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Uptake of Di(2-ethylhexyl) Phthalate (DEHP) by the Plant *Benincasa hispida* and Its Use for Lowering DEHP Content of Intercropped Vegetables

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Supporting Information

ABSTRACT: Uptake of di(2-ethylhexyl) phthalate (DEHP) by the plant *Benincasa hispida* and its use for topical phytoremediation were investigated by cultivation of plants in DEHP-contaminated environments. The results showed that major plant organs of *B. hispida*, including leaves, stems, and fruits, readily absorbed DEHP from the air. The amount of DEHP that accumulated in leaves, stems, and fruits was mainly dependent upon exposure time, and most DEHP accumulated in their inner tissues. A single plant of *B. hispida* with a gourd was able to absorb more than 700 mg of DEHP when it was exposed to DEHP-contaminated air for 6 week. *B. hispida* reduced air DEHP concentration by 65−76% as the air DEHP concentration ranged from 2351 to 3955 $\mu g/m^3$ (high DEHP level) and 85−92% as the air DEHP concentration ranged from 35.1 to 65.3 $\mu g/m^3$ (low DEHP level) in greenhouse experiments. When intercropping of *B. hispida* and *Brassica chinensis* or *Brassica campestris*, *B. hispida* reduced more than 87% of DEHP accumulation in the latter, which indicates that *B. hispida* has excellent use potential for lowering the DEHP content of intercropped vegetables.

KEYWORDS: *Di(2-ethylhexyl) phthalate, Benincasa hispida, uptake, phytoremediation, vegetable*

INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) is widely used as a plasticizer in the manufacture of articles containing polyvinyl chloride (PVC). Because phthalate plasticizers are not chemically bound to PVC, DEHP can leach, migrate, or evaporate into the atmosphere, indoor air, soil, water, foodstuffs, and other materials. Human exposure to DEHP may result from direct contact to DEHP-containing consumer products as well as indirectly through general environmental contamination.[1−3] The United States Environmental Protection Agency (U.S. EPA) and the European Union (EU) have set a limit of 20 and 37 $\mu g/kg$ of body weight day^{-1}, respectively, for maximum safe daily intake of DEHP.[4] Recent studies have shown that high doses of DEHP may cause testicular and liver damage, fetal malformation and death, and liver cancer.[5−9] Contamination of food (e.g., vegetables) by DEHP has been reported in recent years.[10−13] For example, we have previously detected high DEHP concentrations in *Benincasa hispida* fruits[14] and demonstrated that plastic mulch, factories, and greenhouses may cause severe contamination of vegetables by DEHP.[15−17] In China, many plastic factories are located in suburbs surrounded by vegetable plantings, and plastic mulch is extensively used for vegetable cultivation. Therefore, remedy for air of vegetable fields contaminated by DEHP is urgently needed.

Over the last 2 decades, phytoremediation has been successfully used for contaminant removal from water and shallow soils.[18] Considerable efforts have been expended to understand the uptake mechanism of pollutants by plants and their application potential for phytoremediation.[19,20] Our previous studies revealed that the stems and leaves of *B. hispida* accumulated higher DEHP levels compared to other vegetables based on the field-cultivated experiment using plastic mulch film.[14,15] Although these investigations suggest the potential application of *B. hispida* for DEHP removal from contaminated air, DEHP uptake by *B. hispida* has not been systematically evaluated in those reports. In the present study, we investigated the uptake and accumulation of DEHP from contaminated air by *B. hispida* leaves, stems, and fruits, analyzed DEHP distribution in the leaf and fruit inner tissue of *B. hispida*, and used the plant of *B. hispida* for phytoremediation to lower the DEHP content of air and intercropped vegetables.

MATERIALS AND METHODS

DEHP Treatments for Investigation of DEHP Uptake by *B. hispida*. Our previous studies showed that *B. hispida* could strongly accumulate DEHP from PVC mulch film.[13,14] To enhance the uptake, we placed Petri dishes filled with DEHP underneath them as a DEHP source. Plants were initially cultivated in a normal glass greenhouse in 30 cm diameter pots containing 5 kg of soil and fertilizer without DEHP contamination. After fruiting of plants of *B. hispida*, three individual plants with a fruit of 10 cm length were moved to a special glass greenhouse and then treated with DEHP by placing four Petri dishes (5 cm inner diameter) containing a 0.5 cm layer of 99.5% DEHP (10 mL, 9.86 g) underneath each plant. The greenhouse was of 3 × 3 × 3 m in length, width, and height. Each of the side walls had

