Analysis of chemical characteristics of lignite upgrading wastewater and its agricultural utilization

Huifen Zhang, Ji Song, Mi Zhang, Cheng Xiang, Baocai Li and Yi Qin

ABSTRACT

The lignite upgrading wastewater (LUW) produced in the drying and upgrading process of lignite cannot be discharged directly. Conventional wastewater treatment methods are usually costly and unable to achieve efficient utilization of water resources which are rich in activity components. In this study, the water quality analysis showed that LUW belonged to seriously polluted waters with low pH and very high total nitrogen content. Fifty-five compounds, mainly phenols and organic acids, were identified by gas chromatography–mass spectrometry (GC-MS) analysis. The study confirmed that the LUW, after being diluted to an appropriate concentration, could significantly promote the growth of wheat seedlings. The phenols and organic acids were the activity material basis of LUW, which promoted seed germination possibly through playing a role similar to plant hormones and simultaneously enhancing the utilization of nutrient elements. LUW had the natural advantages of directly developing high-end liquid fertilizers in terms of its physical form, chemical composition, biological activity, safety and economy. This study confirmed the feasibility of applying LUW to agricultural field as liquid fertilizer only through simple dilution without other treatments. Applying LUW as liquid fertilizer can not only supply a fertilizer product with low production cost and outstanding efficacy, but also provide an efficient and green way for the treatment of upgrading wastewater, which utilize the LUW as natural resources instead of purifying and discharging.

Key words | agricultural utilization, chemical characteristics, lignite upgrading, GC-MS, wastewater

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HIGHLIGHTS

- The results of chemical characteristic analysis showed that the lignite upgrading wastewater (LUW) belonged to seriously polluted water which cannot be discharged directly, and meanwhile the activity of LUW in promoting wheat seed germination was confirmed for the first time.
- Conventional wastewater treatment methods are usually unable to achieve efficient utilization of water resources which are rich in activity components. Fortunately, this study found that LUW had the natural advantages of directly developing high-end liquid fertilizers in terms of its physical form, chemical composition, biological activity, safety and economy.
- This study confirmed the feasibility of applying LUW to agricultural field as liquid fertilizer only through simple dilution without other treatments, and provided an efficient and green way for treating lignite upgrading wastewater, which utilizes the LUW as natural resources instead of purifying and discharging.

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INTRODUCTION

As a kind of inferior coal resource, lignite can be better utilized only after its quality is improved, such as dehydrating (Li *et al.* 2019). The drying and upgrading technology could reduce the moisture content in lignite and increase the calorific value. In most upgrading processes, a large amount of water rich in volatile components is generated, which is condensed by water vapor from lignite (Mursito *et al.* 2010; Rao *et al.* 2015). Due to the presence of many organic compounds and a few inorganic components, the lignite upgrading wastewater (LUW) commonly cannot be discharged directly (Racovalis *et al.* 2002; Artanto *et al.* 2009). At present, the wastewater produced by lignite upgrading could be treated by physical-chemical or biological methods, and discharged after meeting the requirements of environmental protection or continually used as industrial water (Song *et al.* 2016; Li *et al.* 2018; Maiti *et al.* 2019). However, these wastewater treatment methods are usually complicated or costly. Considering the water resources and the useful ingredients contained in them, it is also not conducive to the efficient utilization of resources to discharge directly or use them as industrial water (Ren *et al.* 2007). Therefore, in the interest of resource use and healthy development of lignite industry, it is necessary to carry out basic research to provide theoretical support for exploring efficient utilization ways of LUW. The objective of this study is to assess the feasibility of applying LUW directly to agriculture as liquid fertilizer without additional treatment through the analysis of chemical characteristics and activity investigation.

Currently, the basic research on the application of LUW in agriculture is blank. In a previous study we obtained a distillate from the degradation solution in the process of oxidizing and degrading lignite to prepare fulvic acids, which was called fulvic acid distillate (FAD) or brown coal distillate (BCD) (Ma *et al.* 2014). FAD exhibited a strong activity in promoting wheat seed germination at a very low concentration and meanwhile could alleviate salt injury in the wheat germination stage, and had been developed into an excellent crop growth regulator for sale. The LUW is very similar to FAD in the source of raw materials, formation process and material form. Accordingly, we speculated that the LUW may also have similar plant physiological activities. In this study, we first systematically analyzed the physicochemical properties and chemical components of LUW, and then investigated its effects on wheat seed germination. At the same time, we compared the activity differences between LUW and FAD, and explored the related mechanism. The effects of LUW on wheat seed germination were studied for the first time, and it may be a more efficient and green way of wastewater treatment to directly utilize the LUW as natural resources instead of purifying and discharging.

