Dynamics Analysis for the Distribution of Polycyclic Aromatic Hydrocarbons in Rice

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Ten species of polycyclic aromatic hydrocarbons (PAHs) in rice were determined. Total PAH levels in unpolished rice ranged from 46 to 77 μg/kg dry weight, which an arithmetic mean value of 58 μg/kg dry weight. Phenanthrene was the most abundant in rice and rice straw. Individual PAH content in unpolished rice was higher than in polished rice. Total PAH contents in unpolished rice was 3-fold in polished rice. PAH concentration ratios for the lighter molecular weights (152–202) to the heavier molecular weights (228–252) in rice and paddy leaf, approximated to 70 : 30. Fluoranthene and pyrene commonly exist in environmental waters. River water and rainwater contained much greater levels of PAHs than groundwater. Fluoranthene was the most abundant and phenanthrene was second most abundant in soil. PAHs in the atmosphere directly contributed to the paddy/rice through atmospheric deposition and PAHs in soil and environmental waters indirectly contributed to the paddy/rice through root uptake and bioconcentration.

Key words —— polycyclic aromatic hydrocarbons levels in rice, contamination, atmosphere, soil, environmental waters

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in air, soil and water. Some PAHs are known to be mutagenic and carcinogenic. Especially, benzo[a]pyrene has been listed as an endocrine disruptor in the "environmental hormone" category. These organic compounds are produced during incomplete combustion from natural and anthropogenic sources. Many human activities result in PAH formation such as industrial processes, vehicle emissions, waste incineration and biomass burning.[1] Evidence indicates that anthropogenic sources frequently outweigh PAH input from almost all natural sources.[2–5]

Although many PAHs are included in the "priority pollutant" listings, there are many uncertainties over the significance of PAHs in relation to their transfer from the natural environment into the human food chain. There is very little information on contemporary levels of PAHs in rice. In this study, we attempted to analyze contemporary PAH levels in rice and also investigated rice contamination with PAHs from the atmosphere, soil and environmental waters.

Based on the premise that the plant leaf contents can provide a 'time integral' of the airborne concentration,[6–9] the paddy leaves were selected as passive samplers to observe contemporary levels of PAHs in the atmosphere. PAHs reach the surface of plant leaves primarily from the atmosphere.[10–12] PAHs in the atmosphere exist in either the gas or the particulate phase or both. Thus, the mechanism of transfer to plant leaves may be through gaseous dry deposition, particulate dry deposition, or wet deposition (including rain, snow and fog). Gaseous dry deposition has been shown to be the main process for many organic contaminants.[13,14] PAHs with a vapor phase component in air are regarded as being subject to an air-plant exchange process, moving toward equilibrium over time. Therefore, whether the leaf attains equilibrium with the air depends on the PAH species and the period of exposure. In addition, the paddy root was used for observing the adsorption of PAHs from soil and water because of root uptake, bioconcentration and translocation.

A limited number of samples from Tokushima

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in Japan were introduced in this work, which included rice, rice straw, soil, river water, rainwater and groundwater.

MATERIALS AND METHODS

Apparatus and Materials —— A GCMS system with a GC-17A gas chromatograph and a GCMS-QP5050A mass spectrometer was used (Shimadzu, Japan). Class-5000 software (Shimadzu, Japan) was used for processing data. A GB-35MS fused silica capillary column (0.258 mm i.d. × 30 m l) for PAH separation was purchased from J&W Scientific (U.S.A.). Helium gas (purity 99.99%) was used as carrier gas. A solid phase microextraction (SPME) fiber holder, with a 7 μm polydimethylsiloxane (Supelco Cat. No. 57302) coating were used for extraction of PAHs from environmental waters. A magnetic stirrer (Sibata MGH-110) was used for SPME extraction to ensure proper mixing of the sample.

Special-grade reagent dichloromethane (DCM), methanol, n-hexane, ethanol, boric acid, citric acid and trisodium phosphate 12-water were purchased from Wako Pure Chemical Industries Ltd., Japan. Acenaphthylene, fluorene, phenanthrene, anthracene, pyrene, and benzo[a]pyrene were also purchased from Wako Pure Chemical Industries, Japan; fluoranthene, benz[a]anthracene and perylene were purchased from Kanto Chemical, Japan; chrysene were purchased from Aldrich Chemical Co., U.S.A.

