Mutagenicity of Surface Soils in Urban Areas of Aichi Prefecture, Japan, and Bangkok, Thailand

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To clarify the contamination levels of surface soil with mutagens in urban areas of Aichi prefecture, Japan, and Bangkok, Thailand, 60 and 67 surface soil samples were collected in Aichi and Bangkok, respectively, and mutagenicities of the organic extracts from these soil samples were examined in the Ames assay using *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100 with and without a mammalian metabolic system (S9 mix). Most of the soil samples collected in both areas showed mutagenicity in TA98 with and without S9 mix and in TA100 with S9 mix. Thirteen (22%) soil samples collected in Aichi showed high mutagenicity (more than 1000 revertants/g of soil) in TA98 with and/or without S9 mix, and six soil samples induced more than 4000 revertants/g of soil in TA98. Mutagenic potencies of most of the soil samples from Bangkok were moderate (100–500 revertants/g of soil) or low (less than 100 revertants/g of soil) in both strains with and without S9 mix, and only one sample induced more than 1000 revertants/g of soil in both strains. In the characterization of mutagens, organic extracts of two and one soil samples from Aichi and Bangkok, respectively, were separated on an octadecylsilyl (ODS) column for HPLC, and mutagenicities of the resulting fractions were examined using *S. typhimurium* YG1024 without S9 mix. For the two soil samples from Aichi, retention times of the distinct mutagenic fractions were similar at 40–74 min, and especially high mutagenicities were detected in three fractions corresponding to dinitropyrene isomers. In contrast, distinct mutagenicities were detected at retention times of 6–29 min for the sample from Bangkok, and marked mutagenicity was found in the fraction corresponding to another nitroarene. These results indicate that surface soils in urban areas in Aichi and Bangkok are commonly contaminated with mutagens, but major mutagenic constituents in the surface soils are different.

Key words —— surface soil, mutagenicity, Ames/*Salmonella* assay, Aichi, Bangkok

INTRODUCTION

In many developed and developing countries, air pollution has been a critical issue for decades. Diverse mutagenic and carcinogenic chemicals have been emitted into ambient air from anthropogenic sources such as motor vehicles[1,2] and industrial power plants.[3,4] Since air pollutants eventually descend to the ground, the surface of the ground is thought to be a depository of air pollutants, and airborne mutagens and carcinogens are assumed to accumulate in the ground, *e.g.*, surface soil. Many researchers have reported that organic extracts from surface soil showed mutagenic and DNA-damaging activity.[5–11] To examine the hypothesis that automobile exhaust contributes to soil mutagenicity, Wesp *et al.*[7] exposed two soils with low levels of mutagenic activities to traffic exhaust at a junction with heavy traffic for 3–26 weeks and found that mutagenic activities of the soils were increased at rates of 4–12 revertants per gram of soil per week in the Ames assay using *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100.

In a previous study,[8] we examined the mutagenicity of 110 surface soil samples collected from five geographically different regions of Japan, *i.e.*, the

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Hokkaido, Kanto, Chubu, Kinki, and Kyushu regions, in the Ames assay and found that most of the surface soil samples had mutagenic activity. Furthermore, it was found that some soil samples, *e.g.*, one from Hekinan in Aichi prefecture, showed remarkably high mutagenicity, and three dinitropyrene (DNP) isomers, *i.e.*, 1,3-, 1,6-, and 1,8-DNP, were detected in soil samples from Hekinan.[8] Hekinan is a constituent city of the Chukyo industrial region, one of the largest industrial regions of Japan in which heavy industries such as metal smelting and the chemical industries are found. Because DNP isomers are representative airborne mutagens and carcinogens formed by incomplete combustion of organic materials such as fossil fuels,[12,13] the soil pollution in Hekinan might be caused by industrial activity such as the combustion of fossil fuels in factories and by motor vehicles used to transport raw materials and products.

Nagoya in Aichi prefecture is the heart of the Chukyo industrial region and Japan's fourth largest city, with a population of about 2.1 million. Enormous amounts of exhaust gas and particulate matter have been emitted from large factories, power plants, and innumerable motor vehicles in this region. Bangkok is the capital of Thailand, with a population of more than 5.5 million and has faced serious air pollution problems in recent decades.[14] Several reports indicate that the major source of air pollution in Bangkok is particulate matters emitted from vehicles.[14–16] These facts and previous studies on soil mutagenicity imply that the surface soils in urban areas in Aichi, *e.g.*, Nagoya, and Bangkok are highly polluted with mutagens and carcinogens. Moreover, the major mutagens in surface soil in these areas are assumed to be different, because the putative major sources of mutagens differ, *i.e.*, emissions from industrial plants and vehicles in Aichi and mostly vehicles in Bangkok. Differences in the climate, *i.e.*, temperate versus tropical, and the chemical properties of soil, including biological activity, in Aichi and Bangkok may also affect the composition of the mutagenic constituents in the surface soil. However, no extensive research has been done and the pollution levels and major mutagens in the surface soils in these areas have not been thoroughly investigated.

The purpose of this study was to clarify the mutagenicity levels in surface soils in urban areas in Aichi and Bangkok and to compare the major mutagens in surface soil samples from these areas. Sixty and 67 surface soil samples were collected in Aichi and Bangkok, respectively, and the mutagenicities of organic extracts from the soil samples were examined in the Ames assay using TA98 and TA100, which have been most commonly utilized to evaluate mutagenicity of the environment. Major mutagens in soil samples from both areas were characterized by the bioassay-directed fractionation technique using column chromatography.