MATERIALS AND METHODS

Materials

The LUW was derived from the lignite upgrading production line of Yunnan Shangcheng Biotechnology Co., Ltd., which used rotary drum drying technology to upgrade lignite. The wastewater is a reddish brown solution obtained by condensation of water vapor evaporated from the lignite. The LUW was diluted 50, 100 and 500 times for seed germination test, and the diluents were recorded as LUW50, LUW100 and LUW500, respectively. The FAD was also taken from Shangcheng Company. Its preparation method is as follows: the powdered lignite is first oxidized and degraded by hydrogen peroxide, and then the degradation solution is concentrated under vacuum; the condensate of water vapor is the FAD, and the concentrate continued to be dried to produce the crude fulvic acid. Other process details can be found in reference Zhu *et al.* (2014). The FAD was diluted 100 times for seed germination test, and the diluents were named FAD100 as the positive control. In another study, we investigated the effect of FAD at different dilution times on wheat seed germination; when diluted 100 times, FAD had the strongest activity of promoting germination (Ma *et al.* 2014). The selected wheat cultivar is Yannong 19, which was examined and approved by Crop Variety Approval Committee of Shandong Province, PR China (Approval Nos. 2001001). The identification of the genotype refers to these papers (Hayden *et al.* 2008; Gismondi & Canini 2013; Gismondi *et al.* 2014). There are two main reasons for choosing wheat as tested crop. On the one hand, as an analogue of LUW, FAD had been proved to promote the germination of wheat, which brought enlightenment to the selection of test objects (Ma *et al.* 2014). On the other hand, wheat is the main food crop of the country where the researchers of this paper belong, and the total amount of organic fertilizer required for its production is also large, which is conducive to the industrial utilization of LUW. Hoagland's nutrient solution was prepared according to Hoagland & Arnon (1950).

Water quality analysis

Conventional pollution indexes of LUW samples were analyzed, including pH, BOD_5, COD_{CR}, total phosphorus, total nitrogen, ammonium nitrogen and nitrate nitrogen. The experimental methods were carried out in accordance with the national or industrial standards of GB/T 6920-86, HJ 505-2009, HJ 828-2017, GB/T 11893-89, HJ 636-2012, HJ 535-2009 and HJ 84-2016 issued by the State Environmental Protection Administration of China. In addition, the concentration of trace elements in LUW was determined, including eight nutrient elements and five harmful elements (heavy metal elements). The elements, Ca, Mg, Fe, Mn, Zn, Cu, B, Cd and Pb, were analyzed by an Inductively Coupled Plasma Optical Emission Spectrometer (Leeman Prodigy). The elements, Mo, Hg, Cr and As, were detected by an Inductively Coupled Plasma Mass Spectrometer (PerkinElmer Elan DRC II).

GC-MS analysis

Sample pretreatment

In order to detect as many chemical components as possible, two sample pretreatment methods were implemented for gas chromatography–mass spectrometry (GC-MS) analysis. On the one hand, we used dichloromethane directly to extract the LUW, and the extract liquid was named Sample 1. On the other hand, a multiple extraction method was designed by adjusting the pH of LUW. The steps of the method were as follows: the pH of three LUW samples with same volume was adjusted to 2.0, 7.0 and 12.0, respectively; then each solution was extracted with dichloromethane; finally, three extracts were merged and recorded as Sample 2. Two samples were filtered with 0.45 μm microporous membrane (nylon) before GC-MS analysis.

Chromatographic conditions

GC-MS analysis was carried out on GC7890B/MS5977A instrument (Agilent, America), fitted with an HP-5 capillary column (30.0 m × 0.25 mm × 0.25 μm). The initial temperature of the column was maintained at 50 °C for 4 min; then rose to 100 °C at a rate of 10 °C/min and held for 1 min; then increased to 190 °C at 13 °C/min and maintained for 1 min; and finally rose to 300 °C at 22 °C/min and maintained for 6 min. The carrier gas was nitrogen with a flow rate of 3 mL/min. The injection volume of the sample was 1 μL. Injector temperature was set at 300 °C and splitless mode was used. Transmission line temperature was 250 °C. Ionization potential of mass selective detector was 70 eV. Ionization source temperature and quadrupole temperature were set at 230 °C and 150 °C, respectively. Scanning mode was full scanning and mass range was 50–550 amu. All isolated compounds were estimated using the NIST08 database.