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four holes (5 cm diameter), two in the upper 30 cm from the top and two in the lower 30 cm from the ground. In the top center of each greenhouse, an electric fan was installed running at 1 rpm to obtain air flow with a very low speed. The plants were allowed to absorb DEHP, which was volatilized from the Petri dishes because of the relatively high vapor pressure (ca. 0.000 03 Pa at 25 °C) of DEHP, especially at a high temperature during culture. The range of temperatures during culture in the greenhouse was from 25 °C (at night) to 44 °C (during a sunny day) during May 18–June 28, 2010. Upon fruit maturation, i.e., after 6 weeks of DEHP exposure, the plants were harvested. All of the parts of the plant were washed by water to remove the absorbed DEHP to the dust of plant surfaces before any advanced treatment. The fruit was sliced into 15 segments, each about 4 cm thick. The peel and flesh of each segment was isolated, freeze-dried, and ground into powder. Every leaf, including petiole, and internodal stem section was independently freeze-dried and ground into powder. All of the fruit, leaf, and stem samples were subjected to DEHP determination.

Tissue Isolation. Fresh leaves (100 g) and gourd flesh of *B. hispida* (100 g) were homogenized with 250 mL of cold extraction medium [0.3 M sucrose, 25 mM tetrasodium pyrophosphate, 10 mM KH_2PO_4, 2 mM ethylenediaminetetraacetic acid (EDTA), and 20 mM ascorbate (pH 7.5)] at 4 °C.[19] The homogenate was filtered and centrifuged to yield precipitates I–V and final supernatant V (Figure 1). The

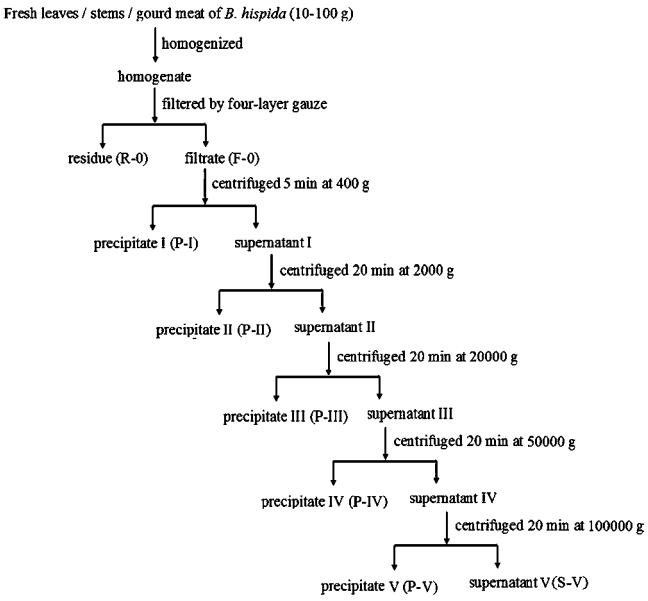

Figure 1. Isolation profile of cellular components in plant tissue homogenates using filtration and centrifugation. Before 20000*g* centrifuge, most of the water in the supernatant was removed by freeze-drying.

precipitates were examined using a transmission electron microscope to identify their main components. The precipitates and supernatant were freeze-dried for DEHP detection.

Transmission Electron Microscope Observations.[22] Each precipitate obtained from the centrifugation described above was treated with 1 mL of fixative containing 2.5% glutaraldehyde and incubated at room temperature for 1 h. Excess fixative solution was then removed, and the precipitate was subjected to three 15 min washes with 1 mL of 1 M phosphate-buffered solution (PBS) at pH 7.2. After the addition of 1 mL of a solution of 1% OsO_4, 0.5% reduced $K_4Fe(CN)_6$, and 1 M PBS at pH 7.2, the sample was incubated in the dark for 1 h. After two 15 min PBS washes, the sample was incubated for 30 min in 30% acetone, followed by 30 min of incubation in 50% acetone, 30 min of incubation in 80% acetone, 30 min of incubation in 90% acetone, and three 30 min washes in anhydrous acetone. The sample was then incubated in a series of graded Epon (liquid epoxy resin)/acetone solutions, as follows: 2 h each at room temperature with Epon/acetone (3:5), Epon/acetone (1:1), and Epon/acetone (2:1), then overnight in fresh 100% Epon at 4 °C in a desiccating chamber, and finally 3 times, 2 h each, at room temperature with 100% Epon. The sample was embedded in Epon and polymerized for 2 days at 60 °C in a drying oven. The Epon block was then separated from the Petri dish by immersion in liquid N_2. The Epon block was sliced into thin sections, 80–90 nm, using a Diatome diamond knife. The sections were transferred to 200-mesh thin bar copper grids and stained with uranyl acetate and lead citrate. Samples were viewed with a Philips Technai 12 electron microscope equipped with a MegaView II charge-coupled device (CCD) camera (Olympus Soft Imaging Soutions GmbH, Germany).