The standard stock solutions of 10 PAH species were prepared in ethanol and refrigerated (4°C) before use. The operative solutions were prepared by diluting the stock solution to a specified concentration with ethanol. For the SPME method, the stock solutions were diluted with distilled water, and the pH level of buffer solutions was adjusted to 6.

Sample Collection and Processing —— Each sample group, such as rice, rice straw, soils and environmental waters, was collected at the same range (shown in Fig. 1). Samples were taken as far as possible away from obviously anthropogenic inputs in order to reflect environmental PAHs levels. For soil sampling, five points of 0–5 cm cores at each site were taken with a stainless steel corer, wrapped individually in aluminum foil, transported to the laboratory in sealed vinyl bags, and stored at 4°C prior to oven drying. The samples of soils, rice straw, unpolished and polished rice were oven-dried 48 hr at 60°C, and then soil, unpolished and polished rice was ground or disaggregated with a mortar and
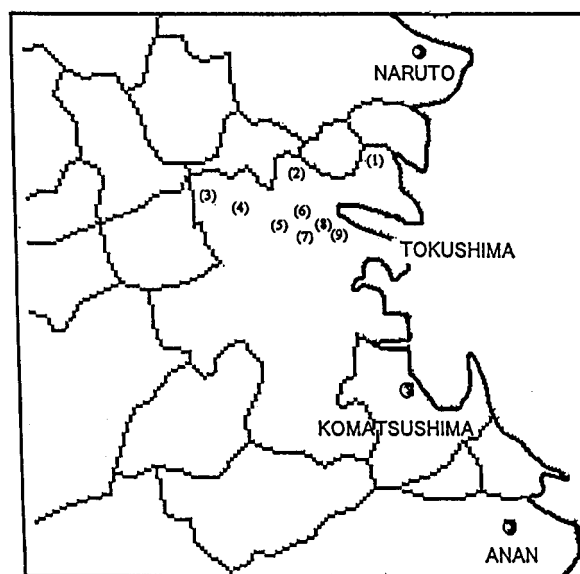

Fig. 1. Sample Location Map
(1) Kawauchi (2) Oujinand (3) Kokufu (4) Kokufu (5) Kuramoto (6) Kasuga (7) Kitatamiya (8) Kamiyoshino (9) Higashiyoshino.

pestle, sieved through a 2-mm mesh, bulked, and stored in glass jars (previously rinsed with DCM) with polypropylene lids prior to extraction, respectively. The leaves and root of rice straw were cut at 2 cm length for extraction. The samples from river water, groundwater and rainwater were extracted by the SPME method.

Extraction of PAHs in Rice Straw and Soil —— A 2 g rice straw (leaf or root) sample and a 20 g sample of oven-dried, sieved soil was put into a Soxhlet extractor (Iwaki, Japan), which had been previously rinsed with DCM. The sample was then extracted over 20 hr[15] with DCM 120 ml. After extraction, the extract was removed from the Soxhlet extractor and dried in a draft chamber. For cleaning extracts, column chromatographic steps with silica gel as column packing were used. The sample was prepared by dissolving the extract in DCM, and then it was charged to the top of the column and eluted with 3 ml of n-hexane followed by 7 ml of DCM-hexane [4 : 1 (v/v)] solution and 5 ml of DCM-methanol [4 : 1 (v/v)] solution. PAHs eluted in the second eluent[16] were collected and dried in the draft chamber. The residue was dissolved in 3 ml DCM and refrigerated at 4°C before analysis.

