http://pubs.acs.org/journal/acsodf

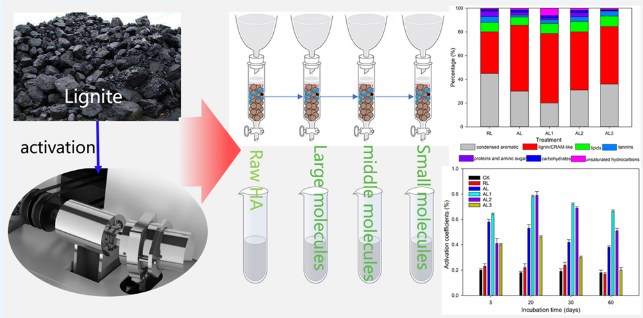
Article

Molecular Characterization of Size-Fractionated Humic Acids Derived from Lignite and Its Activation of Soil Legacy Phosphorus and *Lactuca sativa* Growth-Promoting Performances

Xiaoqi Liu, Xuejiao Zhao, and Jialong Lv*

Cite This: *ACS Omega* 2023, 8, 6838−6846

ABSTRACT: Humic acid (HA) has been widely used in agricultural production processes because it improves soil nutrients and has a growth-promoting effect on plants. Understanding the relationship between the structure and function of HA is the key to efficient utilization of HA in the field of activating soil legacy phosphorus (P) and promoting crop growth. In this work, HA was prepared using lignite as raw materials by the ball milling method. Moreover, a series of HAs with different molecular weights (<10, 10−50, and >50 kDa) were prepared through ultrafiltration membranes. The chemical composition and physical structure characteristics of the prepared HA were tested. The effects of HA with different molecular weights on activating accumulated P in calcareous soil and root promotion of *Lactuca sativa* were investigated. Results showed that HAs with different molecular weights have different functional group structures, molecular compositions, and micromorphologies, and the molecular weight of HA can significantly affect the activation performance on soil accumulated P. Moreover, the HA with low molecular weight more easily enhanced the seed germination and growth of *Lactuca sativa* than that of raw HA. It is expected that more efficient HA can be prepared in the future for the activation of accumulated P and promoting crop growth.



1. INTRODUCTION

Phosphorus (P) is an essential nutrient and one of the most limiting macronutrients for plants growth.[1] The P fertilizers are mainly prepared from phosphate rock (PR), and about 90% of PR is used to prepare P fertilizers for agricultural production.[2] Unfortunately, >80% of the P fertilizers applied to the soil is lost due to leaching, runoff, soil adsorption, and fixation processes, which means that the utilization rate of P fertilizer is very low.[3] Therefore, a large amount of P fertilizers have been continuously applied to the soil in order to meet human demand for food in recent decades. This has caused the depletion of PR resources; according to the current mining speed, the remaining PR resources are only enough to last about 50−100 years.[4] In addition, the accumulated P in the soil has caused soil and water environmental problems, such as soil acidification, soil pollution, and water eutrophication.[5] Activation of legacy P can ameliorate these problems, and more and more related studies have been reported.[6]

The current methods of activating soil accumulated P mainly include phosphate solubilizing microorganisms, phosphatase enzyme, organic acids, biochar, zeolites, humic acids (HAs), lignin, and other materials.[7−9] Some activators have the advantages of a high activation effect and fast response speed such as phosphate solubilizing microorganisms and phosphatase enzyme; however, they are usually more expensive and highly specific.[6] HA is increasingly used for activating soil phosphorus in agricultural production owing to HA itself being beneficial for the soil.

HA, which is the major constituent of soil organic matter, is a complex mixture of macromolecular organic weak acids. It is widely found in lignite, peat, etc.[10] The structure of HA is complicated, as it contains various complexing agents due to more carbon (C) and oxygen (O). It also has the following characteristics: redox properties, adsorption, weak acid, ion exchange, and other physiological activity. It can interact with many inorganics and organics.[11] The development and utilization of HA has become one of the more active fields today.[12,13]

HA stored in lignite is difficult to apply directly for agricultural production due to its low efficiency. Activation is a critical step for improving the work efficiency of HA. The activation enhances the availability of HA so that it can absorbed by crops easily.[14,15] So, HA can be better developed

Received: November 24, 2022
Accepted: February 1, 2023
Published: February 10, 2023

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

and applied in the agricultural field by this way.[16] The activation methods of HA include physical, chemical, and some biological methods. These activation methods can improve the content and physiological activity of HA. Physical activation is mainly done by mechanical disintegration, but the activation efficiency is too low. The chemical activation method requires a strong oxidant, and the extraction process is complex; the reaction period is long as well as costly. Temperature mainly influences the biological method and is time limited, so each method has some limitations.[17,18] In this experiment, the solid phase ball milling method was used for HA activation, where KOH was used as an activator.

Furthermore, there have been an increasing number of reports on the application of HA to vegetables and grain crops. HA successfully increases germination and improves the initial growth of seedlings. Valdrighi reported HA extracted from lignite and lignite could promote the absorption of nutrients by tomato plants.[19] Gau found HA could promote the synthesis of protein in the root cells of barley during the transcription stage.[20] In addition, HA concentration and origin (the substrate from which HA was obtained) can also affect plant growth. Šerá and Novák compared the effect of HA (obtained from different natural sources) on the stimulation of seed germination and initial seedling growth.[21,22] It was found that the seed reaction of lamb's quarters (*Chenopodium album* agg.) depends on the concentration of HA but also the origin (the substrate from which HA was obtained).[21] The same humic substance (concentration of 200 mg/L) was used in the experiment with poppy, hemp, rape, and pepper seeds. Significant effects were confirmed in poppy, where HA (originated from podzol) increased germination to 202%, seedling growth to 236%, and seedling vitality index to 252% compared to the control (100%).[22] HA improved the soil structure, increased the utilization rate of chemical fertilizers, promoted the growth and vigor of root, and increased crop stress resistance, thereby enhancing the yield and quality of crops.[23−25]

In fact, HA has been widely used in agricultural production in recent years. However, there are few comparative studies on the actual value of HA with different molecular weights. Obviously, HA with different molecular weights has different functions due to the different structures. It is necessary to study the relationship between structure and function of HA in the field of activating soil legacy phosphorus (P) and promoting crop growth. In this study, HA was prepared from lignite by the ball milling method under a KOH environment. HA with different molecular weights was separated through a small membrane separator. The contributions of this study are as follows: (i) to understand the molecular composition and physical structure of humic acid with different molecular weights; (ii) to explore the activation of HA with different molecular weights on the soil legacy P and their influence on *Lactuca sativa* growth.

