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Studies on the sources of benzo[*a*]pyrene in grain and aboveground tissues of rice plants

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ABSTRACT

Rice plant pot experiments designed to identify benzo[*a*]pyrene (B[*a*]P) sources in plant tissues were conducted in an air-quality controlled greenhouse built to prevent contamination from B[*a*]P air pollution. Results from quartz sand cultures with control and 50, 100 and 500 μg kg^{-1} of B[*a*]P treatments were compared with those from outdoor field experiments, in which rice plants were exposed to polluted air in the urban area of Shenyang, China. When B[*a*]P was strictly controlled in both air and quartz sand culture medium, the background values of B[*a*]P in rice plant tissues were uniformly very low. There was no significant difference of B[*a*]P contents of rice grain between control and treatments of B[*a*]P in controlled air quality trials. This indicated that the source of B[*a*]P in the rice grains is not from any B[*a*]P in the root culture media. The B[*a*]P content of rice grain, husk, and stem with leaf sampled from outdoor field was up to 7.33-, 9.21- and 27.10-fold higher than corresponding tissues from air-quality controlled conditions. This indicated that polluted air is the main source of B[*a*]P in aboveground tissues. Therefore control of B[*a*]P pollution in ambient air is of prime importance for improving the quality of cereal crops.

1. Introduction

China is now in a stage of accelerating development resulting in massive industrialization and urbanization, and increased exploitation of natural resources (People's Republic of China Yearbook 2006) [1]. Liaoning Province in north-eastern China is referred to as "a cradle of industry of new China". The industrial output of Liaoning is the second highest in the country. However, environmental pollution in Liaoning Province is steadily increasing in urban areas and is now detected in adjacent rural areas. The quality of agricultural production and human health are thereby threatened.

Polycyclic aromatic hydrocarbons (PAHs) are among the most dangerous environmental contaminants due to their toxic, carcinogenic and mutagenic effects. Benzo[*a*]pyrene (B[*a*]P) is one of the priority 16 PAHs listed by the US EPA [2]. PAHs are found in different environmental media in worldwide [3–5]. In Liaoning Province, a recent field investigation organized by the Institute of Applied Ecology, Chinese Academy of Sciences was carried out in the Shenfu Wastewater Irrigation Agricultural Area located between the two major provincial cities of Shenyang and Fushun. The field investigation results showed that the mean B[*a*]P concentration in first 20 cm of soil was 340 μg kg^{-1}, the highest value found being 3180 μg kg^{-1}. Aspects of soil and air pollution in another Irrigation Agricultural Area, "Hunpu" in a suburb of Shenyang was also investigated, and the B[*a*]P concentrations in soil from this area ranged from 80 to 320 μg kg^{-1} [6]. These B[*a*]P concentrations are higher than the environmental standards set in many European countries [5]. Soil pollution by B[*a*]P is now being paid increasing attention [6–10].

In the meantime, many studies report the B[*a*]P pollution in air [11–17]. The B[*a*]P content in ambient air has many sources including the combustion of petroleum, grass, wood and coal. It is mostly absorbed by particulate matter (PM) of various diameters [13]. In the conference on the Regional Quality Management of Air hold by China EPA and US EPA, Vice Director of China EPA, Lijun Zhang has leaked out the information that 48.1% of Chinese cities the air quality are on the condition of median or heavy pollution, and the first pollutant affecting Chinese air quality is particulate matter [18]. B[*a*]P pollution in air is also a problem and is related to the particulate matter pollution in the atmospheric environment.It is a viable hypothesis that environmental pollution of B[*a*]P could affect the

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quality of agricultural products. As one of the world's largest producers of rice, the culture area and production of rice plants in China make up to 23% and 39% respectively of the world totals. Rice plant is the main cereal crop in China. The cultivation area of rice is approximately 28.6 million ha with an annual production of approximately 185 million tons (2001 data).

