

1 **Migration of di-(2-ethylhexyl) phthalate from the plastic film into the air and**
2 **vegetables in the greenhouses**

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12 **Keywords:** DEHP; vegetables; air; greenhouse; cover plastic film; temperature
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17 **Abstract:** The aim of this study was to investigate di-2-ethylhexyl phthalate (DEHP)
18 from plastic film of the greenhouse to the air and the vegetables grown in the
19 greenhouse. This paper presents the migration of di-2-ethylhexyl phthalate (DEHP)
20 by determining the di-2-ethylhexyl phthalate (DEHP) concentration in air and
21 vegetables in greenhouses with the change of temperature in a day and a half year. The
22 determination of di-2-ethylhexyl phthalate (DEHP) content in the air and the
23 vegetables was performed by gas chromatography-mass spectrometry. The results
24 showed that di-2-ethylhexyl phthalate (DEHP) content in the greenhouse air increased
25 with increasing temperature during one day, but di-2-ethylhexyl phthalate (DEHP)
26 content changes in vegetable were different from those in air, and which depends on
27 the exposure time. For the monthly content changes, di-2-ethylhexyl phthalate (DEHP)
28 content in air first increased to a maximum and then decreased with increasing time
29 and temperature. The di-2-ethylhexyl phthalate (DEHP) content changes of six
30 vegetables not only were related with di-2-ethylhexyl phthalate (DEHP) content in air
31 and temperature, but also with varieties of vegetables.
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36 **Introduction**

37 Plastic greenhouses are a form of horticulture which plays a special role for supplying
38 off-season vegetables. As food-safety is increasingly concerned, the safety evaluation
39 of vegetable is not limited to heavy metals and pesticide residues, but also organic
40 contaminants [9]. Contamination of vegetables by the plasticizers is being received
41 serious attention. The most used plasticizer, di-(2-ethylhexyl)phthalate (DEHP) is
42 easily released to environment from plastic products due to its instability, fluidity and
43 volatility[1] since DEHP is not chemically bonded to the polymer of plastics.
44 Therefore, DEHP from the greenhouse film is possible of an organic contaminant of
45 vegetables cultivated in greenhouses.

46 Migration of DEHP from the plastic products to several foods was reported. Zhang et
47 al.[19] reported that meat wrapped with PVC film could be contaminated by DEHP,
48 and the migrated amount of DEHP depended on the temperature and time that the
49 meat wrapped by plastic film. Goulas[7] studied that the migration of DEHP from
50 food-grade polyvinyl chloride film into hard and soft cheeses, and the result showed
51 that the longer the exposure, the higher levels of DEHP in cheese. The uptake of
52 DEHP from plastic mulch film used in field cultivation by 10 vegetable plants was
53 investigated by experiments carried out in pots by Du et al. [5,6]. The results showed
54 that DEHP was transferred from the mulch film to all vegetable plants, and *Benincasa*
55 *hispidia*, *Cucumis sativu*, *Cucurbita moschata* and *Brassica parachinensis* could
56 accumulate high level of DEHP from mulch plastic film giving dietary exposure over
57 or close to the daily intake limit for DEHP. The air DEHP from the plastic production
58 building could cause contamination of vegetables according to a determination of
59 DEHP concentrations in the atmosphere and four vegetable crops cultivated on land
60 surrounding a plastic production factory [6]. However, no data is available concerning
61 the migration of DEHP from the plastic film into vegetables in the greenhouses. This
62 paper presents the migration of DEHP by determining the DEHP concentration in air
63 and vegetables in greenhouses with the change of temperature in a day and a half
64 year.

65 **Materials and methods**

66 **Solvents and reagents**

67 All solvents and reagents used for extraction were of analytical grade and
68 purchased from Huadong Chemicals Company (Hangzhou, China). DEHP and
69 chromatographically pure chloroform for dissolving DEHP standard was purchased
70 from Sigma Shanghai Division (Shanghai, China).

71 **Film material**

72 The film was manufactured by Liaochen Plastic Film (Shangdong, China) and was
73 used for the field-plant experiments.

