

1 **Transformation behaviors and environmental risk assessment of**
2 **heavy metals during resource recovery from *Sedum plumbizincicola***
3 **via hydrothermal liquefaction**

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21

22 **Abstract**

23 Environmentally sound disposal of hyperaccumulator harvests is of critical
24 importance to industrialization of phytoremediation. Herein, transformation behaviors
25 and environmental risk of heavy metals were comprehensively examined during
26 subcritical hydrothermal liquefaction of *Sedum plumbizincicola*. It is concluded that
27 low temperature liquefaction favored resource recovery of heavy oil and hydrochars
28 in terms of higher energy density, improved carbon sequestration and less energy
29 consumption. Heavy metals were mainly distributed into hydrochars and water
30 soluble phase with less than 10% in heavy oil. All metal elements except As could be
31 accumulated in hydrochars by extending reaction time, whereas more than 96% of As
32 was redistributed into water soluble phase. Prolonged liquefaction time facilitated
33 immobilization of Cd, Cr and As in hydrochars, but fast liquefaction favored Pb
34 stabilization. Liquefaction significantly reduced environmental risk level of Cd, Zn
35 and As, but may mobilize Pb and Mn, especially for Mn to very high risk level at 240
36 °C. High temperature with long reaction time tended to inhibit leaching rate of Mn,
37 whereas low liquefaction temperature with short reaction time prevented the leaching
38 of Zn and As from hydrochars. Overall, these findings are essential for downstream
39 upgrading of heavy oil and metals recovery from hydrochars.

40 **Keywords:** Phytoremediation; Hyperaccumulator; Heavy oil; Hydrochar; RAC

41

42 **1. Introduction**

43 In recent years, great attention has been paid to the remediation of heavy metals
44 contaminated soils due to their tremendous human health risks (Eijsackers et al.,
45 2020). Many applicable technologies have been proved to be effective for soil
46 remediation, including engineering techniques (e.g., excavation, leaching or washing,
47 thermal treatment and electro-kinetics) (Hasan et al., 2019; Kollannur & Arnepalli,
48 2019), biological remediation (e.g., microbial immobilization and phytoremediation)
49 (Bandara et al., 2020), and agrochemistry amendment (e.g., chemical immobilization
50 with biochar modifier) (Bandara et al., 2020; Hasan et al., 2019; He et al., 2019b).
51 Particularly, phytoremediation using hyperaccumulators has been considered as one of
52 the most desirable and environmentally sound remediation techniques to remove
53 heavy metals from contaminated soils due to its simplicity, high efficiency, low cost
54 and favorable public acceptance (Keller et al., 2005; Suresh & Ravishankar, 2004).
55 Current research on phytoremediation mainly focuses on enrichment and
56 detoxification mechanisms of heavy metals in plants and intensified measures for
57 phytoextraction (Conesa et al., 2007; Jin et al., 2009; Zhang et al., 2011). For instance,
58 it has just been revealed that efficient Cd phloem transport favored the redistribution
59 of Cd to growing leaves in hyperaccumulator *Sedum alfredii* (Hu et al., 2019b). In
60 addition, organic chelants could improve the dissolution and mobility of metals in
61 soils, thereby distinctly facilitating phytoextraction of heavy metals (Shahid et al.,
62 2014).

63

64 However, since concentration of heavy metals in hyperaccumulators could be more
65 than ten times of that in ordinary plants (van der Ent et al., 2013), inappropriate
66 disposal of heavy metals enriched biomass would not only cause a waste of biomass
67 resources, but also result in secondary pollution due to potential release of heavy
68 metals into surrounding environment (Keller et al., 2005). Although conventional
69 thermal treatment (i.e., incineration, pyrolysis and gasification) is viable for the
70 treatment of heavy metals enriched plants, moisture content of hyperaccumulator
71 harvests and metal volatilization are critical concerns for high recovery efficiencies of
72 metals and environmental safety of biochars (Du et al., 2019; Keller et al., 2005). Up
73 to date, there is still limited technical research on the post-treatment of heavy metals
74 enriched plants (Wu et al., 2013; Zhong et al., 2015). Fortunately, hydrothermal
75 liquefaction (HTL) occurring under 200-350 °C and 4-22 MPa in the presence of
76 water (Li et al., 2018) has been extensively applied to recover valuable resources (e.g.,
77 metals, hydrochar, chemicals and bio-oil) from hyperaccumulators into with mitigated
78 ecological risks (Qian et al., 2018; Yang, 2010; Yang et al., 2010; Zhu et al., 2019).

79

80 At present, tremendous studies have explored the fate and environmental risk of heavy
81 metals in solid residues from HTL of biomass wastes, such as livestock manures (Li et
82 al., 2018), sewage sludge (Leng et al., 2014) as well as lignocellulosic biomass (Chen
83 et al., 2014; Leng et al., 2018). HTL of biowastes, such as sewage sludge (Chen et al.,

