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Importance of Sporopollenin Structure and Accessibility in the Sorption of Phenanthrene by Biota Spores and Pollens

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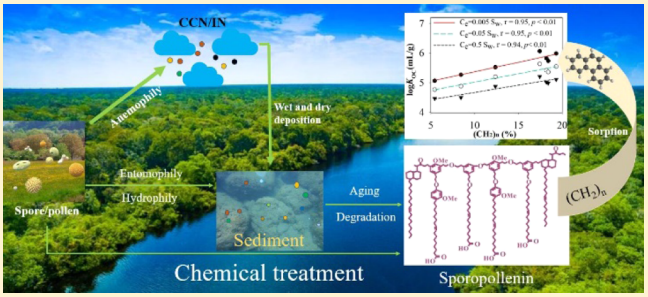
Supporting Information

ABSTRACT: Although spores/pollens are so abundant and ubiquitous in the environment, the role of these natural organic matter concerning fate and transport of organic pollutants in the environment is neglected. Lipid-free fractions and sporopollenins were isolated from seven spores/pollens collected from lower and higher biota species and were characterized by elemental analysis, CO_2 adsorption techniques, and advanced solid-state ^{13}C nuclear magnetic resonance spectroscopy. Then, the sorption isotherms of phenanthrene (Phen) on all the samples were investigated by a batch technique. The sporopollenins were a highly cross-linked polymer including alkyl carbon, poly(methylene) carbon, and aromatic carbon as well as oxygen functionalities; additionally, their sorption capacities (K_{oc}) for Phen reached up to 1 170 000 mL/g, suggesting that some of the sporopollenins were good biopolymeric sorbents for the removal of hydrophobic organic contaminants in aquatic media. A highly significant and positive correlation between the sorption capacity of Phen and the aliphaticity of the sporopollenins suggested that their structure was critical to Phen sorption. Meanwhile, the (O + N)/C atomic ratios and polar groups were significantly and negatively correlated with the sorption capacity of Phen, indicating that accessibility also played a significant role in the sorption process. Moreover, variable correlations between the sorption capacities (K_{oc}) and the micropore volumes of the spore/pollen fractions were observed. This study sheds light on the importance of the polarity, microporosity, and structure of sporopollenins in the sorption process of Phen.



INTRODUCTION

Pollen grains are male microgametophytes of seed plants that generate male gametes (sperm cells). Spores are the single-celled reproductive units of nonflowering plants (such as ferns and mosses), bacteria, fungi, and algae.[1] Pollens and fungal and fern spores are ubiquitous in the environment.[2,3] These particles are emitted directly from the biosphere to the atmosphere by wind and become primary biogenic aerosol particles, which can cause or enhance asthma and allergies, especially when toxic contaminants are attached.[4−6] In airborne sporopollen areas of the American South, atmospheric maximal pollen counts reach up to 15 000 grains/m^3 in winter, which would result in an estimated 15 grains cloud condensation nuclei (CNN)/m^3.[7] Because airborne pollens and fungal spores are not only generated in great abundance but also transported to high altitudes and for long distances,[8] they are significant media for the global transportation of air pollutants. Recently, the observed organic carbon-normalized sorption coefficient (K_{oc}) of phenanthrene (Phen) for the pollen fractions reached up to 670 000 mL/g.[9] Moreover, spores/pollens can act as CNN or ice nuclei to form cloud droplets.[10,11] Furthermore, a portion of these particles was deposited on water and land by wet precipitation and were well preserved in millions of years of strata, with full retention of their morphology in the form of fossil spores (sporopollenins).[12] The refractory nature of sporopollenins makes them important constituents in sedimentary organic carbon or "kerogen".[13,14]

The sporopollenin exine surface possesses phenolic, alkane, and carboxylic acid groups. Accordingly, sporopollenins would be a promising low-cost biosorbent for the removal of heavy metals from aquatic media.[15] Moreover, sporopollenins could also be a cost- and energy-saving alternative to activated carbon to remove environmental contaminants such as pesticides, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls from different sources such as waste water, soil, and sediments.[16] However, only two studies have been conducted on sporopollenins for the removal of hydrophobic organic contaminants (HOCs).[9,16] Although structures of sporopollenins from various lower and higher plant species have been investigated in previous studies,[17−19] the role of the

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Table 1. Weight and OC Contents in the LF, LP, and Sporopollenin Samples

samples	LF wt %	LF/OC %	LP wt %	LP/OC %	sporopollenin wt %	sporopollenin/OC %
G. lucidum spore	89.2	83.8	8.65	14.5	5.11	6.39
D. indusiate spore	99.0	97.5	0.714	0.69	0.848	1.34
L. japonicum spore	98.8	98.6	0.390	0.346	3.72	5.46
L. clavatum spore	91.9	88.5	6.93	10.8	10.9	15.3
C. japonica pollen	57.4	58.1	29.3	39.8	3.10	4.48
R. rugosa pollen	58.5	59.6	27.4	39.0	4.07	5.94
R. chinensis Mill pollen	54.9	54.8	37.1	42.2	2.23	3.27

structures in the sorption of HOCs has not been examined. Moreover, there is a relative dearth of quantitative information available regarding spore/pollen-based sorption of hydrocarbon substances.

Numerous investigators have reported that the chemical structure, microporosity, and polarity of natural organic matter (NOM) influence the sorption capacities of HOCs.[9,20−23] Whether the aliphaticity or aromaticity of NOM is related to the sorption capacities of HOCs is still controversial.[24] Previous studies have indicated the important role of the aromatic domains of NOM in the sorption affinities of HOCs.[25,26] However, the significant contribution of the aliphatic moieties of NOM in the interaction with HOCs has been emphasized in many studies.[20,27,28] The mobile $(CH_2)_n$-type aliphatic structures in NOM could be derived from resistant biopolymers.[29] In addition, the specific surface area (SSA) and micropore volumes of sedimentary organic carbon are dominant characteristics that influence their affinity and capacity for the sorption of HOCs.[23,30] The influence of micropores of sporopollenins has also been investigated together with its structures.