MATERIALS AND METHODS

Materials —— 1,3-DNP (CAS 75321-20-9), 1,6-DNP (CAS 42397-64-8), and 1,8-DNP (CAS 42397-65-9) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 6-Nitrochrysene (NC) (CAS 7496-02-8) was obtained from Aldrich Chemical Company Inc. (Milwaukee, WI, U.S.A.). 1-Nitropyrene (NP) (CAS 5522-43-0) and 3-nitrofluoranthene (NFT) (CAS 892-21-7) were purchased from Wako Pure Chemical Industries (Osaka, Japan). 2-NFT (CAS 13177-29-2), 2-NP (CAS 789-07-1), and 3-nitrobenzanthrone (NBA) (CAS 17117-34-9) were synthesized by the methods described by Kloetzel *et al.*,[17] Bolton,[18] and Enya *et al.*,[19] respectively. 2-Nitrotriphenylene (NTP) (CAS 81316-79-2) and 3,6-dinitrobenzo[*e*]pyrene (DNBeP) were synthesized by the methods of Ishii *et al.*[20] and Watanabe *et al.*,[21] respectively. Sephadex LH-20 was obtained from Amersham Biosciences (Uppsala, Sweden). HPLC-grade acetonitrile and methanol were purchased from Nacalai Tesque Inc. (Kyoto Japan). All other chemicals were of analytical grade.

Collection and Preparation of Organic Extracts from Surface Soil —— Sample collection and preparation of organic extracts were carried out by the methods described previously.[8] Soil samples were collected at parks or roadsides in two areas in Aichi prefecture, *i.e.*, Nagoya and Hekinan and adjacent areas, and in Bangkok and its adjacent provinces between February 2000 and March 2001. Sampling was carried out after a minimum of 7 days of nonrainy weather. Soil from the surface of the ground to about 10 cm deep was dug up, mixed thoroughly, and collected. The soil was dried at room temperature for a few days and screened through a 60-mesh sieve. The sieved soil (< 0.25 mm, 15 g) was extracted twice with methanol (200 ml) using an ultrasonic apparatus (185 W) for 10 min. The extracts were combined, filtered, and evaporated to dryness for the mutagenicity test.

Mutagenicity Test —— All the samples were dissolved in dimethyl sulfoxide (DMSO) and assayed

for mutagenicity using the preincubation method[22] with *S. typhimurium* TA98,[23] TA100,[23] and YG1024.[24] The mammalian metabolic system (S9 mix) contained 0.05 ml of S9, prepared from livers of male Sprague-Dawley rats treated with phenobarbital and β-naphthoflavone, in a total volume of 0.5 ml. The mutagenic potencies of samples were calculated from linear portions of the dose-response curves, which were obtained with three or four doses and duplicate plates at each dose. The slope of the dose-response curve was adapted as the mutagenic potency. When the samples induced two-fold increases over the average yield of spontaneous revertants, the samples were judged positive.

Characterization of Mutagens in Surface Soil ——— For characterization of mutagens in surface soil, organic extracts of surface soils were prepared with a Soxhlet extractor as described previously.[25] The organic extracts were applied to a Sephadex LH-20 column (2×40 cm) and eluted with chloroform/methanol (1 : 1, v/v). Twenty milliliters of eluate was collected as a fraction and 10 fractions were obtained. To examine mutagenicity, an aliquot of each fraction was dried in a stream of nitrogen and redissolved in DMSO. After combining fractions 5–7 and evaporating to dryness, the residue was redissolved in 50% tetrahydrofuran and applied to a YMC-Pack octadecylsilyl (ODS) AM324 column (5 μm particle size, 10×300 mm, YMC, Kyoto, Japan) for HPLC. The materials were eluted with the following gradient system of acetonitrile in distilled water: 0–40 min, 60%: 40–70 min, 60–100%: 70–90 min, 100%, at flow rate of 3 ml/min. Three milliliters of each eluate was collected. An aliquot of each fraction was diluted with DMSO and used for the mutagenicity assay.

A standard solution of nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), containing 1-NP, 2-NP, 2-NFT, 3-NFT, 3-NBA, 6-NC, 2-NTP, 1,3-DNP, 1,6-DNP, 1,8-DNP, and 3,6-DNBeP in ethanol, was injected onto the YMC-Pack ODS AM324 column for HPLC. The materials were eluted with the gradient system of acetonitrile in distilled water described above.

All HPLC experiments were performed at ambient temperature, and the eluates were monitored by absorbance at 254 nm using a Shimadzu SPD-M10AV photodiode array detector.

RESULTS

Mutagenicity of Surface Soil in Aichi Prefecture

Soil samples were collected at 60 sites in Aichi prefecture, Japan. Figure 1 shows the location of the sampling sites. Mutagenicities of the soil samples in *S. typhimurium* TA98 and TA100 are summarized in Table 1. All soil samples showed mutagenicity in TA98 with and/or without S9 mix, and mutagenic potencies of 35 (58%) soil samples were higher without S9 mix than with S9 mix. In TA100, 52 (87%) samples showed mutagenicity with S9 mix, and 25 (42%) samples were mutagenic without S9 mix. Thirteen (22%) soil samples showed high mutagenicity (more than 1000 revertants/g of soil) in TA98 with and/or without S9 mix, but only 7 samples (12%) in TA100 with and/or without S9 mix did. Remarkably high mutagenicity (more than 4000 revertants/g of soil) were detected in TA98 for six soil samples from sites A40, A43, A53, A54, A55, and A58, while only one sample from site A55 showed such high mutagenicity in TA100. The medians of the mutagenicities of the soil samples in TA98 with and without S9 mix were 287 and 282 revertants/g of soil, respectively, and that in TA100 with S9 mix showed the same level of 294 revertants/g of soil. The arithmetic mean values ($\pm$ the standard deviations) of mutagenic potencies of surface soil in TA98 with and without S9 mix were 1305 ± 3661 and 842 ± 1647 revertants/g of soil, respectively, and these values were 7.5-fold and 1.8-fold as high as those in TA100, respectively.

