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Occurrence and Bioaccumulation of Micropollutants in Black Cicada

Haruki Shimazu* and Kazuki Sawai

School of Science & Engineering, Kinki University, Osaka 577-8502, Japan

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Abstract: Seven organophosphoric acid triesters (OPEs) and eight polycyclic aromatic hydrocarbons (PAHs) in black cicadas were measured to determine the concentration levels and to investigate how the cicadas are affected by soil contamination. Adult cicadas, nymphal exoskeletons, and soils were sampled in Higashi Osaka, Japan. Four OPEs and six PAHs were detected in the adult cicadas. The total concentrations of OPEs and PAHs ranged from 27.2 ng/g to 824 ng/g and from 4.30 ng/g to 270 ng/g, respectively. Four OPEs and five PAHs were detected in the nymphal exoskeletons. The total concentrations of OPEs and PAHs ranged from 184 ng/g to 1830 ng/g and from 40.3 ng/g to 970 ng/g, respectively. In the soils, three OPEs and six PAHs were detected at the same concentration levels as those detected in the adult cicadas. Significant correlations were observed in the micropollutant concentrations between soils and adult cicadas, and between soils and nymphal exoskeletons. This trend indicates that black cicadas accumulate OPEs and PAHs from contaminated soils.

Key words: Black cicada, bioaccumulation, organophosphoric acid triesters, polycyclic aromatic hydrocarbons.

1. Introduction

Organophosphoric acid triesters (OPEs) and polycyclic aromatic hydrocarbons (PAHs) are groups of ubiquitous environmental pollutants. Some OPEs are considered to be neurotoxicity. Most PAHs are mutagenic and some are carcinogenic. The pollutants have been regulated from many perspectives in some countries [1-3]. OPEs and PAHs give rise to concerns over their occurrence in the environment.

OPEs have been generally used for organic plasticizers, flame-retardants, and photographic films. PAHs produced primarily as a result of incomplete combustion from anthropogenic sources including emission from cars, incinerators, and factories. Therefore, the occurrences of OPEs and PAHs in the environments have been reported. Kawagoshi et al. [4] reported that a variety of OPEs as well as tributyl phosphate (TBP) and triethyl phosphate (TEP) were detected at 10,000 ng/L levels in the leachates from a sea-based waste disposal site. Shimazu et al. [5] indicated the occurrence of OPEs in the Kurose River. Many kind of studies also have been reported the occurrence of PAHs in the environments throughout the world [6-9].

It is well known that bioaccumulation occurs when an organism absorbs a toxic substance, even if environmental levels of the toxin are not very high. Many toxins such as methylmercury, DDT, chlorophenol, and metals have been reported in the variety of organisms [10-14]. However, little is known about the environmental dynamics of micropollutants between organisms of different species. This study focuses on cicada because cicada has a long life in an insect and there are so many predators such as spiders and birds. The purposes are to determine the concentration levels of OPEs and PAHs in black cicada and to investigate how the cicadas are affected by soil contamination.

***Corresponding author:** Haruki Shimazu, Asso. Prof./Ph.D., research field: environmental chemistry. E-mail: hshimazu@civileng.kindai.ac.jp.

2. Experiment

2.1 Black Cicada

Black cicada (*Cryptotympana facialis*) is an insect of the order Hemiptera, suborder Auchenorrhyncha, in the superfamily Cicadoidea. The cicadas live from the west Japan to the south Japan. The range of the cicada distribution extends to the north gradually. The body length of adult cicada is usually 30 mm to 50 mm long. It is one of the largest species of cicada in Japan.

After mating, the female deposits her eggs into the bark of a twig. When the eggs hatch, the cicada nymphs drop to the ground, where they burrow. Cicada nymphs go through a life cycle that lasts approximately seven years as nymphs for most of their lives. They suck xylem from the roots of tree. Moles, mole crickets, and ground beetles are predators for cicada nymphs. On the other hand, the life of adult cicada ranges from two weeks to one month. Adult cicadas also drink plant sap. They are commonly eaten by spiders, mantises and birds. Another known predator is the cicada killer wasp.