Seed germination test

The plump wheat seeds were disinfected with 10% sodium hypochlorite for 15 min and then washed seven times with sterile distilled water. The seeds were immersed respectively in distilled water, LUW, LUW50, LUW100, LUW500 and FAD100, and sensitized for 2 h. According to the general settings of seed germination test, 150 seeds were randomly selected after being sensitized and used for control group or each experimental group, and a total of 900 seeds were used in this study. Each 150 sensitized seeds from the same batch were put into three petri dishes with 50 grains per dish. The bottom of the petri dishes was covered by a layer of filter paper, and 10 mL Hoagland's nutrient solution was added into each dish. The seeds were cultured in a constant temperature incubator at 25 °C and the average photoperiod was 14 h. The evaporative loss of water was obtained through the weight method and compensated by adding distilled water into petri dishes.

It was the standard of seed germination that the length of the seed radicle was more than the seed length by one half. The germination amount of seeds was observed every day. Germination rate is the ratio of the number of germinated seeds to the total of the tested seeds, and the germination rate of wheat seeds is generally calculated on the seventh day. Germination energy is the ratio of the total number of germinated seeds to the total number of tested seeds in the peak period of germination, and the peak period of wheat seeds in this experiment is the fourth day. After 4 d, the germination energy was calculated in germination fastigium according to the formula: germination energy = (germination amount in 4 d)/(total number of seeds). After 7 d, the germination rate was calculated using the following formula: germination rate = (germination amount in 7 d)/(total number of seeds); for each group, 30 seeds were randomly selected from the germinated seeds, of which the length, fresh weight and dry weight of roots and buds were measured, respectively.

RESULTS AND DISCUSSION

Water quality of LUW

The results of water quality analysis of LUW were shown in Table 1. The pH presented that the LUW was a weak acidic solution. COD_{CR} and BOD_5 values indicated that the contents of reducing pollutants and biodegradable organic matter in LUW were very high. The values of pH, COD_{CR}, BOD_5, ammonium nitrogen and Mn greatly exceeded the limit requirements of Integrated Wastewater Discharge Standard (GB 8978-1996) issued by China Environmental Protection Agency, demonstrating that LUW belonged to the seriously polluted water and cannot be discharged directly. Excessive total nitrogen content can easily cause water eutrophication, but it is conducive to the development of liquid fertilizers. The contents of the four trace elements Ca, Mg, Fe and Mn in LUW were relatively high, and they are nutrient elements beneficial for plant growth. Meanwhile, the concentrations of the five harmful elements Hg, Cd, Pb, Cr and As were extremely low. In addition, the following activity experiments also proved its activity of promoting seed germination. At present, there is no national or industrial standard of liquid fertilizer for seed soaking in China. With reference to the national standard of Foliar Fertilizer with Organic Matter (GB/T 17419-2018), LUW meets the requirements in terms of pH and harmful element limits, and the deficiencies in organic matter content and nutrient content can be solved by additional additions. From the perspective of basic research, LUW can be used directly as a functional liquid fertilizer. However, from the perspective of product development, due to the limitations of relevant standards, LUW can be chosen to be used as an additive or solvent for liquid fertilizers.

Table 1 | The results of water quality analysis of lignite upgrading wastewater

No.	Index	Value	Limit[a]
1	pH	3.43	6–9
2	COD_{Cr} (mg/L)	4,878.05	150
3	BOD_5 (mg/L)	2,128.78	30
4	Total phosphorus (mg/L)	0.36	1.0
5	Total nitrogen (mg/L)	1,623.52	–
6	Ammonium nitrogen (mg/L)	1,347.25	25
7	Nitrate nitrogen (mg/L)	103.24	–
8	Ca (mg/L)	188.00	–
9	Mg (mg/L)	65.30	–
10	Fe (mg/L)	717.00	–
11	Mn (mg/L)	24.30	2.0
12	Mo (μg/L)	2.21	–
13	Zn (mg/L)	0.13	5.0
14	Cu (mg/L)	0.11	1.0
15	B (mg/L)	0.24	–
16	Hg (μg/L)	0.08	50
17	Cd (mg/L)	0.02	0.1
18	Pb (mg/L)	0.07	1.0
19	Cr (mg/L)	0.04	1.5
20	As (mg/L)	0.30	0.5

[a] The limits were quoted from the secondary standard specified in the Integrated Wastewater Discharge Standard (GB 8978-1996) issued by the China Environmental Protection Agency.

GC-MS analysis of LUW

The two samples obtained by two different pretreatment methods were analyzed by GC-MS. The names, retention times, molecular formulas, similarities and area percentages of the components only with higher similarity to the NIST08 database than 80% were detailed in Tables 2 and 3, respectively. The relative content of the components was calculated through the method of peak area normalization, and expressed as area percentage.

