanotubes can cause cell membrane damage, mitochondrial DNA damage and induce granulomas and atherosclerotic lesions; and TiO_2 nanomaterials can cause acute toxicity, oxidative damage and growth inhibition (Farré et al., 2009; Lee et al., 2010).

Table 6.5 Comparison of q_m, K_L and k_2 of SHLA and other humic acid-based adsorbents.

Sample	q_m (mg/g)	K_L (L/mg)	k_2(g/(mg·min))	References
SHLA	46.4-58.6	0.336-0.466	5.75×10^{-3}-9.73×10^{-2}	This study
Humic acid from soil	26.4-38.1	N.A.	N.A.	(Bibak, 1994)
Insolubilized humic acid	2.0	2.58×10^{-3}	N.A.	(Gezici et al., 2007)
Humic acid from lignites	16.7-26.2	4.09×10^{-3}-7.66×10^{-3}	1.25×10^{-2}-7.71×10^{-2}	(Arslan et al., 2007)
Humic acid (commercial)	23.0	N.A.	N.A.	(Li et al., 2010)
Humic acid from sediment	7.55-85.1	0.0295-0.181	N.A.	(Yang et al., 2015)
Humic acid from soil	10.3-61.4	0.017-0.151	N.A.	(El-Eswed and Khalili, 2006)
Humic acid from sediment	39.0-107.9	N.A.	N.A.	(He et al., 2016)
Humic acid-immobilized polymer/bentonite composite	101.3-112.9	0.11-8.44	1.10×10^{-3}-7.12×10^{-2}	(Anirudhan and Suchithra, 2010)
Humic acid modified Ca-montmorillonite	15.3	0.029	1.72×10^{-1}	(Wu et al., 2011)
Humic acid-immobilized surfactant-modified zeolite	19.8-21.5	2.16-3.92	1.50×10^{-2}-7.40×10^{-2}	(Lin et al., 2011)
Humic acid modified Fe_3O_4	0.4-15.6	0.157-2.126	N.A.	(Janoš et al., 2013)
Humic acid modified activated carbon	6.0	93.33	N.A.	(Liu et al., 2014)
Humic acid modified bentonite	23.0	N.A.	N.A.	(Jin et al., 2016)
Humic acid and goethite modified kaolinite clay	1.7	5.26×10^{-3}	2.1×10^{-2}	(Unuabonah et al., 2016)
Humic acid modified Fe_3O_4 nanoparticles	46.3	22.4	N.A.	(Liu et al., 2008)
Humic acid modified HAP nanoparticles	48.7-58.4	2.239-4.380	2.57×10^{-3}-3.45×10^{-3}	(Yang et al., 2016)
Humic acid modified TiO_2 nanoparticles	5.3	0.07	3×10^{-3}	(Mahdavi, 2016)
Humic acid modified carbon nanotubes	68.5	N.A.	N.A.	(Côa et al., 2017)

6.3.6 Desorption and reusability of SHLA

In addition to the removal of metals by adsorption, when considering practical applications of adsorbents in remediation, their reuse should be considered as this impacts on the economics of the process. Hydrochloric acid solution (0.5 M) was chosen as the eluent in this study, and the adsorption-desorption cycles were performed six times on SHLA. The Cu^{2+} removal efficiency for the first cycle was about 75%, indicating the adsorption sites were saturated. If the SHLA is not regenerated by HCl, it is foreseeable that if the SHLA were reused, the removal efficiency would be very low. Figure 6.4 shows the adsorption capacity of Cu^{2+} onto SHLA with six adsorption-desorption cycles and the % desorption achieved by each HCl wash. The Cu^{2+} adsorption capacity of regenerated SHLA gradually and slightly decreased with the number of reuse cycles. Compared with the first adsorption, the Cu^{2+} adsorption capacity reduced by about 10% after five cycles of regeneration, similar to other materials such as tannin-immobilized nanocellulose (6.5% decrease), graphene oxide membranes (10% decrease) and humic acid-modified polymer/bentonite (appr 20% decrease) (Anirudhan and Suchithra, 2010; Tan et al., 2015; Xu et al., 2017) indicating that SHLA could be reused several times and is thus a promising adsorbent for practical application.

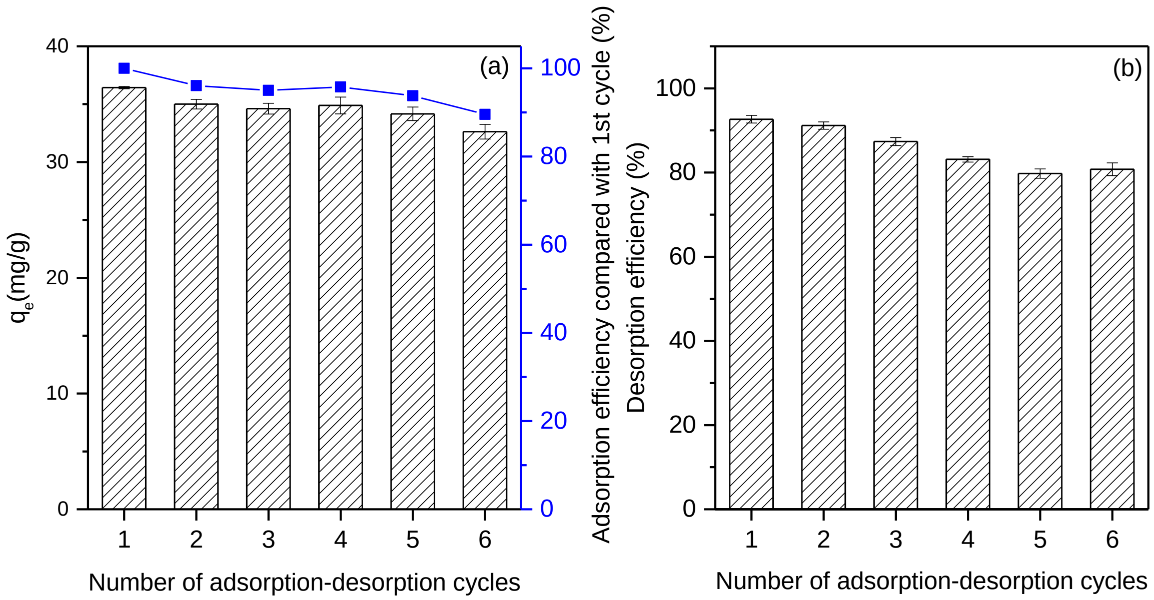

Figure 6.4 Adsorption capacity of Cu^{2+} onto SHLA with 6 adsorption-desorption cycles (a) and desorption efficiency achieved by each HCl wash (b).

6.3.7 Multielement adsorption

The SHLA used in this study was optimized for the removal of Cu^{2+} from solution.

However in cases of real contamination single-metal contamination is unusual and for that reason we also investigated the adsorption of multiple metals (including Cu, Pb, Cr, Ni, Cd, Zn and Co) from solution by the SHLA.

As shown in Figure 6.5, the Cu and Pb removal efficiency of SHLA at three initial concentrations were all above 85%. At low metal concentration, SHLA also had good removal efficiency for Ni and Cr. To compare metal removal ability of SHLA for different metals, the original mass concentration for each metal was converted to mole concentration (Figure 6.S3). In the zones surrounded by black dashed lines in Figure 6.S3, the mole concentration for each metal is similar. At the lower metal concentrations, removal efficiency showed a decreasing trend in the order Cu ≈ Pb > Cr > Ni > Cd > Zn > Co. Although, the relatively lower atomic mass of Cu means that higher mmol/L concentrations are lacking a value for Cu, the other elements still show the same trend of Pb > Cr > Ni > Zn > Co and indicate that the SHLA is well able to adsorb metals other than Cu which is was optimized for. These trends are similar to the differing affinities of metals for humic acid (Irving and Williams, 1948; Kerndorff and Schnitzer, 1980; Pandey et al., 2000). The sequence was also similar to the results from other adsorbents, like chitosan saturated montmorillonite, graphene oxide, titanate nanotubes and carbon nanotubes (Hu et al., 2017; Li et al., 2015; Li et al., 2003; Tan et al., 2015; Wang et al., 2013). With increasing initial metal concentration from 1 mg/L to 50 mg/L, the removal efficiency decreased by < 14% for Cu and Pb, but sharply decreased by 38-64% for other metals. The greater decrease for the other metals reflects the preferential adsorption of Cu and Pb and the saturation of adsorption sites at higher metal concentrations.