2.2 Study Site and Sampling

The study site was the campus of Kinki University located in Higashi Osaka, in a mixed traffic, industrial, and residential area. There are a lot of main roads such as Kinki expressway with an approximate rate of 25,000 cars/d. Higashi Osaka has over 4,000 industry factories, which density is 124.9 factories/km^2. The population and the population density are 504,216 people and 8,158 people/km^2 respectively as of 2010.

20 adult cicada bodies, 20 nymphal exoskeletons, and four soils were collected at the campus of Kinki University (Campus area is approximately 300,000 m^2) in August-September, 2010. The body length and the dry weight of the adult cicadas ranged from 37 mm to 47 mm and from 0.53 g to 1.24 g, respectively. The body length and the dry weight of the exoskeletons were from 29 mm to 37 mm and from 0.20 g to 0.37 g, respectively.

2.3 OPEs and PAHs

Seven OPEs and eight PAHs were determined in Table 1. The OPEs and PAHs are target compounds in this study because many kinds of studies have reported that they are fluently detected in aquatic and airborne environments [11]. Some OPEs and PAHs also are the subject of air quality standard in Japan [15].

Seven OPEs for the extra pure grade were purchased from Tokyo Chemical Industry Co., Ltd. and diluted to make calibration standards with acetone and hexane (for the pesticide residue and polychlorinated biphenyl analysis from Wako pure chemical industries, Ltd.). Eight PAHs for the standard material grade were purchased from Wako pure chemical industries, Ltd. and diluted to make calibration standards with acetone and hexane.

2.4 Analytical Methods and Instruments

The samples for adult cicada and exoskeleton were dried in a dark place for about 10 days and broken into shatters with a mill mixer (Iwatani). Then each sample was put into a cellulose extraction thimble (Whatman) and extracted with 40 mL of dichloromethane (for pesticide residue and polychlorinated biphenyl analysis from Wako pure chemical industries, Ltd.) for 15 min by ultrasonic extraction.

Cleanup was performed on a disposable filter device (Whatman, PURADISC^{TM} 25TF). After cleanup, the extract is concentrated to 5 mL with rotary evaporator and then 0.1 mL under a N_2 flow. Hexane is added to the extract until 2 mL.

Soil samples were dried in a dark place for about 20 days. Then 1 g of the soil was put into a cellulose extraction thimble and extracted with 40 mL of dichloromethane for 15 min by ultrasonic extraction. Cleanup was performed on a disposable filter device. After cleanup, the extract is concentrated to 5 mL with rotary evaporator and then 0.1 mL under a N_2 flow. Hexane is added to the extract until 2 mL.

Table 1 OPEs and PAHs measured in this study.

Chemicals	Abbreviations*	logKow**
OPEs		
Tributyl phosphate	TBP	4.00
Tri-2-butoxyethyl phosphate	TBXP	3.75
Tri-2-chloroyethyl phosphate	TCEP	1.44
Tris (1,3-dichloroisopropyl) phosphate	TDCPP	3.65
Triethyl phosphate	TEP	0.80
Tris (2-ethylhexyl) phosphate	TEHP	4.23
Triphenyl phosphate	TPP	4.59
PAHs		
Anthracene	AN	4.45
Benzo(a)pyrene	BaP	6.13
Benzo(b)fluoranthene	BbF	5.78
Benzo(k)fluoranthene	BkF	6.11
Benzo(ghi)perylene	BghiP	6.63
Dibenzo(ah)anthracene	DahA	6.75
Fluoranthene	FL	5.16
Pylene	PY	4.88

* These abbreviations are used in this study.

** logKow values are obtained from Ref. [16].

Table 2 Analytical conditions for GC/MS.