A recent study from our group on two pollens and their fractions (including sporopollenins) has revealed that the roles of alkyl and aromatic groups predominate in the sorption of Phen.[9] In this study, seven sporopollens from lower and higher biota species were chosen and sequentially fractionated into lipid-free (LF) residue and sporopollenin. Phen was selected to represent a typical class of polycyclic aromatic hydrocarbons. The major objectives of this study were[1] to quantify and compare the sorption affinities for Phen on seven sporopollens and their fractions from various plant species;[2] to examine the relationships among the sorption capacity of Phen, alkyl carbons, and aromatic moieties; and[3] to explore the role of micropores and polarity on the sorption of Phen.

MATERIALS AND METHODS

Spore/Pollen Samples and Isolation of Organic Fractions. The following species (OS) were selected for this experiment: Fungi—*Ganoderma lucidum* and *Dictyophora indusiate*; Pteridophyta—*Lygodium japonicum* and *Lycopodium clavatum*; and Angiospermae—*Camellia japonica, Rosa rugosa,* and *Rhus chinensis* Mill. Fungi and fern spores were purchased from CaSmart, China. Three kinds of pollens were purchased from the Guangdong Institute of Entomology. Herein, all chemicals used for this study were of analytical reagent grade or better. Based on previous separation methods,[9,31] a sequence of isolation steps was used to produce lipid-free (LF) fractions and sporopollenins. In brief, the samples were extracted with dichloromethane and methanol by using ultrasonic extraction techniques to remove lipids (LP). Next, polysaccharides and proteins were hydrolyzed with trifluoroacetic acid (TFA) and HCl. The isolation steps are described in detail in the Supporting Information, and the main steps for the isolation of the spore/pollen fractions are summarized in the flow chart (Figure S1).

Characterization of Spore/Pollen Organic Fractions. C, H, and N elemental analyses were conducted using a vario EL cube elemental analyzer (Elementar, Germany), and the oxygen content of NOM was analyzed with a vario EL III elemental analyzer (Elementar, Germany). During the elemental analyses experiments, each sample was weighed using the Mettler Toledo AL204 balance (Shanghai, China) or the Mettler Toledo XP6 microanalysis balance (Mettler Toledo International Inc., Greifensee, Switzerland) at room temperature. The CO_2 sorption isotherms were measured at 273 K in a relative pressure range of 1×10^{-6} to 0.03 using a Micromeritics ASAP 2460 surface area and pore size analyzer. The calculation of the micropore volume and the SSA was performed using the Dubinin−Radushkevich (DR) model and density functional theory (DFT), respectively, and the nanopore size distribution was determined with DFT.[32] Solid-state ^{13}C nuclear magnetic resonance (NMR) experiments were performed on a Bruker AVANCE III 400 spectrometer (Bruker, Germany) operating at 400 MHz ^{1}H and 100 MHz ^{13}C frequencies. The characterization method of NOM structures is described in detail in the Supporting Information.

Batch Sorption Experiments. The background solutions at pH 7 contained 5 mg/L $NaHCO_3$, 0.01 mol/L $CaCl_2$, and 200 mg/L NaN_3. The initial concentrations of Phen ranged from 10 to 1000 μg/L. The quantities of the investigated adsorbents were weighed to achieve the concentrations of sorbates at apparent equilibrium, which accounted for 30−70% of the initial concentrations. All the sorption isotherms were run according to the reported batch technique,[9] as described in detail in the Supporting Information.

Data Analysis. The sorption data for Phen on the original spores/pollens and their fractions were fitted to the log arithmetic form of the Freundlich isotherm model (FM)

$$\log q_e = \log K_F + n \log C_e \quad (1)$$

where q_e (μg/g) is the equilibrium solid-phase loading of the sorbate; C_e (μg/L) is the equilibrium aqueous concentration; K_F [(μg/g)/(μg/L)^n] is the Freundlich affinity coefficient; and parameter n is the Freundlich exponent. The low n values are indicative of greater surface heterogeneity and adsorption intensity. We described the concentration reliance of the affinity of the sorbate on the sorbents by computing K_{oc} at different concentrations (C_e) of aqueous solubility (S_w) of Phen (at $0.005S_w$, $0.05S_w$, and $0.5S_w$).

RESULTS AND DISCUSSION

Elemental Compositions. As shown in Table 1, the sporopollenin contents accounted for 0.848−10.9% of the bulk

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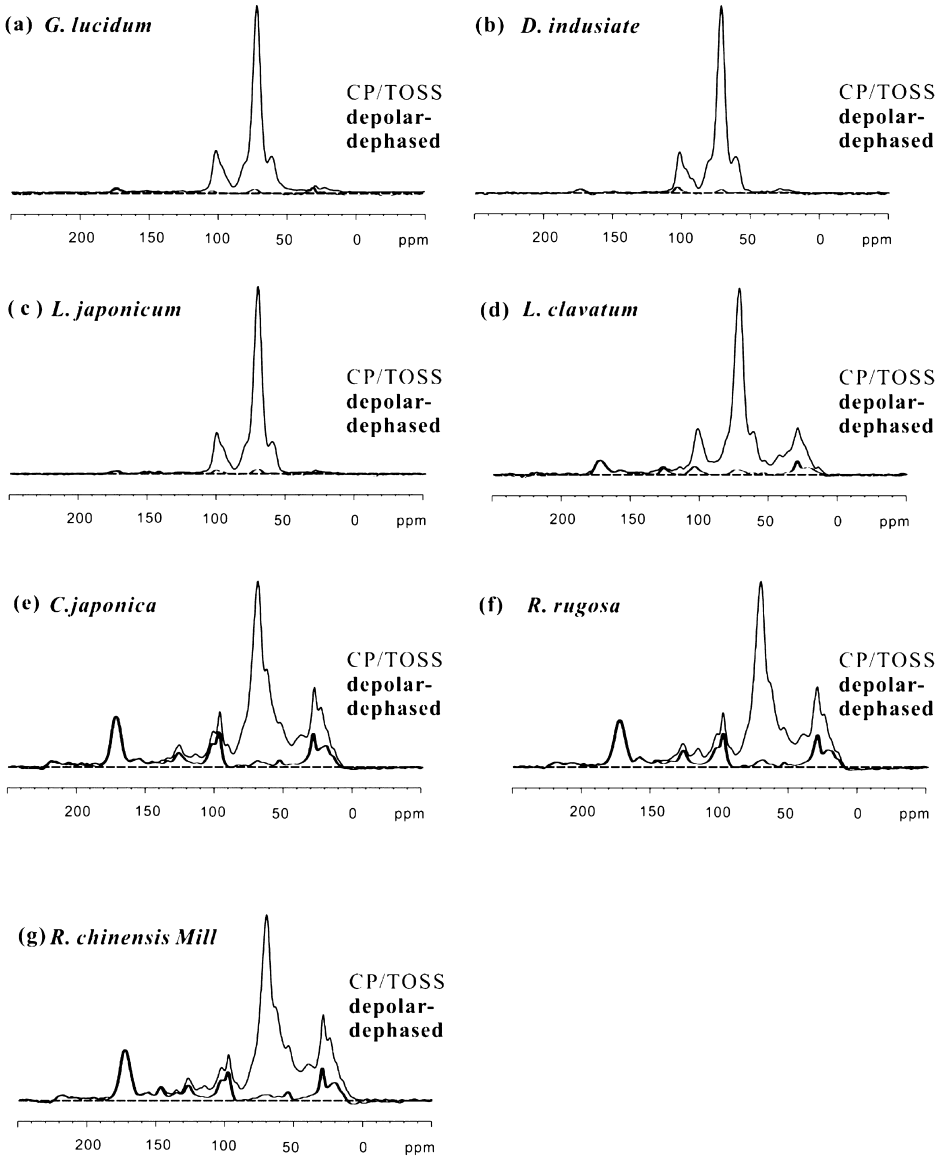