It is very necessary to be aware of the B[*a*]P content in rice grain and its possible sources in order to carry out environmental management to ensure the good quality of rice grain and minimise risk to human health. However a few published reports deal with the mechanism of adsorption and accumulation of B[*a*]P in plants, especially for cereal crops like rice plants [19,20]. Therefore to locate sources of B[*a*]P and eliminate its contamination of rice grain are of major importance for protection of human health. The aim of the present study is to identify the real source of B[*a*]P in the tissues of rice plant and to verify where and how the B[*a*]P is located in rice plant tissues. By understanding the B[*a*]P source for the rice plant, any necessary environmental management and remediation can be conducted effectively.

2. Materials and methods

2.1. Air-quality controlled greenhouse

The "air-quality controlled greenhouse" is the key facility for this study. A commodious greenhouse chamber, located at the campus of the Institute of Applied Ecology (IAE) in Shenyang, China, was established as the air-quality controlled site for rice plant pot experiments. The air in the chamber was controlled technically and stringently as shown in Fig. 1.

Air filters are essential installations in air-quality controlled greenhouses. A series connection of two air filters was used to provide unpolluted fresh air into the air-quality controlled greenhouse continuously throughout the whole growth season. The primary air filter (Model M-1) had a standard air capacity of 2000 m^3 per hour. Its efficiency for removal of particulate matter was 70%. The second stage was a high-grade air filter (Model GB-3), which has a very high efficiency; air capacity 1500 m^3 per hour, air speed at entrance 0.8–1.0 m/s. Its efficiency was 99.91% removal of particulate matter including particle diameters of 0.3–0.6 μm, and 99.91–99.99% removal of oil droplets. The greenhouse was maintained at positive internal pressure for the whole growth season to avoid ingress of untreated air.

In addition, a shower spray with 30 nozzles was established. The shower spray was operated twice each day during rice plant growing season to further clean the air. A second purpose of the shower spray was to lower the temperature in the chamber on the hot summer days. Below the shower spray, glass plates were positioned to prevent the shower watering the experimental pots.

The ambient light source was supplemented using four mercury lamps (400 W each) located underneath the glass plates.

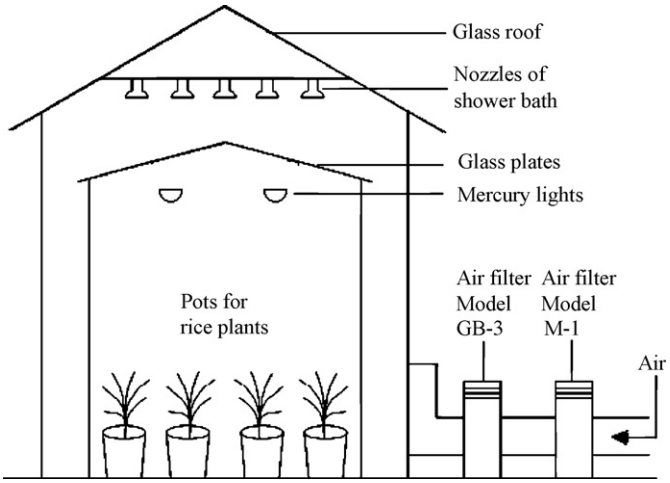


Fig. 1. Air-quality controlled greenhouse.

2.2. Experimental design

Potted rice plants were deployed as shown in Table 1. The culture media for treatments 2–4 was spiked twice (at replanting and again at tilling) with B[*a*]P at the concentrations of 50, 100 and 500 μg kg^{-1} respectively, expressed as 50 μg $kg^{-1} \times 2$, 100 μg $kg^{-1} \times 2$ and 500 μg $kg^{-1} \times 2$. For pots in the field there were covers on the top of each pot to prevent contamination of the culture medium from free ash and dust in air, and also from precipitation. Every cover had three holes to allow the leaves of the growing rice plant access to light and the ambient air.

2.2.1. Air-quality controlled greenhouse group of pots

Rice plants were grown in porcelain pots. Quartz sand was selected as a growth medium, because quartz sand lacks many of the components of natural soils and there are no fine particles; thus allowing the B[*a*]P to be more easily absorbed by plant roots. The treatment 1 (control) had no B[*a*]P added. In treatments 2–4 the quartz sand was spiked with B[*a*]P at the treatments 50 μg $kg^{-1} \times 2$, 100 μg $kg^{-1} \times 2$ and 500 μg $kg^{-1} \times 2$. The B[*a*]P was dissolved in benzene and then spiked evenly through the sand in the pot in the first application. The second spiking was to the top layer, which was then coved by a thin layer of new quartz sand.