Five of six soil samples collected in Minami-ku showed high mutagenicity (more than 1000 revertants/g of soil) and three of six induced more than 4000 revertants/g of soil. Other highly mutagenic soil samples were collected at sites A21, A30, A40, A42, A43, and A48. These sampling sites, including ones in Minami-ku, are in the south of Nagoya, and a number of large factories and power plants are located in the southern costal area. On the other hand, the mutagenic potency of 17 soil samples collected in four districts, *i.e.*, Nishi-ku, Kita-ku, Moriyama-ku, and Meito-ku, which are far from the costal area, was low, and 12 (71%) of them did not induce more than 300 revertants/g of soil. Among the samples from Hekinan and its adjacent areas, samples from sites A57 and A58 were highly mutagenic in TA98 and TA100. The soil sample collected at Minami-ku 5, *i.e.*, site A55, showed the highest mutagenicity among the samples from Nagoya, inducing 26355 revertants/g of soil in TA98 without S9 mix. Among

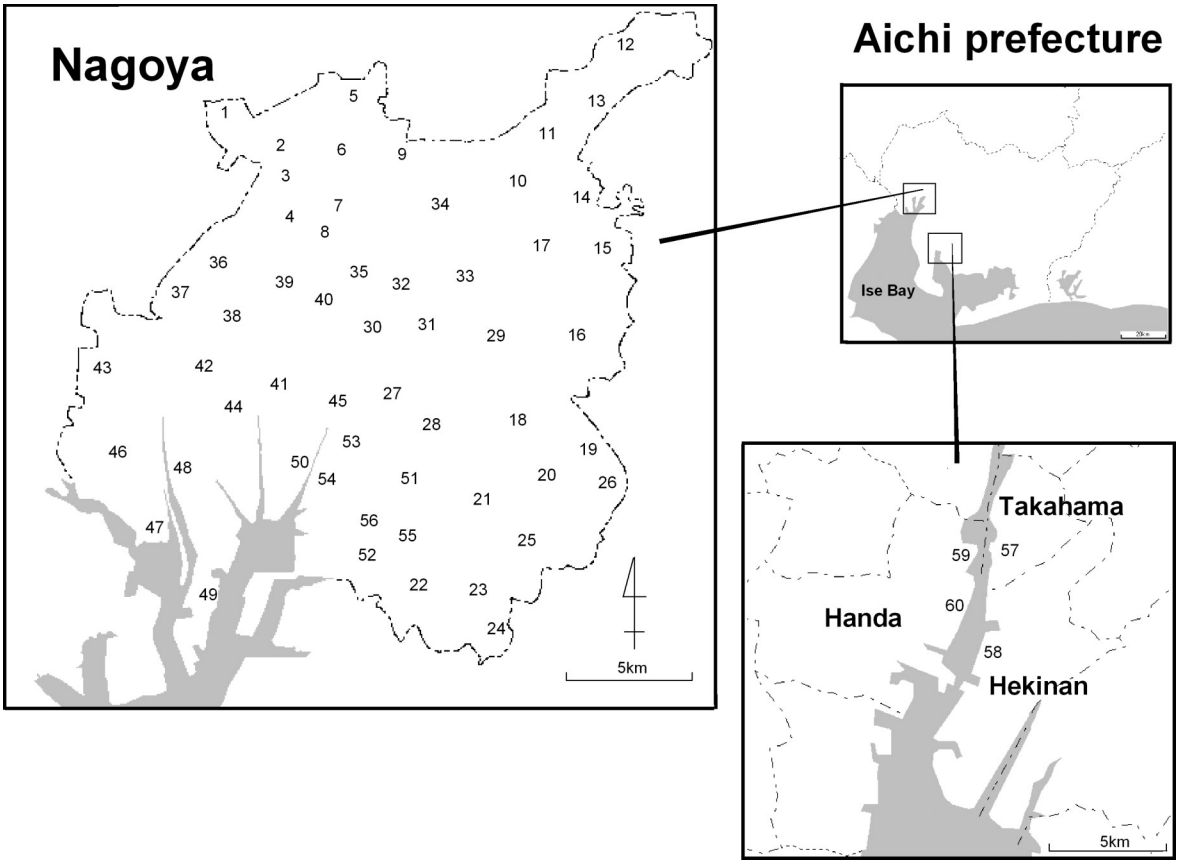

Fig. 1. Map of the Study Area in Aichi

Sampling sites are labeled with Arabic numerals, *i.e.*, 1–60. Identification numbers of surface soil samples consist of a letter standing for the sampling area, *i.e.*, A, and the Arabic numerals as shown in Table 1.

the soil samples from Hekinan and its adjacent areas, the soil from Suma, *i.e.*, site A58, showed the highest mutagenicity in TA98 without S9 mix, inducing 4160 revertants/g of soil.