GC/MS (Agilent Technologies: 5975B inert XL E/CI MSD)	
Separation column	HP-5MS (30 m × 0.25 mm × 0.25 μm)
Injection temperature	250 °C
Injection method	Splitless
Injection quantity	2 μL
Oven temperature program	70 °C (1.5 min) → + 20 °C/min → 180 °C → + 5 °C/min → 280 °C (1 min)
Detector temperature	230 °C
Carrier gas	Helium

Table 3 Detection limits for OPEs and PAHs.

OPEs	[g]	PAHs	[g]
TBP	0.72	AN	3.5
TBXP	9.5	BaP	1.5
TCEP	5.3	BbF	1.6
TDCPP	3.7	BkF	1.8
TEP	1.4	BghiP	4.0
TEHP	0.76	DahA	4.2
TPP	2.5	FL	0.80
		PY	1.0

OPEs and PAHs in these extracts were determined with a gas chromatograph with mass spectrometer (Agilent technologies, 5975B inert XL E/CI MSD). The operation condition for the gas chromatograph with mass spectrometer is shown in Table 2. After qualifying by three typical SIM mass, each pollutant was quantified by the largest SIM mass. The quantification was by an external calibration method. The detection limits were calculated from threefold values of signal-noise ratio in the baseline of chromatogram (Table 3). The recoveries and the variation coefficients for OPEs and PAHs in the pretreatment process for the analysis ranged from 70% to 120% and from 7% to 20%, respectively.

3. Results and Discussion

3.1 OPEs and PAHs in Black Cicada

Four OPEs and six PAHs were detected in the adult cicadas (Table 4). The total concentrations of OPEs and PAHs ranged from 27.2 ng/g to 824 ng/g and from 4.3 ng/g to 270 ng/g respectively. Tributyl phosphate (TBP) and fluoranthene (FL) were detected in all of the adult cicada samples. Triethyl phosphate (TEP), tris (2-ethylhexyl) phosphate (TEHP), anthracene (AN), benz(a)pyrene (BaP), and pyrene (PY) were also detected in over 10 adult cicada samples out of 20. The median concentration and range for TBP, which is maximum value among the detected pollutants, were 281 ng/g-dry and 27.2-768 ng/g-dry, respectively.

Four OPEs and five PAHs were detected in the exoskeletons (Table 4). The total concentrations of OPEs and PAHs ranged from 184 ng/g to 1830 ng/g and from 40.3 ng/g to 970 ng/g respectively. TBP, FL, and PY were detected in all of the exoskeleton samples. TEP and BaP were also detected in over 10 exoskeleton samples out of 20. The median concentration and range for TBP, which is maximum value among the detected pollutants, were 378 ng/g-dry and 125-1800 ng/g-dry, respectively. The concentrations of the pollutants for the exoskeletons were a little bit higher than those of them for the adult cicadas.

The concentrations for the detected OPEs and PAHs in the adult cicadas were compared by sex (Fig. 1). The difference between male and female were not observed in the adult cicadas. In the exoskeletons, it was also not observed.

Table 4 OPEs and PAHs detected in adult cicada and exoskeleton.

	Adult cicada (N = 20)			Exoskeleton (N = 20)		
	Median*	Range	DS**	Median*	Range	DS**
TBP	281	27.2-768	20	378	125-1800	20
TBXP	-	-	0	-	-	0
TCEP	13.2	10.3-18.2	4	31.7	31.7	1
TDCPP	-	-	0	-	-	0
TEP	12.3	2.0-30.9	17	22.8	12.0-45.0	17
TEHP	19.2	4.5-86.7	18	55.8	24.4-69.1	3
TPP	-	-	0	-	-	0
Σ7OPEs	305	27.2-824	20	393	184-1830	20
AN	13.8	4.5-82.4	10	249	13.6-319	7
BaP	26.4	4.7-206	14	41.5	30.6-57.0	12
BbF	8.7	4.5-20.7	3	-	-	0
BkF	5.7	5.7	1	-	-	0
BghiP	-	-	0	-	-	0
DahA	-	-	0	24.1	24.1	1
FL	21.6	4.3-49.0	20	44.7	19.6-574	20
PY	11.9	2.0-27.9	16	26.7	11.5-315	20
Σ8PAHs	68.2	4.3-270	20	103	40.3-970	20

*Median concentrations were calculated after removing the data below the detection limits;
**DS means the number of detected samples;
All units are ng/g-dry.