74 **Plants used for experiments**

75 Some vegetables cultivated in the plastic-film greenhouse including bok choy (*B.*
76 *chinensis*), celery (*A. graveolens*), spinach (*S. oleracea L.*), cabbage (*B. oleracea*),
77 lettuce (*L. sativa*), edible amaranth (*A. mangostanus*), were selected for testing DEHP
78 uptake.

79 **Sampling**

80 **The daily variation of DEHP from the greenhouse films**

81 The edible parts of bok choy (*B. chinensis*) and the air samples in the greenhouses
82 were collected at the same time for DEHP determination. The air samples were
83 collected at 5:00, 8:00, 11:00, 14:00, 16:00, 19:00 and 22:00 in 3 consecutive days by
84 the activated carbon adsorption method[17,18]. And the temperature at each time was
85 recorded. For each DEHP determination, three replications were carried out.

86 **The monthly variation of DEHP from the greenhouse films**

87 From December 1, 2010 to June 10, 2011, the air samples and six vegetables were
88 collected in the tenth day of each month at 9:00 am. Six vegetables were planted in
89 the first day of each month and sampled in the 10th day of next month. The air in the
90 greenhouse was sampled by the activated carbon adsorption method, and the
91 temperature at each time was recorded. For each DEHP determination, three
92 replications were carried out.

93 **Determination of DEHP**

94 **Extraction of DEHP**

95 Vegetable samples were freeze-dried, ground and sieved to less than 0.2 mm. For
96 each sample, 5-10 g was extracted in a Soxhlet extractor for 24 h with 200 mL
97 acetone/dichloromethane (1:1,v/v) in a water-bath at 75 °C . The extracts were
98 reduced to 5.0 mL using a rotary evaporator in a water-bath at 50 °C . The
99 concentrated sample was subjected to clean-up.

100 Air samples: For each sample, 10 g was extracted in a Soxhlet extractor for 24 h with
101 200 mL acetone/dichloromethane (1:1,v/v) in a water-bath at 55 °C . The extracts
102 were reduced to 5.0 ml using a rotary evaporator in a water-bath at 50 °C . The
103 concentrated sample was subjected to clean-up.

104 **Clean-up**

105 The concentrated samples were loaded on a combined column of silica gel and
106 alumina. The glass chromatography column 25 cm long, 1 cm I.D.) , was packed
107 with 3 cm alumina plus 10 cm silica, followed by 2 cm anhydrous sodium sulfate.

108 Dichloromethane (20 mL) was used for elution. The collected fraction was reduced to
109 0.5 mL under a gentle stream of nitrogen.

110 **Gas chromatography-mass spectrometry**

111 The cleaned samples were analyzed by GC-MS [8,13] using a Hewlett-Packard
112 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA, USA) equipped with an
113 HP-5 trace analysis column (30 m, 0.32 mm I.D., 0.25 mm film thickness). The GC
114 oven temperature was held at 150 °C for 3 min, and programmed to increase at 20
115 °C min⁻¹ to 300 °C and finally held at 300 °C for 3 min.

116 The temperature of the injector was 250 °C. Helium was the carrier gas at a linear
117 flow-rate of 20.7 cm s⁻¹. Full scan electron ionization data was obtained as follows:
118 solvent delay 5 min, electron ionization energy 70 eV, source temperature 200 °C,
119 emission current 150 μA, scan rate 4 scan s⁻¹, detector voltage 350 V. The DEHP
120 level in the sample was taken as the average of three injections. The amounts of
121 DEHP were calculated from a calibration curve: $y=0.0014x + 0.0006$ ($r^2=0.9911$) for
122 air samples and $y=0.0008x + 0.0003$ ($r^2=0.9848$) for vegetable samples. The final
123 contents of the vegetables were expressed as μg g⁻¹ based on the amounts of the dried
124 samples.

