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Accumulation of Phthalic Acid Esters in Water Spinach (*Ipomoea aquatica*) and in Paddy Soil

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Municipal sludges are sediments of sewage that has been treated in municipal wastewater treatment plants. The current disposal methods for municipal sludge comprise mainly incineration, landfill, ocean dumping, and agricultural utilization. Unfortunately, sludge usually contains a variety of pollutants including potentially toxic elements (PTEs) and organic pollutants (Jacobs et al., 1987; Webber et al.,1989; Smith, 1996; Mo et al., 2000; Cai et al., 2001; Mo et al., 2001) which can limit agricultural application.

Phthalic acid esters (PAEs), consisting of a benzene ring with two adjacent (ortho-) carboxylic acid side groups, are one of the most widely used and distributed classes of chemicals, mainly as plasticizers but also used in cosmetics, defoaming formulations, and other products. PAEs have been found in sewage sludge, plants, soil, and water (Smith, 1996). They are classed as priority organic pollutants by the United States Environmental Protection Agency (USEPA). Studies have shown that municipal sludges applied as fertilizer may result in elevated concentrations of PAEs in plants and soils (Smith, 1996). However, in most studies analysis has been restricted to a limited number of compounds (Staples et al., 1997) and unrealistically excessive soil PAE concentrations have been used.

MATERIALS AND METHODS

In this study *Ipomoea aquatica* (Forsk.) was grown in pots under glasshouse conditions and municipal sludges were applied. Six PAE compounds were determined by GC-MS to investigate the distribution pattern of PAE compounds in the plant-soil system and the effect of municipal sludge on the accumulation of PAEs in *I. aquatica* and paddy soils.

Municipal sludges were obtained from the Datansha wastewater treatment plant in Guangzhou and Zhen'an wastewater treatment plant in Foshan in Guangdong province, South China. Soils were collected from the farm of South China Agricultural University in Guangzhou. Soil was air-dried and then passed through a 5-mm sieve for subsequent use. Some physicochemical characteristics of soil and sludges are presented in Table 1. The soil had a CEC of 9.1 cmol kg^{-1} and a pH of 6.2.

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Table 1. Physicochemical characteristics of soil and sludges.

	Total (g kg^{-1})				Available (mg kg^{-1})			EC_{25}
	OM	N	P	K	N	P	K	(mS cm^{-1})
Guangzhou sludge	298	29.6	13.3	21.4	125	1393	4712	1.9
Foshan sludge	354	25.0	26.8	10.3	124	1462	6355	2.2
Paddy soil	26	1.3	0.4	15.6	15	45	119	0.2

The pot experiment comprised six treatments: control, chemical fertilizer, Foshan sludge, Foshan sludge+fertilizer, Guangzhou sludge, and Guangzhou sludge+ fertilizer, with five replicates in a randomized complete block design. Pots were fertilized with PAE-containing municipal sludge (10g kg^{-1} soil) and fertilizer (0.20g N, 0.15g P, and 0.20g K kg^{-1} soil) according to the treatment above prior to cultivation and were thoroughly mixed into the soil in each pot (5 kg DW soil per pot). Five days later the soils were dried and ground again. In April 1999, three five-day-old seedlings of *I. aquatica* were planted in each pot. 200ml distilled water per pot was added each day. Insect pests were controlled manually and no chemical pesticides were used during plant growth. Plants were harvested after 39 days. At harvest, soil samples (approximately 300g) were taken from each pot in quadruplicate, air-dried and ground (1-mm sieve).

Analytical grade organic solvent including dichloromethane (DCM), acetone and *n*-hexane (Guangzhou, China, purity >98%) were redistilled before used. All the glass apparatus were washed with K_2CrO_4-H_2SO_4 solution, then washed with tap water and redistilled water, then dried at 300 °C for 4 h before use.

Plants were cut into pieces, washed with DCM and ground and dried at 45°C. Dried plant (~10g) and soil samples (~20g) were weighed into Whatman cellulose extraction thimbles (pre-extracted with *n*-hexane and DCM for 24 h) and extracted in a Buchi Soxhlet extractor for 24 h with 100 ml ether and acetone-DCM mixture (1:1, v/v), respectively. The extracts were reduced to 1.0 ml in a rotating evaporator in a water bath at 50 °C at 50 r min^{-1}, and then transferred to a glass column filled with alumina and silica gel by washing with 40 ml *n*-hexane and then 40 ml DCM.

