The Role of *Phragmites Australis* for Sludge Stabilization

下水処理によって出てくる汚泥の量は近年急激に増加している。この中に含まれる有機汚染物質を吸収するためには葦が有効であることを検証する。

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Abstract

On-site experiment on constructed wetland for surplus activated sludge treatment was presented. The research focused on polycyclic aromatic hydrocarbons (PAHs) accumulation and distribution in *Phragmites australis* during the sludge stabilization process and investigated the effect of PAHs transport in *Phragmites australis* from stabilized sludge by comparison with native ones. Quantitative analysis on the contents of PAHs in native *Phragmites australis* shows that it has obvious PAHs distribution characteristics: highest contents are in leaves, followed by stems and roots, corresponding contents are 2.583, 2.198 and 0.899 mg/kg (DW), respectively. After two-year loading and one-year natural stabilization, tested *Phragmites australis* accumulated PAHs obviously in constructed wetland. The leaf concentration factor (LCF) was slightly higher than the root concentration factor (RCF) and stem concentration factor (SCF) in plant-sediment phase, which were 3.759, 3.518 and 3.368, respectively. The PAHs contents in the root, stem and leaf of tested *Phragmites australis* are 8.13, 3.19 and 3.02 times that in native ones, which were 7.313, 7.002 and 7.814 mg/kg (DW). The RCF and SCF of the low-molecular-weight PAHs (2–3 ring PAHs) predominated (5.02 and 4.93) in all samples taken from constructed wetland, then middle-molecular-weight PAHs (4 ring PAHs)(3.15 and 2.12) and high-molecular-weight PAHs (5–6 ring PAHs)(2.29 and 2.63). LCFs of low-molecular-weight and middle-molecular-weight PAHs had higher values of 4.03 and 4.17, followed by 2.81 of high-molecular-weight PAHs.

Keywords constructed wetland; *Phragmites australis*; polycyclic aromatic hydrocarbons; sludge treatment

Introduction

Wastewater sludge amounts produced in urban sewage treatment increases rapidly with the growing of the wastewater treatment industry, and sludge treatment and disposal become urgent problems to be solved across the world. Wastewater sludge is a kind of fertilizer with abundant nitrogen, phosphorus, potassium, organic matter, and other microelements. However, the pollutants in the sludge also need to be treated suitably. Polycyclic aromatic hydrocarbons (PAHs) tend to accumulate in sediments due to their hydrophobicity and high lipophicity and are on the blacklists of priority pollutants in many countries because of their persistence, lower biodegradability and carcinogenic properties. The surplus sludge produced in industrial wastewater treatment has higher PAHs contents up to 2000 mg/kg (Mo, et al, 2001). Benzo(a)pyrene (Bap) content is less than 3 mg/kg

(DW) in China standards of PAHs in agricultural application) while more serious standards are carried out in some developed countries. For instance, Denmark changed total quantity standard of PAHs from less than 6 mg/kg to less than 3 mg/kg on July 1, 2007. Holland sets the range of PAHs content in uncontaminated soil from 0.02 to 0.05mg/kg (DW) (Song, et al, 2008).

Previous studies have suggested that some plants can be used for remediation of soils contaminated by PAHs (Simon and Kevin 1994; Tao, et al, 2009; Gao, et al, 2011). Constructed wetland technology provides a new way for degradation and transformation of PAHs in surplus sludge (Uggetti, et al, 2010; Cui, et al, 2012). Constructed wetland for sludge treatment is also known as sludge drying reed bed, and its relevant research and application are increasingly extensive, especially in the aspects of sludge dewatering, organic matter stabilization, removal of nitrogen and phosphorus, etc (Cooper, et al, 2004; Uggetti, et al, 2010; Cui, et al, 2011). However, research on PAHs degradation and transformation in sludge is unpublished. A three-year experimental study is presented here in order to systematically investigate PAHs transformation effects in stabilized sludge by constructed wetland.