Mutagenicity of Surface Soil in Bangkok and its Adjacent Provinces

Sixty-seven soil samples were collected in Bangkok and its adjacent areas in Thailand (Fig. 2), and their mutagenicities were examined using *S. typhimurium* TA98 and TA100. As shown in Table 2, most of the samples showed mutagenicity in TA98 with and without S9 mix and in TA100 with S9 mix. The mutagenic potencies of most of the soil samples were moderate (100–500 revertants/g of soil) or low (less than 100 revertants/g of soil) in both strains with and without S9 mix. Fourteen samples showed relatively high mutagenicity, inducing more than 500 revertants/g of soil, and 13 of them were collected at parks or roadsides in heavy traffic areas. The other sample was collected in an industrial park. Only one sample showed high mutagenicity, inducing more than 1000 revertants/g of soil. The medians of the mutagenicities of the soil samples from Bangkok and its adjacent areas in TA98 with and without S9 mix were 217 and 94 revertants/g of soil, respectively, and that in TA100 with S9 mix was 285 revertants/g of soil. The arithmetic mean values of the soil samples in both strains ranged from 112 to 304 revertants/g of soil. The highest mutagenic potencies in both strains were detected for the sample collected at Terdthai Road in Thon Buri prefecture, *i.e.*, site B21. The potencies of this soil sample in TA98 and TA100 were higher without S9 mix than with S9 mix, inducing 1366 revertants in TA98 and 1837 revertants in TA100 per gram of soil, respectively, without S9 mix.

Characterization of Mutagens in Soil from Aichi Prefecture and Bangkok

In the characterization of the mutagens in the surface soils from Aichi and Bangkok, organic materials were extracted from three soil samples collected at sites A55, A58, and B21, which showed

Table 1. Mutagenicity of Organic Extracts from Surface Soil Collected in Aichi Prefecture

ID no.	Sampling site	Sampling date	Mutagenicity (revertants/g of soil)[a)]			
			TA98		TA100	
			−S9 mix	+S9 mix	−S9 mix	+S9 mix
Nagoya City						
A1	Nishi-ku 1	February 24, 2000	109	ND	ND	ND
A2	Nishi-ku 2	February 24, 2000	855	542	243	290
A3	Nishi-ku 3	February 24, 2000	419	334	217	335
A4	Nishi-ku 4	February 24, 2000	265	144	ND	170
A5	Kita-ku 1	February 24, 2000	465	320	122	247
A6	Kita-ku 2	February 24, 2000	291	382	ND	376
A7	Kita-ku 3	February 24, 2000	141	122	ND	ND
A8	Kita-ku 4	February 24, 2000	60	103	ND	356
A9	Moriyama-ku 1	March 2, 2000	188	71	ND	135
A10	Moriyama-ku 2	March 2, 2000	322	172	ND	334
A11	Moriyama-ku 3	March 2, 2000	160	73	ND	ND
A12	Moriyama-ku 4	March 2, 2000	146	186	ND	ND
A13	Moriyama-ku 5	March 2, 2000	232	71	ND	207
A14	Moriyama-ku 6	March 2, 2000	212	124	ND	224
A15	Meito-ku 1	March 2, 2000	271	204	122	ND
A16	Meito-ku 2	March 2, 2000	167	108	ND	181
A17	Meito-ku 3	March 2, 2000	149	56	ND	226
A18	Tenpaku-ku 1	February 25, 2000	404	251	153	229
A19	Tenpaku-ku 2	March 2, 2000	110	66	ND	217
A20	Midori-ku 1	February 25, 2000	194	135	ND	ND
A21	Midori-ku 2	February 25, 2000	427	1205	ND	532
A22	Midori-ku 3	February 25, 2000	474	464	ND	433
A23	Midori-ku 4	February 25, 2000	290	159	ND	73
A24	Midori-ku 5	February 25, 2000	201	64	ND	107
A25	Midori-ku 6	February 25, 2000	168	179	ND	142
A26	Midori-ku 7	February 25, 2000	238	38	ND	206
A27	Mizuho-ku 1	March 2, 2000	219	263	ND	297
A28	Mizuho-ku 2	March 2, 2000	330	418	ND	210
A29	Showa-ku 1	March 2, 2000	132	64	ND	131
A30	Showa-ku 2	March 2, 2000	2500	1185	458	671
A31	Showa-ku 3	March 2, 2000	55	80	ND	86
A32	Chikusa-ku 1	February 24, 2000	71	78	ND	ND
A33	Chikusa-ku 2	March 2, 2000	304	187	246	240
A34	Higashi-ku 1	February 24, 2000	186	231	157	276
A35	Higashi-ku 2	February 24, 2000	787	579	455	408
A36	Nakamura-ku 1	February 24, 2000	196	140	ND	258
A37	Nakamura-ku 2	February 24, 2000	619	310	176	339
A38	Nakamura-ku 3	February 24, 2000	263	212	ND	383
A39	Nakamura-ku 4	February 24, 2000	69	131	ND	ND
A40	Naka-ku	February 24, 2000	7952	1403	1778	732
A41	Nakagawa-ku 1	February 25, 2000	364	442	ND	301
A42	Nakagawa-ku 2	February 25, 2000	2898	1674	ND	1066
A43	Nakagawa-ku 3	February 25, 2000	7818	10488	794	2243
A44	Nakagawa-ku 4	March 2, 2000	302	253	159	300
A45	Atsuta-ku	February 25, 2000	282	370	ND	318
A46	Minato-ku 1	February 25, 2000	304	413	ND	339

a) The organic extracts were obtained from 15 g of soil (< 250 μm) with an ultrasonic extractor. The slope (revertants per gram of soil) of the dose-response curve was calculated using least-squares linear regression from the first linear portion of the dose-response curve. ND = not detected.