2. MATERIALS AND METHODS

2.1. Materials. Lignite was bought from Qingxin Longke Co., Ltd. (Xinjiang, China). *Lactuca sativa* seeds were purchased from Shouguang Fengnong Trading Co., Ltd. (Shandong, China). Quartz sand was bought from Yongda Chemical Reagent Co., Ltd. (Tianjin, China). All chemicals were purchased at the analytical grade. Solutions were prepared with distilled water.

2.2. Activation and Grade of HA. Activated HA was prepared through the ball milling method on a laboratory scale. Briefly, 100 g of KOH was mixed with 2 kg of lignite in a ball-grinding machine, and then, it was milled for 80 min at 65 rpm. The product was activated HA from lignite. 10 g of activated HA was adequately dissolved in 2 L of deionized water. The solution was filtered through a 50 nm ceramic membrane, and filtrate was then filtered through a series of ultrafiltration membranes (10 and 50 kDa). Finally, three molecular weight classes of HA with different molecular weights (<10, 10−50, and >50 kDa) were obtained and labeled as AL1, AL2, and AL3, respectively. The yields were calculated by the percentage of organic carbon retained in each fraction from the original HA. Organic carbon was determined by the potassium dichromate oxidation method.

2.3. Characterization. The morphologies of the HA were investigated by scanning electron microscopy (SEM, QUANTA250, USA) and transmission electron microscopy (TEM, FEI TAIOS, F200X, USA). The functional group of HA was analyzed by a Fourier transform infrared spectrometer (FTIR) (Nicolet IS10, USA) at a scanning range from 4000 to 500 cm^{-1}. The elemental analysis of the samples was obtained by energy-dispersive X-ray spectroscopy (EDX). Negative ion ESI FT-ICR MS analysis was conducted using a Bruker Apex ultra-FT-ICR MS (Bruker, Billerica, MA, U.S.A.).

2.4. Determination of the HA Activating Soil Legacy P Soil. The type of soil used for the experiment was loess soil, and the sampling point (34°28′ N and 108°45′ E m a.s.l) was in the Yangling district, Shaanxi province, China. The 0−20 cm soil was used for testing and had the following properties: pH, 8.16 (1:1 soil/water); $CaCO_3$, 60.4 g/kg; Olsen-P, 27.31 mg/kg; total phosphorus 0. 83 g/kg. The detailed procedure was as follows: 2.5 g of HA was evenly mixed with 250 g of soil passed through a 2 mm sieve in a plastic box. The box was placed in a room at a constant temperature of 25 °C for cultivation. Water was added to the soil to 50% of its water holding capacity. The Olsen-P and P activation coefficient were measured after the soil aged 5, 20, 30, and 60 days. The P activation coefficient was the ratio of Olsen-P to total-P and is expressed as a unitless ratio. Each treatment was replicated three times.

2.5. *Lactuca sativa* Cultivation. First, the organic carbon content of HA was measured using a total organic carbon (TOC) analyzer (Vario TOC, Germany) before preparation of the culture medium. The HA solutions with different organic carbon contents and molecular weights were prepared (listed in Table S1). The pH of the HA solution was adjusted to 6.8 by using 0.02 mol/L sulfuric acid or 0.02 mol/L KOH solution. The germination of *Lactuca sativa* seeds was carried out in a Petri dish (about 9 cm in diameter). A total of 50 seeds were placed in each Petri dish, which contains 2 mL of HA solution of different molecular weights. Deionized water was only used in the control treatment. The Petri dishes were covered with lids and kept in the dark at 25 °C. The seedling growth experiment was carried out using quartz sand. Each Petri dish was filled with 110 g of quartz sand, and 50 seeds were sown on a quartz sand bed. The seeds were covered with 40 g of quartz sand and kept for 30 days in the dark at 25 °C. Deionized water (3 mL) was applied to each pot every 3 days to maintain the plant growth. After culturing, the seeds' germination rate and water uptake rate were recorded every day for five consecutive days. The length of germinated seedlings was measured every day from the third to eighth days. The plant height and seedling fresh weight were

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

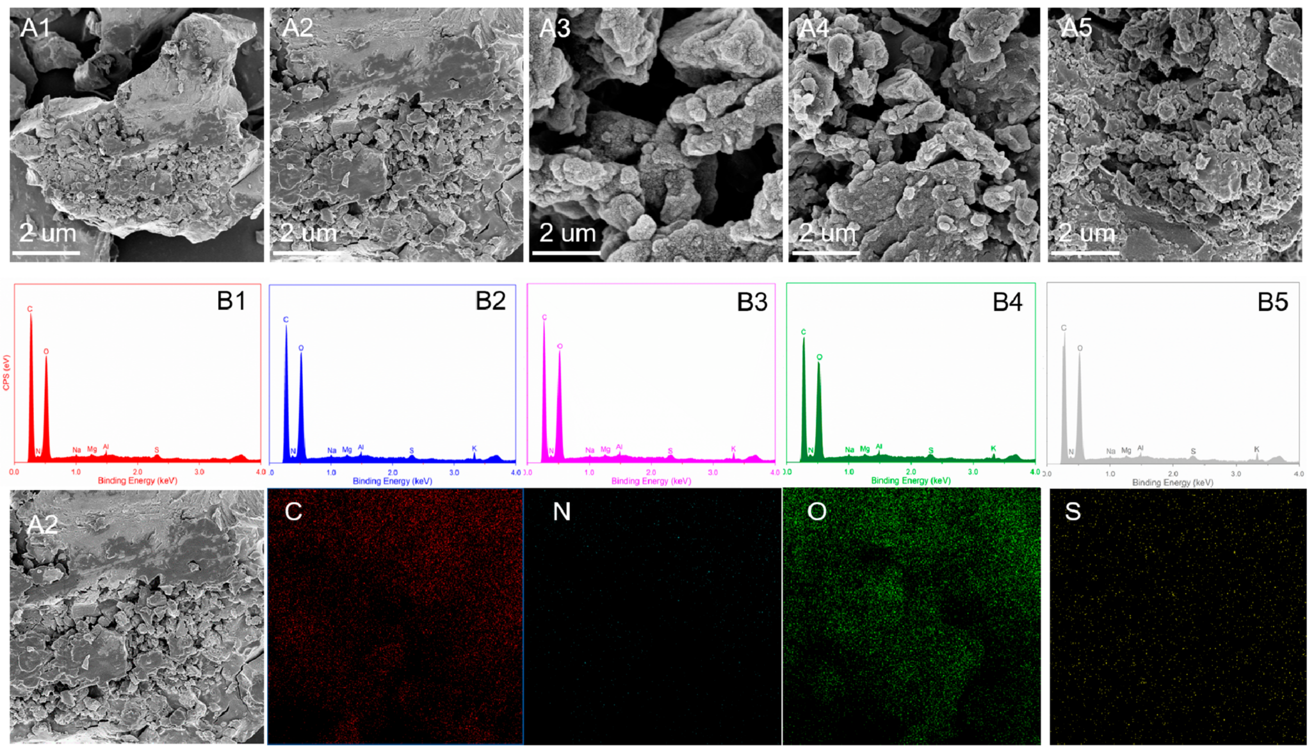


Figure 1. SEM images of RL (A1), AL (A2), AL3 (A3), AL2 (A4), and AL1 (A5); EDX spectra of RL (B1), AL (B2), AL3 (B3), AL2 (B4), and AL1 (B5); EDX-mapping of AL. RL, AL, AL3, AL2, and AL1 represent raw lignite, activated raw HA without grading, and HA with >50 000, 10 000−50 000, and <10 000 kDa. Other charts use the same label.