Extracts were concentrated again under a gentle stream of nitrogen to 0.5 ml and then diluted to the required volume with DCM. Pyrene-d_{10} and aniline-d_5 were used as internal and blank standards. An internal standard of pyrene-d_{10} was added to the samples to give a final concentration of 45.45 μg ml^{-1} before injection. Blank and recovery experiments were conducted.

A composite stock standard solution (500 μg ml^{-1}) contains 6 PAEs, namely dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP),

Table 2. Concentrations of phthalic acid esters in *Ipomoea aquatica* plants (mg kg^{-1} DW).

Compound	Control	Fertilizer	Foshan sludge	Foshan sludge + fertilizer	Guangzhou sludge	Guangzhou sludge + fertilizer
DMP	0.02	0.01	0.01	0.08	0.03	0.03
DEP	0.01	0.20	0.10	0.20	0.12	0.16
DnBP	0.37	0.28	0.18	1.85	0.30	0.48
BBP	1.73	1.05	1.84	0.25	3.33	2.21
DnOP	ND	0.56	0.72	ND	0.49	2.09
DEHP	ND	0.53	0.72	0.93	0.50	2.15
ΣPAE	2.13	2.64	3.57	3.30	4.77	7.11

[a] ND, not detected;

[b] ΣPAE, total content of six PAE compounds.

butyl benzyl phthalate (BBP), di-*n*-octyl phthalate (DnOP) and di(2-ethylhexyl) phthalate (DEHP). The original solution of PAE, pyrene-d_{10} and nitrobenzene-d_5 were purchased from ULTRA Scientific, Inc., North Kindstown, RI, USA.

Quantification of PAEs was carried out by GC-MS (USEPA 8270C). GC-MSD analysis was performed on a Hewlett Packard Model 5890 II gas chromatograph interfaced to a HP 5972 mass-selective detector equipped with the HP MS Chem-station. Separations were achieved on a fused-silica capillary HP-1 column (25 m×0.22 mm i.d.) coated with 0.11 μm film. Helium carrier gas flow-rate was 0.615 ml min^{-1}. The temperature program started at 45 °C, and increased to 200 °C at 6.0 °C min^{-1}, then to 300 °C at 8.0 °C min^{-1} and finally held for 1 min. The solvent delay was 3.00 min and total run time was 39.33min. Injection port temperature was 250 °C, MSD detector temperature was 280 °C. A manual method was used to inject 1.0 μl of sample. Injection was splitless. The electron ionization energy was 70 eV. The MSD was operated in the scan-mode and mass scan ranged from 50 amu to 550 amu.

Identification of individual PAEs was based on the comparison of PAE and retention time data between samples and the standard solution containing six USEPA PAE compounds. External calibration was used for quantification. The retention time of the internal standard was used to correct for variation in retention time. Mass spectra were compared with reference compounds in Pripol.L and Nbs75k.L. This operation was performed automatically by the GC-MS chromatography data system. The instruments were calibrated daily with calibration standards which were <20% for all of target analyses. Recovery of pyrene-d_{10} ranged from 65.42 to 87.74% and none of the PAE compounds was detected in a blank experiment. The detection limits, calculated as signal-to-noise ratio of three, were 1.4 μg L^{-1} for DMP, 0.91 μg L^{-1} for DEP, 0.60 μg L^{-1} for DnBP, 0.39 μg L^{-1} for BBP, 0.49 μg L^{-1} for DEHP, and 0.57 μg L^{-1} for DnOP.