Figure 1. ^{13}C NMR spectra for the identification of functional groups of the seven bulk spores/pollens: thin lines indicate unselective CP/TOSS spectra; thick lines indicate the corresponding dipolar-dephased CP/TOSS spectra.

spores/pollens. The sporopollenin content of *Lycopodium* spore was much higher than that of any of the other samples. It is noted that the sporopollenin content of each of the lower plants is different from that of each of the higher plants. Previous investigations showed that the abundance of sporopollenin isolated via acetolysis was approximately 1% of the total mass of Populus species' pollen and reached up to 45% in spores of ferns.[33] In addition, the abundance of sporopollenin strongly affected the susceptibility of the sporopollen to oxidative destruction, and the susceptibility to oxidation increased when the sporopollenin content decreased.[34] *Lycopodium* spores were least susceptible to oxidation because they had the highest sporopollenin content in 22 different pollen/spore species, and this species was by far the most resistant.[34,35] The lipid yields of *G. lucidum*, *D. indusiate*, *L. japonicum*, and *L. clavatum* spores were 8.65, 0.714, 0.390, and 6.93%, respectively; previous studies reported that the lipid content of these spores was 10.0, 0.700, 0.438, and 9.42%, respectively.[36−39] The slight difference between our data and theirs might relate to the different extraction methods and the corresponding analysis. The lipid content of three pollens was much higher than that of the investigated spore samples, which is likely related to the interspecies differences. After the successive TFA and HCl hydrolyses, the organic carbon content of the NHC fractions decreased significantly (Table 1), indicating that the pollens/spores were rich in proteins, amino acids, and carbohydrates.[37,40]

The contents of C, H, and N of the pollens/spores and their fractions are listed in Table S1. The C contents of the seven bulk sporopollens ranged from 42.2 to 47.4%; their O contents ranged from 35.8 to 48.8%. The O content of pollens, ranging from 35.8 to 39.7%, was lower than that of spores, ranging from 43.2 to 48.8%. For the N content, the opposite result was observed in the bulk sporopollens. The elemental compositions of the LF fractions changed slightly after lipid removal, which was similar to the previous study.[9] It was noted that after removing carbohydrates and proteins, the H/C and (O + N)/C ratios of the sporopollenins decreased significantly compared with those of the LF fractions. In addition, the (O +

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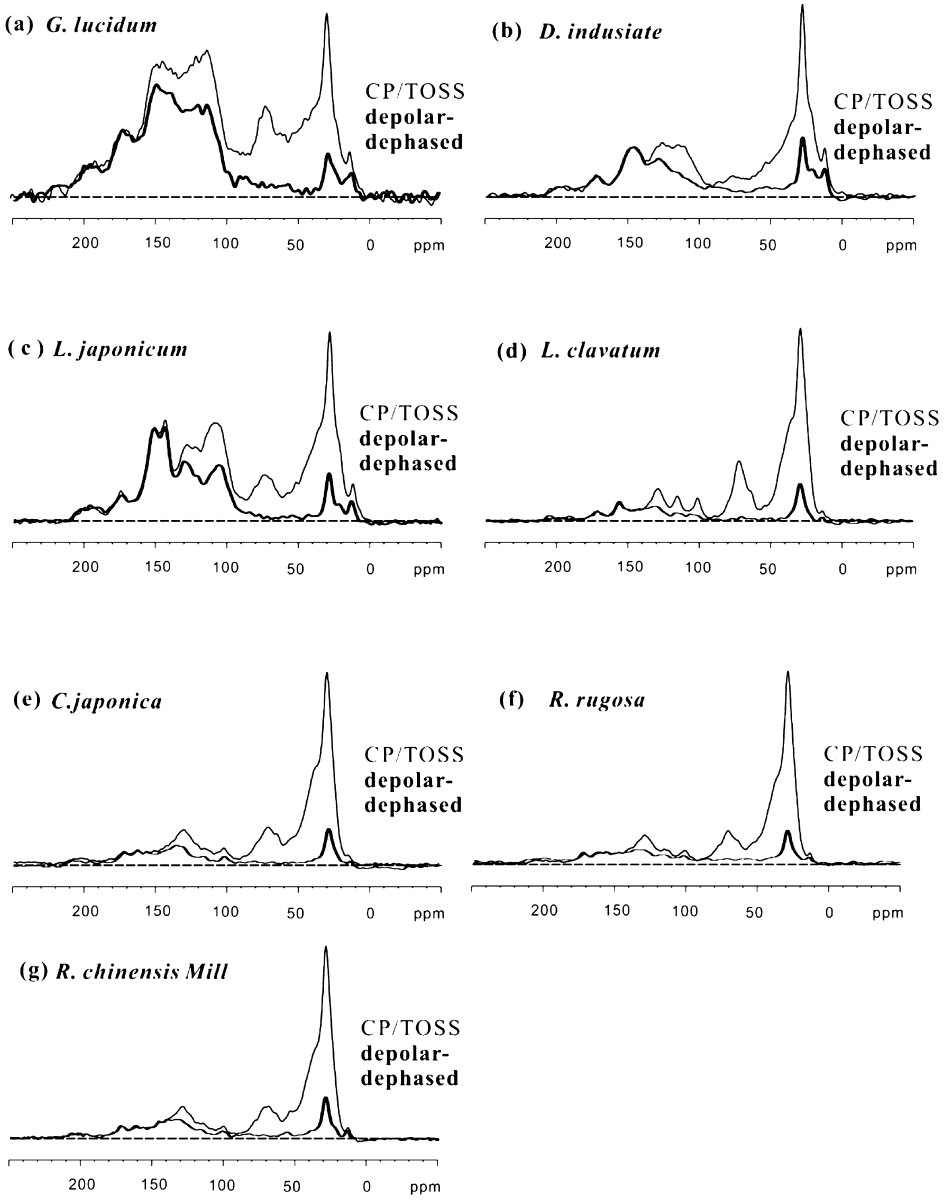