Thirty-six constituents were acquired from Sample 1 obtained by directly extracting LUW. The main components were phenols, of which the number reached 15. The rest was a small amount of acids (6), ketones (4), aldehydes (3) and so on. The total relative content of phenols is the highest, followed by organic acids. The component with highest relative content was p-cresol (7.22%). Forty-three constituents were detected from Sample 2 obtained from LUW by the multiple extraction method, and one-fifth more than Sample 1. The main components were still phenols (12), but the number of acids (3) reduced relatively and the number of hydrocarbons (7) increased relatively. The component with highest relative content in Sample 2 was phenol (13.93%). Nineteen compounds were newly detected in Sample 2 and 12 compounds in Sample 1 were not acquired. In this study, a total of 55 compounds were detected from Sample 1 and Sample 2, and the phenolic components were the most, followed by organic acids.

Table 2 | Chemical constituents of Sample 1 obtained by directly extracting LUW

No.	Compound	Retention time (min)	Molecular formula	Similarity (%)	Area percentage (%)
1	Phenol	6.59	C_6H_6O	91	3.54
2	4-(Methylamino)-butanoic acid	8.13	$C_5H_{11}NO_2$	83	5.53
3	o-Cresol	8.38	C_7H_8O	95	1.56
4	p-Cresol	8.76	C_7H_8O	97	7.22
5	3-Methylbenzyl alcohol	9.51	$C_8H_{10}O$	83	0.35
6	2-Ethylphenol	9.64	$C_8H_{10}O$	93	0.47
7	2,4-Dimethylphenol	9.83	$C_8H_{10}O$	96	0.96
8	4-Ethylphenol	10.25	$C_8H_{10}O$	93	2.51
9	2-Methoxy−4-methylphenol	10.59	$C_8H_{10}O_2$	95	1.39
10	3,4-Dimethylphenol	10.73	$C_8H_{10}O$	93	1.30
11	3-Methoxyphenol	11.41	$C_7H_8O_2$	91	0.70
12	p-Allylphenol	11.46	$C_9H_{10}O$	86	0.48
13	2-Ethyl−5-methylphenol	11.81	$C_9H_{12}O$	87	0.44
14	m-Toluic acid	12.12	$C_8H_8O_2$	91	0.55
15	3-Methoxy−5-methylphenol	12.74	$C_8H_{10}O_2$	94	1.71
16	2,6-Dimethoxyphenol	13.06	$C_8H_{10}O_3$	97	2.32
17	3-Hydroxybenzaldehyde	13.47	$C_7H_6O_2$	93	2.33
18	Vanillin	13.72	$C_8H_8O_3$	97	4.08
19	3,3-Dimethyl−1-indanone	14.12	$C_{11}H_{12}O$	81	1.27
20	Vanillic acid	14.19	$C_8H_8O_4$	81	1.86
21	4′-Hydroxyacetophenone	14.34	$C_8H_8O_2$	93	1.75
22	Acetovanillone	14.67	$C_9H_{10}O_3$	97	3.05
23	6-Acetyltetralin	14.74	$C_{12}H_{14}O$	90	0.89
24	1-Naphthol	14.93	$C_{10}H_8O$	93	1.03
25	2,6-Dimethoxy – 4-allylphenol	15.76	$C_{11}H_{14}O_3$	93	0.47
26	3,5-Dimethoxy – 4-hydroxybenzaldehyde	16.33	$C_9H_{10}O_4$	93	1.47
27	Acetosyringone	16.97	$C_{10}H_{12}O_4$	90	1.15
28	Myristic acid	17.13	$C_{14}H_{28}O_2$	95	0.76
29	Octadecane	17.46	$C_{18}H_{38}$	97	0.28
30	Methyl hexadecanoate	18.71	$C_{17}H_{34}O_2$	98	0.66
31	Palmitic acid	19.02	$C_{16}H_{32}O_2$	99	0.84
32	Stearic acid	20.29	$C_{18}H_{36}O_2$	98	0.34
33	Hexadecanamide	20.40	$C_{16}H_{33}NO$	98	0.28
34	N-Phenyl – 2-naphthylamine	20.74	$C_{16}H_{13}N$	98	0.10
35	Bis(2-ethylhexyl) adipate	21.43	$C_{22}H_{42}O_4$	98	0.74
36	Erucylamide	22.90	$C_{22}H_{43}NO$	97	0.89

Through comparative analysis, it was found that the chemical components in the samples obtained by direct extraction method and multiple extraction method were basically the same in type. But there were some differences in quantity of the compounds with different types, and principal components and their relative content. Especially, phenol content in Sample 2 greatly increased, indicating that some of the phenol may be ionized and not easily

Table 3 | Chemical constituents of Sample 2 obtained from LUW by multiple extraction method