Table 1. Continued

ID no.	Sampling site	Sampling date	Mutagenicity (revertants/g of soil)[a)]			
			TA98		TA100	
			−S9 mix	+S9 mix	−S9 mix	+S9 mix
A47	Minato-ku 2	February 25, 2000	679	890	160	683
A48	Minato-ku 3	February 25, 2000	1435	1240	154	720
A49	Minato-ku 4	February 25, 2000	232	473	82	688
A50	Minato-ku 5	February 25, 2000	270	589	119	666
A51	Minami-ku 1	December 18, 2000	1193	1510	221	404
A52	Minami-ku 2	February 25, 2000	281	565	ND	410
A53	Minami-ku 3	December 18, 2000	3232	4171	422	1431
A54	Minami-ku 4	December 18, 2000	4235	4755	491	827
A55	Minami-ku 5	February 18, 2001	26355	4576	2653	4955
A56	Minami-ku 6 Takahama City	February 18, 2001	1693	678	220	725
A57	Hekikai Hekinan City	February 18, 2001	1437	2944	292	1035
A58	Suma Handa City	February 18, 2001	4160	2457	420	1193
A59	Sunosaki	February 18, 2001	234	588	ND	221
A60	Shiohi	February 18, 2001	233	583	39	189

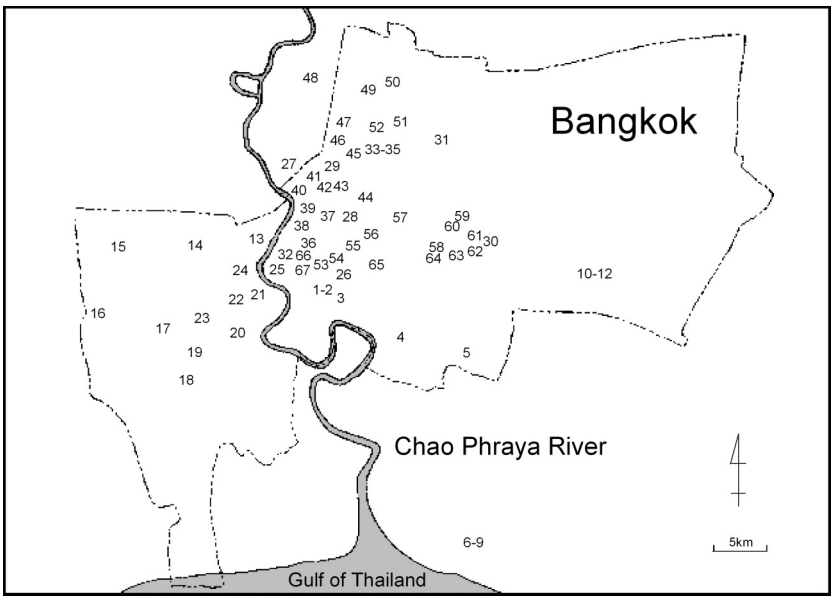

Fig. 2. Map of the Study Area in Bangkok and its Environs

Sampling sites are labeled with Arabic numerals, *i.e.*, 1–67. Identification numbers of surface soil samples consist of a letter standing for the sampling area, *i.e.*, B, and the Arabic numerals as shown in Table 3.

the highest mutagenicities in TA98 without S9 mix among the samples collected in each area (Tables 1 and 2). The mutagenic potencies of the organic extracts of soil samples from sites A55, A58, and B21 in TA98 without S9 mix were 27600, 2990, and 128 revertants/mg of extract, respectively. As shown in Table 3, the soil extracts showed potent mutagenicity in YG1024, which is an *O*-acetyltransferase-overproducing derivative of TA98, without S9 mix, inducing 6610–1120000 revertants/mg of extract. These values were 41-fold to 65-fold higher than those in TA98. To characterize the major mutagens,

Table 2. Mutagenicity of Organic Extracts from Surface Soil Collected in Bangkok and its Adjacent Provinces

ID no.	Sampling site	Sampling date	Mutagenicity (revertants/g of soil)[a)]			
			TA98		TA100	
			−S9mix	+S9mix	−S9mix	+S9mix
B1	Lumpinee 1	November 29, 2000	40	98	ND	170
B2	Lumpinee 2	November 29, 2000	78	131	ND	331
B3	Khlong Toei	November 29, 2000	126	142	219	202
B4	Phra Khanong	November 29, 2000	59	117	ND	243
B5	Prawet	November 29, 2000	135	159	ND	203
B6	Bangpoo 1	November 29, 2000	53	252	ND	185
B7	Bangpoo 2	November 29, 2000	71	144	ND	101
B8	Bangpoo 3	November 29, 2000	ND	117	ND	ND
B9	Bangpoo 4	November 29, 2000	ND	ND	ND	ND
B10	Ladgabang 1	November 29, 2000	405	759	400	549
B11	Ladgabang 2	November 29, 2000	49	117	ND	160
B12	Ladgabang 3	November 29, 2000	ND	94	ND	228
B13	Bang Phlat	November 30, 2000	65	293	175	320
B14	Taling Chan	November 30, 2000	ND	83	ND	180
B15	Utthayan	November 30, 2000	66	ND	ND	ND
B16	Nong Khaem	November 30, 2000	124	181	ND	143
B17	Bang Khae	November 30, 2000	170	617	212	454
B18	Bang Khun Thian	November 30, 2000	ND	167	ND	240
B19	Bang Bon	November 30, 2000	ND	101	ND	ND
B20	Chom Thong	November 30, 2000	42	64	ND	ND
B21	Thon Buri	November 30, 2000	1366	1242	1837	573
B22	Petchakasem 1	November 30, 2000	88	142	ND	199
B23	Petchakasem 2	November 30, 2000	ND	67	ND	147
B24	Bangkok Noi	November 30, 2000	139	292	ND	399
B25	Phra Nakhon	November 30, 2000	ND	32	ND	ND
B26	NaNa	December 6, 2000	121	462	ND	435
B27	Bangkok-Nonthaburi	December 7, 2000	215	298	ND	341
B28	Phrachacheun 1	January 8, 2001	ND	71	ND	161
B29	Phrachacheun 2	January 8, 2001	153	150	ND	ND
B30	Ram Kham Heang	January 8, 2001	114	271	275	255
B31	Ram Inthra	January 8, 2001	463	925	173	275
B32	Nakorn Sawan	January 8, 2001	49	161	ND	215
B33	Chatuchak 1	March 27, 2001	92	278	ND	293
B34	Chatuchak 2	March 27, 2001	320	531	409	493
B35	Chatuchak 3	March 27, 2001	861	868	664	685
B36	Dusit	March 28, 2001	268	333	131	150
B37	Bang Sue 1	March 28, 2001	52	194	137	557
B38	Bang Sue 2	March 28, 2001	127	177	287	472
B39	Bang Sue 3	March 28, 2001	127	245	125	305
B40	Bang Sue 4	March 28, 2001	94	147	ND	316
B41	Bang Sue 5	March 28, 2001	ND	178	ND	359
B42	Bang Sue 6	March 28, 2001	214	426	201	373
B43	Kamphaengpet	March 28, 2001	ND	32	ND	332
B44	Phahon Yothin	March 28, 2001	ND	181	ND	273
B45	Wiphawadi	March 28, 2001	79	226	141	236
B46	Nontha Buri 1	March 28, 2001	188	343	227	362
B47	Nontha Buri 2	March 28, 2001	ND	56	ND	285