Figure 2. ^{13}C NMR spectra for the identification of functional groups of the seven sporopollenins: thin lines indicate unselective CP/TOSS spectra; thick lines indicate the corresponding dipolar-dephased CP/TOSS spectra.

N)/C ratios of three pollen sporopollenins are 0.210−0.230, which was similar to the previous study.[9] The organic matter with a low (O + N)/C ratio indicates high hydrophobicity, meaning that it is easily accessible by nonpolar compounds.

The nitrogen content was lower than 1.3% in the seven sporopollenin samples, suggesting that nitrogen cannot be regarded as an integral element of sporopollenin. Generally, sporopollenin is considered a polymer containing no nitrogen.[12,41,42] Sporadic studies pointing to the presence of N in sporopollenins may be due to contamination of the experimental material during preparation.[43,44] Compared with the LF fractions, sporopollenins of three pollens were almost devoid of nitrogen, which suggested that the proteins were efficaciously hydrolyzed by TFA and HCl. The C, H, and O content of *L. clavatum* sporopollenin was 66.3, 8.11, and 22.5%, respectively. Based on C_{90} units, the molecular formula of one sporopollenin was $C_{90}H_{132}O_{23}$, which was comparable to the results ($C_{90}H_{144}O_{27}$ and $C_{90}H_{124}O_{23}$) in other studies.[1,41]

Structures of Bulk Spores/Pollens and Sporopollenins. The ^{13}C cross polarization/total sideband suppression (CP/TOSS) NMR and CP/TOSS plus dipolar dephasing (CP/TOSS/DD) NMR experiments were performed to investigate the structures of the bulk spores/pollens and sporopollenins, as illustrated in Figures 1 and 2, respectively. Although the ^{13}C CP/TOSS data are semiquantitative, they can still be applied to emphasize the tendencies of generic chemical structural changes with other characterization parameters of NOM.[45] To compensate for the deficiencies of the CP/TOSS technology, we adopted the method previously reported by Huang et al.[46] to correct the data in this study. The corrected data and integration results are shown in Table S2. The corresponding ^{13}C CP/TOSS spectra after DD (thick lines, Figures 1 and 2) solely exhibited signals of nonprotonated carbon and mobile groups, containing rotating

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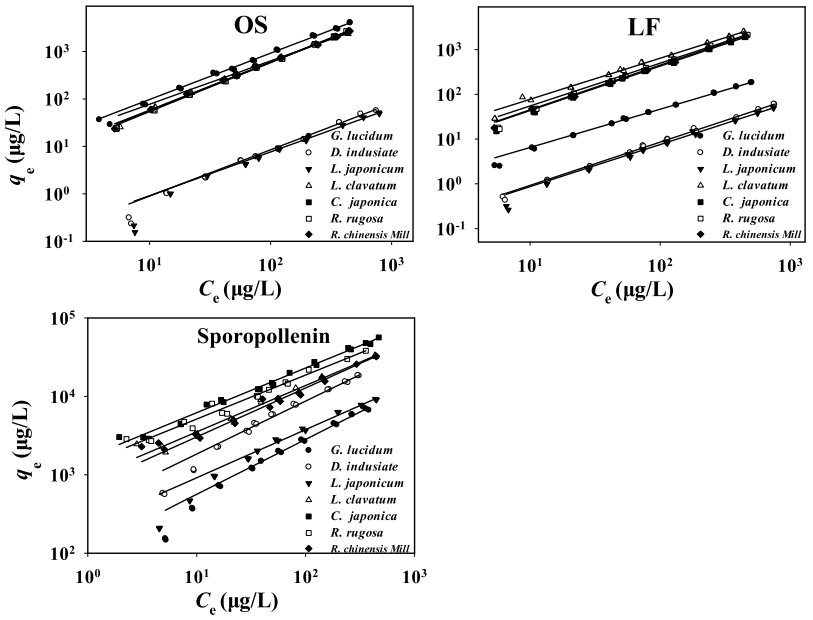

Figure 3. Freundlich sorption isotherms of Phen by the OS, LF, and sporopollenin fractions.

CH_3 groups, which had decreased C−H dipolar coupling owing to their fast motion. The ^{13}C CP/TOSS spectra with DD of the sporopollenins demonstrated that the aromatic region (110−165 ppm) nearly matched that in the corresponding unselective CP/TOSS spectra, indicating that most of the aromatic moieties of the sporopollenins were nonprotonated. As calculated from Table S2, protonated aromatic carbon (aromatic C−H) only accounted for 9.37−27.9% of all aromatic regions in the sporopollenins.