Greenhouse Treatments for Phytoremediation of DEHP-Contaminated Air by *B. hispida*. Four special glass greenhouses (3 × 3 × 3 M), which were the same design as those described in the DEHP Treatments for Investigation of DEHP Uptake by B. hispida section, were used for DEHP treatment and cultivation of *B. hispida*. DEHP treatment was performed by placing Petri dishes filled with DEHP on a metal shelf (2 m × 30 cm), which was 1.5 m from the ground. The metal shelf was a reticular plate, which allowed for the flow of air. The treatment with the high air DEHP concentration was carried out by evenly placing 14 Petri dishes (10 cm inner diameter) filled with 40 mL of DEHP in each dish, while the low air DEHP concentration was obtained by evenly placing 4 Petri dishes (7 cm inner diameter) filled with 20 mL of DEHP in each dish. Plant treatment was as following: 4 similar potted plants of *B. hispida* with a 10 cm length of waxgourd (fruit) were moved into the four corners of the greenhouse treated with the high air DEHP concentration or the low air DEHP concentration. The normal cultivated management for *B. hispida* was performed during the whole experiment (June 3–July 3, 2011). Air sample collection for DEHP determination was conducted in the center of each greenhouse at a height of 0.8 m from the ground. The sampler was fixed in the center of the greenhouse. Each sample were collected for 10 min at a flow rate of 5 L/min with QCD-5000 Intelligent Air samplers (Galaxy Technology Co., Yancheng, China) using methanol as the absorption solution. Air samples were collected simultaneously at the site of the four greenhouses. At each site, three samples were collected at 10 min intervals for a 40 min period (11:00–11:40) on every sampling day. The daily air DEHP concentration of a site was expressed as the mean ± standard deviation (SD) of the three samples collected between 11.00 and 11.40 h.

Cultivation of Vegetables Intercropping with *B. hispida* under the DEHP-Contaminant Air. The above glass greenhouses were used for the cultivation of vegetables intercropping with *B. hispida* on the DEHP-contaminant air in the next year. First, the seedlings of *Brassica campestris* L. and *Brassica chinensis* var. *chinensis* were planted in the soil of the greenhouse for 2 weeks with a distance of 50 cm between the two plant sites and each plant with a vegetable at a total of nine plant sites occupied on half of the land. Then, four similar potted plants of *B. hispida* with a 10 cm length of fruit were moved into the four corners of the greenhouse. After that, four of the Petri dishes (7 cm inner diameter) filled with DEHP were placed on the shelf for DEHP treatment of the space of the greenhouse. Two controls were set; i.e., one greenhouse treated with DEHP was used for planting only *B. hispida*, and the other was cultivated for the two vegetables. DEHP treatment continued 30 days from June 1 to June 30, 2012. After 30 days of DEHP uptake of the plants, each plant in various plant sites of *B. campestris*, *B. chinensis*, and *B. hispida* was harvested and freeze-dried for DEHP determination. The DEHP content for the aerial part of *B. campestris* and *B. chinensis* was expressed as the mean ± SD of the nine samples. The DEHP content for stems + leaves and the fruit of *B. hispida* was expressed as the mean ± SD of the four samples.

DEHP Determination. Soxhlet extraction was used for DEHP extraction of all plant samples. The dried samples (50–500 mg) of the plants were extracted with dichloromethane for 4 h, and the resulting extract was concentrated to near dryness in a rotary vacuum evaporator. The extracts of the plant samples were purified before the gas chromatography–mass spectrometry (GC–MS) injections. A