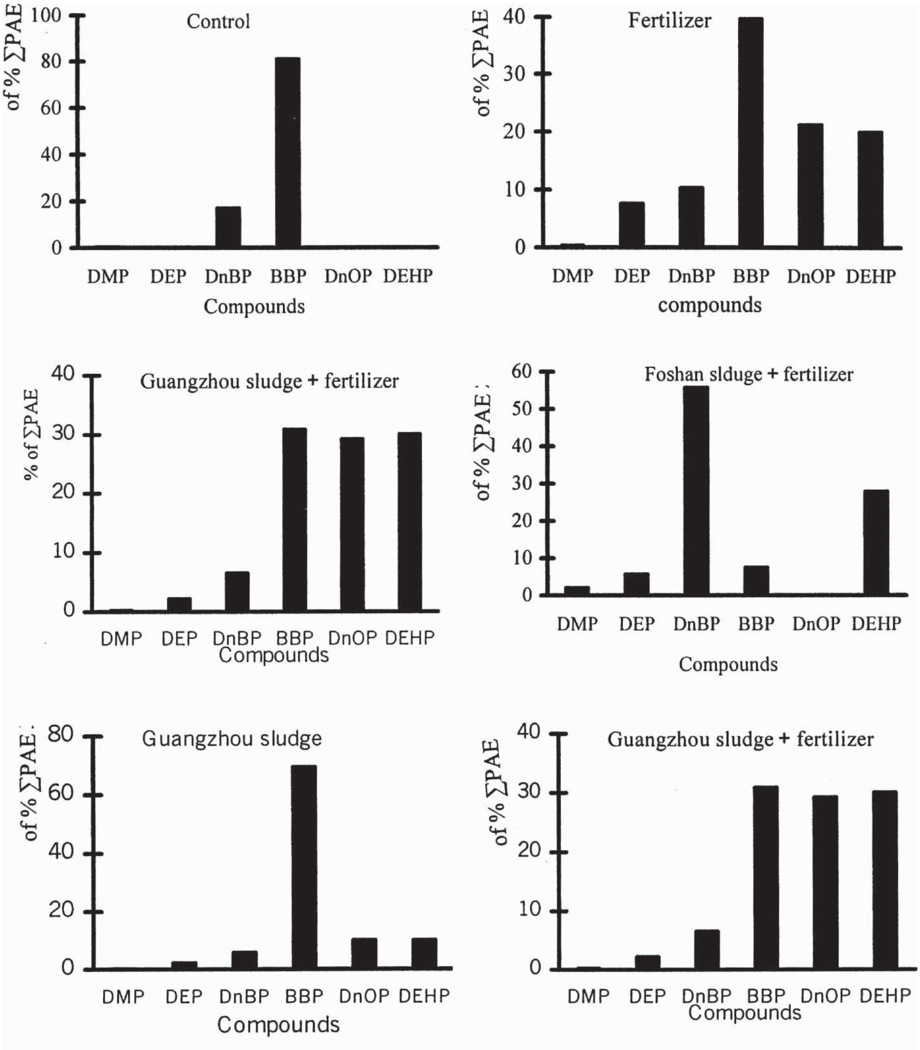

Figure 1. Distribution of individual phthalic acid esters in *Ipomoea aquatica.*

RESULTS AND DISCUSSION

The ΣPAE values in aboveground parts of *I. aquatica* ranged from 2.13 to 7.11 mg kg^{-1} dry weight, decreasing in the order of Guangzhou sludge+ fertilizer > Guangzhou sludge > Foshan sludge+fertilizer > Foshan sludge> Fertilizer >Control (Table 2). Compared to control and those receiving fertilizer only, Σ PAE in plants receiving sludge or sludge+fertilizer increased significantly, especially Guangzhou sludge+fertilizer, in which ΣPAE was twice as high as in the controls. In general, the ΣPAE burden was somewhat higher than the sum of

Table 3. Concentration of phthalic acid esters in soils (mg kg^{-1} D.W.).

Compound	Control	Fertilizer	Foshan Sludge	Foshan sludge + fertilizer	Guangzhou sludge	Guangzhou sludge + fertilizer
DMP	0.287	0.149	0.023	0.159	0.097	0.186
DEP	0.045	0.173	0.004	0.035	0.633	0.084
DnBP	10.7	6.59	1.08	0.932	15.1	2.10
BBP	0.450	0.194	0.514	2.02	0.074	0.394
DnOP	0.221	0.095	0.333	1.58	0.127	0.248
DEHP	5.24	0.469	38.1	26.8	22.1	19.5
ΣPAE	16.9	7.67	40.0	31.6	38.1	22.5

[a] ΣPAE was the total content of six PAE compounds.

16 polycyclic aromatic hydrocarbons (PAHs) in *I. aquatica* plants found by Cai et al. (2002).

