MUTAGENIC ACTIVITY IN ROADSIDE SOILS

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ABSTRACT — Mutagenicity of soils sampled at median strips, roadsides and a park neighboring arterial roads in Kurume City was determined by Ames test. Organic extracts of soils were mutagenic in strains TA98, TA100, YG1041 and YG1042 with and without S9mix. No sample showed mutagenic responses in strains YG3003 or YG7108. Extracts from soils of median strips and beside intersections showed higher mutagenicity and concentrations of PAHs and heavy metals than others, and mutagenic activity of soils correlated significantly with concentrations of PAHs and heavy metals. However, PAHs accounted for less than 12% of total mutagenicity in strains TA98 and TA100 of soil extracts. These extracts showed much higher mutagenicity in YG strains than in TA strains. The results indicate that these soils may be polluted with nitroarenes and aromatic amines.

KEY WORDS: Soil, Mutagenicity, Ames test, Polycyclic aromatic hydrocarbons

INTRODUCTION

Exposure to motor vehicle emissions represents an important cause of concern for possible long-term effects on human health (Kitamori *et al.*, 1982; Matsumoto *et al.*, 1993; Matsumoto *et al.*, 1991; Nakagawa *et al.*, 1983; Tokiwa *et al.*, 1980). The majority of carcinogenic and mutagenic pollutants emitted into the air cause soil and water pollution in addition to air pollution (Knize *et al.*, 1987; Ono *et al.*, 1999). Polluted soil is wind-borne and may be inhaled. Soil pollutants are also absorbed by plants (Nakajima *et al.*, 1994; Suzuki *et al.*, 1992).

Polycyclic aromatic hydrocarbons (PAHs) are mutagenic and carcinogenic, especially in pollutants (Sawicki *et al.*, 1962). To investigate air pollution by PAHs in detail, air must be sampled at many sites, but this is usually difficult due to lack of time and equipment. On the other hand, air pollution is reflected indirectly in soil pollution, and a relationship between concentrations of benzo(a)pyrene in soil and air pollution has been reported (Nishimura *et al.*, 1984). Soil sampling is relatively easy compared to sampling air-

borne particulates, so environmental pollution in an area has been understood by investigating soil pollution (Arashidani *et al.*, 1992). Additionally, the effect of road dust originating from vehicular movement on the heavy metal content of roadside soil has been reported (Nasrullah *et al.*, 1995).

To clarify soil pollution, we examined the mutagenicity of soils at median strips, roadsides and a park neighboring a roadside in Kurume City, Fukuoka Prefecture, Japan, and determined the concentrations of PAHs and heavy metals in these soils.

MATERIALS AND METHODS

Sampling

Soil samples were collected from a depth of 4 cm at 13 sites in Kurume City: 4 sites from median strips and at roadside of Route 210, 2 sites beside Route 322, 2 sites at the intersection of Route 3 and Route 210, 3 sites of the Kurume Hyakunen-kinen Park situated along Route 210, and 2 sites from shrubbery of the General Track and Field Stadium and Gymnasium (Fig.1). Soil sampling was carried out in January 1998.

Fig. 1. Sampling locations in Kurume City, Fukuoka, Japan

(1) median strip of Route 210; (2) roadside of Route 210; (3), (4), (5) park neighboring the roadside of Route 210; (6), (7) median strip of Route 210; (8) roadside of Route 322; (9) shrubbery of the General Track and Field Stadium; (10) shrubbery of the Gymnasium; (11), (12) roadside of the intersection; (13) roadside of Route 322.

The number of cars passing on Route 210 for one day was 48,911 on October 10, 1997.

Sample preparation

Soil samples were prepared for mutagenicity test and for HPLC chemical analysis. Each sample was dried for 2 days at room temperature in the dark. Dried soil was screened through a 60-mesh sieve (250 μm) to remove large gravel and debris, and then 15 g of the sieved soil was sonicated with 200 ml methanol for 10 min and the extract dried by passing it through Na_2SO_4 . This procedure was repeated twice, with extracts combined. The solvent was removed by an evaporator. Samples were then dissolved in dimethyl sulfoxide (DMSO) and sterilized through a 0.45 μm filter.