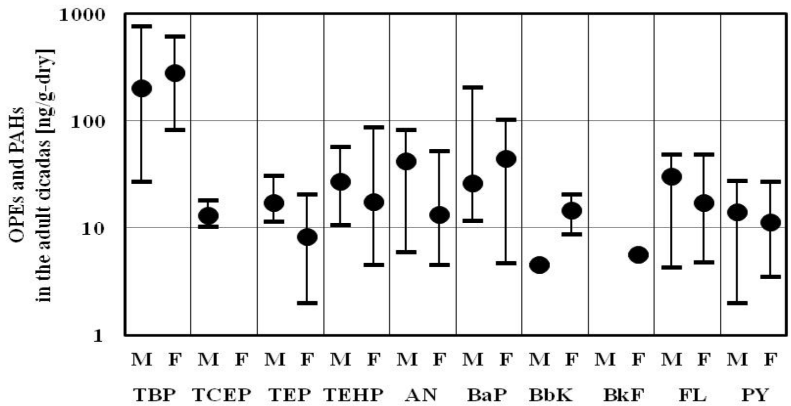

Fig. 1 Comparison of the concentrations for the detected OPEs and PAHs in the adult cicadas by sex. ● show the mean concentrations. The minimum and maximum concentrations are also shown. M and F indicate male and female, respectively.

3.2 OPEs and PAHs in Soil

Three OPEs and six PAHs were detected in the soils (Table 5). The total concentrations of OPEs and PAHs ranged from 92.1 ng/g to 261 ng/g and from 81.8 ng/g to 292 ng/g respectively. TBP, TEP, TEHP, BaP, BbF, BkF, FL, and PY were detected in all of the soil samples. The median concentration and range for TBP, which is maximum value among the detected pollutants, were 175 ng/g-dry and 80.9-239 ng/g-dry, respectively. The concentrations of the pollutants for the soils were almost same with those of them for the adult cicadas.

3.3 Relationship between Micropollutants in Cicada and the Length of Cicada

The relationships between Σ4OPEs in the adult cicadas and the length of adult cicadas, and between Σ6PAHs in the adult cicadas and the length of the adult cicadas are shown in Fig. 2. The proportional

Table 5 OPEs and PAHs detected in soil.

	Soil (N = 4)		
	Median*	Range	DS**
TBP	175	80.9-239	4
TBXP	-	-	0
TCEP	-	-	0
TDCPP	-	-	0
TEP	7.5	5.5-9.0	4
TEHP	9.0	5.7-13.1	4
TPP	-	-	0
Σ7OPEs	192	92.1-261	4
AN	-	-	0
BaP	28.1	8.8-55.8	4
BbF	17.2	6.2-33.5	4
BkF	16.7	8.0-32.2	4
BghiP	-	-	0
DahA	7.9	6.6-9.1	2
FL	55.5	34.3-91.6	4
PY	40.4	24.5-70.0	4
Σ8PAHs	161	81.8-292	4

*Median concentrations were calculated after removing the data below the detection limits;
**DS means the number of detected samples;
All units are ng/g-dry.