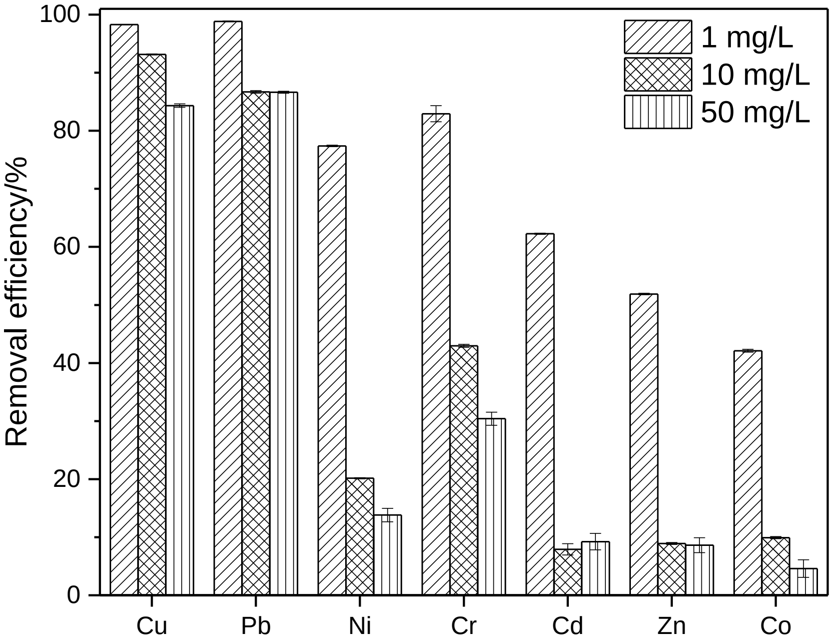

Figure 6.5 Metals removal efficiency of SHLA in multi-metal systems at 3 different initial metal concentrations (mean values, n=3 ± standard deviation; where error bars appear to be missing they are too small to see).

6.4 Conclusions

In this study, a synthetic humic-like acid (SHLA) was prepared by an abiotic humification process optimised on the basis of our previous work to give a high concentration of carboxylic acid functional groups. The adsorption behaviour of aqueous Cu^{2+} onto the SHLA was investigated in detail together with the desorption and reuse potential of the SHLA and the potential adsorption of co-contaminant metals. The SHLA exhibited a range of adsorption efficiencies (27% - 99%) for Cu^{2+} depending on reaction conditions. A pseudo-second-order kinetic model provided the best fit to the experimental data, indicating that chemisorption was most likely the rate-limiting step for adsorption. The equilibrium adsorption data showed good fits to both the Langmuir ($R^2 = 0.9928 – 0.9982$, $p \leq 0.0001$) and Freundlich ($R^2 = 0.9497 – 0.9667$, $p \leq 0.0001$) models. The maximum adsorption capacity (q_m) of SHLA increased from 46.44 mg/g to 58.78 mg/g with increasing temperature from 25 °C to 45 °C. Thermodynamic parameters (ΔG^0=-3.69 - -2.50 kJ/mol; ΔS^0=0.06 kJ/(mol·K); ΔH^0=15.23 kJ/mol) and values of R_L (0.0142-0.3711) and n (3.264-3.527) show that the adsorption of Cu^{2+} onto SHLA was favourable, spontaneous and endothermic in nature.

The Cu^{2+} adsorption capacity of SHLA was much higher than many of the humic acid-based materials reported in the literature, the Cu^{2+} was readily desorbed from the SHLA by HCl allowing the reuse of the SHLA and the SHLA was able to remove co-contaminant metals in addition to Cu^{2+} from solution. Overall, this confirms that SHLA has significant potential for use as an adsorbent for the clean up of contaminated waters.

Appendix 6.Supplementary Materials

Information about preliminary optimization process for SHLAs (Table 6.S1 and 6.S2), assignments of FTIR absorption bands of SHLA 12 (Table 6.S3), additional information about the selection of desorption eluents (Figure 6.S1), intraparticle diffusion model (Figure 6.S2, Table 6.S4) and fitted results of the bi-Langmuir model (Table 6.S5) are shown in supplementary materials.

Acknowledgements

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Appendix 6. Supplementary materials

Table 6.S1 Abiotic humification reaction conditions for 12 SHLAs.

HA Samples	Catechol /mol/L	Glycine /mol/L	Glucose /mol/L	Temperature /°C	pH	MnO_2 addition amount/g	Reaction time
SHLA1	1	1	-	45	7	13.33	240 h
SHLA 2	1	1	1	45	7	13.33	
SHLA 3	0.5	0.5	-	45	7	13.33	
SHLA 4	0.25	025	-	45	7	13.33	
SHLA 5	0.5	1	-	45	7	13.33	
SHLA 6	0.25	1	-	45	7	13.33	
SHLA 7	1	1	-	25	7	13.33	
SHLA 8	1	1	-	35	7	13.33	
SHLA 9	1	1	-	45	6	13.33	
SHLA 10	1	1	-	45	8	13.33	
SHLA 11	1	1	-	45	7	25	
SHLA 12*	0.25	0.25	-	25	8	25	

(*The condition used to synthesize SHLA 12 were determined on the basis of the initial 11 SHLAs' COOH contents.)

The method for extracting and purifying SHLA

The reaction mixture was centrifuged at 10,000 rpm for 10 minutes, and the supernatant filtered through a 0.45 μm polyethersulfone membrane by vacuum filtration. The filtrate was acidified to pH 1 with concentrated HCl and stirred for 24 h. The SHLA fraction precipitated. The slurry was centrifuged at 10,000 rpm for 15 minutes. The precipitate was transferred to a beaker, re-dissolved in 0.1 M NaOH and filtered through a 0.2 μm polyethersulfone membrane twice by vacuum filtration. The filtrate was acidified to pH 1 with concentrated HCl, stirred for 24 h and centrifuged at 10,000 rpm for 15 minutes. The resulting precipitate (SHLA) was dialyzed by a Spectra/Por 6 dialysis membrane (Spectrum Labs, USA, molecular weight cut-off (MWCO) 1000 Da) and freeze-dried.

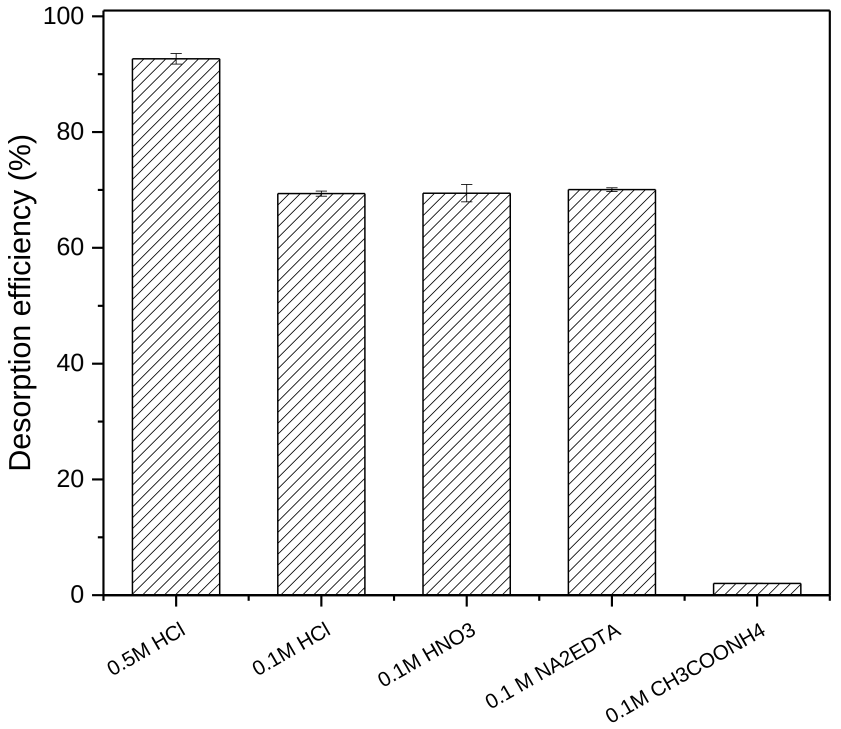


Figure 6.S1 Desorption efficiency for different eluents (SHLA dose 35 mg/35 mL, Cu^{2+} concentration of 50 mg/L, ionic strength of 0.1M $NaNO_3$, pH of 5, and T=25 °C resulting in a Cu^{2+} loading of 36.59±0.15 mg/kg on the SHLA; mean values, n=3 ± standard deviation).