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Table 1. Accumulated DEHP (mg/kg of Fresh Weight; $x \pm d$; $n = 3$) in the Peel and Flesh of Three Fruits

	fruit 1		fruit 2		fruit 3	
segment	peel	flesh	peel	flesh	peel	flesh
1 (top)	118 ± 7.1	36.3 ± 3.3	107 ± 6.5	34.7 ± 2.1	103 ± 15	32.5 ± 4.1
2	125 ± 14	41.0 ± 4.3	115 ± 9.9	39.2 ± 3.5	111 ± 15	37.8 ± 6.7
3	286 ± 35	56.7 ± 9.2	286 ± 26	54.8 ± 6.2	276 ± 26	52.0 ± 6.2
4	332 ± 27	63.0 ± 9.4	322 ± 34	60.5 ± 7.1	314 ± 37	59.1 ± 8.8
5	362 ± 23	64.6 ± 9.0	351 ± 27	62.6 ± 6.0	353 ± 36	63.0 ± 6.8
6	332 ± 36	56.4 ± 9.6	340 ± 30	58.1 ± 4.9	332 ± 44	58.4 ± 8.4
7	275 ± 33	59.8 ± 5.4	276 ± 24	53.7 ± 4.8	279 ± 27	56.2 ± 5.1
8	277 ± 39	58.2 ± 8.7	265 ± 28	53.5 ± 6.6	274 ± 37	55.0 ± 6.0
9	258 ± 33	53.4 ± 5.7	252 ± 24	51.7 ± 4.7	253 ± 31	53.8 ± 6.3
10	210 ± 34	46.9 ± 4.2	217 ± 24	45.8 ± 5.6	231 ± 29	44.8 ± 4.9
11	183 ± 24	44.0 ± 7.6	185 ± 16	43.1 ± 3.8	181 ± 24	42.8 ± 5.2
12	178 ± 14	39.9 ± 6.3	177 ± 17	38.3 ± 3.3	169 ± 20	37.7 ± 7.3
13	175 ± 18	39.6 ± 4.4	170 ± 19	37.6 ± 4.1	166 ± 19	35.6 ± 5.4
14	143 ± 21	34.5 ± 4.5	140 ± 15	32.3 ± 2.7	142 ± 18	32.4 ± 3.4
15 (end)	134 ± 17	28.5 ± 3.8	133 ± 11	26.5 ± 2.8	133 ± 16	26.5 ± 2.9

Table 2. Amounts of DEHP in Various Leaf and Gourd Fractions Following Centrifuge Separation of 100 g of Fresh Material

fractions	R-0[a]	P-I	P-II	P-III	P-IV	P-V	S-V
dry weight (g) of leaf fractions	3.13 ± 0.51[a]	1.34 ± 0.17	0.176 ± 0.019	0.169 ± 0.011	0.255 ± 0.029	0.021 ± 0.002	0.537 ± 0.068
mg/kg in leaf fractions[b]	222 ± 35	4150 ± 450	670 ± 80	1200 ± 130	1520 ± 240	3200 ± 450	107 ± 14
percent occupied to total leaf DEHP (%)	31.2	17.2	8.5	11.9	15.2	8.5	7.5
dry weight (g) of gourd meat fractions	0.830 ± 0.11	0.404 ± 0.070	0.254 ± 0.034	0.194 ± 0.014	0.153 ± 0.010	0.058 ± 0.005	0.197 ± 0.023
mg/kg in gourd meat fractions[b]	175 ± 33	226 ± 29	853 ± 77	1250 ± 212	1050 ± 150	7230 ± 860	238 ± 29
percent occupied to total gourd meat DEHP (%)	8.3	5.2	12.3	13.7	9.1	23.9	26.5

[a]See Figure 1 for the assignment of R-0, P-I–P-V, and S-V. [b]In dry weight; $x \pm d$; $n = 3$.

glass chromatographic column (25 × 1 cm) was packed consecutively with 3 cm alumina, 10 cm silica, and 2 cm anhydrous sodium sulfate. The concentrated extracts above were loaded onto the column and eluted with 20 mL of $CHCl_2$. For GC–MS analysis, the $CHCl_2$ solution was reduced to a volume of 0.1–0.5 mL under a gentle stream of nitrogen.[23] The tissue samples from centrifuge of 2000–100000*g* were directly extracted with $CHCl_2$ for GC–MS analysis.

The purified samples and the prepared air samples were analyzed by GC–MS using a Hewlett-Packard 5890/5971 GC–MSD system (Agilent Technologies, Palo Alto, CA) equipped with a HP-5 trace analysis column (30 m, 0.32 mm inner diameter, and 0.25 μm film thickness).[24] The GC oven temperature was held at 150 °C for 3 min, then increased by 20 °C/min to 300 °C, and maintained at 300 °C for 3 min. The injector temperature was 250 °C. Helium was used as the carrier gas at a linear flow rate of 20.7 cm/s. Full-scan electron impact data were obtained under the following conditions: solvent delay, 5 min; electron impact energy, 70 eV; source temperature, 200 °C; emission current, 150 μA; scan rate, 4 scans s^{-1}; and detector voltage, 350 V.