Typically, concentrations of individual PAE compounds in different treatments varied markedly (Table 2). Among six PAE compounds, BBP showed the highest concentrations, ranging from 0.254 to 3.33 mg kg^{-1}, while those of the other PAE compounds in most treatments were <1.0 mg kg^{-1}, and the lowest content was of DMP, <0.1 mg kg^{-1}. In general, compared to the controls, contents of PAEs in the applied sludge or fertilizer treatments increased to some extent. For example, the concentrations of DEP in Foshan sludge, Foshan sludge+fertilizer, Guangzhou sludge and Guangzhou sludge+fertilizer treatments were 10-fold higher than in the controls. Concentrations of DnOP and DEHP in the controls were below the detection limit, while those in the other treatments were up 0.5 mg kg^{-1} or more (except DnOP in Foshan sludge + fertilizer). The highest content of different PAE compounds was found in different treatments: DMP and DnBP in Foshan sludge + fertilizer, DEP in fertilizer, BBP in Guangzhou sludge, DnOP and DEHP in Guangzhou sludge+fertilizer. Concentrations of individual PAE compounds in *I. aquatica* were lower than reported in oat and tomato plants (Muller, 1993) and higher than found in grains (Kirchmann et al., 1991).

Fig.1 presents the diagrams of PAE as normal percent concentration in *I. aquatica*. As shown, in the controls, Foshan sludge, and Guangzhou sludge, the dominant PAE was BBP which formed more than 50% of ΣPAE. While DnBP was the most abundant compound in Foshan sludge+fertilizer, and the higher molecular weight PAEs, mainly BBP, DnOP and DEHP predominated in fertilizer and Guangzhou sludge + fertilizer.

The ΣPAE values of soils were between 7.67 and 40.0 mg kg^{-1}, and the highest burden was observed in Foshan sludge, up to 40.0 mg kg^{-1}, and decreasing in the order of Foshan sludge+fertilizer > Guangzhou sludge > Guangzhou sludge + fertilizer > Control > Fertilizer (Table 3). Clearly, application of sludge or sludge + fertilizer to soils increased the content of PAE in soil significantly. Moreover, ΣPAE in soils applied sludge or sludge + fertilizer was two to five times higher than that in soils receiving fertilizer.

Table 4. Bioconcentration factor (BCF) of PAEs in *I. aquatica* (based on dry weight).

Compound	Control	Fertilizer	Foshan sludge	Foshan sludge +fertilizer	Guangzhou sludge	Guangzhou sludge + fertilizer
DMP	0.05	0.09	0.57	0.48	0.31	0.14
DEP	0.22	1.18	25.25	5.60	0.19	1.95
DnBP	0.03	0.04	0.17	1.99	0.02	0.23
BBP	3.85	5.42	3.57	0.13	45.0	5.60
DnOP	0	5.93	2.16	0	3.89	8.43
DEHP	0	1.13	0.02	0.03	0.02	0.11
ΣPAE	0.13	0.34	0.09	0.10	0.13	0.32

The loading of soil with individual PAE compounds showed somewhat different trends compared with the plants (Table 3). Among six PAE compounds, the most abundant individual PAE compound was DEHP, which in soil spiked sludge and sludge + fertilizer was 3.0-6.5 fold higher than in the controls. The compound second highest in concentration was DnBP, between 0.932 and 15.1 mg kg^{-1}. Concentration of other PAE compounds in most treatments were <1.0 mg kg^{-1}. Nevertheless, compared to some reported PAE values for soils (Muller 1993; Ruminski, 1995), concentrations of individual PAE compounds in paddy soils in this study were low.

The percent contribution of individual PAEs to ΣPAE is illustrated in Fig 2. It is evident that DnBP in treatment of control and fertilizer, DEHP in those of Foshan sludge, Foshan sludge + fertilizer, Guangzhou sludge and Guangzhou sludge + fertilizer was the abundant compound, respectively.

The bioconcentration factor (BCF), expressed as ratio of PAE concentration in plants to that in soil, was used by the USEPA for risk assessment purposes and expression of plant contamination. BCF values are given in Table 4.

BCF values <1.0 were obtained for all ΣPAE results. Moreover, those for ΣPAE in control, Foshan sludge, Foshan sludge + fertilizer and Guangzhou sludge were 0.1 or so, suggesting slight accumulation of ΣPAE in plants. However, most BCF values higher than 1 were observed for DEP, BBP and DnOP. In general, BCF for PAEs in *I. aquatica* was higher than those for lettuce, carrots and chillies grown on sludge-amended soil (Aranda et al., 1989; Staples et al., 1997). However, if PAE concentrations in plant were expressed on a fresh weight basis, BCF was typically 10 to 20 fold less than that expressed on a dry weight basis depending upon the moisture content of the harvested tissue (O'Connor, 1991).