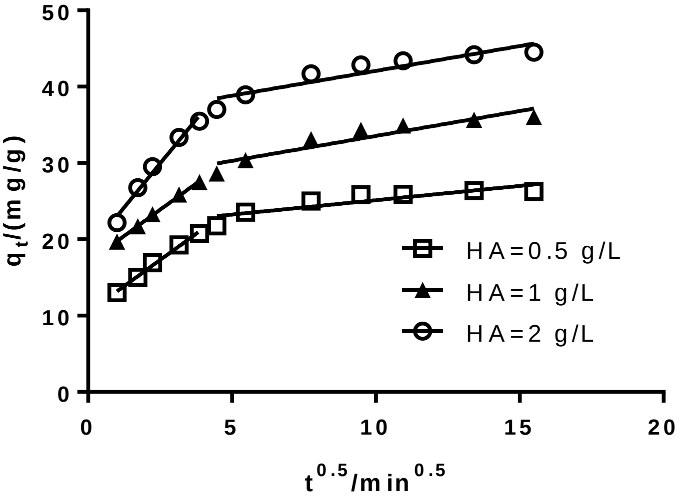
Table 6.S2 Acidic functional group contents of 12 SHLAs.

Sample	COOH (mmol/g)	Phenolic-OH (mmol/g)	Total acidity (mmol/g)
SHLA1	4.10	8.61	12.72
SHLA 2	3.64	12.26	15.90
SHLA 3	4.61	6.72	11.32
SHLA 4	4.83	7.41	12.24
SHLA 5	4.57	9.53	14.10
SHLA 6	4.62	8.92	13.54
SHLA 7	4.81	7.86	12.67
SHLA 8	4.31	8.54	12.85
SHLA 9	4.33	9.29	13.62
SHLA 10	4.66	11.55	16.21
SHLA 11	4.59	14.61	19.19
SHLA 12	5.03	6.55	11.58

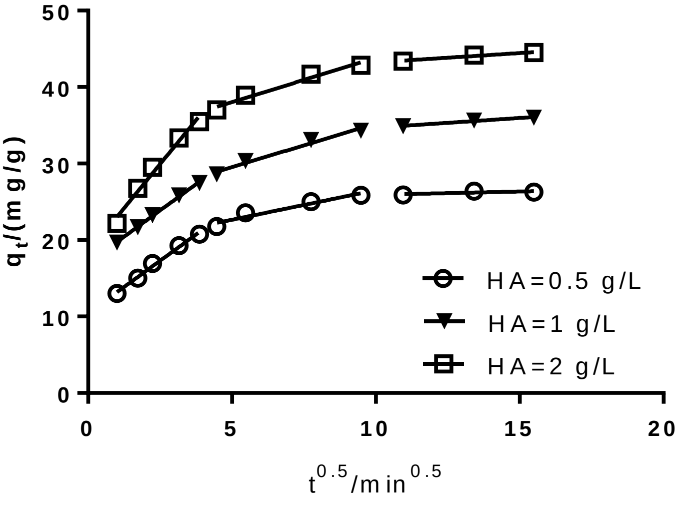
Table 6.S3 Assignments of FTIR absorption bands present in the spectra for SHLA 12 (Stevenson, 1994; Jokic et al., 2004; Fukushima et al., 2009; Hardie et al., 2009; Shiotsuka et al., 2015; Stuart, 2004).

Wavenumber/cm^{-1}	Assignment
2918	C-H stretching of aliphatic structures
2121	C≡C stretching and the presence of cyano group
1717	C=O stretching of carbonyl groups
1579 &1488	C=C ring stretching, symmetric C–O stretch of COO- and N-H deformation and C=N stretching (amide II band)
1382	O-H deformation of phenols, C-H deformation, symmetric and asymmetric stretching of CH_3 and C-O stretch of COO-
1179	C–O stretching and C–O–H deformation of alcohols, phenols and ethers

The adsorption process of Cu^{2+} onto SHLA was divided into both 2 stages and 3 stages at first (Figure 6.S2). Then the intra-particle diffusion rate constant k_{id} and the intercept C calculated from the second portion of plot of q_t versus $t^{0.5}$ were calculated and shown in Table 6.S4. Although there is a slight decrease in p value between the two- and three-stage model, the higher R^2 values of the three stage model lead us to adopt that model for our data.



(a)



(b)

Figure 6.S2 Intraparticle diffusion models for 2 stages (a) and 3 stages (b) with different HA doses.

Table 6.S4 Intraparticle diffusion rate constant (k_{id}) and C values for the adsorption of Cu^{2+} onto SHLA at SHLA doses (estimated values ± standard deviation).

	Two stage model				Three stage model			
SHLA dose (g/L)	k_{id}	C	R^2	p	k_{id}	C	R^2	p
0.5	0.375±0.092	21.4±0.9	0.7691	<0.01	0.773±0.144	18.8±1.0	0.9349	<0.05
1	0.653±0.109	27.0±1.2	0.8774	<0.01	1.134±0.127	23.9±0.9	0.9755	<0.05
2	0.651±0.115	35.56±1.2	0.8649	<0.01	1.155±0.150	32.3±1.1	0.9676	<0.05

Table 6.S5 Fitted isotherms of Cu^{2+} onto SHLA by bi-Langmuir model.

Temperature /℃	$q_{m,1}$ (mg/g)	$K_{L,1}$ (L/mg)	$q_{m,2}$ (mg/g)	$K_{L,2}$ (L/mg)	R^2	p
25	20.77±1.58	4.338±0.719	27.92±1.44	0.0892±0.014	0.9995	<0.0001
35	24.21±2.69	3.807±0.930	37.38±2.23	0.0501±0.012	0.9990	<0.0001
45	26.41±4.66	3.969±1.591	39.98±3.74	0.0442±0.019	0.9971	<0.0001

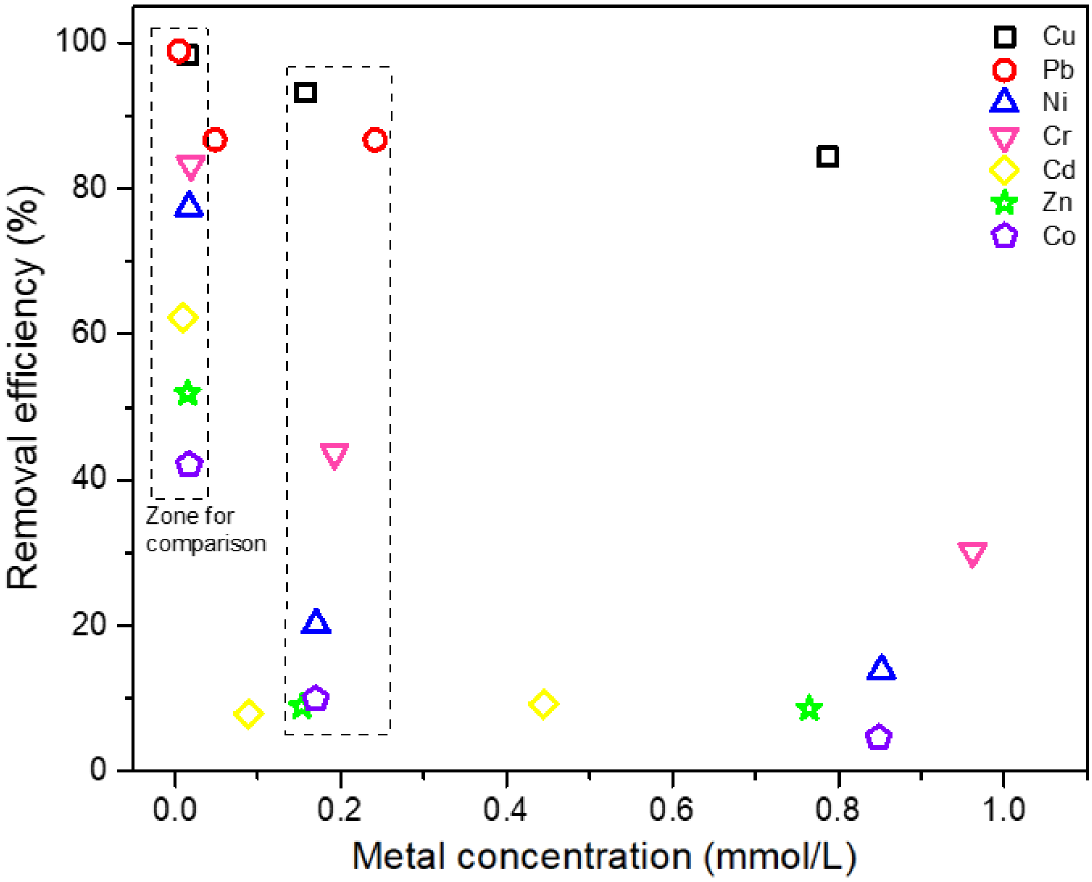


Figure 6.S3 Metals removal efficiency of SHLA in multi-metal systems at different initial metal mole concentrations.