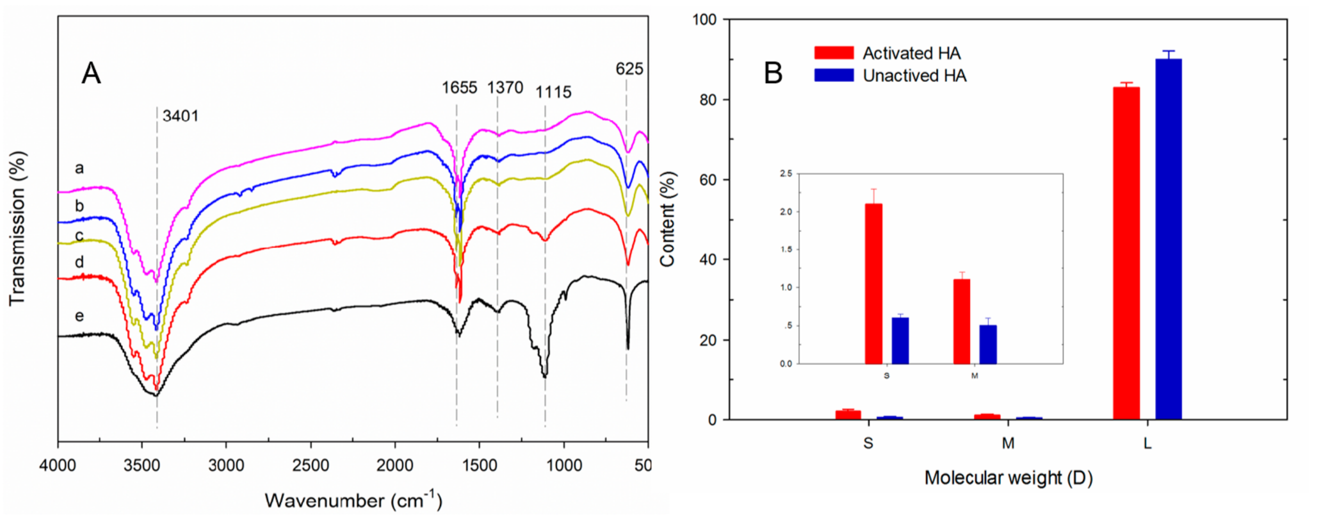


Figure 2. FTIR spectra (A) of RL (a), AL (b), AL1 (c), AL2 (d), and AL3 (e) and molecular weight content distribution (B) of activated HA and unactivated HA, respectively. S (<10 kDa), M (10−50 kDa), and L (>50 kDa).

measured on the 30th day. The root system of *Lactuca sativa* was scanned after 30 days using a root scanner (PERFECTION V700 PHOTO, Japan). The pH of various culture media was determined. After 30 days of cultivation, quartz sand was taken from each treatment Petri dish and baked to a constant weight at 55 °C in an oven, and 2.00 g of dry quartz sand was mixed with 20 mL of deionized water to measure the pH. Each treatment was repeated four times. The microscopic structure of the root tip cells was observed after 30 days using a light microscope (YYS-350E, China). The samples were observed on a light microscope 10 times.

2.6. Statistical Analysis. All experiments were conducted in triplicate or quadruplicate, and the average values were calculated in Microsoft Excel 2016. One-way ANOVA among treatments and the means difference tests (Duncan's multiple range test and least significant difference test) were performed using the Statistical Analysis System (SAS) package version 9.2 (2010, SAS Institute, Cary, NC). Regression equations and coefficients were determined using SAS. The difference among means and correlation coefficients were considered significant at $\alpha < 0.05$.