For the treatments 5 and 7, the soil for the rice plant culture was collected from the botanical garden of the Institute of Applied Ecology, Shenyang, China. The B[*a*]P concentration in the soil was 170 μg kg^{-1}; no further B[*a*]P was added.

2.2.2. Field experiment group of pots

The treatment 6 and 7 pots were placed outdoors in the campus of Institute of Applied Ecology where they were exposed to polluted air in the urban area in Shenyang.

Table 1
The experimental design for the source of B[*a*]P in rice plant tissues

Treatment no.	Culture location	Culture media	B[*a*]P treatment (μg kg^{-1})	Replicate	Culture medium weight per pot (kg)
1	AQCGH[a]	Quartz sand	Control	8	13.5
2	AQCGH[a]	Quartz sand	50 × 2	8	13.5
3	AQCGH[a]	Quartz sand	100 × 2	8	13.5
4	AQCGH[a]	Quartz sand	500 × 2	8	13.5
5	AQCGH[a]	Soil	170	8	10.5
6	FE[b]	Quartz sand	Control	8	13.5
7	FE[b]	Soil	170	8	10.5

[a] AQCGH, air-quality controlled greenhouse.
[b] FE, field experiment.

Table 2
Comparison of air quality in controlled greenhouse and outdoor field

Location	Item			
	B[*a*]P and particulate matter in air			B[*a*]P in dust fallout (μg/m^2 60 days)
	Number of samples	B[*a*]P content (μg/100 m^3)	Weight of PM (mg/100 m^3)	
Controlled air greenhouse	8	ND	1.60	ND
Outdoor field	8	0.91	43.88	110

ND, not detected.

2.3. Rice plant management

Before the replanting of rice seedlings, 6 g of a granular fertilizer (Osmocote plus Controlled Release Fertilizer), containing NPK macro-nutrients as well as necessary trace elements was added to each pot. The pots were top-dressed three times during the experiment with KH_2PO_4 and urea (2 g pot^{-1} per dressing on each occasion). Over the whole growth season, deionized water was used for irrigation. Because of the very careful management, pests and disease were controlled; pesticides were not used.

2.4. Sample treatment, chemical and statistical analysis

Upon maturing, the rice plants were harvested. After harvest the rice plants were washed with copious volumes of tap and deionized water, especially for the roots, to remove sand or soil particles. The rice plant tissues were divided into four groups: rice grain, rice husk, stem with leaf, and roots. The four tissues were ground after drying (in the air-quality controlled greenhouse) and prepared for B[*a*]P analysis using high-performance liquid chromatography HPLC (Hewlett–Packard 1090-II Series) with a fluorescence detector according to the modified method of Song et al. [21]. 5 g dried samples of rice plant tissues were put into centrifuge tubes (50 mL), which had been previously rinsed with dichloromethane. Methanol (20 mL) was added to the samples, which were then extracted for 2 h in ultrasonic bath (KQ-500DB). The extraction procedure was repeated in triplicate. After extraction, the centrifuge tubes were centrifuged for 10 min at 4000 rpm. The combined supernatants were filtered into a flask (200 mL) through a funnel containing anhydrous Na_2SO_4. The extract was concentrated to 5 mL using rotary evaporator (CA 1111) before being transferred to a silica gel column (10 mm i.d. × 100 mm long at the top, 5 mm i.d. × 50 mm long at the bottom). The column was eluted with *n*-hexane (1.5 mL × 3). In this cleanup procedure, only the first 1 mL of eluate collected from the silica column was discarded. The remaining part of the eluate collected was firstly concentrated to near dryness, then transferred and diluted with methanol and brought to exactly 1.0 mL by nitrogen blow-down at room temperature and then for analysis.