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CHAPTER 7

Investigating the use of synthetic humic-like acid as a soil amendment for metal contaminated soil

Chapter 7 Investigating the use of synthetic humic-like acid as a soil amendment for metal contaminated soil

Ting Yang[a*], Mark E Hodson[a]
[a] Environment Department, University of York, Heslington, Wentworth Way, York YO10 5NG, United Kingdom
*Corresponding author. Tel.: +44 (0)7421 236832. Fax: +44 (0)7421 236832.
E-mail address: ty672@york.ac.uk

Abstract

Humic acid can effectively bind several metals and is regarded as a promising soil amendment. In this study, a synthetic humic-like acid (SHLA) was applied as a soil amendment to immobilise metals (Cu, Zn, Ni, Pb, As) in a contaminated agricultural soil (pH: 6.17 ± 0.11; total organic carbon: 5.91±0.40%; Cu: 302.86±3.97 mg/kg; Zn: 700.45±14.30 mg/kg; Pb 323.56±4.84 mg/kg; Ni: 140.16±1.59 mg/kg). With increasing additions of SHLA from 0 to 10% (w/w), the soil pH constantly decreased from 6.17±0.11 to 4.91±0.10 ($p<0.001$), while both total organic carbon (from 6.10 ±0.12% to 10.55±0.18%) and water soluble carbon content (from 171.01±10.15 mg/kg to 319.18±20.74 mg/kg) of soil significantly increased ($p<0.001$). Based on the results of 0.01 M $CaCl_2$-extractable concentration of different metals, SHLA could lower the bioavailability of Cu (from 1.26±0.04 mg/kg to 0.55±0.05 mg/kg), Zn (from 6.74±0.12 mg/kg to 3.26±0.23 mg/kg) and Ni (from 5.16±0.07 mg/kg to 0.12±0.02 mg/kg), but increase the bioavailability of As (from 0.31±0.02 to 1.83±0.09 mg/kg).

***Keywords*:** Synthetic humic-like acid; toxic metals, soil amendment, metal bioavailability

7.1 Introduction

Soil contamination by toxic metals is a serious environmental problem. Various remedial approaches for metal-contaminated soil have been investigated in the past decades, including soil washing, soil amendment, solidification/stabilization, phytoremediation, electrokinetic processes, and bioremediation (Bolan et al., 2014; Leštan et al., 2008; Liu et al., 2017; Rosestolato et al., 2015; Wan et al., 2016; Xia et al., 2017). Of these, soil amendment is regarded as a low disruptive and cost-effective technique (Houben et al., 2012). The amendments applied currently can be classified into two main types: organic amendments (e.g. compost, biochar, manure and humic acid-based materials) and inorganic amendments (e.g. lime, fly ash and natural zeolite) (Beesley et al., 2014; Hartley et al., 2009; Janoš et al., 2010; Pardo et al., 2014; Pukalchik et al., 2017). By adsorption, complexation, precipitation and ion exchange, soil amendments can reduce mobility and bioavailability of toxic metals (Janoš et al., 2010; Kumpiene et al., 2008). Also, soil amendments can improve properties related to soil quality, such as soil organic matter content, biological activity, and nutrient status (Pardo et al., 2014; Pérez-Esteban et al., 2014; Wu et al., 2017).

Due to its abundant oxygen-containing functional groups especially carboxylic and phenolic-OH groups, humic acid can effectively complex with several metals and form stable metal–humic complexes, thereby reducing their mobility and bioavailability (Perminova and Hatfield, 2005; Stevenson, 1994). In addition, humic acid (HA) is also known to be a good plant-growth and mineral-nutrition promoter (Eyheraguibel et al., 2008; Lulakis and Petsas, 1995). Therefore, humic acid-based materials (e.g. lignite, oxihumolite, peat and pure humic acid) have been regarded as promising soil amendments (Clemente and Bernal, 2006; Janoš et al., 2010; Khan et al., 2017; Wang et al., 2017; Wang and Mulligan, 2009; Yu et al., 2017). In addition to occurring naturally, humic acid can be synthesized by abiotic humification processes which have been described in Chapter 2. Humic acid synthesized by abiotic humification is called synthetic humic-like acid (SHLA). Recently, we have reported a SHLA with high Cu complexation ability which can be used as an adsorbent for removing several metal ions from metal-contaminated water and also as a washing agent for removing metals from metal-contaminated soils (Yang and Hodson, 2018a; Yang and Hodson, 2018b). However, studies on how to apply SHLA as a soil amendment are still rare.

In this study, a synthetic humic-like acid which has been studied in Chapter 5 and 6 was applied as a soil amendment. The aim of this study was to investigate the effect of SHLA on soil properties (pH, TOC and DOC) and metal bioavailability under different amendment levels (1, 5 and 10 % (w/w)).

7.2 Materials and methods

7.2.1 Materials

Calcium chloride ($CaCl_2$, >99% purity) was purchased from Fisher Chemical. Ultra-pure water was prepared using a PURELAB Classic ultra-pure system (ELGA, UK) from deionized water. The preparation, optimization and characterization of SHLA are given in Chapter 6 (Section 6.2.2, 6.2.3 and 6.3.1).

7.2.2 Soil sampling and characterization

The soil used in this study (Soil 1) was collected from a farm located on the Stoke Bardolph Estate (GR 52.97104, -1.05515; Nottingham, UK). The physical-chemical characteristics of Soil 1 are given in Chapter 5 (Section 5.2.2 and 5.3.1).

7.2.3 Experiment design

Before incubation, Soil 1 was air dried at room temperature and sieved to 2 mm. At first, 70 g of soil was mixed with 0% (control), 1%, 5% and 10% (w/w) of SHLA, respectively. For each treatment, three replicates of 20 g of the mixture were each placed into 50 ml centrifuge tubes and moistened by adding 30% (w/w) ultra-pure water. Lids were placed loosely on the centrifuge tubes and the mixtures were incubated at 25 °C for 28 days. After incubation, the mixtures were air-dried for later analysis (pH, total organic carbon (TOC), and $CaCl_2$-extractable concentration of Cu, Zn, Ni, Pb and As). To further explore those results the decision was made to also measure water soluble carbon (WSC) in the mixtures.

pH: 8 g of air-dried soil was added to 20 mL deionised water in 50 mL centrifuge tube and shaken for 15 minutes (Rowell, 1994). pH was measured using a Thermo Scientific Orion Model 420 plus pH meter calibrated with pH buffer solutions (pH 4.00, 7.00 and 10.00).

TOC: Air-dry samples were ground in a ball mill and then TOC measured using a vario MACRO C/N analyzer (Elementary, Germany).

Water Soluble Carbon: 4 g of soil was added to 40 mL ultra-pure water and shaken orbitally at 250 rpm for 1 h at room temperature after which time the mixtures were separated by centrifugation at 4500 rpm for 20 min. The supernatant was filtered using a 0.45μm nylon syringe filter (Gilson, UK). The DOC in the supernatants were determined by vario TOC Elemental Analyzer (Elementary, Germany) (Pardo et al., 2014).

$CaCl_2$-extractable metal concentrations: 4 g of soil was added to 40 mL 0.01 M $CaCl_2$ solution in 50 mL centrifuge tube and shaken orbitally at 250 rpm for 2 h at room temperature. Then, the mixtures were separated by centrifugation at 4500 rpm for 20 min. The supernatant was filtered using a 0.45μm nylon syringe filter (Gilson, UK). The metal concentrations in the supernatants were determined by ICP-OES (iCAP 7000, Thermo Scientific, USA) (Houben et al., 2013).