84 2014) and manures (Li et al., 2018), could effectively stabilize the heavy metals with
85 the majority accumulated in the solid fraction based on different types of heavy metals
86 (Li et al., 2018). The mobile fraction of heavy metals in manures was largely
87 transformed into structurally stable state in the solid residues after HTL (Li et al.,
88 2018). In contrast, heavy metals were substantially transferred into bio-oil during
89 individual HTL of lignocellulosic biomass (e.g., rice straw and wood sawdust) (Leng
90 et al., 2018). During HTL of *Sedum alfredii Hance* (Yang et al., 2010), accumulated
91 metals were almost transferred into aqueous phase because of a short reaction time of
92 10-120 s. Although released metal ions (i.e., Zn^{2+} and Pb^{2+}) in the aqueous phase
93 demonstrated catalysis effects on promoting the production of long chain fatty acids
94 (e.g., formic acid and acetic acid) (Qian et al., 2018; Zhu et al., 2019), metals recovery
95 from liquids could be costly. In order to achieve easier recovery of metals, Chen et al.
96 (2019) have separated heavy metals from raw lignocellulosic biomass with up to 90%
97 of metals (e.g., Ce, Cu, Pb and Zn) concentrated in the solid phase by extending
98 reaction time to 30 min during HTL of *Sedum alfredii Hance* at 320 °C. Actually,
99 reduction of released metal ions in *Sedum alfredii Hance* into metallic state in the
100 solid fraction was probably associated with the reducing intermediates from
101 degradation of lignocellulosic biomass (Chen et al., 2019). Besides, most of HTL
102 experiments were conducted in a relatively wide range of temperature and pressure
103 approaching the supercritical HTL region (Chen et al., 2019; Yang et al., 2010). Due
104 to the increasing application of phytoremediation of heavy metals contaminated soils,

105 a mild subcritical HTL condition is desired to treat a large amount of
106 hyperaccumulator harvests with lower energy consumption in terms of economic
107 feasibility and resource recovery. More importantly, there are very limited references
108 in the open literature to investigate the transformation of heavy metals and their risk
109 assessments during HTL of hyperaccumulators although HTL of other bio wastes (e.g.,
110 sewage sludge and manures) has been widely reported.

111

112 Consequently, it is imperative to elucidate transformation behaviors of heavy metals
113 and their risk assessments during resource recovery from hyperaccumulators using the
114 emerging HTL technology. In this study, *Sedum plumbizincicola* (*S. plumbizincicola*)
115 was selected as a typical Zn hyperaccumulator biomass for subcritical HTL. Various
116 mild HTL conditions have been designed to investigate effects of temperature and
117 reaction time on yields of major products and distribution of heavy metals. More
118 importantly, we will elucidate the role of temperature and reaction time in heavy
119 metals transformation and speciation. Furthermore, environmental risks of heavy
120 metals retained in solid residues (i.e., hydrochars) will be comprehensively evaluated
121 through the modified European Community Bureau of Reference (BCR) sequential
122 extraction method and Toxicity Characteristic Leaching Procedure (TCLP).
123 Ultimately, these fundamental findings would shed light on resource recovery and
124 environmentally friendly reutilization of hyperaccumulators using subcritical HTL.

125 **2. Materials and methods**

126 *2.1 Materials*

127 Heavy metals hyperaccumulator *S. plumbizincicola* was collected from a Zn
128 contaminated site in Guangdong Province, China. *S. plumbizincicola* was washed to
129 remove impurities and dried at 85 °C, which was subsequently ground into fine
130 powders less than 0.5 mm and kept in a desiccator before further use. Table 1
131 summarizes the physicochemical properties of *S. plumbizincicola*. Specifically, the
132 concentration of major metals of Zn, Fe, and Cd in the *S. plumbizincicola* was
133 35165.50, 7814.71, and 1222.63 mg/kg, respectively. Particularly, approximately 3.52%
134 of Zn content in this *S. plumbizincicola* was significantly higher than 0.3% for
135 common hyperaccumulators (van der Ent et al., 2013). Other metal and metalloid
136 trace elements were Pb, Mn, Cr, and As with concentration of 371.54, 313.63, 134.19,
137 and 13.87 mg/kg, respectively.

138 *2.2 Experiments*

139 HTL of *S. plumbizincicola* was carried out in a 250 mL autoclave (material: Hastelloy
140 C276, model: HT-250JOC, HTLAB, Shanghai HuoTong Experimental Instrument Co.,
141 Ltd.). Typically, 4 g of *S. plumbizincicola* powders and 80 mL of deionized (DI) water
142 were introduced into a reactor vessel. The vessel was then sealed and purged with
143 nitrogen (N₂) to remove residual air and pressurized to 10 bar. Afterwards, the reactor
144 was heated to a pre-set temperature (i.e., 240, 270, 300, 330 and 360 °C). After being
145 maintained for different reaction times (i.e., 30 to 150 min), the reactor was quenched

146 to room temperature in an ice-water bath.

147

148 The schematic procedure for separation of HTL products is demonstrated in Fig. S1.

149 After cooling down, gaseous product was released and the suspended mixture was

150 filtered through a pre-weighed 0.45 μm PTFE membrane to obtain water soluble and

151 water-insoluble fractions. The water-insoluble fraction was further extracted using

152 acetone to separate acetone soluble organic phase and acetone-insoluble solid fraction.

153 Acetone-soluble fraction was evaporated under reduced pressure at 35 $^{\circ}\text{C}$ to remove

154 acetone thoroughly and obtain the heavy oil (HO), while the solid fraction was oven

155 dried at 105 $^{\circ}\text{C}$ to collect the solid residue which was designated as hydrochar.