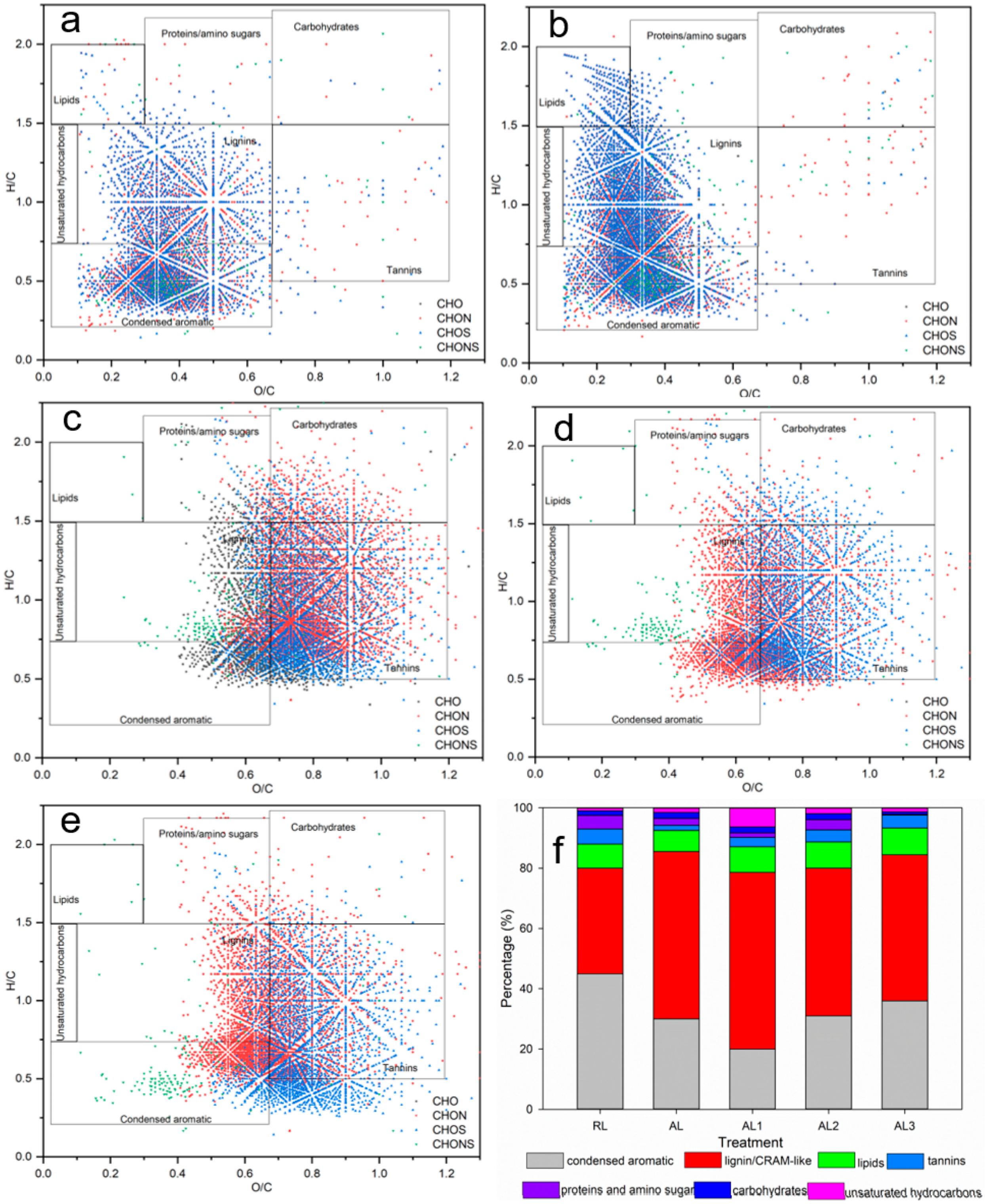


Figure 3. Van Krevelen diagram of humic acid from (a) RL, (b) AL, (c) AL3, (d) AL2, and (e) AL1; (f): bar diagrams exhibit the proportion of major classes in RL, AL, and AL1−3.

3. RESULTS AND DISCUSSION

3.1. Elemental Analysis and Morphology. RL (raw lignite) and ALs mainly contain C, O, N, S, a small amount of Na, and Mg elements. The elemental analysis results showed that AL contained more H, O, and N, but less C (Figure 1B1−B5). The activation process increased the relative content of active elements such as O and H, which was consistent with the more active groups of HAs after activation.[26] In addition, the HAs of small molecular weights contained more higher relative oxygen content (Figure 1B3−B5). After physical−chemical activation, the composition and structure of lignite have undergone great changes. The surface of activated HA presented more developed pore structures. The surfaces of the ALs were relatively rougher, and many small "pinholes" were

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

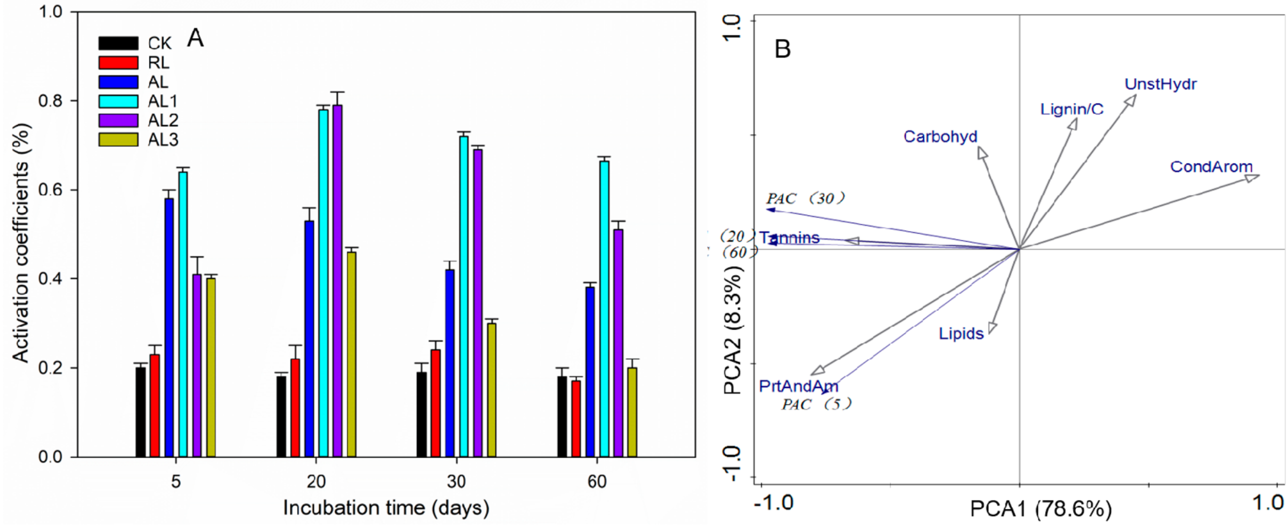


Figure 4. (A) Effects of HA with different molecular weights on soil P; (B) PCA showing the relationship between the main chemical structures and the phosphorus activation coefficient (PAC).

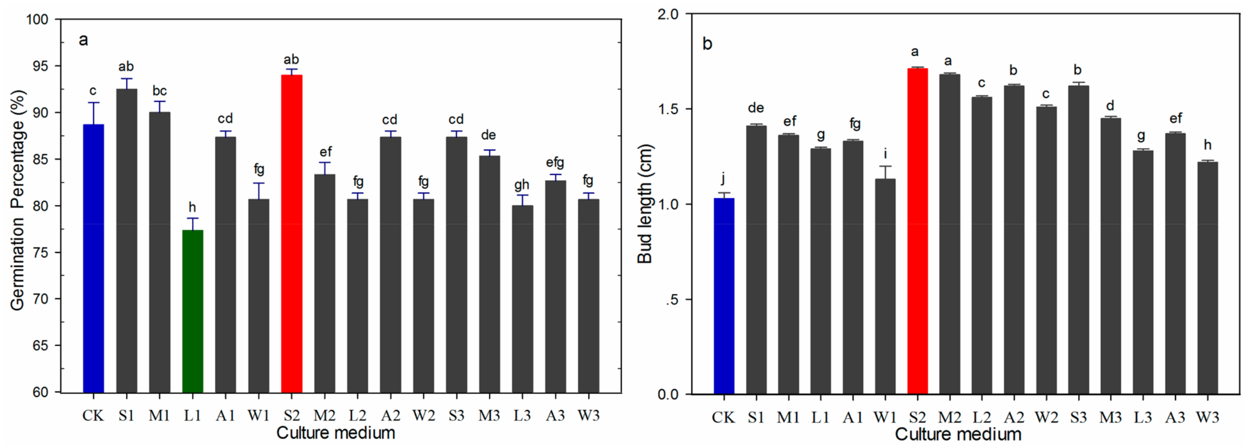


Figure 5. Effect of HA on (a) germination percentage and (b) seedling length of *Lactuca sativa*. CK stands for control treatment. A and W represent the activated and unactivated lignite. S, M, and L represent the HA with molecular weights less than 10 000, 10 000−50 000, and greater than 50 000 Da, respectively. The corresponding numbers (1, 2, 3) show the different carbon concentrations as 100, 300, and 500 mg/L, respectively, in HA solution.