Carbohydrate-like C or other O-alkyls (60−91 ppm) were the dominant components in the bulk spores/pollens, accounting for 38.0−68.4% of all carbon. The O-alkyl content of pollens (38.0−39.6%) was lower than that of spores (50.1−68.4%). Compared with bulk pollens, no alkyl C peak (0−45 ppm) was visible in the spectra of three spores (except *L. clavatum*) (Figure 1a−c). In addition, prominent COO and N−C═O peaks were observed in the spectra of bulk pollens (Figure 1e−g). The spectra of two fungal spores (*G. lucidum* and *D. indusiata*) were virtually indistinguishable, and the same situation also occurred in the gymnosperm pollen. The structures of the pollens were moderately similar to the reported lotus and rape pollens but differed because of the possession of weaker O-alkyl peaks.[9] The removal of lipids or terpenoids, carbohydrates, and proteins from bulk spores/pollens resulted in a redistribution of the ^{13}C NMR signals in alkyl C, O-alkyl, and aromatic C regions. For instance, the signal intensities of alkyl C and aromatic groups significantly increased, whereas those of O-alkyl groups markedly decreased. The alkyl C−O content of *L. clavatum* sporopollenin (16.4%) was much higher than that of the other sporopollenins, ranging from 8.15 to 12.8%. *L. clavatum* was reported to be the most resistant species in most plants.[34,35] *L. japonicum* (*Equisetum*) sporopollenin demonstrated a considerable variety of both aliphatic and aromatic moieties, resulting in a spectrum that contained broad, overlapping peaks (Figure 2c). As shown in Table S2, the aromatic C content (37.0%) of *L. japonicum* sporopollenin was approximately twice that of another pteridophyte (*L. clavatum*) sporopollenin (18.0%). The above results were highly akin to the findings of Hemsley et al.[18] In addition, the $(CH_2)_n$ content (18.2%) of *L. clavatum* sporopollenin was much higher than that of *L. japonicum* sporopollenin (8.4%), even though both *L. japonicum* and *L. clavatum* are classified as "Fern Allies" in the traditional plant taxonomy. Similarly, Wilmesmeier et al.[19] also reported that the aliphatic signals of sporopollenins from the spores of *Equisetum arvense* (Equisetum) were extraordinarily lower than those of *Selaginella selaginoides* (Lycopodiaceae). The NMR spectra of sporopollenins in this investigation showed relative low contents of carbohydrate-like C or other O-alkyls (60−91 ppm), similar to the previous investigations.[17,19,44] Additionally, the selective removal of sugars (and other forms of easily hydrolyzable OM) with an increasing TFA concentration and reaction time was shown to be an efficient approach to reduce the potential for the formation of melanoidin-like material.[31] Thus, the TFA treatment at high temperatures did not change the structure of the sporopollenins.

Recently, a series of fern phylogenetic studies suggested that Lycopodiaceae had been excluded from the fern classification system;[47,48] thus, the prominent difference observed between *L. japonicum* and *L. clavatum* in this study was reasonable. *L. clavatum* sporopollenin differed from other sporopollenins in showing several clear peaks in the aromatic region. This characteristic was observed in the spectrum from *L. clavatum* sporopollenin illustrated by Hemsley et al.[18] However, the spectrum of *L. clavatum* sporopollenin showed a broad, unresolved peak in the aromatic region, similar to that reported by Guilford et al.[17] This discrepancy resulted from the differences in preparatory procedures.[18] The spectra of three pollen sporopollenins were remarkably similar to those of the reported lotus and rape pollens.[9] In addition, the alkyl C (including polymethylene C) content of the three pollen sporopollenins was much higher than that of spores (except *L. clavatum*), and the opposite situation was observed for aromatic C. These differences in the structures will significantly influence their affinities to Phen, which will be analyzed later.

Sorption Isotherms. Phen sorption isotherms for seven sporopollens and their fractions are shown in Figure 3. The Freundlich parameters estimated by the nonlinear least-square

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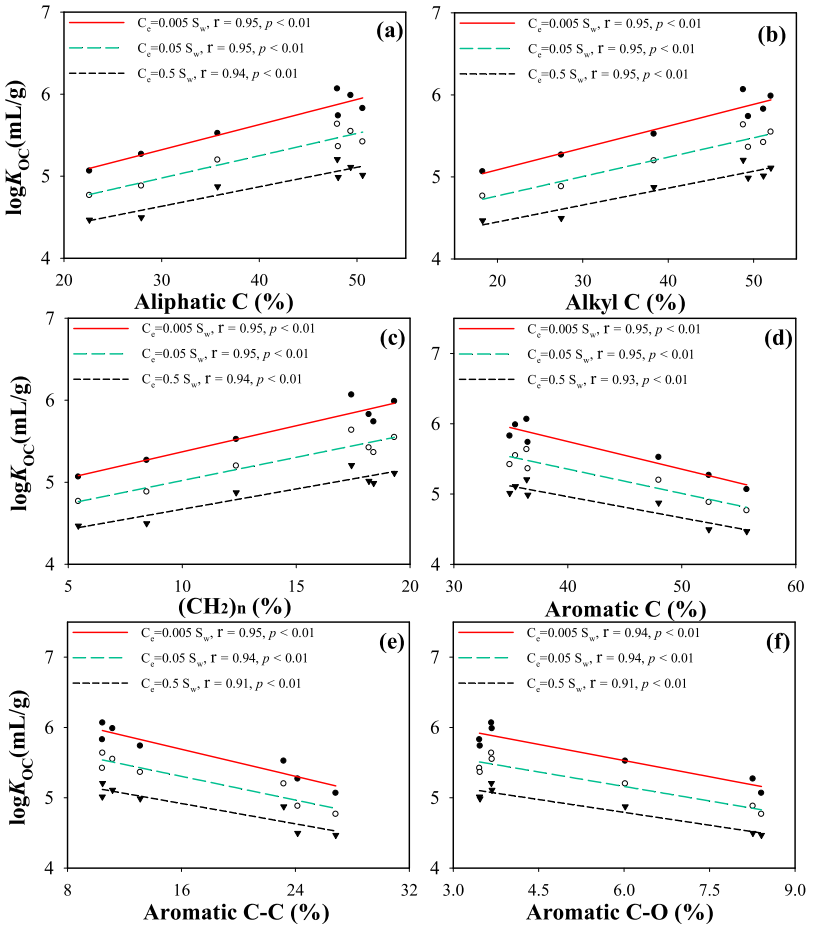