156

157 Yields of HO, hydrochar and others were calculated on a dry basis using Eqs. (1), (2)

158 and (3), respectively. All product yields were described as mean value with standard

159 deviation.

$$\text{HO yield} = \frac{\text{dry weight of HO}}{\text{dry weight of raw biomass}} \times 100\% \quad (1)$$

$$\text{Hydrochar yield} = \frac{\text{dry weight of hydrochar}}{\text{dry weight of raw biomass}} \times 100\% \quad (2)$$

$$\text{Others} = 100\% - \text{HO yield} - \text{Hydrochar yield} \quad (3)$$

160 *2.3 Analytical and evaluation methodologies*

161 Elemental compositions of raw hyperaccumulator biomass, HO and hydrochars were

162 analyzed using CHNS analyzer (Elementar vario EL, Germany). H/C and O/C atomic

163 ratios were calculated based on elemental analysis results and visualized using Van
164 Krevelen diagram. Higher heating values (HHVs) of HO and hydrochars were
165 calculated using the following Dulong formula in Eq. (4) (Xu & Lad, 2008).

$$\text{HHV (MJ/kg)} = 0.3383\text{C} + 1.422(\text{H} - \text{O}/8) \quad (4)$$

166 where C, H, and O represent weight percentages of carbon, hydrogen, and oxygen in
167 HO or hydrochars, respectively.

168

169 Surface functional groups of HO and hydrochars were determined using Fourier
170 transform infrared (FTIR) spectroscopy (Nicolet iS10, Thermo Fisher Scientific,
171 USA). Main organic compounds of HO were analyzed using GC-MS (Agilent
172 Technologies, USA) equipped with a chromatographic column (HP-5MS,
173 30 m × 0.25 mm × 0.25 μm) (refer to Table S1). Concentrations of heavy metals in
174 resulting aqueous phase were determined using an inductively coupled plasma optical
175 emission spectrometer (ICP-OES 5300DV, Perkin Elmer, USA). Prior to ICP-OES
176 analysis, raw biomass and hydrochars were digested in aqua regia under microwave at
177 200 °C for 30 min, which were subsequently diluted to 25 mL using volumetric flask.
178 The modified BCR sequential extraction procedure was employed to examine the
179 speciation of metal elements (i.e., Cd, Cr, Pb, Zn, Fe, Mn, and As) in hydrochars.
180 More specifically, acid soluble/exchangeable (exchangeable and carbonate bound
181 metal) fraction F1 was extracted in step 1; reducible (Fe/Mn oxides) fraction F2 was

182 determined after step 2; oxidizable fraction F3 bound to organic matter was measured
183 in step 3; and residual fraction F4 was obtained in step 4 (Leng et al., 2018). The
184 detailed procedure was described elsewhere (Qian et al., 2018).

185

186 Furthermore, risk assessment code (RAC) (Nemati et al., 2011) and TCLP (Qian et al.,
187 2018) methodologies were applied to quantitatively estimate the potential
188 environmental risk of individual metal element in raw biomass and hydrochars. RAC
189 is determined to classify the environmental risk level of single heavy metal element
190 through percentage of exchangeable and carbonate fractions (F1) in its total content
191 (Du et al., 2019). Specifically, risk level varies from different proportion ranges of F1
192 (RAC value), i.e., no risk for RAC lower than 1%, low risk for RAC range of 1-10%,
193 moderate risk for RAC range of 11-30%, high risk for RAC range of 31-50% and very
194 high risk for RAC higher than 50% (Huang et al., 2011). TCLP is a standard toxicity
195 leaching method recommended by the United States Environmental Protection
196 Agency to detect the dissolution and migration of heavy metal elements in solid media
197 or wastes (Nair et al., 2008). Leaching rate was then calculated to indicate the
198 leachable fraction of each individual heavy metal element in raw biomass and
199 hydrochars using Eq. (5) (Huang et al., 2011).

$$\text{Leaching rate} = \frac{\text{LC}}{\text{TC}} \times 100\% \quad (5)$$

200 where LC and TC represent the leaching content and total content of individual heavy

201 metal in solid samples, respectively.

202 **3. Results and discussion**

203 *3.1 Evolution of products yields during HTL*

204 Fig. 1 depicts effects of temperature and reaction time on variations of product yields
205 during HTL. Reaction temperature is a critical factor to promote HTL of *S.*
206 *plumbizincicola* because major biomass components (i.e., lignin, cellulose and
207 hemicellulose) tend to be decomposed into small molecules at elevated reaction
208 temperature (Dimitriadis & Bezergianni, 2017; Yang et al., 2010). Since all HTL
209 experiments were conducted under subcritical water condition, the HO yield increased
210 gradually with increased temperature, which was nearly doubled from 8.7% to 16.1%
211 when temperature was increased from 240 to 360 °C. Similarly, Xu and Lancaster
212 (2008) and Xu et al. (2018) have previously reported the highest bio-oil yield (ca.
213 24%) from HTL of sewage sludge at 350 °C. In fact, HO would be further cracked
214 into gases and water soluble phase with increased temperature under
215 near/super-critical water region, leading to a decreasing HO yield (Xu & Lad, 2008).
216 In contrast, the hydrochar yield demonstrated a drastically decreasing trend from 44.0%
217 to 19.3% as the temperature was increased to 360 °C due to the gradual degradation of
218 organic compounds. Significant decomposition of cellulose and hemicellulose
219 resulted in a distinct reduction of hydrochar yield during HTL of *Sedum alfredii*
220 Hance at 350 °C (Chen et al., 2019). Meanwhile, the pH value first decreased and then
221 increased to 8.0 at 360 °C with all the aqueous solutions under a basic condition,

222 which was favorable for heavy metals stabilization in hydrochars. Nevertheless, it is
223 worth noting that the optimum HTL temperature is primarily associated with the
224 intrinsic nature of biomass feedstock, reaction solvent, catalysts, and other reaction
225 parameters (Dimitriadis & Bezergianni, 2017).