seen on the surface of Als (Figure 1A1−A5). The different morphology of lignite and activated HA indicated that the structure of activated HA was greatly changed during the activation process. These changes may be attributed to some of the macromolecules becoming excited molecules, which cross the energy barrier and break the molecular chain to become small molecules of HA.[27] As the molecular weight becomes smaller, the surface pores of AL become more and more developed (Figure 1A3−A5), and the abundant fine particles were observed on the surface of HA with small molecules (Figure 1A3−A5). In general, small molecular weight molecules contain more active substances, and their surface structures are looser. This is similar to the research of Tang et al.[16]

3.2. FTIR Analysis. The FTIR spectra of RL, AL, AL1, AL2, and AL3 exhibited a chemical shift after activation and grading (Figure 2A). The spectra of these HAs in lignite were similar, suggesting that the functional group of HA in lignite did not change during the activation and grading. The peaks appearing at 3410 cm^{-1} were attributed to the −OH group. The characteristic peaks of HA occurred at 1612 cm^{-1}, indicating the presence of an −COOH group. Furthermore, the characteristic peaks (1377 cm^{-1}) were observed (Figure 2A), which might have attributed to the $-NO_2$ stretching vibration or υ (C−O) stretching in phenol or −COOH.[28,29] The peaks appearing at 1115 cm^{-1} were attributed to the stretching vibration C−O group. The peaks of 1115 cm^{-1} getting stronger with the molecular weight become smaller, indicating many C−O groups (characteristic group of HA) accumulated. After activation, the content of HA with small molecular weights was increased by 163.38% than that of nonactivated lignite, while the content of large molecular weights was decreased by 6.20% (Figure 2B). This may be due to the grinding and KOH addition during the HA activation process. Grinding reduced the average particle size of lignite HA, increased the surface energy of HA, increased the effective collision probability of molecules, and promoted macromolecular chain breaking into molecules with smaller molecular weight.

3.3. Molecular Characterization. In order to reveal the molecular formulas of HA, the van Krevelen diagram of humic acid was conducted based on elemental H/C and O/C ratios

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

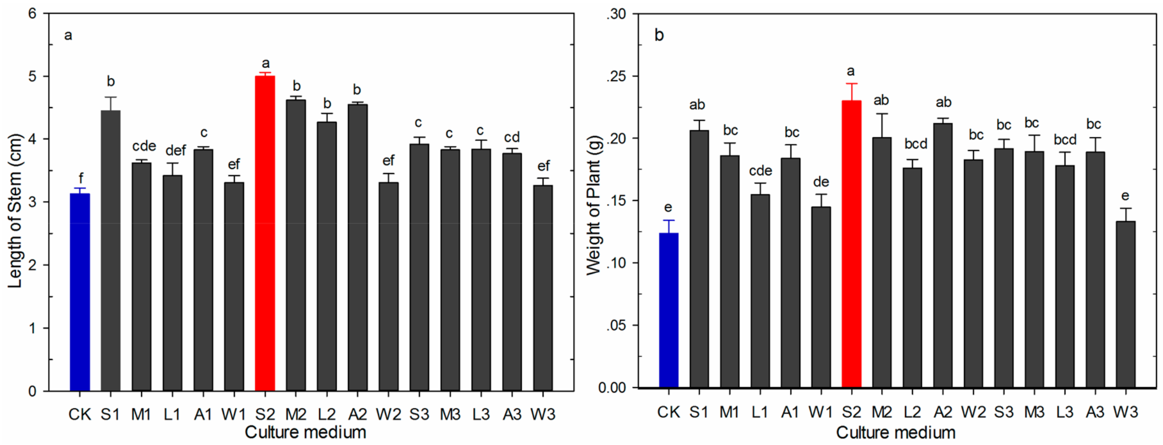


Figure 6. Effect of HA on (a) stem length and (b) fresh weight of *Lactuca sativa*. CK stands for control treatment. A and W represent the activated and unactivated lignite. S, M, and L represent the HA with molecular weights less than 10 000, 10 000−50 000, and greater than 50 000 Da, respectively. The corresponding numbers (1, 2, 3) show the different carbon concentration as 100, 300, and 500 mg/L, respectively, in HA solution.

of samples by ESI-FT-ICR MS. HA in all treatments contain four major types of compounds: CHO, CHON, CHOS, and CHONS. The ratios of H and C (H/C) and O and C (O/C) were low in RL, and the points distributed more dispersedly in the condensed aromatic (Figure 3a), indicating that the molecules in RL mainly exist in the condensed state of macromolecules. However, the points in the activated HA (AL, AL1, AL2, and AL3) were mainly concentrated in the region with high O/C and high H/C (the region relating to lignins, proteins, tannins, and carbohydrates) (Figure 3b−e).

3.4. Effect of HA on the Activation of Soil P. There was no significant difference in the activation coefficient between RL and CK treatment; however, AL can significantly increase the content of soil available P. Activated HAs can activate P via shifting soil pH; H^+ produced by HAs can reduce the concentration of Ca−P minerals and thus increase soluble P concentrations in calcareous soil.[30] The range of activation coefficients in AL (AL, AL1, AL2, and AL3) treatments was 0.39−0.81% (Figure 4A). The activation of soil legacy P was closely related to the molecular weight of HA. The activation of P by small molecular weight HA was more effective. The activation effect of AL1, AL2, and AL3 during the culture period (60 days) reached its maximum at 20 days (Figure 4A). The activation efficiency of soil P was closely related to the composition of HA (Figure 4B). Polymeric aromatic compounds were negatively correlated with the efficiency of P activation, while HA with high tannic acid and amino acid content had higher activation efficiency for P (Figure 4B). The low molecular weight HA could perhaps improve the activity of phosphate solubilizing microorganisms and thereby increase the soil available P.[31] Moreover, low molecular weight organic acids may also promote the release of insoluble organic P.[32] However, the detailed mechanisms need further study.