Materials and Methods

Constructed wetland construction

A constructed wetland system is located in the Dalian Development Zone wastewater treatment plant, Liaoning Province, China, which is composed of sludge pump, sludge feed tank and reed bed. The size of reed bed is 3.0m × 1.0m × 1.3m. The height includes a 0.65 m media layer and 0.65 m super height. The substrata in the reed bed unit comprised a 0.2 m slag layer, a 0.2 m gravel layer, a 0.05 m coarse sand layer, and a 0.2 m fine sand layer from the bottom to the top, while a free board of 0.65 m was allowed for accumulation of dewatered sludge.

The drainage system was made of 3 m perforated PVC pipe with a diameter of 0.2 m and located on the bottom of the bed. Sludge percolate was recycled to the wastewater treatment system through special piping.

Operation and maintenance

The experiment lasted three years: the first year was adjustment phase, the second year was normal operation phase and the third year was natural stabilization phase.

The system started in mid-May of the first year. After *Phragmites australis* was transplanted and the tender plant was cultivated for 25 days, the sludge began to load on the bed intermittently. One cycle was set at seven days with feed sludge amount of 600 L within 30 minutes. The experiment in the first year lasted for 18 periods and stopped when the *Phragmites australis* rotted away. The system operated for 24 periods in the second year. The total thickness of feed sludge reached 8.4 m, and main raw sludge characteristics are average 99.14% water content, 8.14 g/L and 7.04 g/L of TSS and VSS concentrations. Average sludge loading on reed bed was 41.3 kg/m^2.y.

The average PAH content of raw sludge was 5.69 mg/kg, the low-molecular-weight PAHs (2-3 rings Nap, Acy, Ace, Fle, Phe, Ant and Fla) predominated (48.01%), middle-molecular-weight PAHs (4 rings Pyr, BaA, Chr, BaF and BkF) and high-molecular-weight PAHs (5-6 rings BaP, IcP, DaA and DaP.) accounted for 33.14% and 18.85%, respectively.

At the end of the third year, the average PAHs content in stabilized sludge was 2.08 mg/kg, the low-molecular-weight, middle-molecular-weight and high-molecular-weight PAHs accounted for 39.2%, 38.6% and 22.2%, respectively.

PAH analyses

Sample pretreatment: Firstly, the samples were fair-dried naturally and ground through 100 mesh sieve, then weighed 1g sample in 100 ml Erlenmeyer flask, added 2 g of anhydrous sodium sulfate and 1g of copper powder in the sample; Secondly, the sample was dissolved with 60 ml n-hexane - dichlorometh-

ane (1:1) mixed solvent, ultrasonic extraction for 90 min, centrifugal for 30 min (3000 r/min); Thirdly, rotary evaporation; Finally, constant volume to 4ml and set aside for subsequent use.

Sample purification: column chromatography method was used for sample purification. Firstly, weigh 2.0 g silica gel and 3.0 g anhydrous sodium sulfate (burn for 6h at 600°C) to make homogenate in the beaker with a small amount of n-hexane. Then put a small amount of cotton wool and 0.3 g quartz (60-80 mesh, activated at 130°C for 16 h) at the bottom of the column and Infiltrate the column with 10ml hexane; Secondly, fill the column with the sample, which is eluted by 40 ml n-hexane - dichloromethane (1:1) mixed solvent; Thirdly, rotary evaporation (until the eluted solution is dried up); Finally, constant volume to 4 ml under the protection of high purity nitrogen, moreover, the PAHs content is quantified by GC/MS according to external standards method.

GC / MS conditions: GC-2010 gas chromatography / mass spectrometry instrument (Shimadzu, Japan) is used for PAHs determination; the capillary column is made of SE-54 high-resolution paddy quartz. (length of 30.00 m, internal diameter of 0.25 mm, film thickness of 0.25 μm); Temperature program: the initial column temperature is 100° C and held to 280° C with a speed of 5° C/min, then maintain for 30 min; The injection volume is 1 μL; The carrier gas is high purity nitrogen; the column flow rate, makeup gas flow rate and pre-column pressure are 0.88 mL / min, 20 mL/min and 85.6 kPa separately; a split injection method is processed with a split ratio of 10:1; the inlet temperature and detector temperature are both 280 ° C; EI ionization mode is adopted with the ionization energy of 70 eV; the scanning slope of full-scan mode ranges from 100 to 400 amu.

