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

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**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 ℃ for 4 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, Na2EDTA, 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

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, H2SO4, HNO3); 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 MnO2, 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 Cu2+ 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), IR (reduced partition index) and MF (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, Na2EDTA, citric acid and tartaric acid) was conducted.

1. **Materials and methods**

**2.1 Materials**

Catechol (C6H6O2, >99% purity), glycine (C2H5NO2, >99% purity), glucose (C6H12O6, >99% purity), thimerosal (C9H9HgNaO2S, >97% purity), manganese oxide (MnO2, >99% purity), ethylenediaminetetraacetic acid disodium salt dihydrate (C10H14N2Na2O8·2H2O, 98.5-101.5% purity), citric acid (C4H8O7, >99% purity), tartaric acid (C4H8O7, >99% purity) and commercial humic acid (CHA) were purchased from Sigma-Aldrich. Sodium phosphate dibasic dihydrate (Na2HPO4·2H2O, >99% purity), sodium phosphate monobasic dihydrate (NaH2PO4·H2O, >99% purity) and hydrogen peroxide (H2O2, 30% purity) were purchased from ACROS Organics. Calcium chloride (CaCl2, >99% purity), acetic acid (CH3COOH, >99% purity) and ammonium acetate (CH3COONH4, >99% purity) were purchased from Fisher Chemical. Hydroxylamine hydrochloride (NH2OH·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.

**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).

**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% MnO2 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 Na2HPO4 / NaH2PO4 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 MnO2, 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(CH3COO)2 titration methods, respectively (Schnitzer and Kahn, 1972). The phenolic-OH content was calculated by subtracting the carboxylic group content from total acidity. The E4/E6 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 Kcu 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.

**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:



where M0 (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), Elovich (equation 4), power function (equation 5) and Parabolic function (6) models (Almaroai et al., 2013; Xu et al., 2008).











where *Qt* and *Q0* are the mole fraction of metals in soils at time t and t = 0, respectively; *qt* is the mole fraction of metals desorbed by extractants time t; and *k1*, *k2*, *kf*, *b*, *α*, *β*, *kp*, and *C* are constants.

**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 1; Zhang et al., 2017).

Table 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 CH3COOH | 16h, 25 ℃ |
| 2 | Reducible fraction (F2) | 0.1M NH4OH·HCl | 16h, 25 ℃ |
| 3 | Oxidizable fraction (F3) | 8.8M H2O21M CH3COONH4 | 85 ℃ for 1h and room temperature for 16 h. |
| 4 | Residual fraction (F4) | HNO3: HCl =1:3 | See Section 2.2 |

*IR* (reduced partition index) and *MF* (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.





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), *Fi*is the percentage of the considered metal in fraction *i*, and *Fi’* is actual concentration of the considered metal in fraction *i.*

**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), Na2EDTA, 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 Na2EDTA, 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 Na2EDTA, 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.

* 1. **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 < 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 < 0.05 (Sigmaplot 12.5, USA). The IR and MF 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 < 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.

1. **Results and discussions**

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

Table 2 lists the elemental composition, acidic functional group content and E4/E6 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 2. Elemental composition, acidic functional groups content and E4/E6 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) | E4/E6 |
| 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 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 S1 in the supplementary material.



Figure 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 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 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 |
| F1 | 18.73±0.84 | 14.84±0.07 |  |
| F2 | 62.19±5.34 | 86.10±9.56 |  |
| F3 | 173.77±7.70 | 78.48±2.99 |  |
| F4 | 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 |
| F1 | 280.44±8.67 | 368.05±23.81 |  |
| F2 | 214.28±9.26 | 215.11±10.67 |  |
| F3 | 43.25±1.20 | 66.22±9.61 |  |
| F4 | 127.13±18.38 | 134.81±18.01 |  |
| Total Pb | 323.56 ±4.84 | 451.21 ±1.92 | 300 |
| F1 | 0.54±0.06 | 15.42±1.75 |  |
| F2 | 195.48±11.29 | 235.58±13.03 |  |
| F3 | 34.09±2.71 | 51.55±7.18 |  |
| F4 | 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 |
| F1 | 53.15±1.61 | 0.64±0.04 |  |
| F2 | 44.46±2.38 | 1.10±0.03 |  |
| F3 | 13.21±0.29 | 0.69±0.02 |  |
| F4 | 18.71±2.51 | 0.26±0.00 |  |
| Total As | 24.59 ±0.93 | 242.23 ±5.24 | 50 |
| F1 | 4.52±0.23 | 22.61±1.29 |  |
| F2 | 10.86±0.37 | 92.05±4.57 |  |
| F3 | 5.37±0.09 | 21.74±2.38 |  |
| F4 | 1.07±0.01 | 58.42±21.69 |  |

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

**3.2 The effect of washing conditions on % metal removal**

Figure 2 shows % metal removal of the SHLA and Figure 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 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. AsO43−, HAsO42−, and AsO33−)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 Na2EDTA (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 less than 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.

**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 ℃ and a duration of 24 h (Figure 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 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℃; one of these variables was varied at a time.

**3.2.2 pH**

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 ℃ and a washing duration of 24 h (Figure 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).

**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 ℃ for a wash duration of 24 h (Figure 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.

**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 ℃ (Figure 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 (R2), SEE (standard errors of the estimate) and p-value (Table 4). The low R2 values of the first-order (R2: 0.5678-0.8305) and second-order (R2: 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).

**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 ℃ for 4 h.

Table 4. Kinetics parameters (R2, 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 |
| R2 | SEE | p-value |  | R2 | SEE | p-value |  | R2 | SEE | p-value |  | R2 | SEE | p-value |  | R2 | 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 |

**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 3, Fig. 3 for distributions in terms of actual concentration, Fig. 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 F3Zn in Soil 2), but F4 was not be removed (p>0.05 except F4Cu 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).

IR (reduced partition index) and MF (metal bioavailability factor) before and after SHLA washing (Figure 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 IR and low MF values indicate that metals in soils are stable with low mobility and bioavailability (Gusiatin and Klimiuk, 2012). The IR 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 IR value (p<0.001) of all the metals and the IR of Ni had the largest increase (by 0.2 units, p<0.001). Similarly in Soil 2, Cu had the largest IR value (p<0.001) of all the metals and the largest increase in IR value (p<0.01). In Soil 1 the MF 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 MF values of either Zn or As (p ≥ 0.05) in Soil 2, which might be caused by the low F1 extraction efficiency of both Zn and As.



Figure 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 S2 (Supplementary Material).

Figure 4. IR (a,b) and MF (c,d) values before and after SHLA washing of two soils (n=3, mean values ± standard deviation).

**3.4** **Comparison with CHA,** **Na2EDTA, 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), Na2EDTA, citric acid and tartaric acid were used as washing agents with which to compare the performance of SHLA (Fig. 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 N2EDTA. Na2EDTA 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 Na2EDTA, 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 Na2EDTA, 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 Na2EDTA, citric acid and tartaric acid, the molar concentration of SHLA was much lower, which caused the apparent low % metal removal. Accordingly, Figure 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 Na2EDTA 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. Comparison of % metal removal of SHLA, CHA, Na2EDTA, 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).

1. **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, Na2EDTA, 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.

**Supplementary Materials**

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

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