3.5. Effect of HA on Germination of *Lactuca sativa*. The rate of seed germination tended to be stable after 3 days. The higher seed germination rate (94%) was observed in S2 culture medium, while an 88% germination rate was observed in the control treatment (Figure 5a). The molecular weight of HA showed an antagonistic effect on the germination rate. The higher germination rate was recorded in the smaller molecular weight HA treatment. Similarly, Jing et al. investigated that the low molecular weight HA can promote the uptake of nutrients by a maize crop.[33] In addition, HA with 300 mg/L carbon concentration was more favorable for *Lactuca sativa* than that of 100 and 500 mg/L. The highest seed germination rate was observed in S2 culture medium, suggesting that the smaller molecular weight HA with a concentration of 300 mg/L can obviously promote the seed germination. The length of shoots observed after 7 days was shown in Figure 5. The length of germinated seedling grown in S2 culture medium was 66.02% longer than that of CK (Figure 5b), probably due to low molecular weight HA directly having a positive effect on the transcription of root genes and long-term effects on gene expression in shoots.[34] The HA solution of low molecular weight was more favorable to promote the bud length of *Lactuca sativa* seeds under the same carbon concentration. The optimum concentration was 300 mg/L for germination.

3.6. Effect of HA on Growth of *Lactuca sativa*. The height and fresh weight of *Lactuca sativa* were measured in all treatments after 30 days. The plant height in S2 culture medium was 171.54% higher than that in CK (Figure 6a). The "S" culture medium was more favorable than "M" and "L" for the growth of *Lactuca sativa* seedlings. 500 mg/L HA solution has an inhibitory effect on seedling growth (Figure 6a). The plant weight also reflected the same phenomena after 30 days (Figure 6b). In addition, all HA medium had a pH range between 6.3 and 6.8, which was a suitable pH for the growth of *Lactuca sativa* (Figure 7).

3.7. Effect of HA on Root System. HA significantly affected the root system of *Lactuca sativa*. The root system of plants grown in S2 culture medium was healthier. The total root length, surface area, number of root tips, and number of root branches in S2 culture medium were about 2.27, 2.00, 4.00, and 5.11 times that in CK treatment, respectively (Table 1). The optical micrograph of root tip cells for CK, S1, S2, S3, M2, and L2 treatments are shown in Figure 8. The plants grown in S2 culture medium show an expansion in the volume of the cell, and the cells are arranged regularly and loosely (Figure 8). Moreover, the developed root system of *Lactuca sativa* treated with small molecule HA further helped the *Lactuca sativa* to absorb more P.[35]

Quite interestingly, HA with small molecules can enter the root cells and promote the growth of root tip cells, which made the root tip cells larger and arranged loosely and tidy. Thus, it

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

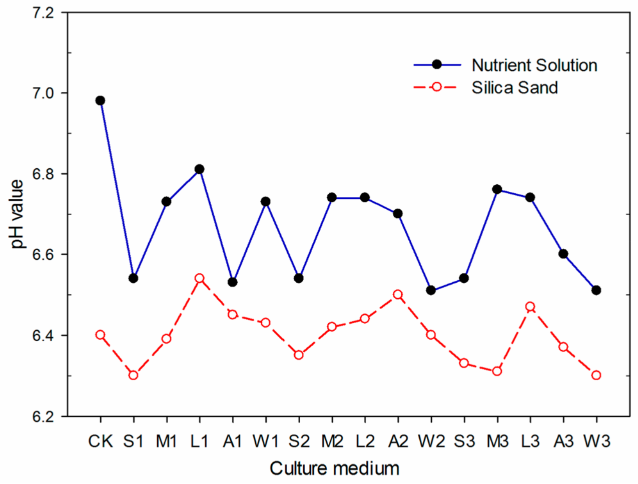


Figure 7. Effect of HA on the pH of silica sand and culture medium.

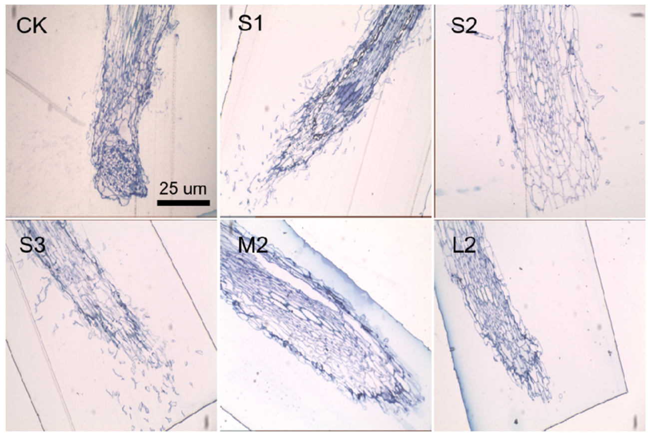


Figure 8. Micrograph of *Lactuca sativa* root tip cells under different culture media. CK stands for control treatment. S, M, and L represent the HA with molecular weights less than 10 000, 10 000−50 000, and greater than 50 000 D, respectively.

made the internal osmotic pressure higher, which is beneficial to the absorption of water and nutrients. On the other hand, HA with small molecules contain many hydrophilic groups, carboxylic acid, aromatic C=C double bond, hydrogen bond associated carbonyl —C=O—, $-NO_2$, C−O, and strongly active aromatic nucleus and other structures. HA with small molecules has more C−O and $-NO_2$ groups, which actively promote plant metabolism.[36,37]

Overall, HA can improve the utilization efficiency of soil P by *Lactuca sativa* due to activating soil legacy P and improving the growth of the root system.

4. CONCLUSION

In this study, lignite-based HA was prepared by ball milling under a KOH environment. Subsequently, three different HAs were separated from activated HA according to the molecular weight. HA with different molecular weights contains different structures. The activation efficiency of HA on soil P was greatly improved with the content of small molecules increased. In addition, HA with low molecular weight had a promoting effect on the root system of *Lactuca sativa*, which was beneficial for roots to absorb more P.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07528.