The recovery of DEHP (see Tables S1 and S2 of the Supporting Information) was investigated by spiking plant (0.5–500 μg/g of fresh weight) and air samples (5–500 μg/m^3) with standards at high and low contents. The recovery of DEHP was in the 76.2–96.9% range for the entire procedure. The DEHP level in the sample was taken as the average of three injections. The amounts of DEHP were calculated from calibration curves $y = 5.2 \times 10^{-6}x + 0.2461$ (concentration range of 0.1–10 μg/mL; $r^2 = 0.9871$) for air and $y = 4.3 \times 10^{-6}x + 3.1626$ (concentration range of 50–500 μg/mL; $r^2 = 0.9901$) for vegetable samples. The final DEHP contents were expressed as mg/kg of fresh weight based on the weight of the fresh samples of plants and μg/m^3 for air samples.

RESULTS AND DISCUSSION

DEHP Uptake by Leaves, Fruits, and Stems. DEHP contents detected in leaves from different positions of three *B. hispida* plants (see the Supporting Information) showed that the DEHP contents in the oldest leaves of plants I, II, and III were 41.6 ± 4.8, 39.6 ± 5.6, and 37.5 ± 4.3 mg/kg of fresh weight, respectively, while the contents gradually decreased in the younger leaves, with the contents in the youngest leaves being only 2.1 ± 0.3, 1.2 ± 0.1, and 0.6 ± 0.08 mg/kg of fresh weight (see Table S3 of the Supporting Information). The oldest leaves had the greatest DEHP accumulations, demonstrating that DEHP uptake in leaves was dependent upon exposure time. Statistical analysis showed no significant difference in DEHP uptake between the individual plants for leaves of identical age, indicating that *B. hispida* leaves absorbed DEHP at a relatively constant rate.

DEHP contents detected in stem segments from three *B. hispida* plants (see the Supporting Information) showed similar trends to those of the leaves. The contents of the lowest segments of plants I, II, and III were 49.5 ± 7.8, 46.6 ± 5.6, and 44.6 ± 4.8 mg/kg of fresh weight, respectively, and the content decreased from the lowest to the top stem segments (see Table S4 of the Supporting Information). When DEHP contents in fresh leaves were compared to those in fresh stem segments from the same plant position, contents were slightly greater in leaves than in stems. If expressed as dry weight, however, the stem segments contained a higher content than the leaves.

DEHP contents detected in the peel and flesh of *B. hispida* fruit (gourds) are shown in Table 1. The two end segments of

the fruit contained lower amounts of DEHP than the middle segment. This may be due to the longer time available for accumulation in the middle segments because, according to our observation, the gourd grew by elongating from the middle toward the two ends. Also, the transportation of DEHP in the fruit should be slow. The highest measured DEHP contents in peel segments were 362 ± 22, 351 ± 27, and 353 ± 36 mg/kg of fresh weight, while those in flesh segments were 64.6 ± 9.0, 62.6 ± 6.0, and 63.0 ± 6.8 mg/kg of fresh weight. More DEHP accumulated in fruit than in leaves and stems. Within a given fruit segment, DEHP levels in the flesh portion were from about $^1/_4$ to $^1/_5$ of that of the peel. The higher DEHP contents detected in the peel, a relatively small part of the fruit (waxgourd), may be related to the high proportion of waxes present that are able to absorb DEHP.