125 **Results and discussion**

126 **The daily variation of DEHP from the greenhouse films**

127 **The daily variation of DEHP in the greenhouse air**

128 The concentration of DEHP in the greenhouse air was determined by gas
129 chromatography-mass spectrometry. The results show that the DEHP content in air
130 increased with the increase of the greenhouse temperature (Fig.1). The air DEHP
131 content was the lowest in 5:00, and then as the temperature rose, the content was
132 gradually increased. To 2 pm when the temperature was the highest point, the DEHP
133 content reached also the highest value. Then as the temperature decreased, the content
134 was slowly declining. It was evident that the DEHP content in the greenhouse air was
135 closely related with the greenhouse temperature.

136 During the day as temperatures rise, the temperature in the greenhouse also will
137 increase. And it also accelerated the release rate of DEHP from the film. That is,
138 DEHP content in air increased, and reached the highest point when the temperature
139 reaches the maximum.

140 As the day time, the temperature gradually decreased. Subsequently the released rate
141 of DEHP from the greenhouse films was affected, which was bound to affect the
142 release of DEHP. Coupled with the greenhouse was not a closed container, or DEHP
143 may also be absorbed by plants or soil in the greenhouse. Therefore, the temperature

144 dropped, the DEHP content in air also declined.

145 Wang et al.[16] reported that the characteristics of distribution of Phthalate esters
146 (PAEs) in vegetable greenhouses. Our results are in agreement with their
147 observations.

148 **The daily variation of DEHP in the greenhouse vegetables**

149 Fig. 2 show that the DEHP content in the greenhouse vegetable was increased from
150 morning to night. For the March 24, March 25, and March 26, 2011 samples, DEHP
151 content were slowly increasing as time went on.

152 DEHP daily changes in vegetables were not consistent with those in the air (Fig.3).

153 DEHP daily content in air increased and then decreased, while the daily content in
154 vegetables was increasing from the morning to the evening.

155 When the DEHP in the air was the highest levels, it does not contain the maximum
156 amount of DEHP in vegetables. This can be inferred that the DEHP concentration in
157 vegetables was related not only with the content of DEHP in the air, but also the time
158 of absorption.

159 **The monthly variation of DEHP from the greenhouse films**

160 **The monthly variation of DEHP in the greenhouse air**

161 With the increase in the greenhouse age, levels of DEHP in the air firstly slowly rose
162 and then rapidly rose and slowly declined followed two months (Fig.4). As time went
163 on, the total amount of DEHP in films was gradually reduced, so that their emission
164 was also reduced. Therefore, the concentration of DEHP in the air gradually
165 decreased after reaching a certain concentration.

166 DEHP content in the air was also associated with the temperature. The higher the
167 temperature, the faster the molecular thermal motion in films, which helps to
168 evaporation and leaching of DEHP from the films. From December 10, 2010 to April
169 10, 2011, with the gradual increase in ambient temperature, which will affect the
170 micro-climate in the greenhouse, the concentration of DEHP of the air in the
171 greenhouse increased slowly to reach maximum concentration in April. With levels of
172 DEHP of the films decreasing, which will reduce its total dissolution, and thus the
173 concentration of greenhouse air was gradually decreased (Fig.4). During May and
174 June, although the temperature is relatively high, but the total DEHP content of films
175 gradually reduced, which also reduced the emission of DEHP, and the number of
176 open-air greenhouse were added, so levels of DEHP in air decreased. Zheng et al. [23]
177 reported the migration of the PAEs from the PVC plastic products in the water
178 environment. Wang et al.[16] studied the variations of the PAEs levels from winter to
179 spring. These results are similar with our findings.

180 **The monthly variation of DEHP in the greenhouse vegetables**

181 Fig.5 show that the content changes of DEHP in *L. sativa*, *S. oleracea L.* and *A.*
182 *mangostanus* were somewhat regular. With increasing age of the greenhouse, the
183 DEHP content in the three vegetables also increased before May 10, 2011. However,
184 the levels of DEHP in the six vegetables had decreased in varying degrees from May
185 10, 2011 to June 10, 2011. This result is consistent with the variation of DEHP levels
186 in the greenhouse air. There are two reasons to explain this result. One is that the total
187 DEHP of the membrane decreased; second is that the greenhouse are required to
188 increase ventilation times because of the high temperature.