Mutagenic assay

Mutagenicity assay was carried out by Ames test (Maron and Ames, 1983). The test was performed in

duplicate with or without S9mix. An S9 fraction of phenobarbital / 5,6-benzoflavone-pretreated male Sprague-Dawley (SD) rat liver was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). The S9mix contained 50 μl of S9 in a total volume of 500 μl . Test strains were *Salmonella typhimurium* TA98, TA100, YG1041, YG1042, YG3003, and YG7108. The TA strains were kindly supplied by Dr. Bruce N. Ames, University of California, Berkeley, and the YG strains by Dr. T. Nohmi of the National Institute of Health Sciences. The TA98 strain is useful for detecting frame-shift mutagens and the TA100 strain for detecting base pair substitution mutagens. Both YG1041 and YG1042 strains have been used recently for sensitive detection of the mutagenicity of aromatic nitro compounds and aromatic amines (Hagiwara *et al.*, 1993). The YG3003 strain is useful for sensitive detection of some oxidative mutagens (Sera *et al.*, 1996) and the YG7108 strain for sensitive detection of mutagenic alkylating agents (Yamada *et al.*, 1995). Revertant colonies were

counted using an automatic colony counter (Colony Analyzer CA-9, Toyo sokki Co. Ltd., Tokyo, Japan). When the dose-response curve showed a 2-fold or greater increase over the spontaneous revertants, the mutagenicity of the sample was judged to be positive. Numbers of revertants per plate of positive controls were 604 (TA98 -S9mix, AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide 0.1 μ g), 837 (TA98 +S9mix, B(a)P: benzo(a)pyrene 2.5 μ g), 643 (TA100 -S9mix, AF-2 0.0025 μ g), 673 (TA100 +S9mix, B(a)P 1.25 μ g), 37 (YG1041 -S9mix, AF-2 0.025 μ g), 73 (YG1041 +S9mix, B(a)P 1.0 μ g), 588 (YG1042 -S9mix, AF-2 0.1 μ g), 205 (YG1042 +S9mix, B(a)P 1.0 μ g), 678 (YG3003 -S9mix, 4NQO: 4-Nitroquinoline-1-oxide 1.0 μ g), 251 (YG3003 +S9mix, B(a)P 1.0 μ g), 683 (YG7108 -S9mix, MNNG: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine 0.05 μ g), and 637 (YG7108 +S9mix, B(a)P 1.0 μ g).

Quantification of PAHs

Chemical substances were quantified by HPLC with fluorescence detection by Waters LC-Module 1 (Waters Corporation, Tokyo, Japan), Mightysil ODS column (5 μ M particle size, 250 $\times$ 4.6mm i.d., Kanto Chemical Co., Inc., Tokyo, Japan), and Waters470 scanning fluorescence detector, with a mobile phase of 90% methanol+10% water, 40°C column temperature, 0.8 ml/min flow rate, injection volume of 20 μ l, and wavelength (Ex, Em) for benzo(a)pyrene (370 nm, 420 nm), benzo(a)anthracene (290 nm, 415 nm), pyrene (335 nm, 375 nm), perylene (440 nm, 475 nm), chrysene (305 nm, 380 nm), benzo(k)fluoranthene (365 nm, 410 nm) and benzo(ghi)perylene (365 nm, 410 nm). (These chemicals were of analytical reagent grade from Wako Pure Chemical Industries, Osaka, Japan.)

Quantification of heavy metals

Concentrations of heavy metals in soils were determined by an inductively coupled plasma mass spectrometer (ICP-MS, Perkin Elmer Elan-5000). Each soil sample was dried for 2 days at room temperature in the dark, and the dried soil was then screened through a 60-mesh sieve (250 μ m), with 5 g of sieved soil added to 10 ml HNO₃ and 20 ml HCl and condensed on a hotplate at 140°C. The solution was added to 10 ml HNO₃, heated again and filtered through No. 5B filter paper (Toyo Roshi Co. Ltd., Tokyo, Japan). Concentrations of Cu, Cr, Pb, V, and Zn in the solution were determined by ICP-MS.

RESULTS AND DISCUSSION

Mutagenic activity of soil extracts in strains TA98 and TA100

Mutagenic activity of organic extracts from soils in strains TA98 and TA100 with and without S9mix are shown in Table 1. All samples showed mutagenic responses toward strains TA98 with and without S9mix. However, mutagenicity in TA100 was weak, regardless of whether S9mix was added or not. All samples showed higher mutagenicity in strain TA98 than in strain TA100. This result indicates that the soil in Kurume City is polluted with the frame-shift type of mutagens rather than the base-substitution types. These results are consistent with a previous observation by Watanabe *et al.* (1998). However, because mutagenicities in strain TA100 with and without S9mix were also shown, the soil in Kurume City is polluted with many kinds of mutagens. Extracts from soils 1, 6, and 7 of the median strip and soil 11 of the intersection in particular showed higher mutagenic

Table 1. Mutagenic activity of soil extracts in strains TA98 and TA100.