226

227 HO yield achieved a maximum value of 12.4% after 90 min (Fig. 1). It suggests that
228 short reaction time may enhance HO yield during subcritical HTL (Karagöz et al.,
229 2004; Qu et al., 2003) and macromolecular intermediates might be degraded in
230 subsequent reactions via recombination and secondary cracking (Brand et al., 2014),
231 resulting in a dramatic decrease of HO yield with prolonged residence time (Yin et al.,
232 2010). In this study, a longer reaction time favored the decomposition of hydrochar
233 and led to a slightly increased yield of HO and other products (e.g., organic acids
234 (Qian et al., 2018) and gases), which was in agreement with a constantly decreasing
235 pH with the prolonged reaction time. Due to a relatively high ash content of 26.7% in
236 *S. plumbizincicola*, this HO yield was not comparable with that from HTL of
237 low-lipid microalgae, lignite, wheat straw and plastic wastes (Hu et al., 2019a; Wang
238 et al., 2014; Yu et al., 2014). Enhanced HO yield is anticipated through manipulated
239 reaction conditions, such as addition of catalysts (Muppaneni et al., 2017), reductive
240 reaction atmosphere (Yin et al., 2010), or co-liquefaction with other biomass
241 feedstocks (Xu et al., 2019). Nonetheless, HO upgrading is necessary for downstream
242 industrial applications. Further cleaning and activation of hydrochars is essential to

243 fabricate functional materials. Besides, separation and re-utilization of mixed gases
244 (e.g., CO₂, CO and H₂) as carbon source as well as energy carriers will be of crucial
245 importance towards circular economy.

246 *3.2 Characterization of HTL products*

247 *3.2.1 Elemental analysis and HHV*

248 As a result of severe biomass decomposition from 240 to 360 °C, C and H contents in
249 hydrochars decreased notably from 37.37% and 4.81% to 19.61% and 2.11%,
250 respectively, but O content increased steadily from 29.27% to 50.21% (Table 2).
251 Despite a sudden increase to 1.11% at 270 °C, N content decreased to 0.40% at 360 °C,
252 implying possible polymerization within this carbonization region. Consequently,
253 HHV of hydrochars decreased remarkably from 14.28 MJ/kg at 240 °C to 0.71 MJ/kg
254 at 360 °C. On the whole, prolonged reaction time at 240 °C facilitated the hydrolysis
255 and degradation of cellulose and hemicellulose, resulting in decreasing C and H
256 contents but increasing O content in hydrochars. However, N content was
257 accumulated to 1.17% after 150 min. This was probably ascribed to formation of
258 N-containing compounds via reaction of sugars with amino acids intermediates during
259 hydrothermal carbonization (He et al., 2019a). Thus, N might be retained in
260 hydrochars via solid-solid conversion and polymerization (He et al., 2019a).
261 Ultimately, HHV of hydrochars slightly decreased to 13.07 MJ/kg after 150 min under
262 240 °C.

263

264 After HTL at 240 °C, C and H content in HO was 77.23% and 9.94%, respectively,
265 whereas relatively low N and O content was 1.81% and 11.02%, respectively. This
266 HO exhibited the highest HHV of 38.30 MJ/kg, which was similar to 37.80 MJ/kg for
267 biocrude oil from HTL of swine manure under 370 °C for 30 min (Lu et al., 2018).
268 Despite an increased C content up to 78.57% at 360 °C, O and H content decreased
269 distinctly from 12.80% and 8.72% to 10.28% and 8.46%, respectively, indicating
270 improved dehydration and decarboxylation from 270 to 360 °C. Interestingly, N
271 content (2.69-3.08%) in HO under severe temperature (> 270 °C) was much higher
272 than 1.81% in HO at 240 °C due to significant polymerization. HHV of these HO
273 samples fluctuated around 36 MJ/kg. With extended reaction time at 240 °C, C and H
274 contents in HO decreased but N and O contents slightly increased, leading to a
275 gradually reduced HHV to 36.78 MJ/kg after 150 min. Hence, HO could be recycled
276 as biofuels with better fuel quality via HTL of pristine *S. plumbizincicola*, yet
277 hydrodeoxygenation and denitrification are necessary because of higher contents of
278 nitrogen and oxygen as compared to only 0.3% of N and 1.0% of O in conventional
279 crude oil (Lu et al., 2018).

280

281 Evolution of H/C and O/C atomic ratios (Fig. 2) indicated that primary reactions
282 during liquefaction process were dehydration, decarboxylation, and demethanation
283 (He et al., 2013). Moreover, dehydration and demethanation reactions favored
284 hydrochars formation, while decarboxylation and dehydration processes were mainly

285 responsible for HO generation in HTL of *S. plumbizincicola*. Specifically, O/C atomic
286 ratio for hydrochars increased obviously from 0.59 to 1.92 from 240 to 360 °C but
287 H/C atomic ratio was almost a constant of 1.2 after a sharp reduction at 270 °C. It is
288 speculated that demethanation was more severe than dehydration and decarboxylation
289 along with hydrochars formation in high temperature region, retaining high O content
290 therein. As a result of typical carbonization via dehydration and decarboxylation
291 reactions, prolonged reaction time at 240 °C led to a gradually decreasing H/C atomic
292 ratio in hydrochars from 1.55 to 1.36 after 150 min but O/C atomic ratio (ca. 0.60)
293 presented negligible variation. As for HO, higher temperature (> 270 °C) could cause
294 decreasing H/C and O/C atomic ratios from 1.39 and 0.13 at 270 °C to 1.29 and 0.10
295 at 360 °C, respectively, implying the occurrence of enhanced decarboxylation and
296 dehydration. In addition, extended reaction time at 240 °C had more obvious impact
297 on H/C atomic ratio than that on O/C atomic ratio regarding HO formation. The
298 notable reduction of H/C atomic ratio may be ascribed to demethanation and
299 dehydration in prolonged carbonization. Overall, a mild temperature HTL was
300 recommended to recover HO and hydrochars in terms of higher energy density,
301 improved carbon sequestration and less energy consumption.