The recoveries from the procedure above-mentioned were checked against spiked PAH standards. Recoveries were in the range of 67–88% depending on the PAHs species. Data presented here are the means of duplicate analyses. Reproducibility was calculated on replicate analyses giving an error of

Table 1. The Levels of Individual PAHs and Total PAHs in Unpolished Rice

PAH Species		Sampling sites								
(molecular weight)		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Acenaphthylene	(152)	1	3.7	1.8	2.2	3.1	2	2.9	2.4	2.8
Fluorene	(166)	3	5.1	3.5	4	5.9	3.5	5.3	6.4	6.9
Phenanthrene	(178)	14	16	12	19	18	9.4	11	24	25
Anthracene	(178)	1.4	4.2	2.4	17	4.6	2	2.7	3.3	3.2
Fluoranthene	(202)	3.6	7.7	5.8	3	5.5	4.4	10	8	12
Pyrene	(202)	3.5	5.1	4.9	2.5	3.8	3.1	3.8	4.4	10
Benz[a]anthracene	(228)	16	3.6	2.9	2.3	2.7	2.1	3.3	3.4	6
Chrysene	(228)	1.8	5.9	3.6	2.6	2.2	2.4	3.1	3.2	3.4
Benzo[a]pyrene	(252)	5.1	0.9	5	2.4	2.8	1.3	2.6	3.5	3
Perylene	(252)	3.4	13	8.8	5	4.3	16	8.3	6	5
TotaPAHs		53	65	51	60	53	46	53	65	77

Concentration is expressed as μg/kg dry weight.

about 26%.

Extraction of PAHs in Polished and Unpolished Rice —— A 50 ml solution of 1 N KOH/ethanol was added to a 20 g sample, then refluxed for 1 hr at 80°C for alkali decomposition. After the alkali solution was filtered, the residue on the filter paper was washed with 20 ml of a 1 : 1 solution of hexane : ethanol followed by 30 ml n-hexane. The solution and 50 ml distilled water were removed into a first separating funnel, where PAHs were extracted for 10 min. The hexane phase was removed into a second separating funnel. 50 ml n-hexane was added to the first separating funnel for PAHs extraction once more. This hexane phase was also removed into the second separating funnel. The combined hexane phase in the second separating funnel was washed with 50 ml and 25 ml distilled water, and then removed into a tall horn beaker. This hexane solution was treated with Na_2SO_4 (anhydrous) for dehydration, and concentrated to 2 ml. Cleanup was performed as in the rice straw and soil procedure. Recoveries were in the range of 60–85% depending on the PAHs species. Reproducibility was an error of about 29%.

Extraction of PAHs in Environmental Waters by SPME —— 4 ml buffer solution and 1 ml water sample were placed into a 10 ml vial and the vial was sealed with a septum. The needle was passed through the septum and the plunger depressed, immersing the fiber to samples. Extraction took 15 min. under proper stirring. When the adsorption equilibrium was attained, the fiber was drawn into the needle, and the needle was withdrawn from the vial. Finally, the needle was introduced into the 300°C injector port of the GCMS, where the adsorbed PAHs

were thermally desorbed for 5 min and entered the GCMS column for quantification and analysis. Recoveries were in the range of 71–114% depending on the PAHs species. Reproducibility was an error of about 13%.

Analytical Methods —— The total analysis time was 40 min for PAHs detected by GCMS. The selective ion-monitoring mode (SIM) for all target analytes and standards was performed instead of full scanning analysis. The quantification of target analytes was carried out with a computer integrator. The concentration of PAHs was calculated by means of the calibration curve of each species. Phenanthridine was selected as internal standard. Target species include the 9 EPA priority PAHs compounds plus perylene. The detection limits were judged to be in the range of 0.0005–0.004 μg/ml, depending on the PAHs species.

Regarding statistical treatment of data, arithmetic means were calculated to the concentration data of each sampling area. Information on the absolute concentrations and relative abundance of the compounds for the different areas was then prepared.

RESULTS AND DISCUSSION

PAHs Concentrations in Rice

Table 1 shows the levels of PAHs detected in unpolished rice samples. The total PAHs content in rice ranged from 46 to 77 μ/kg dry weight with an arithmetic mean value of 58 μg/kg dry weight. Phenanthrene was the most abundant in most cases. For analyzed PAHs, the arithmetic mean value of acenaphthylene, fluorene, anthracene, pyrene,

Table 2. Comparision of PAHs Levels between Unpolished and Polished Rice

PAHs Species (molecular weight)		Unpolished Rice	Polished Rice
Acenaphthylene	(152)	2.4±0.8	1.5±0.6
Fluorene	(166)	4.8±1.4	1.8±0.4
Phenanthrene	(178)	16.5±5.5	2.9±2.4
Anthracene	(178)	4.5±4.8	1.6±0.1
Fluoranthene	(202)	6.7±3.0	2.5±1.2
Pyrene	(202)	4.6±2.2	1.6±0.3
Benz[a]anthracene	(228)	4.7±4.4	1.5±0.5
Chrysene	(228)	3.1±1.2	2.0±0.1
Benzo[a]pyrene	(252)	3.0±1.4	0.4±0.5
Perylene	(252)	7.8±4.3	1.5±0.7
Total PAHs		58 ±9.5	19 ±2.6

Concentration is expressed as mean ± S.D. (μg/kg dry weight).

