

Analysis for Effect of Chemical Pollutants on Wetland Ecology

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Wetlands play an important role in maintaining ecological balance and promoting the development of surrounding economy. Chemical pollutants in industrial wastewater cause serious damage to the wetland ecosystem. For this, taking the polycyclic aromatic hydrocarbons (PAHs), one typical chemical organic pollutant in industrial wastewater as examples, this paper studies the effects of PAH on plants and microorganisms in wetlands. Then, the experimental simulation was conducted to study the degradation and absorption effects of wetland soil sediments and wetland plant (*Spartina alterniflora*) on PAHs, and also analyse the effects of PAHs on total biomass and microbial active enzymes in wetlands. The results show that the wetland ecosystem has significant absorption and degradation effects on chemical pollutants. The average removal rate of PHE is 26%, and the average removal rate of PYR is 20%; the content of PHE and PYR in leaves of *Spartina alterniflora* (*S. alterniflora*) is much smaller than that of root system, indicating that the root system of *S. alterniflora* is the main part of absorbing PAHs. PAHs pollution will cause serious damage to the distribution and content of microorganisms in the wetland. PHE causes the microbial content in the leaves of *S. alterniflora* to decrease by more than 35%, while PYR causes it to decrease by more than 26%. The plant-microbial combined treatment system in the wetland system can resist the harmful effects of chemical pollutants to a certain extent, and the bacteria in the plant roots are more sensitive to the response of chemical pollutants. This can provide a new research idea for the microbe-plant joint degradation of chemical pollutants in wetland ecosystems.

1. Introduction

Wetland is the third largest ecosystem in the world except forests and oceans. It has the functions of regulating climate, purifying environment, preventing floods and droughts, maintaining biodiversity and ensuring environmental and ecological balance (Hensel and Silliman, 2013). Meanwhile, various vegetations in the wetland can absorb the difficult degradation products such as water chemical pollutants and heavy metals etc. (Kulkarni et al., 2018). Wetlands play an important role in protecting the environment and promoting economic development.

In recent years, with the rapid development of industry, industrial and domestic wastewater has been directly discharged into rivers, lakes and seas, resulting in nutrient enrichment of water, and toxic metals and organic pollutants have been deposited in wet soil and nearby water bodies, seriously damaging the wetland ecosystems (Cuong et al., 2005; Liu et al., 2014). Polycyclic aromatic hydrocarbons (PAHs) are typical organic pollutants of water chemical pollutants, which are highly toxic, difficult to remove, and hydrophobic (Raza et al., 2013). It is of great practical significance to study the effects of PAHs on the wetland ecological environment, including the growth of animals and plants and also the changes in microbial activity (Wang et al., 2017; Kimbrough and Dickhut, 2006).

Studies have found that plants and microorganisms in the wetland system all play a role in removing PAHs. Plants use roots system to absorb organic matter such as PAHs and transfer them to the ground (Tian et al., 2016); microorganisms such as algae, rhizosphere bacteria, etc., are to degrade the absorbed PAHs into harmless organics or inorganics (Hong et al., 2008; Launen et al., 2008). Plant-microbe chemical contaminant removal systems have important implications for the migration, degradation and repair of PAHs (Oliveira et al., 2014; Watts, Ballesterio and Gardner, 2006; Khan et al., 2013).

Spartina alterniflora is a common plant in coastal wetlands with strong reproductive capacity and adaptability (Wan et al., 2009; Gao et al., 2014; Hong et al., 2015). Some researchers have applied *S. alterniflora* to the

migration and transformation of various inorganic elements (C, P, S, etc.) in wetland systems, but at present, the research on absorption and migration effect of *S. alterniflora* on organic pollutants such as PAHs is still largely in blank state.

Taking the polycyclic aromatic hydrocarbons (PAHs), one typical chemical organic pollutant in industrial wastewater as examples, this paper studies the effects of PAH on plants and microorganisms in wetlands. The experimental simulation was conducted to study the degradation and absorption effects of wetland soil sediments and wetland plant (*Spartina alterniflora*) on PAHs, and also analyse the effects of PAHs on total biomass and microbial active enzymes in wetlands. The research results can provide a new research idea for the microbe-plant joint degradation of chemical pollutants in wetland ecosystems.