a) The organic extracts were obtained from 15 g of soil ($< 250\ \mu$m) with an ultrasonic extractor. The slope (revertants per gram of soil) of the dose-response curve was calculated using least-squares linear regression from the first linear portion of the dose-response curve. ND = not detected.

Table 2. Continued

ID no.	Sampling site	Sampling date	Mutagenicity (revertants/g of soil)[a)]			
			TA98		TA100	
			−S9mix	+S9mix	−S9mix	+S9mix
B48	Nontha Buri 3	March 28, 2001	116	303	155	260
B49	Lak Si	March 28, 2001	114	195	ND	219
B50	Don Mueang	March 28, 2001	286	733	ND	454
B51	Chaeng Wattana	March 28, 2001	118	627	154	497
B52	Prasrimahathat	March 28, 2001	269	364	210	349
B53	Ratchaprarop	March 29, 2001	71	172	ND	456
B54	Din Daeng	March 29, 2001	105	258	154	233
B55	Prachasongkro	March 29, 2001	78	330	ND	584
B56	Ratchadaphisek	March 29, 2001	183	441	ND	335
B57	Lad Phrao 1	March 29, 2001	184	373	176	682
B58	Lad Phrao 2	March 29, 2001	93	217	ND	682
B59	Nawamin	March 29, 2001	199	428	177	369
B60	Sri Burapha	March 29, 2001	ND	221	ND	199
B61	Seri Thai 1	March 29, 2001	239	464	163	417
B62	Seri Thai 2	March 29, 2001	134	383	170	400
B63	Lamsalee	March 29, 2001	ND	99	ND	268
B64	Bang Kapi	March 29, 2001	69	94	ND	202
B65	Huai Khwang	March 29, 2001	217	332	ND	548
B66	Ratchathewi 1	March 29, 2001	121	249	180	613
B67	Ratchathewi 2	March 29, 2001	169	322	357	404

each extract was analyzed with the bioassay-directed fractionation method using YG1024 as a test strain because the mutagenicity of the sequentially fractionated organic extracts was examined in this analysis and an adequately sensitive test strain was needed for the mutagenicity assay.

The soil extracts were separated into 10 fractions by column chromatography using Sephadex LH-20 resin. The yield and mutagenicity of each fraction are shown in Table 3. For the soil sample from site A55, the mutagenic potency of fraction 5 was the highest among those of 10 fractions, followed by fractions 6 and 7. The total of the contribution ratios of fractions 5, 6, and 7 to the mutagenicity of the soil sample from site A55 was 89%. Similar phenomena were found for the sample from site B21, and the total of the contribution ratios of fractions 5, 6, and 7 was 76%. For the sample from site A58, fraction 6 showed the most potent mutagenicity, followed by fractions 7 and 5. The total of contribution ratios of these three fractions accounted for 87%.

For the characterization of mutagens in each soil sample, these three fractions were combined and applied to a YMC-Pack ODS AM324 column for HPLC. The eluate was separated into 1-min fractions, and resulting fractions were tested for mutagenicity (Fig. 3). As shown in Fig. 3, numerous fractions from each soil extract showed mutagenicity in YG1024 without S9 mix. For the two samples collected in Aichi, *i.e.*, sites A55 and A58, most of the distinctly mutagenic fractions were observed at retention times of 40–74 min, and three major mutagenic fractions were detected at retention times of 43–45, 46–47, and 49–51 min. For the organic extract of soil from site A55, the fractions with retention times of 54–55, 64–65, and 69–70 min also showed potent mutagenicity. Similarly, the fraction with retention times of 61–62 min showed potent mutagenicity for the sample from site A58. In contrast, for the sample collected at site B21, most of the distinctly mutagenic fractions were observed at retention times of 6–29 min, and the major mutagenic fraction was detected at 33–34 min.