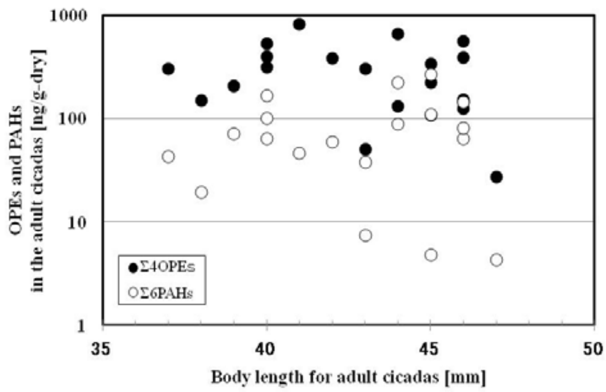

Fig. 2 Relationships between Σ4OPEs in the cicadas and the length of the cicadas, and between Σ6PAHs in the cicadas and the length of the cicadas. Σ4OPEs are the total concentrations of TBP, TCEP, TEP, and TEHP. Σ6PAHs are the total concentrations of AN, BaP, BbF, BkF, FL, and PY.

relationships were not observed in both Σ4OPEs and Σ6PAHs. In each OPE and PAH, the proportional relationship was not also observed. Nymphal cicadas seem to move underground at depths ranging from about 30 cm down to 2.5 m. These trends are probably because individual cicadas move and don't stay at the same point.

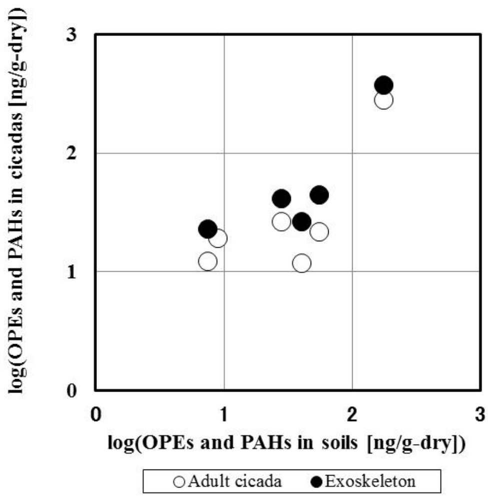

Fig. 3 Relationships in the mean concentrations for the detected OPEs and PAHs between adult cicadas and soils, and between nymphal exoskeletons and soils.

3.4 Relationship between OPEs and PAHs in Cicada and Those in Soil

For OPEs and PAHs detected in both the cicadas and the soils, the relationships between the mean concentrations in the adult cicadas and those in the soils, and between for the mean concentrations in the exoskeletons and those in the soils are shown in Fig. 3. Though there are five chemicals in the adult cicada and six chemicals in the exoskeleton, the mean concentrations for OPEs and PAHs in the cicadas tend to increase with those in the soils. This may indicate that black cicadas accumulate OPEs and PAHs from soils contaminated with these pollutants.

4. Conclusions

Seven organophosphoric acid triesters (OPEs) and eight polycyclic aromatic hydrocarbons (PAHs) in black cicadas were measured to determine the concentration levels and to investigate how the cicadas are affected by soil contamination. Adult cicadas, nymphal exoskeletons, and soils were sampled in Higashi Osaka, Japan. Four OPEs and six PAHs were detected in the adult cicadas. The total concentrations of OPEs and PAHs ranged from 27.2 ng/g to 820 ng/g and from 4.30 ng/g to 270 ng/g respectively. The concentrations showed little difference between male and female cicadas. Four OPEs and five PAHs were detected in the nymphal exoskeletons. The total concentrations of OPEs and PAHs ranged from 180 ng/g to 1830 ng/g and from 40.0 ng/g to 970 ng/g respectively. The concentrations in the nymphal exoskeletons were a little bit higher than the concentrations in the adult cicadas. In the soils, three OPEs and six PAHs were detected at the same concentration levels as those detected in the adult cicadas. Significant correlations were observed in the micropollutant concentrations between soils and adult cicadas, and between soils and nymphal exoskeletons. This trend indicates that black cicadas accumulate OPEs and PAHs from soils contaminated with these pollutants.

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