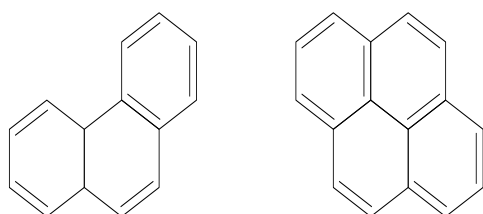
2. Experimental materials and methods

2.1 Experiment material:

For the elementary substance of typical PAHs: phenanthrene (PHE) and hydrazine (PYR), their chemical structures are shown in Figure 1. Deuterated PAHs: naphthalene-d8, 蒽 acenaphthene-d10, phenanthrene-d10, and chrysene-d12; the remaining reagents were of analytical pure. *S. alterniflora* and wetland sediments were taken from a wetland reserve in the southern provinces of China.

2.2 Preparation of test samples

After preparing certain concentration of PHE and PYR mixed solution, the solution was poured into 200g of sediment, which mixture was then added to 50kg of sediment and mixed thoroughly. Then, it stood for 15d in the room before the PHE and PYR concentration in the mixed sediment was measured. Besides, a number of *S. alterniflora* were selected and planted into the culture pots, and the rhizosphere (RS) and non-rhizosphere (NRS) parts were labelled. The planted *S. alterniflora* was divided into three groups for comparison. The first group of the sediment was not added with PHE and PYR, as the control group; the second group was added with 10 mg/kg PHE and 100 mg/kg PHE respectively, which were recorded as PHE10 and PHE100; the third part was added with 10mg/kg PYR and 100mg/kg PYR, respectively, as PYR10 and PYR100. The culture environment was room temperature, 50% relative humidity, and culturing for 70 days.



(a)PHE
S.

(b)PYR

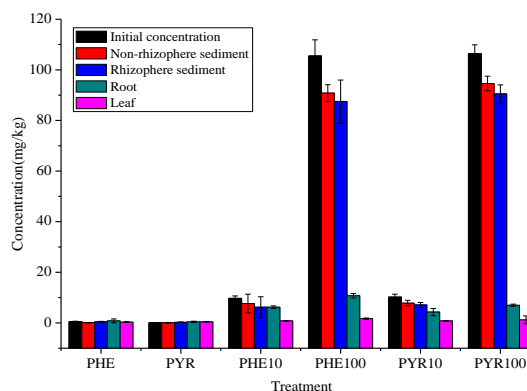


Figure 2: Changes in concentration of PHE and PYR in the

Figure 1: Chemical structures of PHE and PYR *alterniflora*-sediment wetland system at initial time and after 70 days of culture

Determination of PAHs Content: The *S. alterniflora* and sediments in the above three groups were ground and sieved to obtain samples respectively, and then the PAHs content was measured.

The microbial structure was determined by fatty acid labelling method; the activity of various biological enzymes in the sediment was determined by TTC reduction method. The extracted data were analysed using software SPSS.

3. Experimental results and analysis

3.1 Distribution of PAHs in wetland systems

Figure 2 shows the changes in the concentration of PHE and PYR in the *S. alterniflora*-sediment wetland system at the initial time and after 70 days of culture. It can be seen from the figure that compared with the initial concentration, the concentration of PHE and PYR in the RS sediments is significantly reduced, the average removal rate of PHE is 26%, and the average removal rate of PYR is 20%; the removal rate PHE and PYR in NRS sediments is significantly less than that of the RS sediments, because the presence of more species of microorganisms in the RS sediments is more useful for the degradation of PAHs. When the content of PAHs increases gradually, the monitoring concentrations of PHE and PYR in leaves and roots of *S. alterniflora* also increase gradually. It can also be observed from the figure that the content of PHE and PYR in the leaves of *S. alterniflora* is much smaller than that in the root system, indicating that the root system of *S. alterniflora* is the main part to absorb PAHs.

Table 1 lists the microbial content in the roots and leaves of *S. alterniflora* and their enrichment factors for PHE and PYR. It can be seen from the table that with the increase of PAHs content, the enrichment factors of roots and leaves are gradually reduced, and the enrichment factors of PHE and PYR in roots are much larger than those of leaves.