Results and Discussion

PAHs distribution in native *Phragmites australis*

Native *Phragmites australis* samples were taken at the end of plant growth season and analyzed. The PAHs contents were presented in Fig. 1.

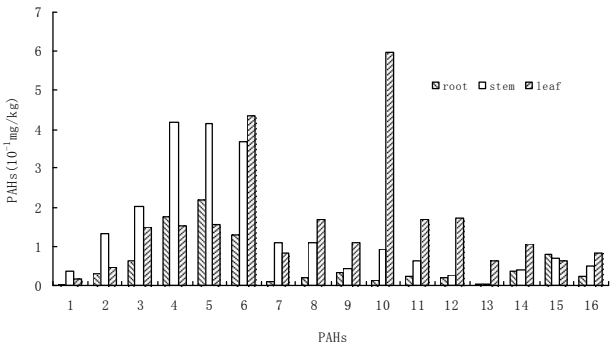

Fig. 1 PAHs distribution in native *Phragmites australis*

Table 1 Percentages of Low, middle and high molecular weight PAHs in total PAHs/%

PAHs	root	stem	leaf
Low-molecular-weight	70.4	76.9	40.5
Middle-molecular-weight	12.9	15.4	47.1
High-molecular-weight	16.7	7.7	12.4

PAHs in *Phragmites australis* are clarified into three kinds according to their fumed rings. Low-molecular-weight PAHs include 2-ring and 3-ring Nap, Acy, Ace, Fle, Phe, Ant, Fla; middle-molecular-weight PAHs include 4-ring Pyr, BaA, Chr, BaF and BkF. High-molecular-weight PAHs include 5-ring and 6-ring BaP, IcP, DaA and DaP. From Figure 1 and Table 1 we can get the content distribution characteristics in native *Phragmites australis* as follows:

The contents of low-molecular-weight PAHs in roots and stems were 70.4% and 76.9%, respectively, indicating a predominant distribution. While middle-molecular-weight and high-molecular-weight PAHs contents were relatively low, being 12.9%, 16.7% (in roots) and 15.4%, 7.7% (in stems), respectively. Middle-molecular-weight and low-molecular-weight PAHs predominated in *Phragmites australis* leaves the contents of 47.1% and 40.5%, but high-molecular-weight PAHs left only contents of 12.4%.

Phragmites australis stems had relatively higher content of low-molecular-weight PAHs (including Phe, NaP, Acy, Ace, Fle, Phe) than that in roots and

leaves. However, middle-molecular-weight and high-molecular-weight PAHs (including Pyr, BaA, Chr, BbF, BkF, BaP, IcP, BgP) contents in leaves were much higher than that in roots and stems, moreover, Chr was the most effectively adsorbed by *Phragmites australis*.

These characteristics of native *Phragmites australis* may result from the atmospheric PAHs. Previous research has showed that (44±18)% of PAHs in atmosphere was adsorbed and purified by plants (Simonich, and Hites 2004). The results in this research showed that stems and leaves exposed in atmosphere play the role of adsorption PAHs from atmosphere directly, mainly including low-molecular-weight PAHs such as Ace, Fle, Phe, Ant and Chr. The high PAHs content found in leave suggested that these PAHs were derived from atmospheric deposition in gaseous or particulate forms, and that atmospheric fall-out might be the main input pathway for PAHs in these plants. While low contents in the roots might have resulted from the relative low PAHs content in the sediments at the *Phragmites australis* sample site (Guo, Pei, Yang, et al 2011).

Distribution of PAHs in *Phragmites australis*

Distribution of PAHs in tested *Phragmites australis* in the second and third years were presented in Table 2 and Table 3. Total concentrations of PAHs in different *Phragmites australis* parts were compared in Table 4.