DEHP Accumulation in Inner Tissues of Leaves and Fruits. The retention of accumulated DEHP in plant tissues was also analyzed. Table 2 list DEHP accumulation levels in the samples (R-0, P-I–P-V, and S-V) following filtration and centrifugation of tissues. To determine what cellular tissue types were present in P-I–P-V, the precipitates were examined under transmission electron microscopy. As seen in Figure 2, precipitates P-I, P-II, and P-III, obtained after centrifugation of leaf material at 400*g*, 2000*g*, and 20000*g*, contained primarily cell walls, chloroplasts (ca. 2 μm width and 5 μm length), and mitochondria (ca. 0.1–1.0 μm width and 1.5–2 μm length), respectively. The major components of precipitates P-IV and P-V, generated from centrifugation at 50000*g* and 100000*g*, were Golgi complex particles (ca. 0.3–0.5 μm) and ribonucleoprotein particles (less than 0.2 μm), respectively. These identifications were based on particle size as well as the similar conclusions by Xiao.[21] The results observed for gourd samples (Figure 2) were different from those obtained for leaves. No chloroplast and mitochondria were apparent in precipitates P-I, P-II, and P-III. The substances obtained at 400*g*, 2000*g*, and 20000*g* were variously sized membrane fragments. The precipitates from centrifugation at 50000*g* and 100000*g* were portions of the Golgi complex based on the same criteria used to evaluate leaf-derived material. DEHP contents (mg/kg of dry weight) in the precipitate fractions were 222 ± 35 (fibers and insoluble wax), 4150 ± 450 (cell wall), 670 ± 80 (chloroplasts), 1120 ± 130 (mitochondria), 1520 ± 240 (Golgi complex particles), and 3200 ± 450 (ribonucleoprotein particles), while the soluble constituent had a DEHP content of 107 ± 14 mg/kg of dry weight (Table 2). The DEHP contents in membrane fragments from the gourds ranged from 226 ± 29 to 1250 ± 210 mg/kg of dry weight, while Golgi complex and ribonucleoprotein particles contained DEHP contents of 1050 ± 150 and 7230 ± 860 mg/kg of dry weight, respectively (Table 2). There was a high content of DEHP in the ribonucleoprotein particles, implying that they may adsorb DEHP.

Percentages of DEHP found in various leaf or fruit flesh tissue fractions following centrifugation are also shown in Table 2. In leaves, 31.2% of the accumulated DEHP was located in the fiber and insoluble wax fraction (R-0), with 17.2, 8.5, 11.9, and 8.5% distributed in cell walls, chloroplasts, mitochondria, and nucleoproteins, respectively. For the fruit flesh, only 8.3% of DEHP was distributed in the fiber and insoluble wax portion, while 40.3, 23.9, and 26.7% were found in membrane fragments (P-I–P-IV), nucleoproteins, and soluble constituents, respectively. On the basis of the above data, it is clear that only a small proportion of absorbed DEHP was present in soluble constituents, indicating that accumulated DEHP was retained in *B. hispida* plants.

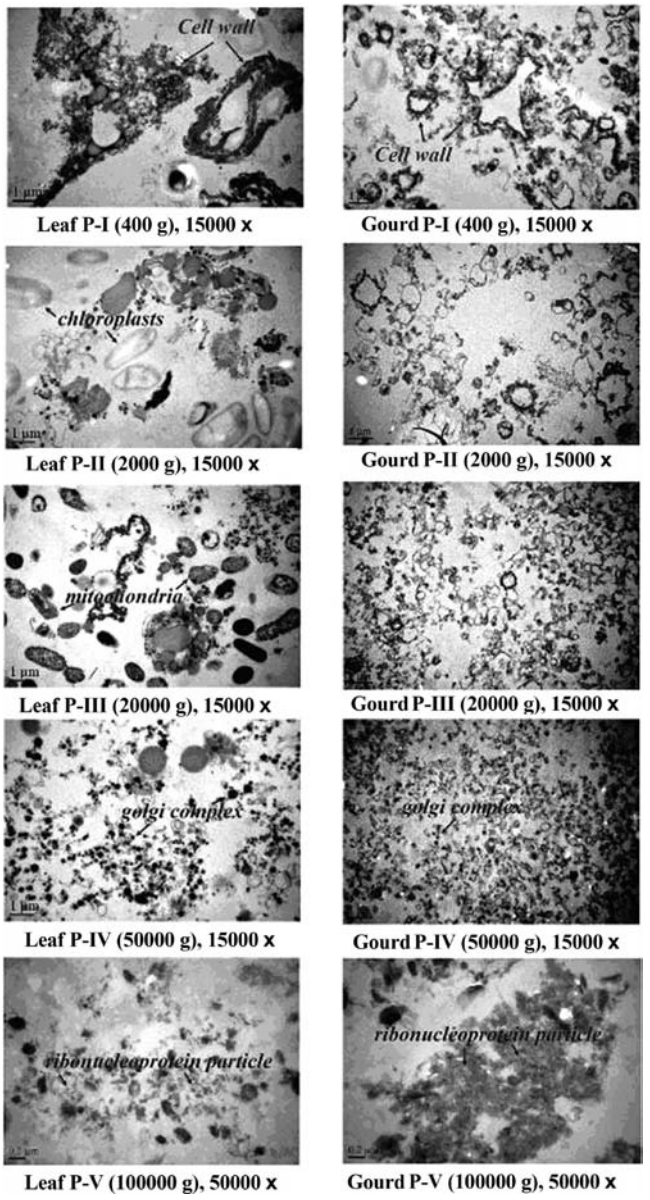

Figure 2. Transmission electron microscope photographs of various fractions obtained from centrifuge separation.