Figure 4. Correlations among log K_{oc} (mL/g) values and concentrations of aliphatic C, alkyl C, $(CH_2)_n$, aromatic C, aromatic C−C, and aromatic C−O derived from CP/TOSS ^{13}C NMR spectra of the sporopollenins (a−f).

regression method with Sigmaplot 12.0 (Systat Software, USA) for all isotherms are summarized in Table S3. The Freundlich model fits the data quite well for twenty-one sorbents (R^2 > 0.990). The results indicated that the sorption isotherms of bulk pollens were linear, and their nonlinear coefficient (n) values (1.01−1.03) were very similar to that of magnetized ragweed pollens (1.01) in the sorption of Phen in another study.[16] However, the n values of the bulk spores were slightly nonlinear (n = 0.914−0.975) and were slightly lower than the n value of bulk pollens. Significant nonlinearity was observed in the sporopollenins, and their n values were in the range 0.562−0.701 (Table S3), which were slightly lower than the n values of the reported two pollens (0.644 and 0.737).[9] The nonlinearity generally increased in the following sequence: original sample (OS) < LF fraction < sporopollenin. The nonlinearity factor n is related to sorption site energy distribution on the heterogeneous glassy or condensed NOM domain.[28,49] Thus, the smaller n values in the sporopollenins indicated a more condensed and rigid structure and a broader distribution of the sorption energy.

Log K_{oc} (mL/g) at C_e = 0.5S_w ranged from 2.20 to 4.29 for the bulk spores/pollens and from 2.21 to 4.09 for the LF fractions. The sorption of Phen on the bulk samples and the LF fractions generated almost linear isotherms, indicating that the partitioning was a primary sorption mechanism. After the extractable lipids were removed from the bulk spores/pollens, the K_{oc} of the LF fractions demonstrated only an insignificant change (except for *G. lucidum*), which suggested that extractable lipids may not be the dominant sorption medium for Phen in biosorbents. After the removal of proteins and carbohydrates, the highest K_{oc} (mL/g) at C_e = 0.5S_w was observed in the sporopollenins, and the average K_{oc} for the sporopollenins was 15.1 times higher than that of the LF fractions. The protein and carbohydrates containing more polar groups (e.g., carboxyl and hydroxyl) could provide substantial hydrophilic sites for water cluster formation at the sorbent surface through H-bonding, which limited HOCs from approaching the adsorption sites and partitioning on condensed domains, thus reducing their accessibility.[22] Moreover, the concentration-dependent OC-normalized sorption coefficient K_{oc} value of sporopollenins for Phen decreased as a function of C_e because of the isotherm nonlinearity. Regardless of C_e levels, the pollen sporopollenins exhibited much higher sorption affinity than spore sporopollenins (except *L. clavatum*), and the same trend was also observed for the LF fractions (Table S3). The significant difference in the sorption capacity might be due to their different structures and compositions of pollens/spores of lower and higher plant species. The single log K_{oc} of Phen for the pollen sporopollenins ranged from 5.74 to 6.07 mL/g at C_e = 5.6 μg/L, which was higher than that of the lotus pollen sporopollenins (5.68 mL/g),[9] and was practically comparable to that of modified black carbon (5.52−6.08 mL/g).[32] As in this laboratory experiment, producing a small quantity of sporopollenins is expensive. However, in a large-scale batch, the abovementioned experiment procedures may be modified

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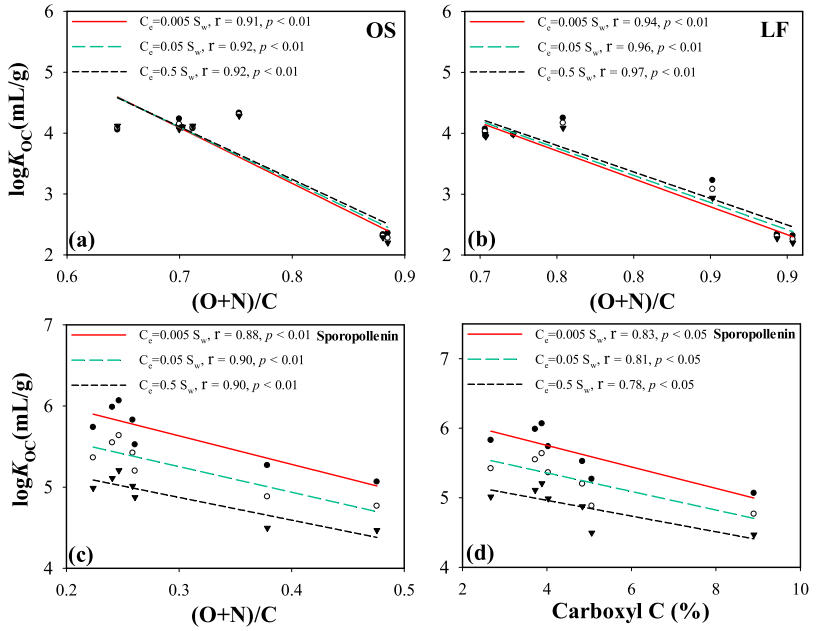

Figure 5. Correlations between log K_{oc} (mL/g) values and the polarity index [(N + O)/C] for the OSs, LF fractions, and sporopollenins (a–c). Correlation between log K_{oc} (mL/g) values and the concentration of carboxyl C derived from CP/TOSS ^{13}C NMR spectra of the sporopollenins (d).

to attain the low-cost requirements, such as designing assembly line and using industrial or chemical pure reagents. We also compared the maximum sorption capacity observed (1.17×10^6 mL/g) with that of commercial activated carbon or biochar. The maximum sorption capacity was comparable to that of any commercially available sorbents. More details about the abovementioned comparisons can be found in the Supporting Information. Therefore, the abovementioned results suggested that the investigated sporopollenins were potential low-cost biopolymeric sorbents for the removal of HOCs from aquatic media.