Table 3. The Levels of Individual PAHs and Total PAHs in Rice Straw

| PAH Species (molecular weight)		Rice Straw Leaves	Rice Straw Root
Acenaphthylene	(152)	20.5± 1.0	23.8± 0.3
Fluorene	(166)	41.7±11.1	33.0±10.2
Phenanthrene	(178)	96 ±13.4	70.4±23.6
Anthracene	(178)	34.8± 3.8	16.6± 0.7
Fluoranthene	(202)	64.4±10.5	46 ± 1.2
Pyrene	(202)	40.8± 6.7	33.1± 4.3
Benz[a]anthracene	(228)	33.0± 3.2	29.1± 4.1
Chrysene	(228)	23.6± 0.7	21.7± 2.1
Benzo[a]pyrene	(252)	19.9± 2.5	18.0± 1.4
Perylene	(252)	34.7± 3.3	37.9± 3.6
Total PAHs		409.4±40.4	329.6±48.0

Concentration is expressed as mean ± S.D. (μg/kg dry weight).

benz[a]anthracene, chrysene and benzo[a]pyrene in rice ranged from 2 to 5 μg/kg dry weight, except for phenanthrene, fluoranthene and perylene. Individual PAHs concentration in unpolished rice was higher than in polished rice and total PAHs concentrations in unpolished rice was 3-fold in polished rice (shown in Table 2). The PAHs contents ratio in terms of lighter molecular weight (152–202) to heavier molecular weight (228–252) species in unpolished and polished rice, approximated to 70 : 30 in both cases.

The Influence of PAHs in the Atmosphere on Rice

Table 3 reports the levels of PAHs in rice straw. Phenanthrene was the most abundant and fluoranthene was the second most abundant. PAHs distribution in the paddy leaf was characterized by higher amounts of all PAHs detected relative to phenanthrene. The ratio of the lighter molecular
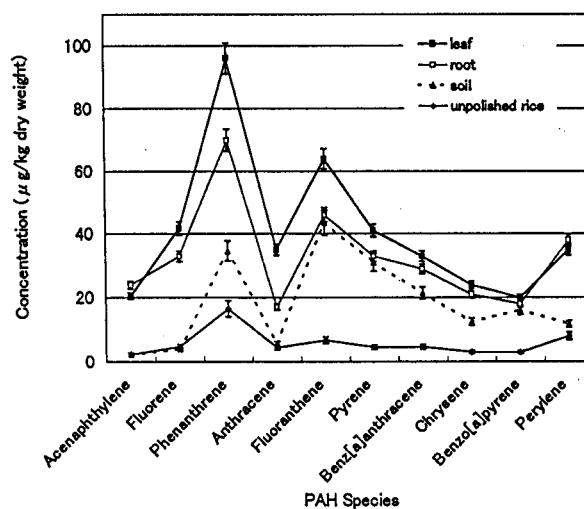
Fig. 2. Comparison of the Concentration Percent of Individual PAHs in Paddy Leaf, Paddy Root, Unpolished Rice and Soil

weight (152–202) to heavier molecular weight (228–252) PAHs in paddy leaf (73 : 27) was similar to that in rice (70 : 30). The mobility of light molecular weight PAHs through the atmosphere was greater than that of the heavy molecular weight PAHs because of the greater gas phase component. Conversely, heavy molecular weight PAHs were considered to be only particle-bound and will be subject to deposition processes that effectively remove them from the atmosphere as air masses move away from source regions.[7,9] In addition, there are many sources of PAHs in the atmosphere[1] and emitted PAHs should be mixed during their passage through the atmosphere away from source regions to other locations where the rice straw/rice was collected. Selective deposition and photolytic reaction processes may occur in the atmosphere to change this mixture as it became subject to atmospheric transport. The regional differences of PAHs in rice straw/rice are feasible in terms of the collective effect of these processes. In this work, although PAHs data for paddy leaf and unpolished rice varied with region, a PAHs level in paddy leaf were much higher than in unpolished rice. It should be noted that the distribution status of both PAHs was quite consistent (shown in Fig. 2). This indicates that PAHs levels in the atmosphere directly influenced PAHs levels in rice.