In the results, all calculated values (see below) are quoted as mean values ± standard deviations.

7.2.4 Statistical analysis and quality control

The initial TOC values and those after 28 days were analyzed using paired t-tests (for each SHLA addition amount) with significance set at $p \leq 0.05$ (Sigmaplot 12.5, USA). Other data were analyzed using a one-way ANOVA (Sigmaplot 12.5, USA). The post-hoc Tukey test was used to determine differences between pairs of treatments with significance set at $p \leq 0.05$.

The ICP-OES detection limits for the metals were 0.002 mg/L for Cu, 0.002 mg/L for Zn, 0.002 mg/L for Pb, 0.001 mg/L for Ni and 0.004 mg/L for As which were equivalent to c. 0.02 mg/kg for Cu, 0.02 mg/kg for Zn, 0.02 mg/kg for Pb, 0.01 mg/kg for Ni and 0.04 mg/kg for As.

7.3 Results and discussions

7.3.1 Soil pH

Figure 7.1 shows the pH of soils 28 days after addition of different amounts of SHLA. Generally, the SHLA amendment decreased the soil pH ($p<0.01$), due to the weak acid nature of humic acid. As the mass of SHLA increased, pH values decreased ($p<0.001$). The pH of the SHLA (pH = 2.4) suggests that in many applications SHLA could cause a reduction in pH. In some cases this might be beneficial, for example, if the SHLA was applied to alkaline soils such as saline-sodic soil. However, in other cases a decrease in soil pH may not be wanted and in such cases, application with a base such as biochar or lime would need to be considered (Forján et al., 2016; Li et al., 2016; Meng et al., 2018). In these cases laboratory trials investigating the interactions of the SHLA – neutralizing agent would need to be carried out. The pH value of SHLA is 2.4 which is lower than that of natural humic acids (pH: 3.4-7.8) (Chen and Elimelech, 2007; Fatehah et al., 2014; Paul et al., 2015). This is likely due to the high acid group content of the SHLA. For example, the pH of commercial humic acid used in this study is 5.52 and the acid group content is 3.87 mmol/g, however the acid group content of SHLA is 19.19 mmol/g.

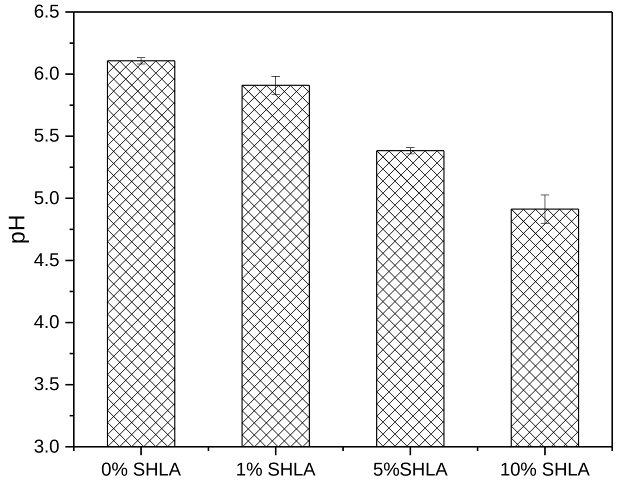

Figure 7.1 The pH of soils treated with different amounts of SHLA (0, 1%, 5% and 10 % (w/w)) after 28 days (n=3, mean value ± standard deviation).

7.3.2 TOC and WSC of soil

In addition to immobilizing potentially toxic metals, another aim of applying organic amendment can be to increase the organic matter content of soil. Figure 7.2 shows the soil TOC and WSC for the different SHLA treatments. As the amount of SHLA increases, the TOC content of Soil 1 significantly increases ($p<0.001$). There was no significant change in TOC between the start and end of the incubation for each treatment ($p>0.05$), indicating that the SHLA was relatively stable in the soil consistent with other studies that indicate that humic acid is stable in soils for at least 6 months (Clemente and Bernal, 2006). The soil WSC content also increased significantly with increasing additions of SHLA ($p<0.001$).

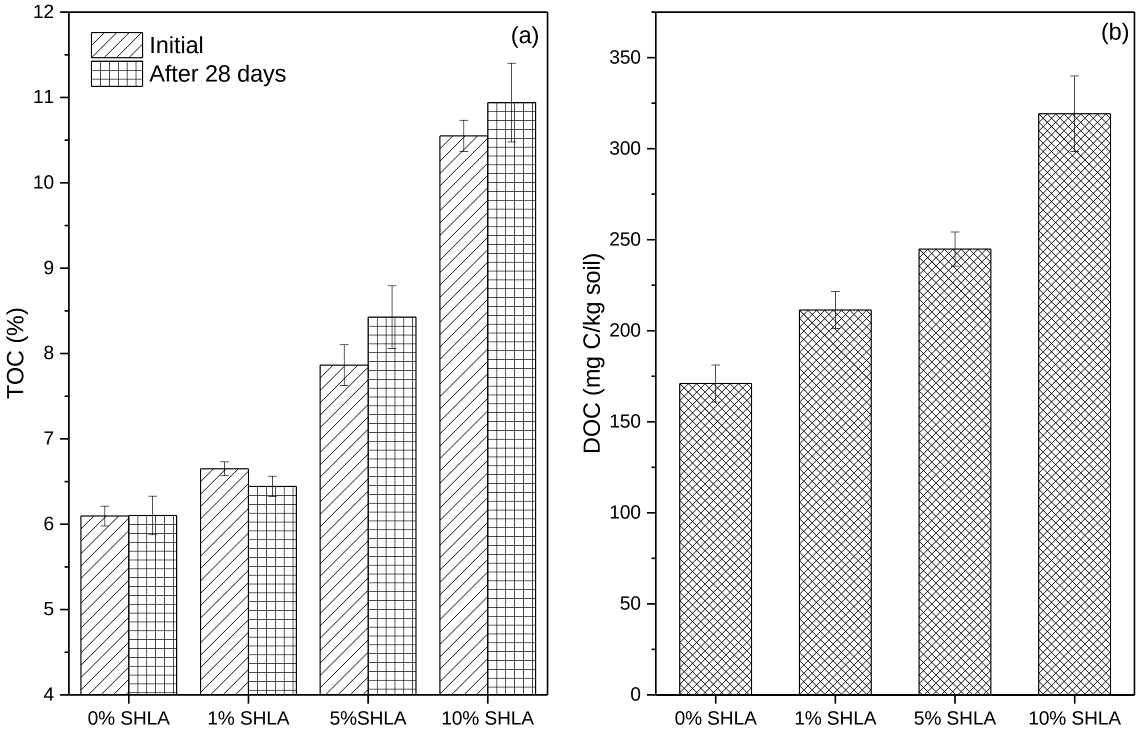

Figure 7.2 The TOC (a) and WSC (b) of Soil 1 treated under different SHLA addition amount (0, 1%, 5% and 10% (w/w)) (n=3, mean value ± standard deviation).

7.3.3 Metal solubility and bioavailability in soil

The 0.01 M $CaCl_2$ extraction was used to determine the effect of the SHLA amendments on metal solubility, bioavailability and mobility in the soil (Figure 7.3) (Houben et al., 2013).