To compare concentrations of B[*a*]P in rice plant tissues for different treatments, the data were analyzed using the analysis of variance (ANOVA) routine provided SPSS-version 11.5, and the P value used to assess the statistical significance and quantitative differences [22].

3. Results and discussion

3.1. Air quality as measured in controlled greenhouse and at the outdoor field plot

The concentration of B[*a*]P and the weight of particulate matter in ambient air were determined from samples collected in the air-quality controlled greenhouse and outdoor field plot. In the whole growth season air quality was as shown in Table 2.

In the controlled air greenhouse, except for a little amount of particulate matter (1.60 mg/100 m^3), neither B[*a*]P nor dust fallout were detected. In contrast the B[*a*]P, particulate matter and dust fallout were determined at 0.91 μg/100 m^3, 43.88 mg/100 m^3 and 110 μg/(m^2 60 days), respectively in the outdoor field site, which are high enough to be considered as distinct B[*a*]P pollution [23]. The monitoring data showed that the two air filters had a very high efficiency in removing the fine particle fraction of the kind to which most B[*a*]P are bound [24]. The high efficiency can also be demonstrated by the scanning electron micrographs of filter papers and surface of rice husk, shown in Figs. 2 and 3.

Fig. 2 is for the air sampling filter paper. In the outdoor field, many particles of a range of diameters are deposited on the filter paper. In contrast, in the controlled air sample, the surface of the filter paper fibres are still clearly visible.

Fig. 3 shows the image from the scanning electron microscope of husk (lemma and palea) that enclosed a rice grain at maturity.

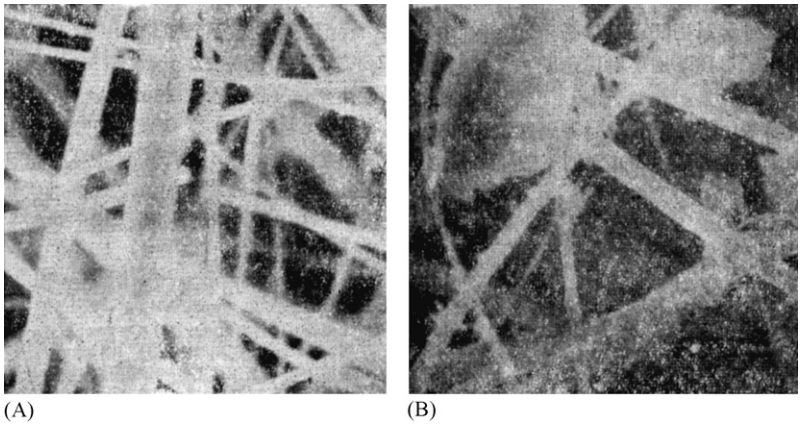
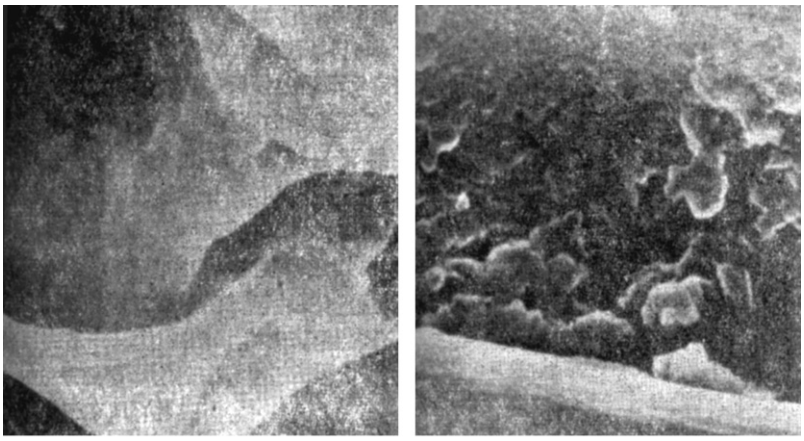


Fig. 2. Scanning of electron micrographs of filter paper for the air sampling (magnification 3000×): (A) in the air quality control greenhouse; (B) in outdoor field.



(A) (B)

Fig. 3. Scanning electron micrographs on outer surface of lemma of mature rice grain (magnification 10,000×): (A) in the air quality control greenhouse; (B) in outdoor field.