189 Among the 6 vegetables, three kinds of vegetables (*L. sativa*, *B. chinensis* and *A.*
190 *graveolens*) can be strong to absorb DEHP in air, followed by the other two
191 vegetables (*S. oleracea L.* and *A. mangostanus*). While a vegetable (*B. oleracea*) is
192 the weakest ability to absorb DEHP. The results concluded that the DEHP content in
193 vegetables is related with the vegetable varieties. And different vegetables have
194 different absorption or adsorption ability of DEHP. Wang Jiawen et al. [17] reported
195 the DEHP pollution of vegetable from farmland and differences between vegetables
196 species. contaminated by DEHP. Wang et al.[16] studied that the distribution of
197 Phthalate esters in the greenhouse vegetables in the different ages. In this regard, our
198 results are basically the same with their.

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202 **Author contributions statements**

203 Xiaowei Fu wrote the main manuscript text and Bin Huang prepared figures 1–3. All
204 authors reviewed the manuscript.

205 **Competing financial interests**

206 The authors declare no competing financial interests.

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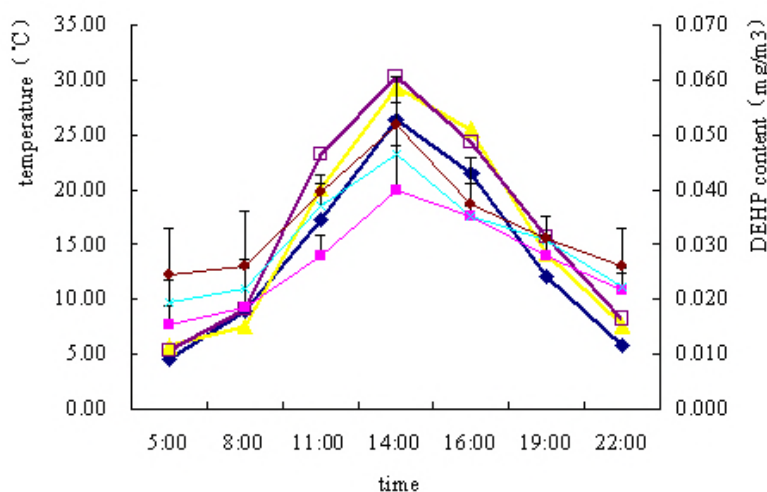
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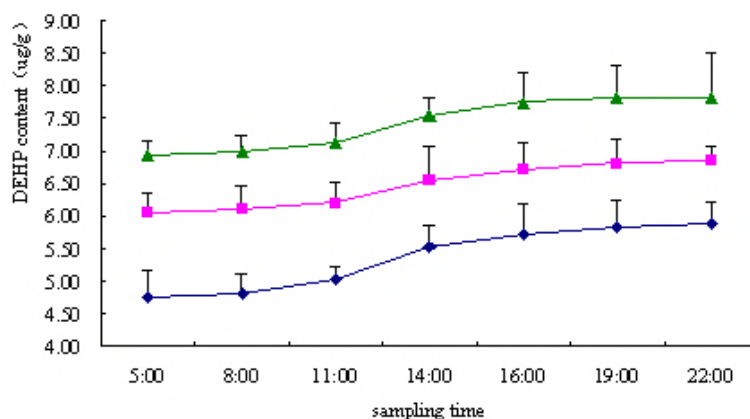
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264 Figure 1 The change of temperature and DEHP content of air in the greenhouse during a day.