302 *3.2.2 Variations of functional groups in hydrochars and HO*

303 According to FTIR spectra in Figs. S2a and S2b and previous identification peaks
304 (Özçimen & Ersoy-Meriçboyu, 2010), the band at 3400 cm⁻¹ was attributed to –OH
305 stretching vibration in hydroxyl or carboxyl groups. Comparable intensity of this peak

306 for hydrochars and raw biomass indicated largely retained hydroxyl group in
307 hydrochars. Intensities of peaks at 2918 cm^{-1} and 2850 cm^{-1} , associated with aliphatic
308 carbon $-\text{CH}_x$ stretching vibration in hydroxyl or carboxyl groups, became more
309 intense with elevated reaction temperature and extended reaction time. Remarkable
310 reduction of the relative intensity of hydrochars was observed at 1645 cm^{-1} with
311 higher reaction temperature. The band at 1645 cm^{-1} could be ascribed to the stretching
312 vibration of $-\text{C}=\text{O}$ in ketone and amide groups. This reduction was probably related to
313 decarboxylation reaction during HTL of *S. plumbizincicola*. The peak at 1423 cm^{-1}
314 was assigned to the $-\text{C}=\text{C}$ stretching in aromatic carbon rings, which presented a
315 remarkable increase after HTL. The distinct increase of relative intensity at 1034 cm^{-1}
316 may be attributed to $-\text{C}-\text{O}-\text{R}$ in aliphatic ethers and alcohol $-\text{C}-\text{O}$ stretching, or
317 $-\text{Si}-\text{O}$ stretching from SiO_2 (He et al., 2013; Yuan et al., 2011a). The increased peaks
318 of $650\text{-}900\text{ cm}^{-1}$ were attributed to the out-of-plane bending vibration of C-H
319 structure from aromatics (Zhou et al., 2010), suggesting that high aromaticity after
320 HTL of *S. plumbizincicola*.

321

322 In Figs. S2c and S2d, broad band of $-\text{OH}$ or $\text{N}-\text{H}$ stretching vibration between 3200
323 and 3700 cm^{-1} confirmed the presence of alcohols and phenolic groups in HO. C-H
324 stretching vibrations at 2920 and 2850 cm^{-1} and C-H deformation at 1454 cm^{-1} were
325 ascribed to $-\text{CH}_3$ and $-\text{CH}_2$ groups in alkenes and alkyl aromatic compounds. C=O
326 vibration at 1683 cm^{-1} was attributed to the existence of carboxylic acids, aldehydes,

327 or ketones. Aromatic skeletal vibrations at 1604 and 1513 cm^{-1} as well as C-H
328 bending vibrations between 900 and 650 cm^{-1} were relevant to aromatics and their
329 derivatives. C-H bending vibrations at 1454 and 1375 cm^{-1} , together with C-O
330 bending vibration at 1270 cm^{-1} , could originate from fats and esters. Therefore, the
331 HO mainly consisted of phenolic derivatives, ketones, aldehydes, carboxylic acids and
332 alcohols. In fact, chemical composition of HO from *S. plumbizincicola* was extremely
333 complicated and detailed organic compounds in HO were determined by GC-MS as
334 summarized in Table S1. The majority of components were macromolecular organics
335 ($> \text{C}_{12}$), including esters, carboxylic acid and alcohols. Almost no light components
336 were found in HO, which may be present in the water soluble phase due to their
337 hydrophilic properties.

338 *3.3 Migration and distribution of heavy metals during HTL*

339 Fig. 3 illustrates effects of hydrothermal temperature and reaction time on the
340 redistribution of seven major metal elements (i.e., As, Mn, Fe, Cd, Cr, Pb and Zn).
341 Evolutions of heavy metal concentrations in raw biomass and hydrochars are further
342 depicted in Fig. 4. Generally, metal elements were mainly distributed into hydrochars
343 and water soluble phase, whereas HO only contained less than 10% of each element.
344 Especially, more than 95% of As was transformed into water soluble phase regardless
345 of the temperature and reaction time. However, more than 75% of As was transferred
346 into solid residues after HTL of swine manure in the range of 220 to 370 $^{\circ}\text{C}$ (Lu et al.,
347 2018). Albeit a higher As concentration than 13.87 mg/kg in raw biomass, all

348 hydrochars contained the lowest As concentration as compared with other six
349 elements, with its peak value of 113.31 mg/kg at 300 °C.

350 *3.3.1 Effect of reaction temperature*

351 Increasing temperature (< 300 °C) facilitated transformation of metal elements except
352 As from water soluble phase into hydrochars with less than 5% of variation in HO
353 (Fig. 3a). At 270 °C, Cr and Pb in hydrochars achieved 91.4% and 93.8%, respectively.
354 However, heavy metals except As were redistributed into water soluble phase and HO
355 when temperature was elevated from 300 to 360 °C. This may be associated with
356 accelerated release of heavy metals bonding to organic components in hydrochars
357 resulting from its intensive degradation under severe temperature (Shi et al., 2013a;
358 Yuan et al., 2011b). More than 60% of Mn, Fe, Cd, Cr, Pb and Zn was retained in
359 hydrochars with about 30% in water soluble phase and 5-7% in Ho at 360 °C. Thus,
360 higher temperature HTL (> 330 °C) may not benefit the removal of heavy metals from
361 HO as well as its recovery from water soluble phase.