The pollution of PHE and PYR shall cause serious damage to the distribution and content of microorganisms in the wetland. In Table 1, the microbial content in the leaves decreases by more than 35% after PHE treatment; while after PYR treatment, the microbial content in the leaves is reduced by more than 26%. The enrichment factor can be used to effectively determine the tolerability of wetland plants to PAHs and the migration ability of PAHs in plants.

Table 1: Microbial content in the roots and leaves of *S. alterniflora* and their enrichment factors for PHE and PYR

Treatment	Root	Leaf	Total biomass	RCF		LCF	
				PHE	PYR	PHE	PYR
Control	1.23±0.08	2.28±0.55 ^a	3.46±0.48	1.95±1.82	2.75±2.71	0.69±0.20	0.79±0.49
PHE10	0.95±0.13	1.56±0.39 ^b	2.51±0.29	0.42±0.03	-	0.28±0.27	-
PHE100	1.41±0.22	1.17±0.12 ^b	2.54±0.36	0.14±0.02	-	0.01±0.01	-
PYR10	1.29±0.73	1.82±0.57 ^{ab}	3.13±1.244	-	0.43±0.31	-	0.18±0.08
PYR100	1.70±0.24	1.55±0.17 ^{ab}	3.32±0.22	-	0.09±0.02	-	0.02±0.01

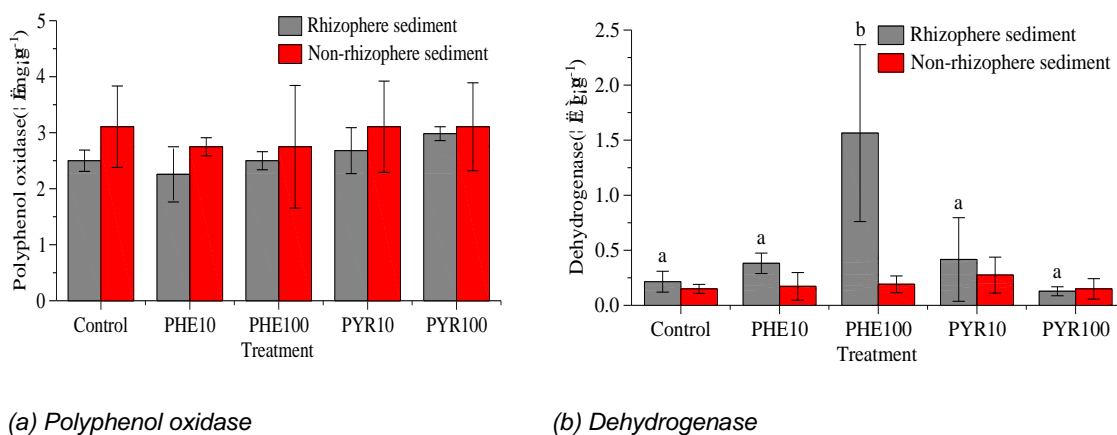


Figure 3: Effects of PHE and PYR on biological enzymes in sediments

3.2 Effects of PAHs on fatty acid and bio-enzyme activity

Figure 3 shows the effects of different PHE and PYR contents in the sediment on the activities of various bio-enzymes in microorganisms: polyphenol oxidase in Figure 3 (a) and dehydrogenase in Figure 3 (b). It can be seen from the figures that as the PHE content increases, the activity of dehydrogenase in the sediment near the RS of *S. alterniflora* increases gradually; when the PHE added amount is 100mg/kg, the activity of dehydrogenase in the sediment is about 7 times that of the control group; the activity of dehydrogenase in

NRS sediments is not obvious under the influence of PHE. After addition of PYR in RS sediments and NRS sediments, there was no significant change in dehydrogenase activity.

After the addition of PYR in the RS sediments, the activity of polyphenol oxidase was significantly enhanced, but it was not changed basically in the NRS sediments.

When the concentration of aromatic hydrocarbons in the sediment around the RS of *S. alterniflora* increases, the roots of *S. alterniflora* shall secrete organic carbon and other nutrient secretions for promoting the microbial quantity and activity, increasing the activity of polyphenol oxidase and dehydrogenase, and thereby accelerating the mineralization process of PAHs.