Carbon concentration in different culture solutions (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Jialong Lv − *College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China;* orcid.org/0000-0001-9337-8376; Email: ljlll@nwafu.edu.cn

Table 1. Effects of HA on the Root Morphology of *Lactuca sativa*

culture medium[a]	length (cm)[b]	surface area (cm^2)[b]	average diameter (mm)[b]	volume (cm^3)[b]	number of tips[b]	number of branches[b]
CK	13.5 ± 0.4j	1.6 ± 0.10g	0.4 ± 0.02ab	0.03 ± 0.00 d	45.0 ± 4.33g	46.0 ± 16.74e
S1	32.7 ± 1.17b	3.3 ± 0.27b	0.3 ± 0.01abc	0.38 ± 0.12 abc	176.0 ± 5.51bc	155.0 ± 18.33c
M1	23.8 ± 1.58cde	2.7 ± 0.09 cd	0.4 ± 0.01abc	0.06 ± 0.02d	121.0 ± 25.65de	116.0 ± 4.93 cd
L1	14.4 ± 0.74ij	1.7 ± 0.13g	0.4 ± 0.02ab	0.05 ± 0.00 d	62.0 ± 11.22fg	68.0 ± 25.31de
A1	17.7 ± 0.48ij	2.1 ± 0.11efg	0.4 ± 0.01abc	0.05 ± 0.22 d	55.0 ± 11.06fg	63.0 ± 18.67de
W1	14.1 ± 0.21ij	1.7 ± 0.08g	0.4 ± 0.01ab	0.02 ± 0.00d	62.0 ± 3.67fg	61.0 ± 10.93de
S2	44.2 ± 2.22a	4.8 ± 0.29a	0.3 ± 0.02abc	0.66 ± 0.26a	225.0 ± 29.69a	281.0 ± 48.91a
M2	27.4 ± 1.57c	2.7 ± 0.18c	0.3 ± 0.01c	0.58 ± 0.17ab	150.0 ± 19.22 cd	119.0 ± 6.43 cd
L2	16.7 ± 0.33ghij	2.1 ± 0.09efg	0.4 ± 0.02a	0.41 ± 0.08abc	64 ± 0.41fg	68.0 ± 17.85de
A2	22.4 ± 0.35def	2.4 ± 0.05cde	0.3 ± 0.00bc	0.14 ± 0.01 cd	105.0 ± 9.45def	99.0 ± 1.67de
W2	19.6 ± 1.16fgh	2.1 ± 0.08efg	0.3 ± 0.01abc	0.41 ± 0.03 abc	95.0 ± 12.86efg	88.0 ± 4.93de
S3	32.2 ± 1.41b	3.5 ± 0.03b	0.02 ± 0.02bc	0.31 ± 0.05bcd	208.0 ± 6.06ab	212.0 ± 18.41b
M3	24.4 ± 1.93 cd	2.7 ± 0.23 cd	0.3 ± 0.01abc	0.26 ± 0.02 cd	88.0 ± 18.59efg	118.0 ± 20.22 cd
L3	19.2 ± 0.28fgh	2.2 ± 0.15def	0.4 ± 0.02abc	0.14 ± 0.01 cd	77.0 ± 4.37efg	87.0 ± 5.03de
A3	20.5 ± 1.37efg	2.2 ± 0.15def	0.3 ± 0.03abc	0.20 ± 0.01 cd	77.0 ± 18.10efg	82.0 ± 5.61de
W3	16.2 ± 1.23hij	1.8 ± 0.24fg	0.3 ± 0.02abc	0.08 ± 0.02d	82.0 ± 4.81efg	80.0 ± 10.35de

[a]CK represents deionized water. Letters S, M, and L were HA with <10 000, 10 000−50 000, and >50 000 kDa, respectively. Letters W and A indicate the weathered lignite and activated raw HA without grading, respectively, and the carbon concentrations of culture medium are labeled 1 (100 mg/L), 2 (300 mg/L), and 3 (500 mg/L); e.g., L3 represents the culture medium (carbon concentration was 500 mg/L) configured by >50 000 kDa HA. [b]Different letters within the same column indicate a significant difference at the $P < 0.05$ level. The value after the ± is SE.

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846

Authors

Xiaoqi Liu − *College of Natural Resources and Environment, Northwest A&F University, Yangling, Shaanxi 712100, China*

Xuejiao Zhao − *College of Chemistry and Material Science, Shandong Agricultural University, Tai'an, Shandong 271018, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07528

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the National Key R&D Program of China (Grant No. 2017YFD0200205) and The Project of Shaanxi Agricultural Science and Technology Innovation Drive (NYKJ-2020-YL-21).

■ REFERENCES

(1) Simpson, R. J.; Oberson, A.; Culvenor, R. A.; Ryan, M. H.; Veneklaas, E. J.; Lambers, H.; Lynch, J. P.; Ryan, P. R.; Delhaize, E.; Smith, F. A.; Smith, S. E.; Harvey, P. R.; Richardson, A. E. Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. *Plant Soil* **2011**, *349*, 89−120.

(2) Smil, K. V. Phosphorus in the environment: natural flows and human interferences. *Annu. Rev. Environ. Resour* **2000**, *25*, 53−88.

(3) Gustafsson, J. P.; Mwamila, L. B.; kergoat, K. The pH dependence of phosphate sorption and desorption in Swedish agricultural soils. *Geoderma* **2012**, *189−190*, 304−311.

(4) Sattari, S. Z.; Bouwman, A. F.; Giller, K. E.; van Ittersum, M. K. Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 6348−6354.

(5) Choudhury, A. T.; Kennedy, I. R.; Ahmed, M. F.; Kecskés, M. L. Phosphorus fertilization for rice and control of environmental pollution problems. *Pak. J. Biol. Sci.* **2007**, *10*, 2098−2105.

(6) Zhu, J.; Li, M.; Whelan, M. Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: A review. *Sci. Total Environ.* **2018**, *612*, 522−537.

(7) de Campos Bernardi, A. C.; de Oliveira, C. R. Improved alfalfa phosphate utilization using zeolite amendments in low pH soil. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 1307−1317.

(8) Yang, F.; Sui, L.; Tang, C.; Li, J.; Cheng, K.; Xue, Q. Sustainable advances on phosphorus utilization in soil via addition of biochar and humic substances. *Sci. Total Environ.* **2021**, *768*, 145106.

(9) DeForest, J. L.; Dorkoski, R.; Freedman, Z. B.; Smemo, K. A. Multi-year soil microbial and extracellular phosphorus enzyme response to lime and phosphate addition in temperate hardwood forests. *Plant Soil* **2021**, *464*, 391−404.

(10) Hayes, M. H. B.; Swift, R. S.; Wardle, R. E.; Brown, J. K. Humic materials from an organic soil: A comparison of extractants and of properties of extracts. *Geoderma* **1975**, *13*, 231−245.

(11) Francioso, O.; Sànchez-Cortés, S.; Tugnoli, V.; Marzadori, C.; Ciavatta, C. Spectroscopic study (DRIFT, SERS and 1H NMR) of peat, leonardite and lignite humic substances. *J. Mol. Struct.* **2001**, *565−566*, 481−485.

(12) Audette, Y.; O'Halloran, I. P.; Paul Voroney, R. Kinetics of phosphorus forms applied as inorganic and organic amendments to a calcareous soil. *Geoderma* **2016**, *262*, 119−124.