The Influence of PAHs in Environmental Waters and Soil on Rice

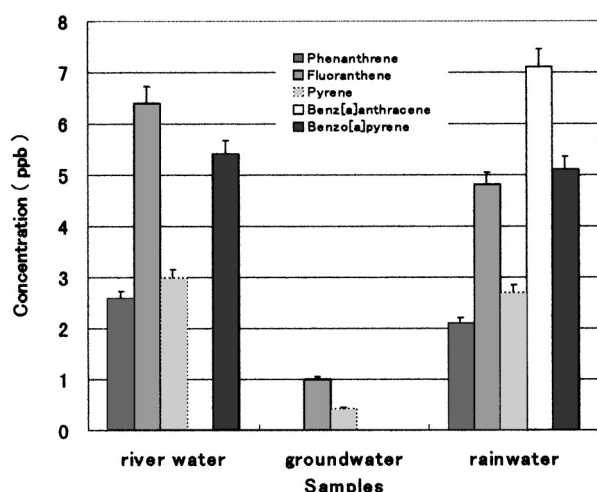River water and groundwater are often used for irrigating paddy fields, in addition to rainwater,

Fig. 3. The Distribution of PAHs Detected in Environmental Waters

Concentration is expressed as mean ± S.D.

which naturally irrigates farmland. In this study, PAHs in these water samples were determined under SPME/GCMS optimum conditions. Five species PAHs, phenanthrene, fluoranthene, pyrene, benz[*a*]anthracene and benzo[*a*]pyrene were detected. The levels of these compounds were between 0.4–6.4 ppb. Figure 3 shows that fluoranthene and pyrene a commonly present in environmental water. Phenanthrene, benz[*a*]anthracene and benzo[*a*]pyrene were also observed in river waters and rainwater samples besides fluoranthene and pyrene. Only fluoranthene and pyrene were detected in groundwater samples and both were at lower levels. This can be attributed to PAHs in surface soil migrating to subsurface soil with water, and some PAHs can finally dissolve into groundwater after subsurface sorption. Consequently, environmental waters may provide PAHs to the paddy.

As soil may be viewed as a complex mixture of minerals and organic material, it is an effective sorbent for organic compounds. The nature of organic matter in soil, the mineral composition and the soil moisture content influence soil sorption capacity. The adsorption of solutes on mineral surfaces is affected by competition of solvents for occupancy of free sites. Polar solvents such as water occupy the adsorbing sites on mineral surfaces, and nonpolar organic compounds such as PAHs are not adsorbed significantly.[17] In dry soil, PAHs sorption on mineral surface is dominant.[18,19] In this work, soil samples had lower PAHs levels than in rice straw (shown in Fig. 2). Because soil and paddy are simultaneously affected by atmospheric deposition,

and other sources such as river water and rainwater also raised PAHs levels in soil, one possible explanation is the biological activity of microbes in soil resulted in PAHs degradation.[20] In contrast to the case with rice straw, fluoranthene was the most abundant and phenanthrene was the second most abundant in soil. Soil may catalyze oligomeric reactions of phenanthrene to form higher molecular weight aromatic products under a variety of conditions.[17] Figure 2 shows that PAHs distribution in soil and paddy root is quite similar. PAHs in soil may be provided to the paddy leaf and rice through root uptake and bioconcentration.

In general, the analytical results demonstrate that PAHs in the atmosphere directly contributed to paddy/rice through atmospheric deposition and PAHs in soil and environmental waters (river water and rainwater) indirectly contributed to the paddy/rice through root uptake and bioconcentration. PAHs of lighter molecular weight are dominant in rice.

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