Table 4 shows the retention times of representative mutagenic nitro-PAHs, which have been detected in surface soil and/or ambient air, under the same HPLC conditions as used for the soil extracts. Retention times of 1,8-, 1,6-, and 1,3-DNP were 44.2, 46.6, and 50.7 min, respectively, and these retention times corresponded to those of major mutagenic fractions for the soil samples collected at sites A55 and 58 in Aichi. Retention times of 3-NFT and 3,6-

Table 3. Yield and Mutagenicity of the Soxhlet Extracts and the Fractions of Surface Soil from Aichi and Bangkok

ID no.	Sampling site	Sample	Yield (mg)	Mutagenicity (revertants/mg)	Recovery of total mutagenicity (%)
A55	Aichi	Soxhlet extract	142.6	1120000	100
		Fr. 1	4.5	ND	0
		Fr. 2	46.7	5450	0.2
		Fr. 3	36.2	3440	0.1
		Fr. 4	36.4	11000	0.3
		Fr. 5	10.0	12300000	77
		Fr. 6	3.0	5500000	10
		Fr. 7	2.5	1360000	2
		Fr. 8	1.7	272000	0.3
		Fr. 9	1.4	51400	< 0.1
		Fr. 10	0.4	ND	0
A58	Aichi	Soxhlet extract	84.9	194000	100
		Fr. 1	3.2	ND	0
		Fr. 2	33.5	ND	0
		Fr. 3	29.1	ND	0
		Fr. 4	11.6	ND	0
		Fr. 5	2.4	425000	6
		Fr. 6	2.0	5550000	67
		Fr. 7	0.8	2790000	14
		Fr. 8	0.1	1170000	0.7
		Fr. 9	0.2	29000	< 0.1
		Fr. 10	0.4	16000	< 0.1
B21	Bangkok	Soxhlet extract	123.3	6610	100
		Fr. 1	9.3	ND	0
		Fr. 2	7.7	ND	0
		Fr. 3	35.7	ND	0
		Fr. 4	26.4	ND	0
		Fr. 5	4.1	108000	54
		Fr. 6	5.5	24700	17
		Fr. 7	4.5	8890	5
		Fr. 8	3.9	2820	1
		Fr. 9	0.4	ND	0
		Fr. 10	0.9	ND	0

ND = not detected.

DNBeP, *i.e.*, 54.9 and 64.1 min, were also consistent with those of the potent mutagenic fractions in the sample from site A55. The retention time of 3-NBA, *i.e.*, 33.5 min, corresponded to that of the major mutagenic fraction for the sample from site B21 in Bangkok. However, UV absorption spectra of the chemicals in the major mutagenic fractions in these three soil samples were too weak to compare with those of authentic nitro-PAHs.

DISCUSSION

In this study, we examined the mutagenicities of 60 and 67 soil samples collected in Aichi prefecture and Bangkok and its adjacent provinces, respectively, in the Ames assay. The *Salmonella* mutagenicity test is the most commonly utilized bioassay in environmental mutagenesis studies of complex mixtures such as soil[26] and surface water,[27] and TA98 and TA100 are the most common test strains in such studies.[26–29] Most of the soil samples collected in Aichi and Bangkok showed mutagenicity in TA98 with and without S9 mix and in TA100 with

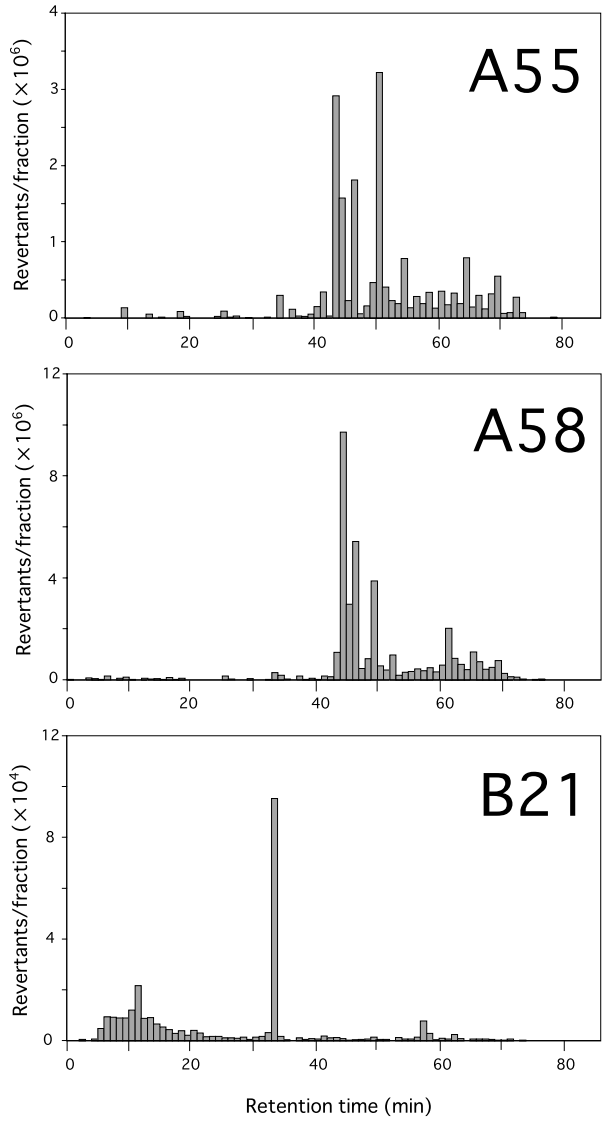

Fig. 3. HPLC Profiles of Mutagens in Surface Soil Samples Collected at Nagoya (A55), Hekinan (A58), and Bangkok (B21)

HPLC was performed on a YMC-Pack AM324 column, eluted with the following gradient system of acetonitrile in distilled water: 0–40 min, 60%; 40–70 min, 60–100%; and 70–90 min, 100%, at flow rate of 3 ml/min. The mutagenicity of 1-min fractions was tested in *S. typhimurium* YG1024 without S9 mix.