Uptake and Accumulation versus Plant Biomass. The amount of DEHP accumulated relative to plant biomass is a critical factor when evaluating its potential use in phytoremediation of DEHP-contaminated environments. The amount of DEHP accumulated in leaves, stems, and gourds of the three individual plants is given in Table 3. The total biomass of the three individual plants was 13.6, 12.3, and 12.7 kg (fresh weight), 90% of which was derived from the gourds. Measured amounts of accumulated DEHP in the three individual plants were 796, 737, and 723 mg, with more than 96% found in the gourds. From these results, it can be seen that the fruit is the most important organ absorbing and accumulating DEHP in *B. hispida*. Consequently, if *B. hispida* is to be successfully used for phytoremediation of DEHP-contaminated environments, cultivated plants must be grown to maturity, so that large fruits are produced.

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Table 3. Accumulated DEHP in the Three Individual Plants of *B. hispida*[a]

	plant I		plant II		plant III	
item	fresh weight (kg)	DEHP accumulation (mg)	fresh weight (kg)	DEHP accumulation (mg)	fresh weight (kg)	DEHP accumulation (mg)
leaves	0.61	15.1	0.56	14.5	0.52	14.0
stem	0.52	10.7	0.48	9.8	0.47	9.3
gourd	12.5	770	11.3	712	11.7	723
total	13.63	796	12.3	737	12.7	747

[a]Data were obtained by calculating the weight of the leaves, segments of gourds and stems, and their average values of the DEHP content.

In China, most of plastic factories are located in suburbs where vegetables are cultivated. In a previous study, we detected aerial DEHP levels as high as 12 $\mu g/m^3$ at a distance of 0.2 km from a plastic factory.[14] At those levels, a single gourd can remove the DEHP from 6×10^5 m^3 of air.

Topical Phytoremediation of DEHP-Contaminated Air by *B. hispida*. Figure 3 showed the air DEHP concentrations

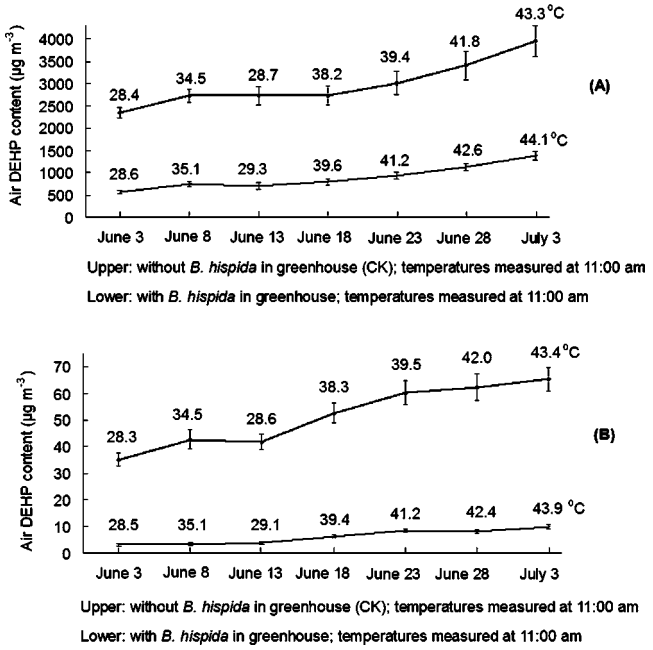

Figure 3. Air DEHP concentrations and the temperatures measured during 30 days as *B. hispida* was/was not cultivated in the greenhouse: (A) treatment of high DEHP concentration and (B) treatment of low DEHP concentration.