Figure 4 shows the changes in microbial content in the RS sediments and NRS sediments of *S. alterniflora* after treatment with PHE and PYR. From the control group, the contents of microorganisms in the RS sediments of *S. alterniflora* were Gram-negative bacteria, Gram-positive bacteria, actinomycetes and fungi from large to small. After PHE treatment, the total biomass decreased in the RS sediments, and the Gram-negative bacteria decreased by about 25%; when the PHE content was 10 mg/kg in the NRS sediments, the total biomass in the sediment increased by 21.6. %, and when the PHE content is 100 mg/kg, the total biomass decreased by about 15.2%. After PYR treatment, the total biomass in the RS and NRS sediments did not change much.

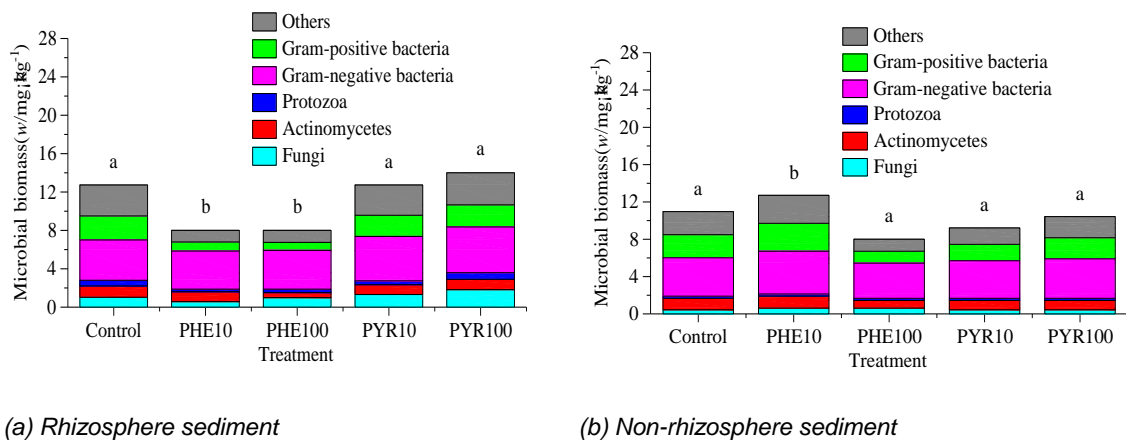


Figure 4: Effects of PHE and PYR on microbial content in rhizosphere sediments and non-rhizosphere sediments

Figure 5 shows the effect of PHE and PYR on microbial DNA abundance in sediments. It can be seen from Figure 5 that after treatment with PHE and PYR, the DNA abundance of microbes in the underground roots of the *S. alterniflora* and the RS sediments increased significantly, indicating that the PAHs has a greater impact on the microbial populations and structure, but there was small changes in DNA abundance of microbes in NRS sediments.