Our results show that content of PAE in *I. aquatica* and paddy soil increased to some degree when sludges were applied to paddy soil. Moreover, ΣPAE in paddy soil was significantly higher than in *I. aquatica*. In general, plant uptake of sludge-borne PAEs can occur by uptake from the soil solution (liquid phase transfer), with translocation from roots to shoots, absorption by roots or shoots of

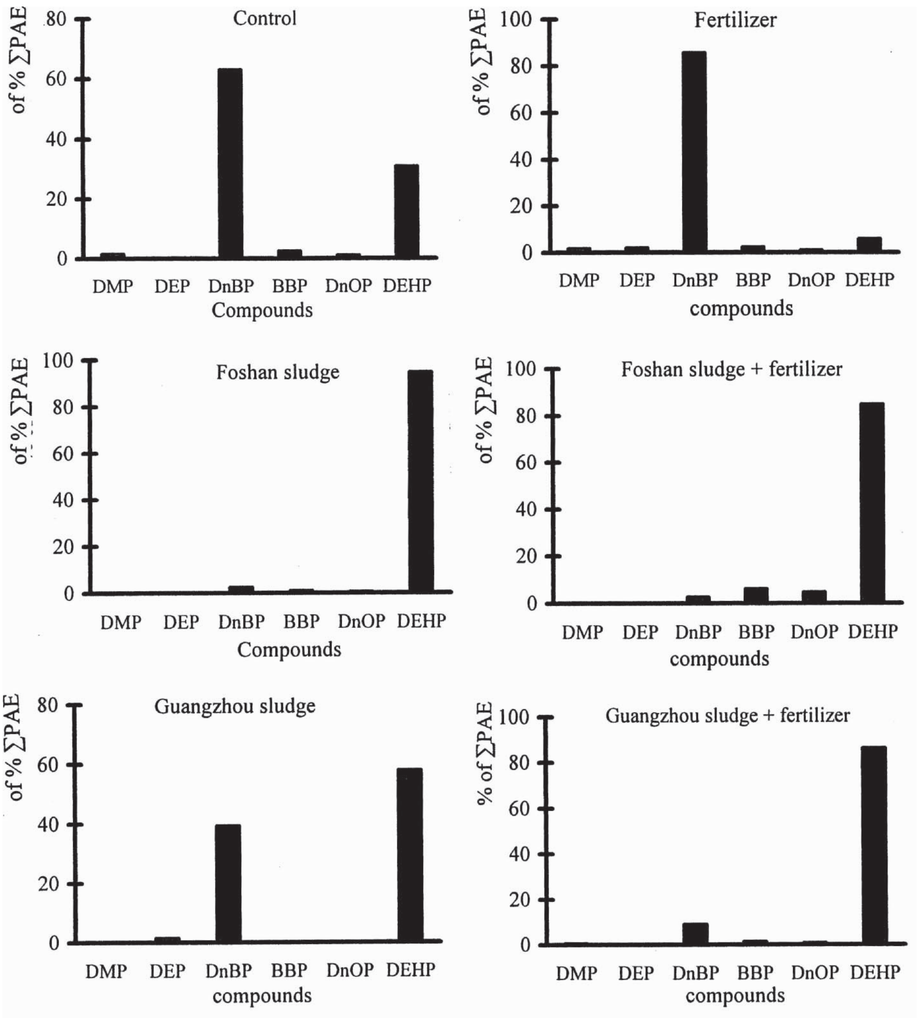

Figure 2. Distribution of phthalic acid esters compounds in paddy soil.

volatilized PAE (vapor phase transfer) from the soil and partitioning of bound PAE directly into plant tissues from soil particles or aerosols deposited on leaves (Patterson 1990). However, there was difference among the PAE compounds, plants and soils under different treatments. Among six PAE compounds, BBP and DEHP predominated in *I. aquatica*, and DnBP and DEHP in paddy soil. Uptake and accumulation of PAE compounds are typically affected by the physico-chemical properties of the compounds, environmental conditions, and plant characteristics (Patterson, 1990). The final fate of PAE compounds in sludge-amended soils depends on the interaction of their behavior and the factors above.

However, in some research work uptake of organic pollutants via the soil pathway has not been detected (Muller, 1993). More research should be conducted to determine whether or not accumulation of sludge-borne PAEs occurs in vegetables.

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