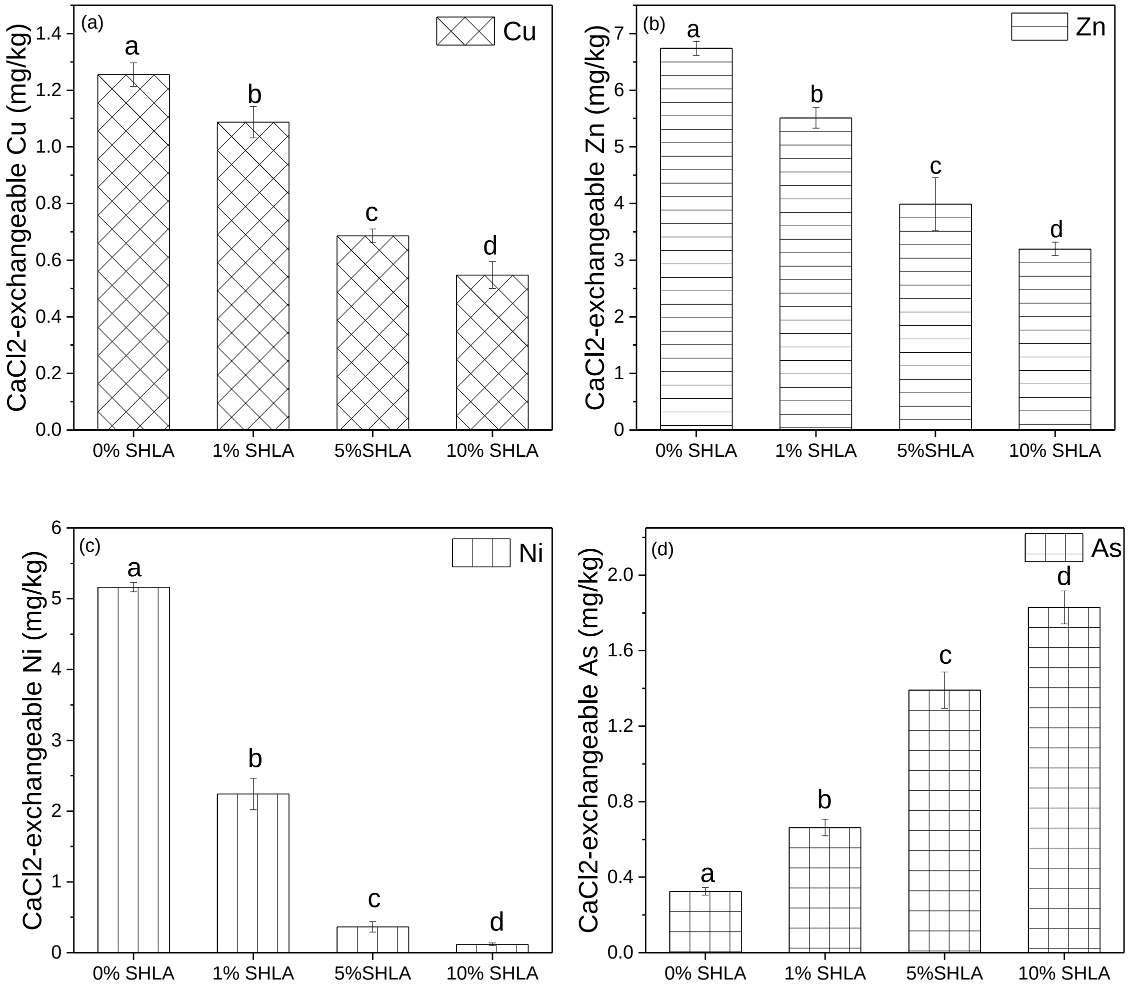

Figure 7.3 The 0.01 M $CaCl_2$-extractable metal concentrations (Cu(a), Zn(b), Ni(c), As(d)) in Soil 1 after 28 days incubation (n=3, mean value ± standard deviation). Different letters over columns indicate significant differences ($p < 0.05$).

The addition of SHLA decreased the $CaCl_2$-extractable concentrations of Cu ($p<0.05$), Zn ($p<0.001$) and Ni ($p<0.001$), indicating SHLA could lower the bioavailability of Cu. Chapter 6 (Section 2.8 and 3.7) demonstrates that SHLA is an efficient adsorbent for Cu, Zn and Ni at pH 5 and for copper its adsorption efficiency remains high at pH 3. The decrease of extractable Cu, Zn and Ni is despite the decrease in soil pH is therefore likely due to adsorption of the metals by the SHLA. However, the $CaCl_2$-extractable concentration of As increased ($p<0.001$) with increasing masses of added SHLA. As shown in Figure 7.2, the WSC content of Soil 1 increased with increasing addition of SHLA. The presence of WSC may compete with As for adsorption sites on mineral surfaces of soil, hence increasing the bioavailability of As (Grafe et al., 2001; Lin et al., 2008). The $CaCl_2$-extractable concentrations of Pb in the soils with and without SHLA were below detect limitation.

7.4 Conclusions

In this study, a synthetic humic-like acid (SHLA) was applied as a soil amendment to immobilse metals *in situ* in a metal-contaminated soil. As the amount of SHLA increased from 0 to 10%, soil pH decreased ($p<0.001$), and both TOC and DOC of the soils increased ($p<0.001$). Despite these changes which would be expected to increase metal solubility/mobilty, 0.01 M $CaCl_2$-extractions suggest that the SHLA amendments lowered the bioavailability of Cu, Zn and Ni, most likely due to adsorption onto the SHLA. In contrast the bioavailability of As increased, most likely because the soil WSC content increased with increasing addition of SHLA which may compete with As for adsorption sites on mineral surfaces of soil.

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Clemente, R., Bernal, M.P., 2006. Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. Chemosphere, 64, 1264-1273.

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CHAPTER 8

General conclusions and future study

Chapter 8 General conclusions and future study

8.1 General conclusions

The main aim of the PhD project is to investigate the possibility of applying synthetic humic-like acid (SHLA) in environment remediation (water/wastewater treatment and soil remediation). The project includes three stages. At the first stage (Chapter 3), the objective is to answer the question whether SHLA can be used for binding metals or not. After getting the answer "yes" for the first stage, at the second stage (Chapter 4), the main objective is to answer questions under which abiotic humification conditions SHLA with better metal binding ability can be synthesized and which structural characteristics of SHLA will affect its metal binding ability. Then the optimal humification conditions to produce SHLA with a high binding ability are achieved and the optimal SHLA is synthesized. At the third stage, the optimal SHLA is applied in three ways: as a soil washing agent (in aqueous form, Chapter 5); as an adsorbent for water/wastewater treatment (in solid form, Chapter 6); as a soil amendment (in solid form, Chapter 7). The structure of PhD project please refer to Figure 1.1 in the Introduction section. The conclusions in detail are listed as follows.

In Chapter 3, a synthetic humic-like acid (SHLA) is prepared under the condition depending on the previous research. Copper ion is chosen as the representative toxic metal. Then the complexation ability of SHLA for Cu^{2+} and the influence of solution physico-chemical factors on the complexation reaction (ionic strength, pH, temperature and humic acid concentration) are investigated. The results show that the SHLA have a stronger complexation ability than most natural humic acids. The log K and complexation efficiency of SHLA are higher at higher pH, lower ionic strength, higher temperature and higher SHLA concentration, and the effects of solution physico-chemical factors on the complexation reaction are similar to natural humic acids. In conclusion, Chapter 3 demonstrates that SHLA can be used for binding metals.

In Chapter 4, the main aim is to achieve the optimal conditions to produce optimal SHLA with better metal binding ability. Therefore, the effect of the varying humification conditions on the complexation ability of the SHLA for Cu^{2+} are 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, E_4/E_6). Specifically, 11 synthetic humic-like acid (SHLAs) are 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 MnO_2 catalyst (1.3-2.5% w/v). Chapter 4 demonstrates that the SHLA, which has a higher content of carboxylic groups, more aromatic structures and a higher degree of humification, will have a better Cu complexation ability. The optimal abiotic humification conditions to synthesize SHLA with better copper complexation ability are determined as follows: at a lower temperature (25 °C), pH 8, low precursor concentrations (glycine: catechol = 0.25M:0.25M) and a larger proportion of catalyst (2.5% w/v).

Before the application related chapters, the optimal synthetic humic-like acid is prepared under the optimized abiotic humification condition and then applied in three ways.

In Chapter 5, the optimal SHLA in liquid form is used as a soil washing agent to remove metals (Cu, Zn, Ni, Pb, As) from two contaminated agricultural soils. The effects of washing conditions on removal efficiency are investigated and optimal extraction conditions were achieved (pH=9, L/S ratio of 80:1 mL/g, initial SHLA concentration of 1500 mg/L, contact time of 360 min). Under these conditions, a single washing can remove 45.2% (equal to 136.98 mg/kg) of Cu, 34.6% (equal to 242.21 mg/kg) of Zn, 42.2% (equal to 59.16 mg/kg) of Ni and 15.6% (equal to 50.52 mg/kg) of Pb from Soil 1, and 30.6% (equal to 74.37 mg/kg) of Cu, 28.1% (equal to 236.24 mg/kg) of Zn. 14.6% (equal to 35.35 mg/kg) of As and 18.1% (equal to 81.78 mg/kg) of Pb from Soil 2, respectively. In terms of removal efficiency on molar basis, SHLA was more effective than some common washing agents, like Na_2EDTA, citric acid, commercial humic acid and tartaric acid. Considering the environment and human health risk, reduced partition index (I_R) and metal bioavailability factor (M_F) are calculated based on the BCR analysis, and the results indicate synthetic humic-like acid can effectively reduce bioavailability and environmental/human health risk of metals. In summary, SHLA shows great potential as a soil washing agent.