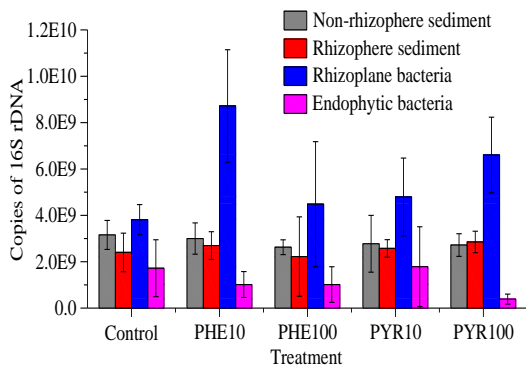


Figure 5: Effects of PHE and PYR on microbial DNA abundance in sediments

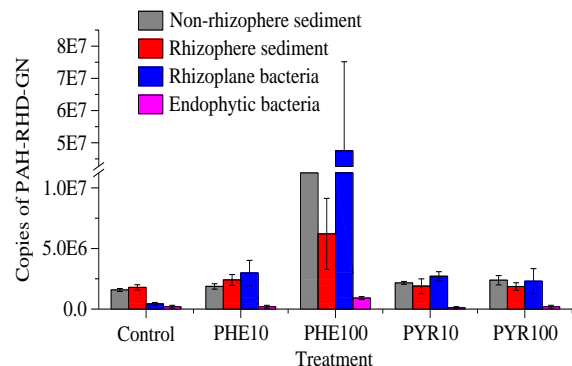


Figure 6: Effects of PHE and PYR on Gram-negative bacteria dioxygenase DNA

Figure 6 shows the effect of PHE and PYR on Gram-negative bacteria dioxygenase DNA. It can be seen from the figure that after treatment with PHE and PYR, the DNA abundance of the rhizosphere bacteria was 1.9×10^6 - 4.8×10^7 , which was significantly higher than the bacterial DNA abundance in the root of *S. alterniflora*. When treated with 100mg/kg PHE, the bacterial DNA abundance in the RS sediments of *S. alterniflora* was the highest, which was 100 times higher than that of the blank control group, followed by that in the RS and root of *S. alterniflora* treated with 100 mg/kg PYR; when treated with 10mg/kg of PYR, the bacterial DNA in the RS and root of *S. alterniflora* remained essentially unchanged.

Figure 7 shows the effect of PHE and PYR on Gram-positive bacteria dioxygenase DNA. It can be seen from the figure that the DNA abundance of the bacteria in the RS sediment of *S. alterniflora* was 8.4×10^6 - 1.8×10^8 , and the DNA abundance of the bacteria in *S. alterniflora* was 1.47×10^6 - 2.5×10^6 . The RS bacterial DNA abundance is much higher than the root bacteria.

The degradation of PAHs by *S. alterniflora* mainly depends on the absorption of microorganisms in the roots and RS. According to this study, the degradation of PAHs by bacteria in the underground roots of plants in the wetland system has further potential for development. The degradation and absorption of PAHs is significantly related to the type and content of plants in the wetland. Therefore, for different types of chemical pollutants, the corresponding plants or microorganisms should be prepared in the wetlands for absorption. The research results can provide a new research idea for the microbial-plant joint degradation of chemical pollutants in wetland ecosystems.

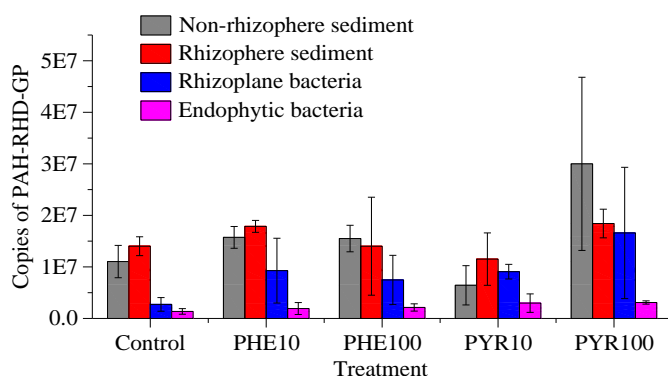


Figure 7: Effect of PHE and PYR on Gram-positive bacteria dioxygenase DNA

4. Conclusions

Chemical pollutants have serious harm to the ecological environment of wetlands. Taking the polycyclic aromatic hydrocarbons (PAHs), one typical chemical organic pollutant in industrial wastewater as examples, this paper studies the effects of PAH on plants and microorganisms in wetlands. Then, the experimental simulation was conducted to study the degradation and absorption effects of wetland soil sediments and wetland plant (*S. alterniflora*) on PAHs, and also analyse the effects of PAHs on total biomass and microbial active enzymes in wetlands. The research conclusions are as follows:

(1) The wetland ecosystem has significant absorption and degradation effects on chemical pollutants. The average removal rate of PHE is 26%, and the average removal rate of PYR is 20%; the content of PHE and PYR in leaves of *S. alterniflora* is much smaller than that of root system, indicating that the root system of *S. alterniflora* is the main part of absorbing PAHs. PAHs pollution will cause serious damage to the distribution and content of microorganisms in the wetland. PHE causes the microbial content in the leaves of *S. alterniflora* to decrease by more than 35%, while PYR causes it to decrease by more than 26%.

(2) The plant-microbial combined treatment system in the wetland system can resist the harmful effects of chemical pollutants to a certain extent, and the bacteria in the plant roots are more sensitive to the response of chemical pollutants. This can provide a new research idea for the microbial-plant joint/combination degradation of chemical pollutants in wetland ecosystems.

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