No.	Compound	Retention time (min)	Molecular formula	Similarity (%)	Area percentage (%)
1	3-Methyl−2-cyclopenten−1-one	6.07	C_6H_8O	90	0.56
2	Phenol	6.62	C_6H_6O	91	13.93
3	Dipentene	7.34	$C_{10}H_{16}$	89	0.76
4	Benzyl alcohol	7.56	C_7H_8O	96	0.37
5	o-Cresol	8.15	C_7H_8O	95	2.12
6	p-Cresol	8.52	C_7H_8O	96	6.55
7	Guaiacol	8.57	$C_7H_8O_2$	94	3.20
8	2,4-Dimethylphenol	9.74	$C_8H_{10}O$	95	0.64
9	Decamethylcyclopentasiloxane	9.85	$C_{10}H_{30}O_5Si_5$	91	0.37
10	3-Ethylphenol	10.18	$C_8H_{10}O$	91	1.08
11	4-Ethylphenol	10.34	$C_8H_{10}O$	87	0.94
12	2-Methoxy−4-methylphenol	10.57	$C_8H_{10}O_2$	95	1.08
13	3,4-Dimethylphenol	10.68	$C_8H_{10}O$	91	0.63
14	2-Methyl−1-indanone	12.55	$C_{10}H_{10}O$	86	0.38
15	1,2,3,4-Tetrahydroquinoxaline	12.91	$C_8H_{10}N_2$	90	0.22
16	2,6-Dimethoxyphenol	13.02	$C_8H_{10}O_3$	97	1.07
17	3-Hydroxybenzaldehyde	13.42	$C_7H_6O_2$	93	0.71
18	Tetradecane	13.57	$C_{14}H_{30}$	96	0.36
19	Vanillin	13.63	$C_8H_8O_3$	96	2.11
20	3,3-Dimethyl−1-indanone	14.08	$C_{11}H_{12}O$	87	0.55
21	Vanillic acid	14.15	$C_8H_8O_4$	87	0.94
22	Acetovanillone	14.61	$C_9H_{10}O_3$	97	1.53
23	6-Acetyltetralin	14.70	$C_{13}H_{18}$	90	0.49
24	2-Ethoxy – 4-anisaldehyde	15.57	$C_{10}H_{12}O_3$	80	0.38
25	Hexadecane	15.66	$C_{16}H_{34}$	96	0.33
26	4-Allylsyringol	15.74	$C_{11}H_{14}O_3$	87	0.20
27	3,5-Dimethoxy – 4-hydroxybenzaldehyde	16.31	$C_9H_{10}O_4$	86	0.67
28	3,4,5-Trimethoxybenzaldehyde	16.96	$C_{10}H_{12}O_4$	81	0.18
29	Acetosyringone	16.97	$C_{10}H_{12}O_4$	83	0.27
30	Octadecane	17.46	$C_{18}H_{38}$	97	0.19
31	Methyl hexadecanoate	18.71	$C_{17}H_{34}O_2$	98	0.18
32	Palmitic acid	19.01	$C_{16}H_{32}O_2$	99	0.46
33	1-Eicosene	19.73	$C_{20}H_{40}$	93	0.06
34	Octadecanoic acid, methyl ester	20.08	$C_{19}H_{38}O_2$	86	0.07
35	Stearic acid	20.29	$C_{18}H_{36}O_2$	98	0.30
36	Hexadecanamide	20.41	$C_{16}H_{33}NO$	93	0.16
37	Docosane	20.48	$C_{22}H_{46}$	96	0.10
38	Cyclohexadecane	20.53	$C_{16}H_{32}$	95	0.09
39	Bis(2-ethylhexyl) adipate	21.42	$C_{22}H_{42}O_4$	95	0.42
40	Anti Oxidant 2246	21.56	$C_{23}H_{32}O_2$	97	0.28
41	2-ethylhexyl hydrogen phthalate	22.05	$C_{16}H_{22}O_4$	87	0.52
42	3-Methylheneicosane	22.21	$C_{22}H_{46}$	80	0.17
43	Erucylamide	22.90	$C_{22}H_{43}NO$	95	0.75

extracted by direct extraction method. Another significant difference was that the 4-(methylamino)-butanoic acid, which was an amphoteric compound with high relative content in Sample 1, was not detected in Sample 2. When liquid–liquid extraction is carried out, pH will affect the presence of acidic, alkaline and amphoteric compounds in solution (molecular or ionic forms), thereby influencing their distribution ratio in the water phase and organic phase. This should be the root cause of the distinction between the analysis results of the two samples and the identification of more abundant chemical components from Sample 2. The above analysis showed that the sample preparation method combining direct extraction with multiple extraction could obtain more chemical composition information of LUW.