Table 3
B[*a*]P content of rice plant tissues in air-quality controlled greenhouse μg kg^{-1} (coefficient of variation % in brackets, $n = 8$ for all samples)

Treatment			Rice grain	Rice husk	Stem with leaf	Root
No.	Concentration[a]	Media				
1	Control	QS[b]	0.16 (54)	1.0 (18)	3.6 (14)	13.1 (41)
2	50 × 2	QS[b]	0.21 (28)	2.6 (34)	4.8 (19)	1,704 (91)
3	100 × 2	QS[b]	0.17 (29)	1.0 (29)	4.6 (20)	3,679 (87)
4	500 × 2	QS[b]	0.22 (22)	2.8 (36)	6.9 (15)	12,055 (104)
5	170	Soil	0.15 (54)	1.9 (46)	4.1 (20)	50.4 (39)

[a] Concentration in μg kg^{-1}.
[b] Quartz sand.

The outer surface of the lemma is very clear to show that in the air-quality controlled sample the cell construction on surface of the lemma is clearly discernible, in contrast, in sample from the outdoor field the surface of the lemma was fully covered by particulate matter.

3.2. B[a]P content in rice plant tissues grown in the air-quality controlled greenhouse and outdoor field

The B[*a*]P content in rice plant tissues, i.e. rice grain, rice husk, stem with leaf, and root are shown in Tables 3 and 4. In the air-quality controlled greenhouse, there was no air pollution; in treatment 1, there was no B[*a*]P added to the quartz sand either. The B[*a*]P content in the rice grain, rice husk, stem with leaf and root in treatment 1 were only 0.16, 1.00, 3.60 and 13.10 μg kg^{-1}, respectively. These data are considered as the background values of B[*a*]P for corresponding tissues of rice plants from the other treatments.

3.3. B[a]P content in rice grain

The B[*a*]P content in rice grain among the treatments 1, 2, 3, 4 and 5 show no significant different by the SPSS statistic analysis. The detailed results of statistical analysis for the mean differences are shown in Table 5.

The pairwise comparisons showed that no significant differences were found at the 0.05 level. This statistical analysis indicated that even in quartz sand culture with the highest B[*a*]P treatment no. 4 (500 μg kg^{-1} × 2), rice grain B[*a*]P content is not significantly different from that of grain grown under the control treatment (no. 1). In other words, even a B[*a*]P treatment of (500 μg kg^{-1} × 2) does not affect rice quality of the plants grown in the air-quality controlled condition. Upon rice grain maturation, the B[*a*]P concentration in the quartz sand of this treatment (no. 4) was still measured at up to 216.65 μg kg^{-1}.

In soil culture treatment (no. 5), the B[*a*]P concentration in soil at rice plant seedling replanting and at maturation were 170 and 89.76 μg kg^{-1}, respectively, however the B[*a*]P concentration in the mature rice grains was not significantly different to grain grown under the control treatment (no. 1) conditions. These experimental results indicate that the pollution of the culture media (quartz sand or soil) does not contribute B[*a*]P to the rice grain even when the culture media contains a high concentration of B[*a*]P; and even when the B[*a*]P is freshly added to the media and the culture media has a low ability to bind B[*a*]P.

The result is that B[*a*]P in soil or other potting materials, like quartz sand, does not contribute B[*a*]P into the rice grain. B[*a*]P is one of the heavier PAH molecules with $M = 252.3$ g mol^{-1}. Its BCF (bioconcentration factor) is relatively low, and these characteristics of B[*a*]P control the transferring mechanism. Similar interpreta-

Table 4
B[*a*]P content of rice plant tissues in outdoor field μg kg^{-1} (coefficient of variation % in brackets, $n = 8$ for all samples)

Treatment		Rice grain	Rice husk	Stem with leaf	Root
No.	Media				
6	Quartz sand (no B[*a*]P added)	1.5 (49)	23.3 (23)	185.0 (29)	78.3 (52)
7	Soil (contains B[*a*]P 170 μg kg^{-1})	1.1 (31)	17.5 (40)	111.1 (48)	52.1 (68)