Macroscopically speaking, it is found in this research that low-molecular-weight PAHs predominated in *Phragmites australis* root and stem, including relatively high content of BbF. However, *Phragmites australis* leaf had higher content of middle-molecular-weight and high-molecular-weight PAHs, especially Chr.

The distribution characteristics of PAHs in tested *Phragmites australis* in the second year can be described that the PAHs content in root increased as time goes until the maximum value in November, while PAHs contents in stem and leaf increased until the maximum value in October, both showed the ability of *Phragmites australis* for PAHs bioaccumulation.

Both plant species and PAHs content in environment affect the PAHs content in plants, and PAHs content differentiate in different parts of the same plant. It is found that plant near the industrial zone has a high total content (in root) of 12.3 mg/kg (Lee, et al, 1990). In this research, the tested *Phragmites australis* obtained bioaccumulation rate of 813% (root), 319% (stem) and 302% (leaf) compared to the native ones. Apparently, PAHs were taken up by roots and then transferred to the stem and then leaf within plants. Chr was detected in the highest content of 1.524mg/kg in root while Nap was the lowest (0.090 mg/kg): Ant was the highest (1.246 mg/kg) in stem, while Nap was the lowest (0.095 mg/kg); Phe was the highest (1.395 mg/kg) in leave and Fla was the lowest (0.062 mg/kg).

Bioaccumulation ability of *Phragmites australis*

PAHs can be taken up by roots and leaves and translated within plants. The PAHs accumulation ability can be described using the following equation:

$$RCF = C_{root}/C_{sediment}$$
$$SCF = C_{stem}/C_{sediment}$$
$$LCF = C_{leaf}/C_{sediment}$$

Where $C_{root,}$ $C_{stem,}$ $C_{leaf,}$ $C_{sediment}$ represent the contaminant content in plant root, stem, leaf and sediment.

PAH concentration factors in *Phragmites australis* root, stem and leaf in the third year are listed in the Table 5.

The calculated RCF values range from 0.837 (BaA) to 11.991 (Phe), SCFs range from 0.739 (BbF) to 13.707 (Phe), LCFs range from 0.739 (Fla) to 15.127 (Phe). 87.5% of PAHs accumulated in the tested *Phragmites australis*. The low-molecular-weight PAH concentration factors in *Phragmites australis* root, stem and leaf are 5.02, 4.93 and 4.03, respectively, higher concentration factors may result from relatively high hydrophobicity and bioavailability of PAHs. The middle-molecular-weight PAH concentration factors in *Phragmites australis* root,

Table 2 Distribution of PAHs in tested *Phragmites australis* in the second year (10^{-1}mg/kg)

NO	PAHs	root			stem			leaf		
		Sep	Oct	Nov	Sep	Oct	Nov	Sep	Oct	Nov
1	NaP	0.111	0.111	0.482	0.773	0.807	0.715	0.523	0.485	0.724
2	Acy	0.387	0.387	0.597	2.291	1.692	1.614	0.525	0.95	1.104
3	Ace	1.255	1.255	2.118	2.395	3.193	2.832	2.209	1.746	1.246
4	Fle	2.131	2.131	3.092	4.289	5.329	5.034	3.397	3.865	3.916
5	Phe	2.812	2.812	3.559	4.988	5.04	5.875	2.316	2.358	1.283
6	Ant	2.912	2.912	4.76	3.969	4.842	4.512	5.151	5.23	5.234
7	Fla	0.795	0.795	1.173	1.271	3.153	2.392	1.289	1.103	2.079
8	Pyr	0.900	0.900	1.073	1.268	1.353	1.474	1.781	2.437	2.823
9	BaA	0.909	0.909	1.885	1.048	0.954	0.752	1.181	2.498	1.362
10	Chr	0.256	0.256	0.995	1.68	1.862	1.681	6.01	6.064	6.516
11	BbF	1.069	1.069	2.143	1.848	3.112	1.119	1.94	1.823	2.727
12	BkF	0.691	0.691	1.702	0.772	0.765	1.196	2.392	4.289	2.291
13	BaP	0.192	0.192	0.339	0.58	0.47	0.545	0.714	0.794	1.473
14	IcP	1.198	1.198	1.044	1.118	1.649	1.555	1.761	1.633	1.57
15	DaA	1.051	1.051	1.051	0.877	1.84	1.478	0.778	1.874	0.951
16	BgP	0.534	0.534	0.815	0.652	1.508	0.766	1.533	1.619	1.522