In Chapter 6, the optimal SHLA in solid form is used as an adsorbent to remove metal ions from contaminated water. The adsorption behaviours of aqueous Cu^{2+} onto the SHLA including adsorption efficiency and adsorption kinetics, together with isotherm fits and thermodynamics are investigated together with the desorption and reuse potential of the SHLA and the potential adsorption of co-contaminant metals. The SHLA exhibits a range of adsorption efficiencies (27%–99%) for Cu^{2+} depending on reaction conditions. The pseudo-second-order kinetic model fits best to the experimental data, indicating that chemisorption is most likely the rate-limiting step for adsorption. The equilibrium adsorption data shows good fits to both the Langmuir and Freundlich models. Thermodynamic parameters and values of R_L and n show that the adsorption of Cu^{2+} onto SHLA was favourable, spontaneous and endothermic. The Cu^{2+} adsorption capacity of SHLA is much higher than many of the humic acid-based materials reported in the literature and the SHLA can be regenerated by HCl. Besides, SHLA is able to remove co-contaminant metals from solution. A final experiment using a multi-metal solution (the concentration of each metal:10 mg/L-100 mg/L) indicates adsorption efficiencies of up to 84.3–98.3% for Cu, 86.6–98.8% for Pb, 30.4–82.9% for Cr, 13.8–77.4% for Ni, 9.2–62.3% for Cd, 8.6–51.9% for Zn and 4.6–42.1% for Co. In summary, Chapter 6 demonstrates that SHLA has significant potential for use as an adsorbent for the clean up of contaminated waters.

In Chapter 7, the optimal SHLA in solid form is used as a soil amendment to immobilize toxic metals in metal-contaminated soil. SHLA can lower the bioavailability of Cu (from 1.26±0.04 mg/kg to 0.55±0.05 mg/kg), Zn (from 6.74±0.12 mg/kg to 3.26±0.23 mg/kg) and Ni (from 5.16±0.07 mg/kg to 0.12±0.02 mg/kg), but increase the bioavailability of As (from 0.31±0.02 to 1.83±0.09 mg/kg). Besides, the addition of SHLA can increase TOC and WSC of soil but decrease the soil pH.

Overall, this PhD project demonstrates that the structural characteristics of synthetic humic-like acid can be manipulated by changing abiotic humification conditions and that abiotic humification processes can provide a potential and promising way to synthesize humic acids with appropriate metal binding abilities. Synthetic humic-like acid has significant potential for application as an adsorbent, soil washing agent and soil amendment.

8.2 Future study

8.2.1 Consideration of cost

In order to decrease the uncertainty of the reaction system, all SHLAs was synthesized by pure chemicals in this study, which might cause high cost in the practical application of wastewater treatment or soil remediation. Thus, a simple cost estimation is calculated here. The yield of optimal SHLA is 73.3% (Chapter 6, Section 6.3.1). If just considering the cost of raw materials (Table 8.1), the cost for producing SHLA ranged from £1100/t to £5380/t. In details, producing 1t of SHLA needs 0.81t of catechol, 0.55t of glycine, 0.74t of MnO_2 and 29.46 t of water.

The low cost of SHLA/t

= 0.81t catechol*low cost of catechol/t +0.55t glycine*low cost of glycine/t +0.74t MnO_2*low cost of MnO_2/t +29.46 t water*price of water/t

= 0.81t catechol*£754/t +0.55t glycine*£754/t +0.74t MnO_2*£75/t +29.46 t water*£0.97/t ≈£1100/t

The high cost of SHLA/t

=0.81t catechol*high cost of catechol/t +0.55t glycine*high cost of glycine/t +0.74t MnO_2*high cost of MnO_2/t +29.46 t water*price of water/t

= 0.81t catechol*£4525/t +0.55t glycine*£2262/t +0.74t MnO_2*£603/t +29.46 t water*£0.97/t ≈£5380/t

Table 8.1 Cost of raw materials

Raw materials	Price*
Catechol	£754-4525
Glycine	£754-2262
MnO_2	£75-603
Water	£ 0. 97/t
EDTA	£1131-3922

(*The price data for catechol, glycine, MnO_2 and EDTA were collected from https://www.alibaba.com/; the price of tap water is from Thames Water: http://www.tapwater.org/faqs; the original price of catechol, glycine, MnO_2 and EDTA were in US dollars and the exchange rate for calculation was £1=$1.326)

A simple cost estimation for 4 different approaches to remediate 1 t metal-contaminated soil is also calculated as follows (Table 8.2). The four approaches include soil amendment (10% SHLA & 1% nanoscale zero-valent iron (NZVI)), soil washing (EDTA and SHLA under optimal conditions), phytoremediation, and disposal in the hazardous landfill.

In terms of soil amendment, NZVI, another promising amendment to remediate toxic metals and organic pollutants (Jiang et al., 2018), is chosen to as a representative of nanomaterials to compare with SHLA. For Cu, Zn and Ni, NZVI can reduce their bioavailability as well as SHLA (Xue et al., 2018). Besides, NZVI can decrease bioavailability of As while SHLA has the opposite effect (Gil-Díaz et al., 2017). As for the price, the cost of NZVI is several times higher than that of SHLA (Table 8.2). As for the toxicological effect, recent studies show that NZVI can adverse effects to soil microorganism (e.g. microbial activities and community structure), and thus toxicological effects of NZVI should be considered during application (Fajardo et al., 2012; Tilston et al., 2013). Humic acid is one of important components of soil organic matter, then the application of SHLA amendment might contribute to soil organic matter and have low adverse effect. In terms of soil washing, the widely used EDTA is chosen to compare with SHLA. From Table 8.2, the cost for SHLA washing is similar to EDTA washing if just considering the cost of washing solution. As mentioned in Chapter 3, EDTA can lead to adverse health and environmental effects. However, if SHLAs were used in soil washing, any residual SHLA left in the soil could contribute to soil organic matter. Thus, the use of

SHLA has the potential to be environmentally friendly compared to using EDTA though clearly before its widespread use its application would need to be assessed for any possible toxic side effects.

The next question is what technique is suitable for a real environment case. Compared with soil amendment (10% SHLA), soil washing (SHLA under optimal conditions) and disposal in the hazardous landfill, the cost of phytoremediation is the lowest, but the disadvantage is that application duration of phytoremediation is much longer than soil washing and amendment. Compared with soil washing and amendment approach, disposal in landfill is cheaper. The disadvantages of landfilling are that it will occupy amounts of space and the contaminations still remain in soil which is also a limitation for soil amendment. The metal-removal/immobilization level is also worth considering when choosing the remediation technique. For example, when applying SHLA as soil amendment in Soil 1, SHLA can immobilize 56.4% of bioavailable Cu, 52.6% of bioavailable Zn and 97.7% of bioavailable Ni (based on the $CaCl_2$-extractable metal concentration). SHLA amendment can change the metal species of soil rather than remove metals from soil, and the immobilized metals might be activated when environmental conditions change. When washing Soil 1 with SHLA, SHLA can remove 93.4% of F1-Cu, 48.2 of F1-Zn and 85.0% of F1-Ni. Soil washing can remove metals directly from soil, but the cost goes up accordingly.

For real remediation case, to what level the soil needs to be remediated, how much money the case can spend and duration of the remediate case should be of integrated consideration. Besides, in practical application, costs such as operational costs, set-up and break down costs and disposal costs should be considered together with washing agent or amendment cost.