(13) Wang, Y.; Whalen, J. K.; Chen, X.; Cao, Y.; Huang, B.; Lu, C.; Shi, Y. Mechanisms for altering phosphorus sorption characteristics induced by low-molecular-weight organic acids. *Can. J. Soil Sci.* **2016**, *96*, 289−298.

(14) Shen, Y.; Jiao, S.; Ma, Z.; Lin, H.; Gao, W.; Chen, J. Humic acid-modified bentonite composite material enhances urea-nitrogen use efficiency. *Chemosphere* **2020**, *255*, 126976.

(15) Afzal, M. Z.; Yue, R.; Sun, X.-F.; Song, C.; Wang, S.-G. Enhanced removal of ciprofloxacin using humic acid modified hydrogel beads. *J. Colloid Interface Sci.* **2019**, *543*, 76−83.

(16) Tang, Y.; Wang, X.; Yang, Y.; Gao, B.; Wan, Y.; Li, Y.; Cheng, D. Activated-lignite-based super large granular slow-release fertilizers improve apple tree growth: synthesis, characterizations, and laboratory and field evaluations. *J. Agric. Food Chem.* **2017**, *65*, 5879−5889.

(17) Doskočil, L.; Grasset, L.; Válková, D.; Pekař, M. Hydrogen peroxide oxidation of humic acids and lignite. *Fuel* **2014**, *134*, 406−413.

(18) Vlčková, Z.; Grasset, L.; Antošová, B.; Pekař, M.; Kučerík, J. Lignite pre-treatment and its effect on bio-stimulative properties of respective lignite humic acids. *Soil Biol. Biochem.* **2009**, *41*, 1894−1901.

(19) Valdrighi, M. M.; Pera, A.; Scatena, S.; Agnolucci, M.; Vallini, G. Effects of humic acids extracted from mined lignite or composted vegetable residues on plant growth and soil microbial populations. *Compost Sci. Util.* **1995**, *3*, 30−38.

(20) Gau, R. J.; Yang, H. L.; Chow, S. N.; Suen, J. L.; Lu, F. J. Humic acid suppresses the LPS-induced expression of cell-surface adhesion proteins through the inhibition of NF-kappaB activation. *Toxicol. Appl. Pharmacol.* **2000**, *166*, 59−67.

(21) Šerá, B.; Novák, F. The effect of humic substances on germination and early growth of Lamb's quarters (Chenopodium album agg.). *Biologia* **2011**, *66*, 470−476.

(22) Šerá, B.; Novák, F. Stimulation of seed germination and early growth by humic substances on poppy, pepper, rape, and hemp. *Biologia* **2022**, *77*, 641−648.

(23) Khalili, S.; Khandan-Mirkohi, A. Humic acid improves morpho-physiological and biochemical traits of Phyla nodiflora. *Acta Physiol. Plant.* **2021**, *43*, 133.

(24) Kurochkina, G. N. The Effect of humic acid adsorption on the coagulation stability of soil suspensions. *Eurasian Soil Sci.* **2020**, *53*, 62−72.

(25) Yang, M. H.; Zhang, L.; Xu, S. T.; McLaughlin, N. B.; Liu, J. H. Effect of water soluble humic acid applied to potato foliage on plant growth, photosynthesis characteristics and fresh tuber yield under different water deficits. *Sci. Rep.* **2020**, *10*, 7854.

(26) Tang, Y.; Yang, Y.; Cheng, D.; Gao, B. Value-Added Humic Acid Derived from Lignite Using Novel Solid-Phase Activation Process with Pd/CeO2 Nanocatalyst: A Physiochemical Study. *ACS Sustainable Chem. Eng.* **2017**, *5*, 10099−10110.

(27) Rivas, M. E. Ball Milling Towards Green Synthesis: Applications, Projects, Challenges. *Johnson Matthey Technology Review.* **2016**, *60*, 148−150.

(28) Lu, M.; Zhang, Y.; Su, Z.; Jiang, T. The NMR and spectral study on the structure of molecular size-fractionated lignite humic acid. *Resources, Environment and Sustainability* **2020**, *2*, 100004.

(29) Pantano, G.; Santos, A.; Bisinoti, M. C.; Moreira, A. B. Spectroscopic characterization of humic substances isolated from sediment of an area of sugarcane cultivation. In *Functions of Natural Organic Matter in Changing Environment*; Xu, J. M., Wu, J. J., He, Y., Eds.; Springer: Dordrecht, 2013; pp 209−214.

(30) Hue, N. V. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. *Soil Sci.* **1991**, *152*, 463−471.

(31) Turner, B. L. Resource partitioning for soil phosphorus: a hypothesis. *J. Ecol.* **2008**, *96*, 698−702.

(32) Giles, C. D.; Hsu, P. C.; Richardson, A. E.; Hurst, M. R. H.; Hill, J. E. Plant assimilation of phosphorus from an insoluble organic form is improved by addition of an organic anion producing Pseudomonas sp. *Soil Biol. Biochem.* **2014**, *68*, 263−269.

(33) Jing, J.; Zhang, S.; Yuan, L.; Li, Y.; Lin, Z.; Xiong, Q.; Zhao, B. Combining humic acid with phosphate fertilizer affects humic acid structure and its stimulating efficacy on the growth and nutrient uptake of maize seedlings. *Sci. Rep.* **2020**, *10*, 17502.

(34) Mora, V.; Olaetxea, M.; Bacaicoa, E.; Baigorri, R.; Fuentes, M.; Zamarreño, A. M.; Garcia-Mina, J. M. Abiotic stress tolerance in

plants: exploring the role of nitric oxide and humic substances. In *Nitric Oxide in Plants: Metabolism and Role in Stress Physiology*; Khan, M. N., Mobin, M., Corpas, F. J., Eds.; Springer: Cham, 2014; pp 243−264.

(35) Sun, B. K.; Tanji, Y.; Unno, H. Extinction of cells of cyanobacterium Anabaena circinalis in the presence of humic acid under illumination. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 823−828.

(36) Zhang, S.; Su, J.; Ali, A.; Zheng, Z.; Sun, Y. Enhanced denitrification performance of strain YSF15 by different molecular weight of humic acid: Mechanism based on the biological products and activity. *Bioresour. Technol.* **2021**, *325*, 124709.

(37) Zherebtsov, S. I.; Malyshenko, N. V.; Votolin, K. S.; Shpakodraev, K. M.; Ismagilov, Z. R. Biological activity of native and modified humic acids. *Solid Fuel Chem.* **2020**, *54*, 191−195.

https://doi.org/10.1021/acsomega.2c07528
ACS Omega 2023, 8, 6838−6846