362

363 When temperature was elevated from 240 to 360 °C, the total concentration of Cd, Pb,
364 Fe and Mn in hydrochars gradually increased from 1586.88, 490.00, 7753.75 and
365 213.75 mg/kg to 3228.13, 927.50, 14531.25 and 593.13 mg/kg, respectively (Fig. 4a).
366 It might be related to the reduction of hydrochar yield at higher temperature. Although
367 concentration of Cr, Zn and As slightly decreased when temperature was above 300
368 °C, their corresponding concentration was as high as 194.38, 103168.75 and 62.55

369 mg/kg at 360 °C, respectively. The decreasing As concentration after 300 °C was
370 probably ascribed to intensive As dissolution and facilitated hydrolysis or
371 decomposition of organic macromolecules under higher temperature HTL (Leng et al.,
372 2014; Shi et al., 2013a; Shi et al., 2013b). Nevertheless, Cr, Zn and As in hydrochars
373 after HTL at 360 °C was 1.45, 2.93 and 4.51 times of that in raw biomass, respectively,
374 suggesting that highly accumulated heavy metals in hydrochars could be favorable for
375 metals recovery.

376 *3.3.2 Effect of reaction time*

377 Unlike As, subcritical HTL at 240 °C promoted the accumulation of Mn, Fe, Cd, Cr,
378 Pb and Zn in hydrochars with prolonged reaction time (Fig. 3b). Especially, Pb and Zn
379 were distinctly redistributed from water soluble phase into hydrochars (>90%) with
380 only 3% in HO after 90 min. Mn showed the most remarkable increase from 51.3% to
381 82.1% within 150 min, but Fe, Cd and Cr in hydrochars only slightly increased with
382 time. Under subcritical hydrothermal condition, intermolecular and intramolecular
383 hydrogen bond fracture was likely to occur in these materials bound to metals.
384 Although heavy metals bound with biomass may be altered to ionic state and migrate
385 into the aqueous phase, they could then react with reducing organic intermediates
386 therein via redox reactions, thereby resulting in further precipitation in the form of
387 metal oxide or metal-complexes in hydrochars (Qian et al., 2018). Nevertheless, the
388 unique redistribution of As in water soluble form (> 96%) was probably associated
389 with its speciation in arsenate (Wang et al., 2015) which can be readily degraded into

390 aqueous phase through hydrolysis and demethylation during subcritical HTL in this
391 study. Since *S. plumbizincicola* is not arsenic hyperaccumulator with a low As content
392 (13.87 mg/kg), As released into water soluble form could be limited.

393

394 As concentration in hydrochars decreased obviously from 44.49 to 5.63 mg/kg by
395 increasing reaction time from 30 to 150 min (Fig. 4b). Conversely, the concentration
396 of other six metal elements (i.e., Cd, Cr, Pb, Zn, Fe and Mn) increased to 2118.13,
397 135.0, 645.63, 73425, 10021.25 and 418.75 mg/kg, respectively, after 150 min of
398 HTL at 240 °C. Zn concentration in hydrochars was accumulated by almost 2.1-fold
399 but As concentration was reduced by 59.4% as compared with that in raw *S.*
400 *plumbizincicola*.

401 *3.4 Fractionation of heavy metals during HTL*

402 Bioavailability and eco-toxicity of heavy metals in the environment mainly depend on
403 their chemical speciation (Rauret et al., 1999). The chemical speciation of heavy
404 metals can be determined through selective sequential extraction analysis. In this
405 study, the modified BCR sequential extraction was used to assess the risk of heavy
406 metals released from hydrochars for its sound disposal and re-utilization. Figs. 5 and 6
407 have comprehensively depicted chemical speciation of seven elements in hydrochars
408 after HTL. In raw *S. plumbizincicola*, bioavailable fractions (F1+F2) accounted for
409 nearly 99.0%, 97.7% and 99.0% in Cd, Zn and Fe, respectively, but the stable residual
410 fraction F4 was predominant in Cr (68.9%) and Mn (70.1%). F3 bound to organic

411 matter was the most abundant speciation in Pb (64.3%) and As (44.0%). Moreover, As
412 exhibited comparable F1 (23.5%) and F4 (29.6%), but F2 was the other major fraction
413 of Pb in raw biomass.

414 *3.4.1 Effect of reaction temperature*

415 Varied reaction temperature induced dramatic fractionation variations of heavy metals
416 during HTL (Fig. 5). These elements demonstrated distinctly different transformation
417 behaviors. With the increasing temperature from 240 to 360 °C, the predominant
418 bioavailable fractions (F1+F2) of Cd in hydrochars decreased significantly to 7.7%
419 with F3 being the majority fraction, whereas that percentage (F1+F2) was 76.1% in
420 Zn although the decreasing F1 was partially transformed into F2 and F3. Below 270
421 °C, F1 and F2 in Fe were obviously transformed into F3 (34.4%) and F4 (50.1%),
422 however, F2 and F3 became the dominant speciation because gradually decreasing F4
423 was substantially evolved into F2 under near-critical water condition (300-360 °C),
424 i.e., from 50.1% at 270 °C to 21.3% at 360 °C. Increased temperature facilitated the
425 transformation of F2 and F3 into F4 (76.7-82.9%) of Cr with less than 20% of F3. On
426 the contrary, F4 of Mn was remarkably transformed into bioavailable fractions with
427 the highest (F1+F2) of 97.4% at 240 °C. Under higher temperature above 300 °C, the
428 F1+F2 fraction then decreased to 71.7% but F3 was apparently increased to 26.2% at
429 360 °C. Despite the predominant F3 of Pb and As in raw biomass, reaction
430 temperature caused quite different effects on chemical speciation. Specifically, F3 of
431 Pb was dramatically transformed into F2 (72.8-76.9%), whereas F1 and F4 of As were