and the temperatures measured during 30 days from June 3 to July 3 as *B. hispida* was/was not cultivated in the greenhouse. In Figure 3A, we can see that the air DEHP concentration in the greenhouse ranged from 564 ± 45 to 1380 ± 350 $\mu g/m^3$ as *B. hispida* was cultivated, while the content ranged from 2350 ± 1120 to 955 ± 350 $\mu g/m^3$ as *B. hispida* was not cultivated. Figure 3B exhibits that the air DEHP concentration in the greenhouse ranged from 3.16 ± 0.45 to 9.80 ± 0.83 $\mu g/m^3$ as *B. hispida* was cultivated in it, while the content ranged from 35.1 ± 2.8 to 65.3 ± 4.8 $\mu g/m^3$ as *B. hispida* was not cultivated in it. The percentage of DEHP decrease by *B. hispida* was 65−76 and 85−92% when *B. hispida* was cultivated in greenhouse with high and low air DEHP concentrations, respectively (Table 4). The greenhouses used for the present study were a semiopen type, which provided an environment inner greenhouse close to the normal field of crops and vegetables. Therefore, the effect of

Table 4. Effects of the Plants of *B. hispida* on the Decrease of Air DEHP Concentrations ($\mu g/m^3$) in the Greenhouses from 11:00 to 11:40 am

	high DEHP content		low DEHP content	
	concentration in the air without *B. hispida*	DEHP decreased by *B. hispida* (%)	concentration in the air without *B. hispida*	DEHP decreased by *B. hispida* (%)
June 3	2350 ± 110	76	35.1 ± 2.8	91
June 8	2720 ± 150	73	42.6 ± 3.7	92
June 13	2730 ± 180	72	41.8 ± 3.2	91
June 18	2730 ± 210	71	52.6 ± 4.1	88
June 23	3000 ± 260	69	60.3 ± 4.5	86
June 28	3410 ± 320	67	62.4 ± 5.3	87
July 3	3960 ± 350	65	65.3 ± 4.8	85

the *B. hispida* decreasing air DEHP concentration can be used for topical phytoremediation of DEHP-contaminated air.

Decrease Effect of *B. hispida* on the DEHP Accumulation of the Intercropping Vegetables. We tried an intercropping of *B. hispida* with two vegetables *B. chinensis* and *B. campestris* to further confirm the phytoremediation of *B. hispida* for DEHP-contaminated air in a greenhouse in which the DEHP treatment was the same as the greenhouse with a low air DEHP concentration (Figure 3B). Table 5 showed the DEHP content of two vegetables cultivated in the greenhouses for 30 days with and without *B. hispida* for intercropping. The detected DEHP contents of *B. chinensis* and from the intercropping greenhouse were only from about $^1/_9$ to $^1/_{10}$ of those from the *B. campestris* unintercropping greenhouse. In comparison, the content of the gourd and stems + leaves from the intercropping and un-intercropping greenhouse was very close, which indicates that *B. hispida* could uptake most of the air DEHP in the greenhouse. Obviously, *B. hispida* played a role of phytoremediation on the growth environment of the two vegetables. Therefore, cultivation of vegetables by intercropping with *B. hispida* in the vicinity of plastic factories may be a feasible means of carrying out local phytoremediation of DEHP-contaminated environments of vegetable fields, although the best way to resolve the pollution of DEHP from plastic factories should be controlled at their sources.

In conclusion, the amount of DEHP that accumulated in leaves, stems, and fruits was dependent upon exposure time, and most DEHP accumulated in their inner tissues. A single plant of *B. hispida* gourd with a fruit was able to absorb more than 700 mg of DEHP as it exposures to DEHP-contaminated air for 6 week. In greenhouse experiments, *B. hispida* could remove 65−76 and 85−92% of air DEHP at high and low DEHP level treatments, respectively. Intercropping of *B. hispida* and vegetables could reduce more than 87% of DEHP accumulation in vegetables. Therefore, *B. hispida* has excellent

Table 5. DEHP Content (mg/kg of Fresh Weight) of Two Vegetables Cultivated in the Greenhouses for 30 Days (June 3−July 3) with and without *B. hispida*

			B. hispida	
treatment	*B. campestris*	*B. chinensis*	gourd	stems + leaves
without *B. hispida*	2.8 ± 0.81	3.3 ± 0.72		
with *B. hispida*	0.30 ± 0.04	0.41 ± 0.05	30.2 ± 4.9	12.4 ± 2.8
only *B. hispida*			32.7 ± 5.7	14.0 ± 2.6
percent DEHP decrease (%)	89.3	87.6		

potential for use in phytoremediation of DEHP-contaminated air to lower the DEHP content of intercropped vegetables.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details for the recovery data of the spiking samples and the contents of DEHP in leaves and stem segments from the different positions of the three *B. hispida* plants. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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