Impact of the Alkyl and Aromatic Structure on Phen Sorption. In this study, the relative role of aliphaticity and aromaticity of sporopollenins in the sorption of Phen was investigated. As discussed above, the alkyl C (including polymethylene C) content of three pollen sporopollenins was much higher than that of spores (except *L. clavatum*), and the same trend was also observed in their sorption capacities. The analysis of the Pearson correlation demonstrated that the log K_{oc} values of Phen were positively correlated with the $(CH_2)_n$ group, alkyl C, and aliphatic C content of the sporopollenins, as shown in Figure 4a–c. The role of aliphaticity in the sporopollenin can account for approximately 90.0% of the total sorption variability for Phen, which suggested that aliphaticity in the sporopollenin played a dominant role in Phen sorption. In addition, sporopollenins tended to exhibit a high sorption affinity for Phen as demonstrated in the aforementioned results. Therefore, the TFA treatment at high temperatures gave no impact on hydrolyzing alkene/alkane domains of sporopollenins. A previous study showed that the ^{13}C peak of pyrene was correlated with the aliphatic ^{1}H values of cutin, and the 2D ^{1}H–^{13}C heteronuclear correlation spectra demonstrated that the more hydrophobic aliphatic regions on cutin were preferable for pyrene sorption.[29] In addition, Mao et al.[20] noticed that the aliphatic carbon fraction, rather than the aromatic one, was significantly correlated with Phen sorption. The authors indicated that the sorption capacity of Phen was significantly correlated with the content of the amorphous nonpolar aliphatic domains. The hydrophobicity and relatively low density of the amorphous poly(methylene) regions were analogous to those of alkane solvents. The poly(methylene) domains were therefore proposed as an ideal environment for the partitioning of HOCs.[20]

A strong negative trend was observed by plotting the log K_{oc} values of Phen with aromatic C, nonprotonated aromatic carbon, and aromatic C–O groups (Figure 4d–f), which suggested that the sporopollenins rich in aromatic carbon in the pollens/spores exhibited weaker sorption capacity for Phen than did the sporopollenins rich in aliphatic carbon. Previous measurements showed that the aromaticity of young organic matter was inversely correlated with the K_{oc} values of Phen or pyrene.[27,28] The abovementioned results are different from those obtained from previous bulk pollens and their fractions reported by Zhang et al.;[9] their reported sorption capacities of Phen for bulk pollens were low because the aromaticity and aliphaticity of bulk pollens were intrinsically low, and the sorption capacities for the isolated fractions were higher because their aromaticity and aliphaticity were intrinsically higher. Thus, positive relationships among aliphatic and aromatic structures and sorption capacities for the OS and their fractions were observed in the reported literature. Moreover, the nonlinearity factors (n) were negatively correlated with concentrations of aliphatic C, alkyl C, and $(CH_2)_n$ groups (Figure S2a–c), and a positive correlation between n values and aromatic C or nonprotonated aromatic carbon was also observed (Figure S2d,e), which indicated the importance of aliphatic structures in the sporopollenins for isotherm nonlinearity. In addition, the analyses of the Pearson correlation demonstrated that the atomic ratio of hydrogen to carbon positively correlates with the sorption capacity of Phen and negatively correlates with the n values (Figure S3a,b), which again indicated that aliphaticity was crucial to the sorption of Phen by the sporopollenins. However, poor correlations between log K_{oc} of Phen by the bulk pollens/

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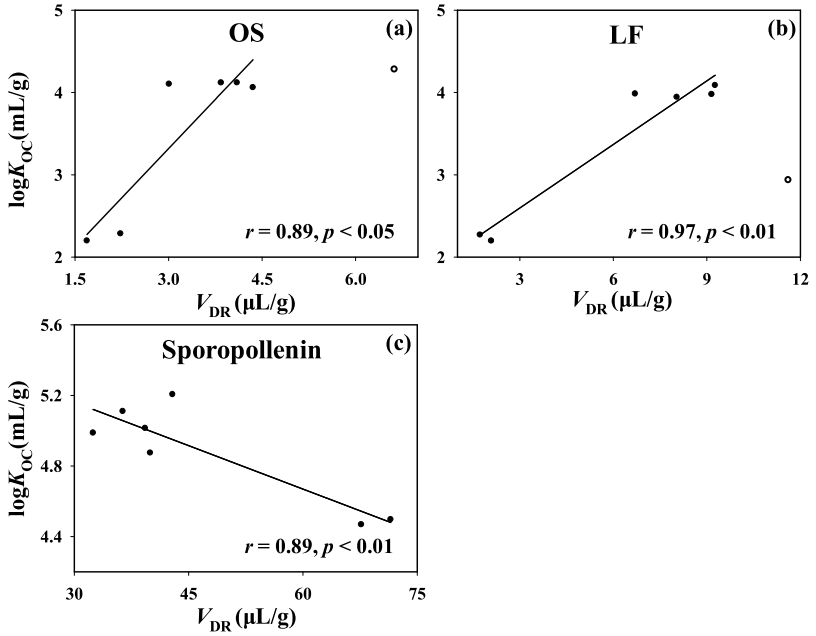

Figure 6. Correlations between V_{DR} (μL/g) and log K_{oc} (mL/g) at $C_e = 0.5S_w$ for the OS, LF, and sporopollenin fractions (a−c). One outlier (*G. lucidum*) is shown in open circles and is not included in the correlation equation.

spores and their aliphatic or aromatic components were observed (Figure S4a−d), which may be ascribed to the low aromatic or aliphatic C content and less accessibility to the aliphatic domains because of a large quantity of polar O-alkyl components (38.0−68.4%). Thus, this result might not seem surprising considering that the sorption capacity of sporopollenins was remarkably elevated because of the increased accessibility of aliphatic structures that were previously blocked by O-alkyl components.

Impact of Accessibility on Phen Sorption. The relationship between the polarity index [(N + O)/C] and log K_{oc} of Phen for the sporopollenins was significantly negative, as illustrated in Figure 5c. Negative correlations were also observed in the bulk spores/pollens and the LF fractions (Figure 5a,b). A similar trend was also observed in other plant biopolymers.[21] The polarity could be an important parameter to regulate the sorption activity for sporopollenins, as suggested by the negatively linear relationship between the sorption affinity and carboxyl C (165−190 ppm) content (Figure 5d). There was disagreement in the literature on whether the majority of carboxyl groups in NOM are connected directly to aliphatic chains or aromatic rings.[50] In this study, the aromatic C content of sporopollenins was significantly and positively related to its carboxyl C content (Figure S5), suggesting that the increased number of carboxylic groups adjacent to aromatic components led to the decreased sorption of Phen. The aromatic carbon of NOM, including carboxyl groups, might be more polar than the glassy and resistant aliphatic carbon.[28] In addition, the nonlinearity factors (n) were positively correlated with the carboxyl C content (Figure S6), which indicated that the sporopollenins rich in polar structures preferentially generated linear isotherms.