A large number of phenolic and acidic substances can inhibit the growth of microorganisms and reduce the biodegradability of wastewater (Kahru *et al.* 1996; Abarian *et al.* 2019). Phenols are a class of protoplasmic toxic substances, which has been listed as one of 129 priority pollutants by the National Environmental Protection Agency of the United States (Santos *et al.* 2004; Basha *et al.* 2010). Phenolic substances are not only toxic but also difficult to degrade (Basha *et al.* 2010; Zhang *et al.* 2019). On the one hand, wastewater rich in phenolic substances will pollute the environment if it is directly discharged without treatment. On the other hand, there are also problems of high cost or secondary pollution when industrial treatment is performed. Combined with the water quality analysis, the results of this study indicated that LUW is characterized by toxicity and high pollution. In view of the above characteristics, such wastewater needs to use a more advanced wastewater treatment process in order to achieve its efficient and stable discharge up to standard, but this will inevitably increase costs (Maiti *et al.* 2019). Fortunately, the phenols and organic acids with small molecular weight derived from lignite often have many excellent biological activities (Calvo *et al.* 2014; Qin *et al.* 2016). Considering the source of the sample, it suggested that these compounds in LUW are just part of fulvic acids or humic acids in lignite. So, it may be a more efficient way of wastewater treatment to directly utilize the LUW as natural resources instead of purifying and discharging.

Effects on wheat seed germination

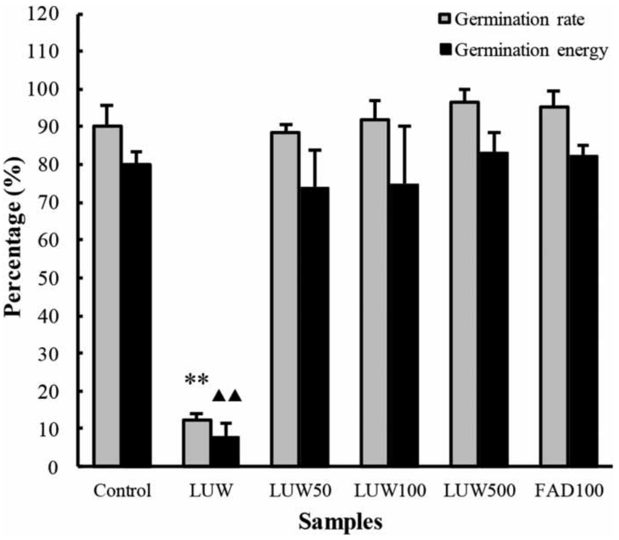

Figure 1 | Effects of different samples on wheat germination rate and germination energy. Results were expressed as mean ± S.D ($n = 3$). $^{**}P < 0.01$ vs. Control; $^{▲▲}P < 0.01$ vs. Control; Student's *t*-test.

As shown in Figure 1, the LUW without dilution presented a significant inhibitory effect on both germination rate and germination energy compared with the control group ($P < 0.01$). After dilution, the inhibition disappeared immediately, and the values of the two indexes increased with the increase of dilution multiple. The germination rate of LUW100 group and LUW500 group as well as the germination energy of LUW500 group was higher than those of control groups, but there was no significant difference. The germination rate and germination energy of the FAD100 group were larger than those of control groups, but there was also no significant difference. FAD100 did not produce as strong activity as in previous experiments, which may be caused by different batches of raw coal or wheat seeds (Zhu *et al.* 2014).

Figure 2 indicates that the LUW without dilution also had significant inhibitory effects on bud length and root length relative to the control group ($P < 0.01$). After dilution, the inhibitory effect changed into a promoting effect. The effect of LUW50 on bud length was significant ($P < 0.05$), but not on root length. The effect of LUW500 on root length was very significant ($P < 0.01$), but not on bud length. This may be related to the content of nitrogen in LUW. With the increase of dilution multiplied, the nitrogen content in LUW decreases. A small amount of nitrogen will first satisfy the growth of roots, resulting in an increase in the ratio of root to bud. FAD100 could increase the bud length and root length, but the promotion effect was not significant.