Table 8.2 Cost estimation for 4 different approaches to remediate 1 t metal-contaminated soil

Approach	Soil amendment with 10% SHLA	Soil amendment with NZVI (1%)	Soil washing by SHLA (0.15%, L/S 80:1)	Soil washing by EDTA (0.15%, L/S 80:1)	Phytoremediation with *Pteris vittata* L (Wan et al., 2016)	Disposal in hazardous landfill
	SHLA (required mass and price): 100 kg→£110-538	NZVI (required mass and price): 10 kg→£33-128/kg	SHLA (required mass and price): 120 kg→£132-646 Water: 80 m^3→£78	EDTA (required mass and price): 120 kg→£180 -624 Water: 80 m^3→£78	2 year-project; *Pteris vittata* L £ 28/m^3*	Landfill tax: £88.95/t (from 2018 April**) Disposal fee: £50-150/t*** VAT:20%
Total	£110-538	£330-1280	£210-724	£258-702	£28	£167-287

(*soil bulk density is set to 1g/cm^3 and the original price about phytoremediation was in US dollars and the exchange rate for calculation was £1=$1.326; ** Guidance Landfill Tax rates from UK government: https://www.gov.uk/government/publications/rates-and-allowances-landfill-tax/landfill-tax-rates-from-1-april-2013; ***Soilutions Company website: https://blog.soilutions.co.uk/2012/01/18/how-much-does-contaminated-soil-disposal-cost/)

In future study, reducing the cost of SHLA producing process should be one of the most worth considering issue. Humification precursors, catalyzer and reaction conditions are three main components of abiotic humification process, which can be regarded as key points for reducing production cost. For humification precursors and catalyzers, to reduce the cost of raw materials, waste containing polyphenols, sugars, amino acids or metal oxides can be considered as precursors or catalyst for abiotic humification process in the future study. For example, Brunetti et al. show that SHLA can be produced from olive oil mill wastewater catalyzed by MnO_2 (Brunetti et al., 2008). Some waste residue, like steel slag and municipal solid waste incineration residue, can be used as a catalyst to enhance abiotic humification process (Kim and Osako, 2004; Nishimoto et al., 2013; Qi et al., 2012). The reuse of waste can not only reduce the cost but also reduce negative impacts of growing amounts of waste on the natural environment. Besides, increasing the SHLA yield is another way to reduce cost, which can be achieved by changing reaction conditions. In this study, the optimal yield is 73.3% depending on the results of 12 different reaction conditions. More research on optimizing the reaction conditions can be conducted for further increasing the yield of SHLA. For example, we can investigate if the nanosized metal oxides have stronger catalytic ability compared with traditional metal oxides. In this thesis, we studied 12 humification reaction conditions (3 temperatures, 3 pH values, 5 precursor concentration ratios, 2 precursor systems). In future, the effect of reaction conditions on SHLA yield and characteristics can be expanded depending on the current 12 reactions, like trying new precursor system, expanding pH and temperature range.

8.2.2 Application in the soil system

Soil system is a complex system, including air, water, inorganic and organic compounds and microorganism (Sparks, 1995). In this study, when applying SHLA as soil amendment or washing agent, we mainly focus on the removal or immobilization characteristics of toxic metals. But the application of SHLA may have effects on other soil properties. For example, when applying organic amendments (e.g. compost, manure and sewage sludge) in soil, they will affect soil chemical properties, physical properties and biological properties simultaneously (Diacono and Montemurro, 2011; Wu et al., 2017). Specifically, for soil chemical properties, organic amendments can increase soil organic matter, CEC, EC and nutrients levels (N, P and K) and change soil pH (Leroy et al., 2008; Pukalchik et al., 2017; Wu et al., 2017). For soil physical properties, organic amendments can increase soil aggregate stability, water holding capacity, soil porosity and percolation (Annabi et al., 2011; Leroy et al., 2008; Luna et al., 2017). For soil biological properties, organic amendments can increase enzyme activities and soil microbial diversity (Innangi et al., 2017; Liu et al., 2017; Liu et al., 2017). So, what effects will SHLA have on the whole soil system when working as soil washing agent or amendment? It will be an interesting research topic. For example, previous studies showed HA amendment can enhance plant nutrient uptake, and HA with low molecular weight and high COOH content can enhance nutrient uptake more effectively (Piccolo et al., 1992; Zhang et al., 2014). Qian et al indicate HA with low molecular weights has more carboxylic groups than that with large molecular size (Qian et al., 2015). As shown in Chapter 5, 6 and 7, the optimal SHLA has higher COOH content (5.03 mmol/g) than those used in the mentioned articles (COOH content of 2.5-4.4 mmol/g) and might have low molecular weights according to Qian's research. Thus, SHLA might enhance plant nutrient uptake effectively. Besides, HA can increase antioxidant enzymatic activities and HA with more auxin-like structures derived by phenolic acid can enhance antioxidant enzymatic activities more strongly (Elmongy et al., 2018; Nagasawa et al., 2016). The optimal SHLA might have higher phenolic acid derived auxin-like structures due to high content of COOH and phenolic-OH content and thus can be used to enhance antioxidant enzymatic activities. As for the chemical properties, the current thesis has demonstrated that SHLA can change increase soil organic matter content and decrease soil pH (Section 7.3.1 & 7.3.2 in Chapter 7).

Some organic amendment (e.g. manure and straw) can also increase greenhouses gases emissions (GHG) from soil via priming effect, methanogenesis, nitrification, and denitrification (Thangarajan et al., 2013). However, humic acid has high microbiological stability and plays an important role in C sequence. Recent studies show that humic acid can work as a suppressor for methanogenesis and mitigate methane emission from peat bog and soil. It is reasonable that SHLA, with a similar structure to natural HA, can also work for mitigating methane (Blodau and Deppe, 2012; Büyükkeskin et al., 2015). Further studies can be considered about what effect SHLA will have on soil GHG emissions.

Another limitation of this study is about the soil samples used, of which both are light-contaminated agricultural soils. As shown in Chapter 5, the nature of soil will affect the metal removal efficiency of SHLA. Hence, if applied SHLA in the heavy-contaminated soil, how is the metal removal performance of SHLA? Tsang et al has applied 100 mg/L commercial HA (from Sigma-Aldrich) to wash a heavily contaminated soil (Cu: 2100 mg/kg; As: 3300 mg/kg) in L/S ratio of 80:1 (ml:g) and it can remove 735 mg/kg Cu and 264 mg/kg As (Hartley et al., 2014; Tsang and Hartley, 2014). In Chapter 5, the same commercial HA is used as a washing agent with which to compare the performance of SHLA. The results show % metal removal for all metals by SHLA was 2.2–15.2 times higher than that of commercial HA. Thus, the metal removal performance of SHLA might be also effectively in the heavy-contaminated soil. It should be considered later. In addition, all experiments in this study are in lab-scale, and it will still have a long way to go from lab-scale to field-scale then to practical application.

8.2.3 To remediate organic contamination

Except for inorganic contaminants, humic acid can also react with organic pollutants via physical absorption, hydrogen bond, charge transfer, covalent bond and distribution, etc (Perminova et al., 2005; Stevenson, 1994). Several studies show that humic acid and humic acid-based materials can be used as adsorbents to remove dye (e.g. methylene blue, rhodamine B, Midlon Black), pesticides (e.g. lindane, atrazine, diuron and thiram), phenanthrene, ethylene dibromide and 3,5-dichlorophenol from contaminated water (Chiou et al., 2000; Filipe et al., 2009; Janoš et al., 2005; Shenvi et al., 2015; Wang et al., 2017; Wang et al., 2011; Wen et al., 2007). Humic acid can also serve as soil washing solution to remove polycyclic aromatic hydrocarbons, thiophenes and petroleum hydrocarbons from soil (Conte et al., 2005; García-Díaz et al., 2015). If SHLA is applied to remediate organic contamination, how will SHLA perform? It is interesting and worth being studied. For example, one of the mechanisms why HA modified materials (e.g. HA-modified pillared clay, HA-modified hydroxyapatite, and HA-modified Fe_3O_4) has stronger cationic dyes adsorption efficiency than unmodified ones is that the carboxylic and phenolic groups of HA causes a net negative charge, then the adsorption through electrostatic interaction between cationic dyes and HA modified materials can be enhanced (Peng et al., 2012; Vinod and Anirudhan, 2003; Wang et al., 2017; Zhang et al., 2013). Part of humic acids mentioned in the above articles are commercial HA with COOH content of 1.01-1.22 mmol/g and phenolic-OH content of 2.65-6.45 mmol/g (Fetsch and Havel, 1998), which are lower than the optimal SHLA used in this study (COOH content: 5.03 mmol/g and phenolic-OH content: 6.55 mmol/g; Table 6.1 in Chapter 6). It is reasonable that the performance of mentioned composite materials could be improved further if a SHLA was used rather than a commercial humic acid.

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