432 mainly converted into the highest F3 (89.0%) in hydrochars at 330 °C. Due to unique
433 existence of heavy metals bound with cellulose and hemicellulose in *S.*
434 *plumbizincicola*, above findings were quite different from those reported from HTL of
435 sewage sludge and mixture of sludge and lignocellulosic biomass (Huang & Yuan,
436 2016; Leng et al., 2018; Zhai et al., 2016). Alkaline was suggested to stabilize heavy
437 metals in HTL of sewage sludge, especially at pH of 11 (Zhai et al., 2016), however,
438 increased basic conditions (pH < 8) at high temperature did not demonstrate obvious
439 influence on fractionation of heavy metals, indicating more severe alkaline may be
440 desired to verify that.

441 *3.4.2 Effect of reaction time*

442 The prolonged reaction time generally presented similar impact on the fractionation of
443 heavy metals with that caused by increased temperature during HTL (Fig. 6).
444 Apparently, longer reaction time could promote a high conversion of F1 and F2 to
445 about 84.0% of F3 in Cd with merely 0.5% of F4 and 15.5% of bioavailable fractions
446 (F1+F2) after 150 min. Although F1 of Zn was partially converted into F2 and F3
447 with increased time, total F1+F2 percentage was still high up to 85% in hydrochars
448 with the remaining F3, indicating higher mobility of Zn and its potential
449 environmental risk. In comparison, F1 and F2 percentage of Fe did not change too
450 much (around 21%) with increased time during HTL, while the main chemical
451 speciation F3 increased but F4 decreased with the prolonged time. Despite high F4
452 percentage of Cr and Mn in raw biomass, their speciation during HTL exhibited

453 obviously different transformation behaviors with almost 80% of F4 for Cr and more
454 than 90% of F1+F2 for Mn regardless of the reaction time. Thus, Cr tended to be
455 immobilized and stable but Mn may be more labile in hydrochars after HTL. With
456 respect to Pb and As with high F3 in *S. plumbizincicola*, F3 was still the predominant
457 speciation of As and became nearly a constant of ca. 87% with only 11.9-12.8% of F1
458 when the reaction time was extended to 60 min. F2 of Pb exhibited distinct evolution
459 into F1 and F4 during HTL at 240 °C within 60 min, however, a longer time than 60
460 min could trigger intense F3 transformation to F2, which may render a higher
461 bioavailable risk of Pb. Hence, prolonging HTL time may benefit the immobilization
462 of Cd, Cr, Fe and As in hydrochars, but a short HTL reaction was desirable for Pb.
463 Unfortunately, HTL transformed F4 of Mn into large percentage of F1 and F2.

464 *3.5 Risk assessment of heavy metals in hydrochars*

465 In pristine *S. plumbizincicola*, RAC of Cd, Cr, Pb, Zn, Mn and As was 41.5%, 0, 3.4%,
466 78.2%, 7.9%, 23.5%, respectively. This implied that these metal elements may pose
467 different risk levels, e.g., very high risk for Zn, high risk for Cd, moderate risk for As,
468 low risk for Pb and no risk for Cr and Mn in raw biomass. Except Mn and Pb, HTL
469 could dramatically reduce the environmental risk level of Cd, Cr, Zn and As
470 regardless of the reaction temperature and time (Fig. 7). Since the chemical speciation
471 of Cr in both *S. plumbizincicola* and hydrochars was in F2, F3 and F4 fraction, RAC
472 value for Cr was zero, indicating no possible risk of Cr in all hydrochars.

473

474 According to Fig. 7, either increased temperature or prolonged time could lead to
475 distinct reduction of risk level of Cd from high risk to low risk with RAC value below
476 7%. Although HTL triggered an increase in RAC for Pb, especially high up to 16.9%
477 at 240 °C for 30 min, its risk was still within the low risk range and higher
478 temperature or longer reaction time could further reduce its RAC to 4.8-6.6%
479 (270-360 °C for 30 min) and 4.6-7.6% (60-150 min under 240 °C), respectively.

480

481 RAC of Zn and Mn in hydrochars declined under higher temperature, which was
482 lower than 40% when temperature was above 330 °C. Besides, 61.4% of RAC for Zn
483 at 240 °C was gradually reduced to 47.3% after 150 min. Unfortunately, RAC of Zn in
484 all hydrochars implied its potential high risk level after HTL. HTL activated potential
485 risk of Mn in hydrochars to high level or even very high level (at 240 °C) although
486 Mn in raw biomass may pose no risk. In fact, higher temperature at 330 °C might
487 cause a certain decrease of RAC to 30.5% which was approaching the moderate risk
488 range. Alternatively, despite high RAC of 62.9% for Mn in hydrochars from HTL at
489 240 °C for 30 min, a low RAC of 4.7% was achieved when the reaction was extended
490 to 150 min. Thus, a long reaction time is recommended for a lower environmental risk
491 of Mn in hydrochars during low temperature HTL. Regarding As in hydrochars, RAC
492 of As in raw biomass declined with either increased temperature or prolonged reaction
493 time. Interestingly, high temperature HTL at 360 °C induced an increase of RAC to
494 18%, while RAC of As was less sensitive to reaction time. Hence, desirable HTL

495 condition of 330 °C and 120 min may be beneficial to realize a low risk level of As in
496 hydrochars.