Table 5
Tukey's highest significant differences [HSD] showing multiple comparisons for B[*a*]P content of rice grain

Treat pair	HSD	Treat pair	HSD	Treat pair	HSD	Treat pair	HSD	Treat pair	HSD
1, 2	−0.0563	2, 1	0.0563	3, 1	0.0125	4, 1	0.0625	5, 1	−0.0038
1, 3	−0.0125	2, 3	0.0437	3, 2	−0.0437	4, 2	0.0062	5, 2	−0.0060
1, 4	−0.0625	2, 4	−0.0062	3, 4	−0.0500	4, 3	0.0500	5, 3	−0.0163
1, 5	0.0038	2, 5	0.0600	3, 5	0.0163	4, 5	0.0662	5, 4	−0.0662

tions have been reported from PAH concentration studied with fruits, potato and leafy vegetables [25].

It was found that in the outdoor field experiment, the rice grain B[*a*]P contents were as high as 1.5 (treatment 7) and 1.1 μg kg^{-1} (treatment 8) respectively in quartz sand and soil culture, which are 9.38-fold and 7.33-fold larger than in grain grown under clean air conditions (treatments 1 and 5). Selected the B[*a*]P contents in rice grain from Tables 3 and 4, Fig. 4 was drawn to show the effects of the two culture condition on the B[*a*]P content in rice grain. The experimental results combined with the monitoring data of air quality from Table 1, indicate that the B[*a*]P content in rice grain is from air pollution.

3.4. B[a]P content of rice husk, stem with leaf and root

3.4.1. B[a]P contents of rice husk

Rice husk B[*a*]P content is significantly different (at $P = 0.05$) between the control (treatment no. 1) and treatment no. 4 (500 μg kg^{-1} × 2 B[*a*]P added). Only in the highest treatment regime (no. 4) does the media B[*a*]P contribute B[*a*]P to the rice husk under air-quality control conditions, as shown in Table 6 (upper part). But rice husk B[*a*]P in the outdoor field trial, is up to 23.3 and 17.5 μg kg^{-1} in quartz sand and soil culture, respectively; 23.3-fold and 9.21-fold increases from corresponding quartz sand and soil culture results from the air-quality controlled greenhouse. Again, experimental results combined with the monitoring data of air quality shown in Table 1, leads the conclusion that the B[*a*]P in rice husk is also mainly from air pollution.

3.4.2. B[a]P contents of rice stem and leaf

The B[*a*]P content of stem and leaf not only is significantly different (at 0.05 level) between treatment 4 (500 μg kg^{-1} × 2, B[*a*]P treated) and all other treatments (nos. 1, 2, 3 and 5), but that difference is still significant at $P = 0.01$ level. That means the B[*a*]P in quartz sand has contributed to the B[*a*]P content of stem and leaf shown in Table 6 (lower half).

In contrast, the field experiment shows B[*a*]P content in rice stem and leaf of up to 185.0 and 111.1 μg kg^{-1} in quartz sand and soil culture, respectively. These values are 51.39-fold and 27.10-fold higher than those of the corresponding quartz sand and soil culture in the air-quality controlled greenhouse. Again, experimental results combined with the monitoring data of air quality shown in Table 1, indicate that the B[*a*]P in rice stem and leaf is also mainly from air pollution.

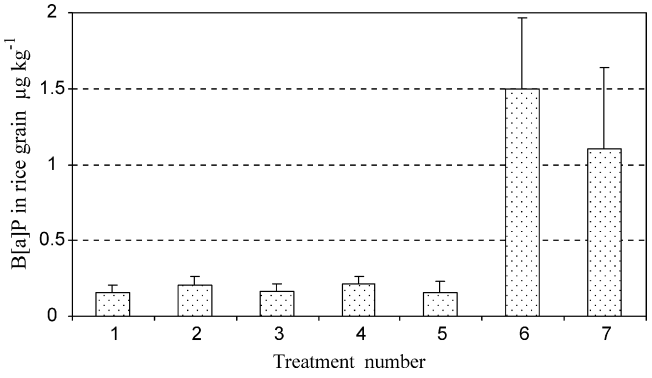


Fig. 4. Content of B[*a*]P in rice grain in different culture conditions and different treatments. (Treatment nos. 1–5 in controlled air greenhouse, nos. 6–7 in outdoor field. For details of conditions, refer to Table 1.)