As presented in Figure 3, the LUW without dilution produced significant inhibitory effect on the increase of fresh

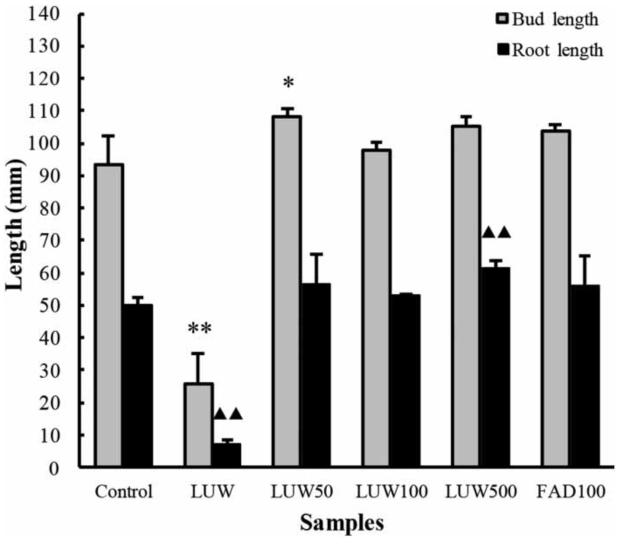

Figure 2 | Effects of different samples on wheat bud length and root length. Results were expressed as mean ± S.D ($n = 30$). *P <0.05 vs. Control; **P <0.01 vs. Control; ▲▲P <0.01 vs. Control; Student's t-test.

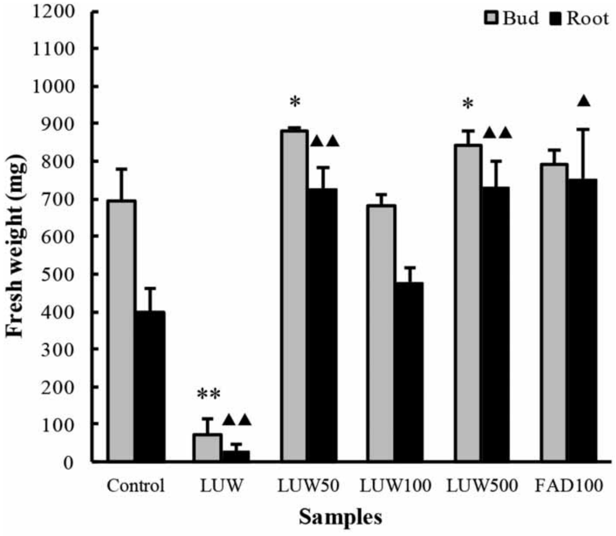

Figure 3 | Effects of different samples on fresh weight of wheat bud and root. Results were expressed as mean ± S.D ($n = 30$). *P <0.05 vs. Control; **P <0.01 vs. Control; ▲P <0.05 vs. Control; ▲▲P <0.01 vs. Control; Student's t-test.

weight of buds and roots compared with the control group ($P < 0.01$). After dilution, both LUW50 and LUW500 significantly promoted the increase of bud fresh weight ($P < 0.05$). LUW100 still inhibited the increase of bud fresh weight, but the effect was not significant. After dilution, the three diluents all promoted the increase of root fresh weight, and the effects of LUW50 and LUW500 reached very significant levels ($P < 0.01$). At the same time, the promoting effect of FAD100 on root fresh weight began to become significant ($P < 0.05$). The effect of each sample on the dry weight of buds and roots was similar to that on fresh weight (Figure 4). The difference is that the effect of diluents on dry weight of buds is more prominent than that on wet weight of buds. The promoting effect of LUW100 on dry weight of buds had reached a significant level ($P < 0.05$), and LUW500 showed a very significant effect ($P < 0.01$). The effect of dilutions on dry weight of roots was also weaker than that on wet weight, and even LUW100 inhibited the increase of root dry weight. In comparison, LUW diluted 50 times (LUW50) had the strongest effect on both the fresh and dry weight of buds and roots.

DISCUSSION ON MECHANISM

This study indicated that LUW without dilution had a strong inhibitory effect on wheat seed germination and seedling growth, but it could significantly promote the growth of wheat seedlings after dilution. When diluted 50 times, LUW had the strongest comprehensive activity and was stronger than FAD. Among the identified components, 4-(methylamino)-butanoic acid, 1,2,3,4-tetrahydroquinoxaline, hexadecanamide and erucylamide can be used as nitrogen sources for plants (Franklin *et al.* 2017; Ganeteg *et al.* 2017). As important allelochemicals, a large number