497

498 Furthermore, Table 3 presents leachable metal concentrations of raw biomass and
499 hydrochars using TCLP extraction method. As aforementioned, the chemical
500 speciation of Cr was mainly in more stable F3 and F4 fraction, thus the leachable Cr
501 concentration was below the detection limit for all samples. However, leachable
502 concentrations of Zn and Mn in hydrochars were notably higher than those in raw
503 biomass, while the concentration of other metal elements (e.g., Cd, Pb, Fe and As) in
504 leachate was much lower after HTL, excluding high As leaching concentration for
505 hydrochars from HTL above 330 °C. Other than extremely higher Zn leaching
506 concentration, leaching concentration of all the other metal elements in hydrochars
507 was basically below its corresponding international standard limit recommended by
508 USEPA. Consequently, Zn may exhibit a high leaching toxicity risk but other heavy
509 metals could be considered safe after leaching. More specifically, leaching
510 concentration of Cd in hydrochars decreased dramatically with the increased
511 temperature, whereas the concentration of Fe and As increased in higher temperature
512 region. Interestingly, leaching concentration of Pb, Zn and Mn from hydrochars
513 demonstrated a peak value after HTL under 240 to 360 °C, which was 4.82, 45780.32,
514 224.41 mg/L, respectively. On the other hand, during HTL at 240 °C from 30 to 150
515 min, leaching concentration of Pb, Zn, Fe and As increased but the opposite trend was

516 found for Cd and Mn. Nevertheless, taking into account the leaching toxicity, it is
517 suggested that HTL at 300 °C should be avoided and HTL at 240 °C for 30 min was
518 sufficient to meet the standard limit for most of the heavy metals in hydrochars,
519 except Zn with a remarkably high content in raw biomass.

520

521 Apart from the leaching toxicity, leaching rates of heavy metals in hydrochars from
522 HTL under different conditions are also summarized in Fig. 8. Although these results
523 suggested some consistency with aforementioned leaching concentrations, they could
524 help to clearly quantify the potential leachability of individual metal element in
525 materials. Overall, leaching rate was less sensitive to temperature and reaction time
526 for Cd, Pb and Fe in hydrochars. In particular, leaching rate of Cd, Pb, Fe and As was
527 substantially reduced after HTL but it did not fluctuate intensively with increased
528 temperature. Due to an extremely high Zn content in raw biomass, its leaching rate in
529 hydrochars was still high up to 33.6% even at 360 °C. Surprisingly, HTL facilitated
530 the leaching rate of Mn which decreased with increased temperature. High
531 temperature and long reaction time may inhibit the leaching rate of Mn but played a
532 detrimental role in leaching rate of As. In fact, HTL under relatively low temperature
533 around 270 °C with moderate reaction time of 60 min was preferable to reduce As
534 leaching from hydrochars. Low temperature (240 °C) and short time (30 min) HTL
535 favored the prevention of Zn leaching from hydrochars. It is worth noting that Pb
536 exhibited a very low leaching rate less than 0.45% regardless of temperature and time,

537 which was somehow inconsistent with its increased F1 and F2 fractions in hydrochars
538 as discussed in Section 3.4. This may indicate that mobile and easily bioavailable
539 metal fractions may not necessarily result in a higher leaching rate for some elements.

540 **4. Conclusions**

541 This study has investigated transformation behaviors and environmental risk of heavy
542 metals during subcritical HTL of *S. plumbizincicola*. During HTL at 240 °C, a
543 maximum HO yield of 12.4% was obtained after 90 min and the highest HHV of
544 38.30 MJ/kg for HO was found within 30 min. Overall, low temperature HTL favored
545 the recovery of HO and hydrochars and heavy metal elements were mainly distributed
546 into hydrochars and water soluble phase. Increasing temperature facilitated
547 transformation of F2 and F3 into F4 (76.7-82.9%) for Cr, while F4 in Mn was
548 transformed into higher percentage of F1+F2 (e.g., 97.4% at 240 °C) for various
549 temperature and time. F1 and F4 in As was largely converted to 89.0% of F3 at 330 °C.
550 The prolonged liquefaction time facilitated immobilization of Cd, Cr and As in
551 hydrochars, but fast liquefaction favored Pb stabilization. Specifically, long reaction
552 time promoted conversion of F1 and F2 to 84.0% of F3 in Cd after 150 min, but
553 F1+F2 percentage in Zn was almost 85% in hydrochars. Based on RAC evaluation, Cr
554 exhibited no potential risk in all hydrochars. The liquefaction significantly reduced
555 environmental risk level of Cd, Zn and As, but may mobilize Pb and Mn, especially
556 for Mn to very high risk level at 240 °C. Regarding leaching toxicity, HTL at 240 °C
557 for 30 min was sufficient to stabilize all heavy metals excluding Zn in hydrochars.

558 High temperature with long reaction time inhibited leaching rate of Mn, whereas low
559 liquefaction temperature with short reaction time prevented the leaching of Zn and As
560 from hydrochars.

561 **CRedit author statement**

562 Chao He: Conceptualization, Writing - Review & Editing, Supervision, Project
563 administration, Funding acquisition. Zhao Zhang: Investigation, Writing - Original
564 Draft. Candie Xie: Data Curation. Apostolos Giannis: Writing - Review & Editing.
565 Zhe Chen: Resources. Yetao Tang: Funding acquisition. Rongliang Qiu: Funding
566 acquisition.

567 **Declaration of Competing Interest**

568 The authors declare no competing interest.

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