Table 6
Multiple comparisons of B[*a*]P content of rice plant tissues by Tukey HSD analyses (with word A or B means having significant different between the treatment pair)

Treatment pair	Treatment pair	Treatment pair	Treatment pair	Treatment pair
Multiple comparisons of B[*a*]P contents of rice husk				
1-2	2-1	3-1	4-1A	5-1
1-3	2-3	3-2	4-2	5-2
1-4A	2-4	3-4	4-3	5-3
1-5	2-5	3-5	4-5	5-4
Multiple comparisons of B[*a*]P contents of rice stem and leaf				
1-2A	2-1A	3-1	4-1B	5-1
1-3	2-3	3-2	4-2B	5-2
1-4A	2-4A	3-4B	4-3B	5-3
1-5	2-5	3-5	4-5B	5-4B

A: significant difference at $P = 0.05$; B: significant difference at $P = 0.01$.

In the air-quality controlled greenhouse, the B[*a*]P content of rice stem and leaf from the soil culture treatment (no. 5) is not significantly different compared with control (treatment no. 1), this indicates that B[*a*]P in soil is not readily taken up by the rice plant roots. Similar research results have been reported for other plants [26]. A study on the accumulation of B[*a*]P in ryegrass found that the ryegrass shoot B[*a*]P concentration tended to increase with soil B[*a*]P, but the differences were not significant [27]. Another study on the fate of ^{14}C-labelled B[*a*]P discharged to soil by the way of contaminated by sewage sludge found that the B[*a*]P is poorly transferred to wheat seedlings after a 45-day period of growth [28]. Other studies of the living biomass show that PAHs found in the plants originated mainly from aboveground sources [29].

3.4.3. B[a]P contents of rice plant root

In the quartz sand culture at the air-quality controlled greenhouse, B[*a*]P concentration of roots increased sharply with the treatment rate of B[*a*]P. Root B[*a*]P concentrations were significantly correlated with the B[*a*]P application rate in the quartz sand experiments. For example at 500 μg kg^{-1} × 2 B[*a*]P treatment (no. 4), the B[*a*]P concentration of root is up to 12,055 μg kg^{-1}. The reason is that the B[*a*]P sorption and binding ability by quartz sand is extremely weak, therefore B[*a*]P is easy absorbed and/or adsorbed by the roots in the media.

4. Conclusion

Under stringently controlled air quality conditions and quartz sand culture, benzo[*a*]pyrene contents of rice grain, husk, stem with leaf and root are only 0.16, 1.00, 3.60 and 13.10 μg kg^{-1}, respectively. The very limited amounts of B[*a*]P are at background values and considered as endogenous.

With air-quality control and quartz sand as the culture media and the highest treatment of 500 μg kg^{-1} of B[*a*]P twice during

the growing season (once at replanting and once at tilling), there is no significant difference of B[*a*]P in rice grain compared with that grown from the control pot. Although there is a significant difference ($P = 0.05$) of B[*a*]P content in rice husks between the highest B[*a*]P treatment and control pot under air-quality controlled conditions, the values of B[*a*]P content of rice husks are in the same order of magnitude. Although there is a highly significant difference ($P = 0.01$) of B[*a*]P in rice stems and leaves between the highest B[*a*]P treatments and control pot in air-quality controlled condition, the values of B[*a*]P content of rice stems with leaves are also in the same order of magnitude.

The B[*a*]P content in above-ground tissues of rice plants under air-quality control conditions are much lower than those from outdoors exposed to air pollution; the difference of B[*a*]P contents is over one order of magnitude. It is thus evident that air pollution contributes much more than does the pollution of culture media. The findings of this research indicate that control of B[*a*]P pollution in air is important for improving the quality of rice grain produced near air pollution sources.

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