Table 3 Distribution of PAHs in *Phragmites australis* in the third year (10^{-1}mg/kg)

NO	PAHs	root	stem	leaf
1	NaP	0.90	0.95	0.82
2	Acy	2.26	4.37	1.22
3	Ace	3.30	3.80	3.60
4	Fle	4.80	4.38	4.00
5	Phe	10.9	12.46	13.75
6	Ant	11.84	12.05	7.55
7	Fla	5.70	2.46	0.62
8	Pyr	2.78	2.49	2.57
9	BaA	1.26	3.24	13.95
10	Chr	15.24	3.82	6.90
11	BbF	0.96	1.76	5.44
12	BkF	3.15	4.23	3.48
13	BaP	3.70	8.60	7.80
14	IcP	2.39	2.34	3.57
15	DaA	2.22	1.68	1.72
16	BgP	1.73	1.39	1.15

Table 4 Total contents of PAHs in tested *Phragmites australis* (10^{-1}mg/kg)

Part	Tested *Phragmites australis*				Native *Phragmites australis*
	The second year			The third year	
	Sep	Oct	Nov	Nov	
root	17.205	26.827	36.447	73.13	8.99
stem	29.819	37.569	33.54	70.02	21.93
Leaf	33.5	38.768	36.821	78.14	25.83

Table 5 PAH concentration factors in *Phragmites australis* root, stem and leaf (mg/kg, DW)

NO.	Compounds	RCF	SCF	LCF
1	NaP	1.125	1.188	1.025
2	Acy	1.642	3.175	0.886
3	Ace	2.562	2.950	2.795
4	Fle	4.111	3.751	3.426
5	Phe	11.991	13.707	15.127
6	Ant	6.507	6.622	4.149
7	Fla	7.194	3.105	0.782
8	Pyr	3.067	2.747	2.836
9	BaA	0.837	2.152	9.267
10	Chr	9.513	2.385	4.307
11	BbF	0.403	0.739	2.286
12	BkF	1.929	2.591	2.131
13	BaP	1.840	4.276	3.878
14	IcP	2.724	2.667	4.069
15	DaA	2.290	1.733	1.774
16	BgP	2.308	1.854	1.534
Total average		3.518	3.368	3.759

stem and leaf are 3.15, 2.12 and 4.17, respectively. And the values of high-molecular-weight PAH are 2.29, 2.63 and 2.81, respectively. Although PAHs are easily taken up by root and leave, the translation mechanism within *Phragmites australis* was not clear (Dettenmaier, et al, 2009). Based on the result of this research, we conclude that tested *Phragmites australis* can accumulate PAHs effectively compare to native ones, higher PAH contents in soil corresponds to higher PAH contents in *Phragmites australis*. Especially we can get the conclusion that PAHs content increase in tested *Phragmites australis* stem and leaf may result from root adsorption and translation in plant.

Conclusions

PAHs in sludge can be effectively accumulated by *Phragmites australis* in constructed wetlands. After two years of sludge loading period and one year of natural stabilization period, the PAH contents in tested *Phragmites australis* root, stem and leaf increased notably, reaching 7.313, 7.002 and 7.814 mg/kg (DW), corresponding to 8.13 times of native ones in roots, 3.19 times in stems, 3.02 times in leaves, respectively. Low-molecular-weight PAHs are more easily accumulated than middle-molecular-weight and high-molecular-weight PAHs. Sixty four percent of PAHs in stabilized sludge by constructed wetland were removed.

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