Table 4. Retention Times of Nitro-PAHs

Sample	Retention time (min)
3-Nitrobenzanthrone (NBA)	33.5
1,8-Dinitropyrene (DNP)	44.2
1,6-Dinitropyrene (DNP)	46.6
1,3-Dinitropyrene (DNP)	50.7
1-Nitropyrene (NP)	53.8
3-Nitrofluoranthene (NFT)	54.9
2-Nitrofluoranthene (NFT)	55.9
2-Nitropyrene (NP)	58.1
6-Nitrochrysene (NC)	62.3
2-Nitrotriphenylene (NTP)	63.0
3,6-Dinitrobenzo[*e*]pyrene (DNBeP)	64.0

Aliquots of the mixed ethanol solution of nitro-PAHs were applied on a YMC-Pack ODS AM324 column and eluted with the following gradient system of acetonitrile in distilled water: 0–40 min, 60%; 40–70 min, 60–100%; and 70–90 min, 100%, at flow rate of 3 ml/min.

S9 mix. About 40% of the soil samples from Aichi and Bangkok were mutagenic in TA100 without S9 mix. More than 20% of the soil samples from Aichi showed high mutagenicity (more than 1000 revertants/g of soil) in TA98 and/or TA100, and remarkably high mutagenicity (more than 4000 revertants/g of soil) was detected in TA98 for six soil samples. These highly mutagenic soil samples were collected within 10 km from the exceedingly industrialized costal area in which many types of industry including heavy industries such as metal smelting and the chemical industries are found. For the soil samples from Bangkok, most of their mutagenic potencies were moderate (100–500 revertants/g of soil) or low (less than 100 revertants/g of soil) in both strains, and relatively high mutagenicity was detected for soil samples collected at parks or roadsides in heavy traffic areas. In a review of published data on the mutagenicity of soil, White and Claxton[30] divided the compiled data on *Salmonella* mutagenic potencies into three site categories, *i.e.*, rural/agricultural, urban/suburban, and industrial, and compared their geometric mean values. Analysis of variance (ANOVA) revealed a significant relationship between site category and mutagenic potency ($p < 0.0001$) in TA98 and TA100 with and without S9 mix, and a significant difference between rural, urban, and industrial sites. The geometric mean value of the published data in TA98 without S9 mix for urban/suburban sites ($n = 219$) was 430 revertants/g of soil, and this value was much higher than that for rural/agricultural sites ($n = 125$, geometric mean value = 57 revertants/g of soil).[30] The corresponding geometric mean value of the mutagenic potencies of soils from Aichi in this study was 400 revertants/g of soil, and was comparable to that of the published data for urban/suburban sites. These results imply that surface soils are highly contaminated with mutagens, which may be emitted by anthropogenic sources such as industrial activities and motor vehicles, in urban areas of Aichi and Bangkok, and that the mutagenic potencies of surface soil were

markedly higher at some sites in Aichi as compared with other published data.

To characterize the major mutagens in surface soil, extractable organic materials were prepared from soil samples collected at two different sites in Aichi and one site in Bangkok. All of the organic extracts showed remarkably higher mutagenicities in YG1024 than in TA98 without S9 mix. YG1024 is an *O*-acetyltransferase-overproducing derivative of TA98 and is highly sensitive to the mutagenicity of aromatic nitro- and hydroxyamino-compounds without S9 mix.[24] These results suggest that these three soil samples were all contaminated with aromatic nitro- and hydroxyamino-mutagens. However, the retention times of distinctly mutagenic fractions for the two soil samples from Aichi were different from those for the sample from Bangkok on a YMC-Pack ODS AM324 column. Moreover, for the two soil samples from Aichi, especially high mutagenicity was detected in the fractions corresponding to 1,8-, 1,6-, and 1,3-DNP, and potent mutagenicities were observed in several other fractions. For the sample from Bangkok, highly mutagenic activity was observed in the fraction corresponding to 3-NBA. These results indicate that most of the mutagenic constituents in the surface soils from Aichi and Bangkok are different, whereas many of them in the two soil samples from Aichi may be common. In a previous study, we revealed that the mutagenic potencies of surface soils in the Kinki region of Japan toward TA98 without S9 mix were significantly correlated with the amount of 1,3-, 1,6-, and 1,8-DNP isomers, and the mean value of the total percent contributions of these DNP isomers to the mutagenicity of surface soil was about 25%.[11] 3-NBA was detected in diesel exhaust particles and would be formed by the atmospheric reaction of benzanthrone with nitrogen oxides.[19] Benzanthrone is a major component of aromatic ketones in extractable organic matter from airborne particles.[31] The differences in mutagenic constituents in surface soils in Aichi and Bangkok may reflect the dissimilarity in major sources of air pollution, *i.e.*, industrial activities in Aichi and motor vehicles in Bangkok. Biological conversion and decomposition of chemicals including mutagens by bacteria and ultraviolet rays, which would occur after emission, may also affect the differences in mutagenic constituents in these soils. DNP isomers and 3-NBA are among the most potent bacterial mutagens that have been identified so far in the literature[19,32] and have been shown to be carcinogenic in experimental animals.[13,33] UV absorption spectra of major mutagens separated from these soil samples from Aichi and Bangkok were too faint to compare with those of authentic aromatic nitro compounds. Larger amounts of soil samples are needed to identify the major mutagens in these soil samples.

Several mutagens and carcinogens have been detected in surface soil, but the major mutagens in surface soil remain unclear. We found many mutagenic fractions for which the retention times were different from those of 1,3-, 1,6-, and 1,8-DNP by HPLC fractionation of soil extracts. Research to identify major mutagens other than these DNP isomers is underway in our laboratory.

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