265 March 24, 2011; ◆temperature; ■DEHP content

266 March 25, 2011; ▲temperature; ×DEHP content

267 March 26, 2011; □temperature; ○DEHP content



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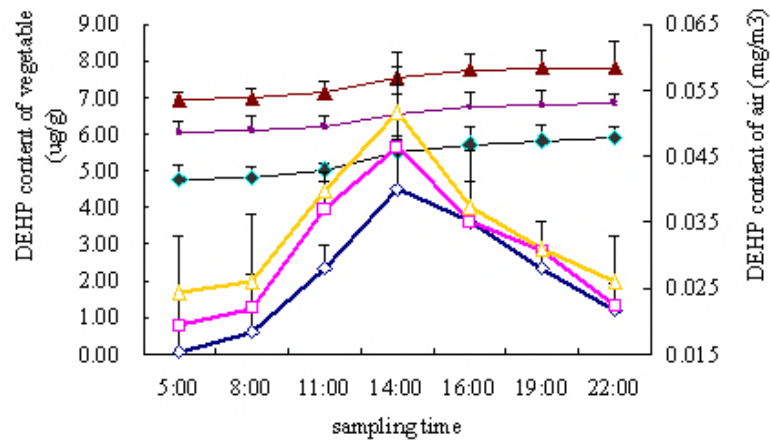
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◆March 24, 2011; ■March 25, 2011; ▲March 26, 2011

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Figure 2 The change of DEHP content of *B.chinensis* cultivated in the greenhouse.

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Figure 3 The comparison between the changes of DEHP content of air and *B.chinensis* in the greenhouse.

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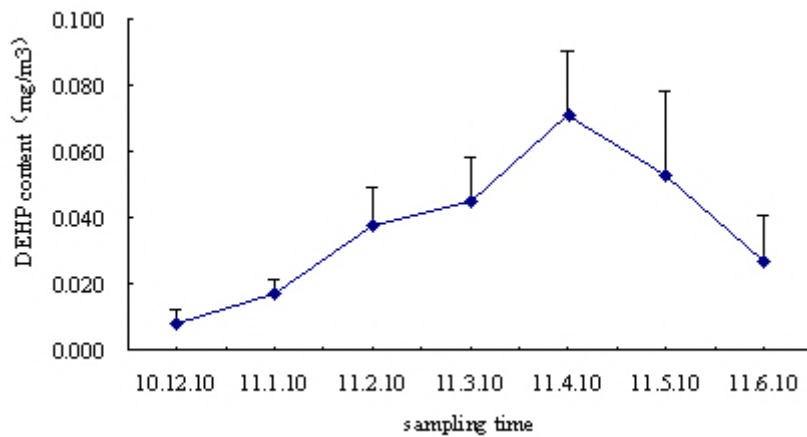
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March 24, 2011; ◇ air; ◆ *B.chinensis*

March 25, 2011; □ air; ■ *B.chinensis*

March 26, 2011; △ air; ▲ *B.chinensis*

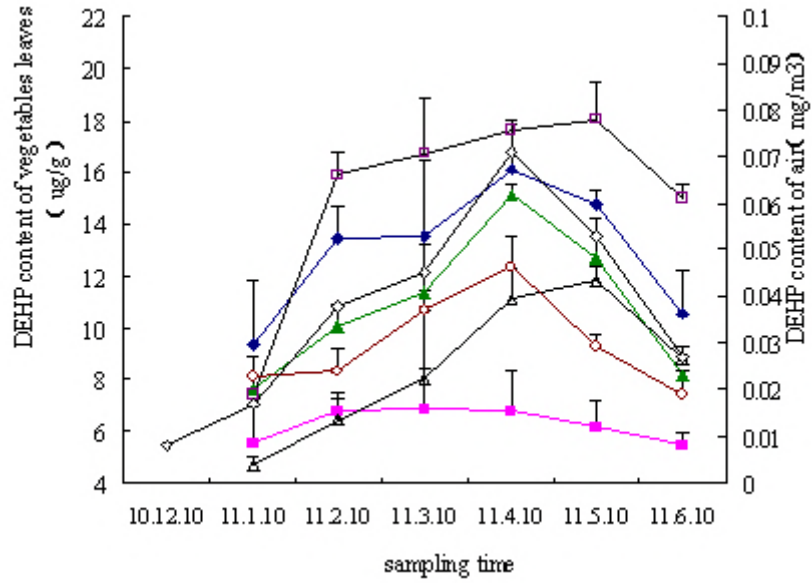


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Figure 4 The monthly variation of DEHP content of air in the greenhouse.

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284 Figure 5 The monthly variation of DEHP content of the different vegetables from the greenhouse.

285 (◆ *B. chinensis*; ■ *B. oleracea*; ▲ *S. oleracea*; △ *A. graveolens*; □ *L. sativa*; ○ *A. mangostanus*;

286 ◇ air)