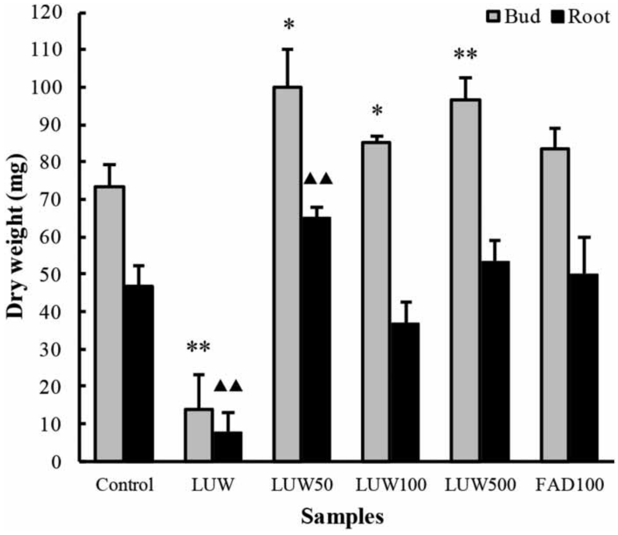

Figure 4 | Effects of different samples on dry weight of wheat bud and root. Results were expressed as mean ± S.D ($n = 30$). *P <0.05 vs. Control; **P <0.01 vs. Control; ▲▲P <0.01 vs. Control; Student's t-test.

of simple phenols, N-phenyl-2-naphthylamine and other components will affect the activity of key enzymes required for seed germination, cell differentiation and seedling growth (Einhellig 1995). At high concentrations, phenols and N-phenyl-2-naphthylamine may strongly inhibit or even poison seed germination and seedling growth. After dilution, the concentration of harmful substances decreases significantly and toxicity is greatly reduced. Carboxylic acids and nitrogen-containing compounds, as analogues of plant hormones or nutrients, begin to play a role and show a low-volume and high-efficiency effect, which is consistent with previous research results (Chou & Leu 1992; Xue *et al.* 2012). In addition, when diluted to a certain extent (such as 500 times), allelochemicals in LUW may stimulate the seed to produce growth hormone and promote the growth of seedlings (Roshchina & Roshchina 1993).

Furthermore, the activity of LUW diluted 500 times is equivalent to or even better than that of FAD diluted 100 times. The above discussion about the results of GC-MS revealed that the phenols and organic acids in LUW were part of fulvic acids or humic acids in lignite to some degree. Further, it has now been confirmed that LUW and FAD have similar biological activities, which provides support for the above inference. Considering the homology of the two, they may have the same mechanism in regulating the germination of wheat seed; that is, the activity mechanism of LUW may be similar to plant hormones and their analogues (Zhu *et al.* 2014; Qin *et al.* 2016). Meanwhile, LUW contains many trace elements that are beneficial to seed germination, and Fe, Ca, Mg, and Mn are especially high in content. Some studies have shown that the complexation of oxygen-containing functional groups (such as carboxyl groups and phenolic hydroxyl groups) belonging to humic substances with metal ions can improve the availability of some trace elements, thereby improving the activity of many enzymes (Rashid 1974; Chen *et al.* 2004). For example, it has been found that fulvic acids (FA) overcome the speed-limiting step of Fe transport from soil solution to plant roots through diffusion, and the efficiency of Fe-FA as a fertilizer is much greater than that of $FeCl_3$ (Pandeya *et al.* 1998). The coexistence of phenols and organic acids with nutrient elements is very helpful for LUW to stimulate seed germination. Therefore, it suggested that the phenols and organic acids, as the main organic components in LUW, can play a role similar to plant hormones, and at the same time enhance the utilization of nutrient elements by seeds, and ultimately promote the germination of seeds.

CONCLUSION

The results of water quality analysis showed that many environmental indicators of LUW exceeded the standard greatly, and LUW belonged to seriously polluted waters which cannot be discharged directly. LUW contained many trace elements and especially Fe, Ca, Mg, and Mn were high in concentration. Meanwhile, the contents of the five harmful elements Hg, Cd, Pb, Cr and As were extremely low. Fifty-five compounds, mainly phenols and organic acids, were identified by GC-MS analysis using the specific sample preparation method. This study also confirmed that LUW without dilution had a strong inhibitory effect on wheat seed germination and seedling growth, but it could significantly promote the growth of wheat seedlings after dilution and the comprehensive activity was the strongest when diluted 50 times. The results of chemical characteristic analysis suggested that it might be necessary to select more advanced wastewater treatment process to achieve the efficient and stable discharge up to standard, but this will inevitably bring about technical and cost pressures. Fortunately, the results of this study also showed that LUW has the natural advantages of directly developing liquid fertilizers such as seed-soaking fertilizers, foliar fertilizers and drip fertilizers in terms of its physical form, chemical composition, biological activity, safety and economy. This study confirmed the feasibility of applying LUW to agricultural field only through simple dilution without other treatments, and provided an efficient and green method for treating lignite upgrading wastewater. The results of this study also provided theoretical basis and technical support for the research on activity mechanism of LUW in the later stage and the development process of high-end organic fertilizers such as field experiments.

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