After removal of lipids or terpenoids, carbohydrates, and proteins from the bulk spores/pollens, the O-alkyl and total polar C content significantly decreased, and the sorption capacities of sporopollenins remarkably increased. The elevated affinity for Phen with decreasing polarity could be attributed to the better compatibility (similarity of solubility parameters) of the organic matter partition phase with nonpolar sorbate molecules.[21] Negative correlations among log K_{oc} of Phen and the alkyl C−O and polar C content are highly significant for the bulk spores/pollens and sporopollenins ($p < 0.001$, Figure S7a,b). The sorption nonlinearity factors (n) were positively related to the alkyl C−O and polar C content for the bulk spores/pollens and the sporopollenins ($p < 0.01$, Figure S7c,d). These correlations again indicated that accessibility was a crucial factor affecting the sorption of Phen by the biopolymer samples.

Microporosity of the Samples and Its Impact on Phen Sorption. As mentioned above, sporopollenins showed an excellent sorption capacity toward Phen, and sporopollenins have also been noted as a porous material in the study.[41] However, few articles have described the role of the porous characteristics of sporopollenins in the sorption of Phen. Thus, this study presented a preliminary attempt. The CO_2 sorption isotherms of these sporopollens and their fractions are illustrated in Figure S8. The micropore volumes and CO_2-SSA values ranged from 1.69 to 71.5 μL/g and from 4.22 to 178 m^2/g, respectively, based on the DR model, as shown in Table S4. The SSA and V_o (or V_{DFT}) of the isolated fractions generally increased in the sequence OS < LF fraction < sporopollenin, indicating that the micropore volumes and surface areas increased after the removal of lipids, carbohydrates, and proteins. *G. lucidum* spores consistently showed a larger SSA than each of the other bulk samples, LF fractions, and sporopollenins. The micropore size distribution (PSD) for the samples is illustrated in Figure S9. The PSD calculated by the DFT model indicated that the predominant micropore volume contribution was from the pores 0.4−0.7 nm in width. The different sporopollens showed different pore size characteristics in the OS and LF fractions (Figure S9a,b). However, a uniform pore size characteristic was observed in the sporopollenins (Figure S9c). The sporopollenins presented a similar micropore size distribution ranging from 0.4 to 0.7 nm and from 0.7 to 0.9 nm in width, which may be related to

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the similar structures in the sporopollenins, with alkyl and aromatic C as the major components.

The sorption capacities (log K_{oc}) of the tested bulk spores/pollens and LF fractions (except the outlier of *G. lucidum*) were significantly and positively related to their micropore volumes based on the DR model, as illustrated in Figure 6a,b, which indicated that the micropore filling mechanism was crucial to the sorption of Phen by NOM.[23,30] However, the sorption capacities of the tested sporopollenins were negatively related to their micropore volumes ($p < 0.01$, Figure 6c). One reason is that the aromaticity of sporopollenins was the highest in all the fractions, compared with the OS and LF fractions; the spacings between aromatic interlayers may not be penetrated by Phen molecules, but the smaller CO_2 molecules could enter all the aromatic interlayers. Therefore, the sorption capacities of the sporopollenins eventually decreased with increasing aromaticity. Another reason is the difference between the dry-state, CO_2-determined microporosity and the wet-state, Phen-available microporosity on NOM. In dry conditions, the carboxylic groups (buried in inner micropores) on the edge of aromatic components were possibly conducive to adsorbing CO_2 molecules. However, in wet conditions, the carboxylic groups connected to aromatic components might prevent the Phen molecules from entering the micropores of sporopollenins that contained abundant aromatic carbon. The aforementioned phenomenon also explained why a strong negative trend was observed by plotting the log K_{oc} values of Phen with the aromatic C and nonprotonated aromatic C content (Figure 4d,e). Additionally, the DR model is used as earlier studies found that the micropore volumes from gas adsorption agree well with sorption of organic solutes.[51] Thus, we also evaluated the sorption of Phen on sporopollenins using the DR model. More details about the DR model can be found in the Supporting Information. The sorption isotherms of Phen were well fitted to the DR model (Table S5 and Figure S10). Similarly, we also found that the micropore volumes V_{DR} (μL/g) from the CO_2 gas adsorption were negatively related to the adsorption volumes (Q_0) of Phen calculated by the DR model (Figure S11), which is similar to that presented by the Freundlich model.

In summary, this study demonstrated the significantly high sorption capacity of sporopollenins derived from various pollens/spores for Phen. The K_{oc} for *C. japonica* sporopollenin reached up to 1 170 000 mL/g. The data reported in this study emphasized the importance of aliphatic components and accessibility of NOM in the sorption of Phen by regulating the compatibility of the sorbate to the sorbent. Moreover, the statistical analysis demonstrated that the sorption capacities were differentially correlated with the microporosity. This study helps to further understand the role of the various molecular interactions of NOM with Phen in the sorption process. More investigations are needed to confirm the sorption mechanism of sporopollenins by investigating other plant pollens/spores and other HOCs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b03911.

Additional details of the isolation of organic fractions; characterization method of NOM structures; sorption models; batch sorption experiments; flow chart for the separation of each organic fraction; Freundlich and the DR sorption isotherms of Phen; correlations among the sorption parameters and the structural parameters for the sporopollens and sporopollenins; correlations between V_{DR} and the Q_0 of Phen for the sporopollenin; correlation between concentrations of carboxyl C and aromatic C content; percentages of isolated fractions for the bulk sporopollens and their fractions; elemental compositions of the original sporopollens and their fractions; functional group percentages of the original sporopollens and sporopollenins; Freundlich isotherm parameters of the original sporopollens and their fractions; DR model parameters for the sorption of Phen on sporopollenins; and CO_2 adsorption results of the original sporopollens and their fractions (PDF)

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