Sample No.	Mutagenic activity (net revertants/g dry soil)			
	TA98		TA100	
	-S9mix	+S9mix	-S9mix	+S9mix
1	528	679	— ^a	424
2	327	394	—	302
3	95	79	—	—
4	151	71	—	—
5	221	125	—	—
6	541	649	198	343
7	903	808	195	386
8	598	750	231	437
9	289	349	—	203
10	463	604	367	550
11	721	840	234	366
12	432	570	—	306
13	724	279	—	—

^a The revertants were less than twice those of the control. Spontaneous controls: TA98 -S9mix, 41; TA98 +S9mix, 38; TA100 -S9mix, 126; TA100 +S9mix, 134 revertants per plate.

responses toward strains TA98 and TA100 with S9mix than other samples did.

PAHs concentrations

Table 2 shows PAHs concentrations in extracts from soils measured by HPLC fluorescence. Extracts of soils 1, 6, and 7 from the median strip and soils 2, 8, 11 and 12 from roadsides showed higher PAHs concentrations than others and also showed higher mutagenicity. However, PAHs concentrations accounted for less than 12% of the total mutagenicity of soil extracts in strains TA98 and TA100. These low contribution ratios indicate that the soils contain other chemicals.

Heavy metal concentrations

To investigate the effects of automobiles and particulate matter from roads on the soils, we measured heavy metal concentrations (Nasrullah *et al.*, 1995). Table 3 shows this result. Nasrullah *et al.* (1995) and Sato and Kanoh (1991) reported that Cu is emitted mainly from engines and tail pipes, Pb and Mn are found in car emissions, Zn is a particulate type of matter from tires, Cr is contained in bodies of cars, V is emitted when oil combusts, and Ca is contained in the particulate matter of roads. Extracts of soils 1, 6, and 7 of the median strip and soils 11 and 12 of the intersection in particular contained higher heavy metal concentrations than other samples. Compared with the results

in Table 1, extracts of soils that showed higher mutagenicity than others also contained higher heavy metal concentrations. This result is similar to the relationship between mutagenic activity and PAHs concentrations of soils. Soil mutagenicity appears to reflect the effect of automobiles. The Mn concentrations of soils were not connected with mutagenicity, but Mn is a component peculiar to soil in Kyushu (Sato and Kanoh, 1991).

Comparison of B(a)P / B(ghi)P content ratios

Fig. 2 shows a comparison of benzo(a)pyrene (B(a)P) / benzo(ghi)perylene (B(ghi)P) content ratios in soils. Sawicki *et al.* (1962) reported that a B(a)P / B(ghi)P ratio of 0.6 or less indicates pollution from auto exhaust. As shown in Table 1 and Fig. 2, soil extracts that showed a higher mutagenicity than others had less than 0.6 ratios. Auto exhaust thus appears to be the main cause of soil mutagenicity.

Mutagenic activity of soil extracts in strains YG1041, YG1042, YG3003, and YG7108

The mutagenic activity of organic extracts from soils in strains YG1041, YG1042, YG3003, and YG7108 with and without S9mix are shown in Table 4. Although the PAHs that we detected accounted for less than 12% of total mutagenicity in strains TA98 and TA100 of soil extracts, mutagenicity assay in these YG

Table 2. Quantitative determination of PAHs in soil extracts.

Sample No.	ng/g dry soil						
	Benzo(a)pyrene	Perylene	Pyrene	Benzo(a)anthracene	Chrysene	Benzo(k)fluoranthene	Benzo(ghi)perylene
1	34.2	66.7	111.2	25.5	227.5	16.3	113.9
2	23.5	n.d.	45.5	15.8	52.5	12.5	60.5
3	n.d. ^a	n.d.	13.9	5.1	n.d.	4.0	8.0
4	n.d.	n.d.	n.d.	3.4	n.d.	2.3	2.3
5	n.d.	n.d.	17.1	2.3	n.d.	1.5	4.0
6	40.7	92.9	273.7	3.2	385.9	17.0	255.0
7	36.3	73.2	219.0	2.9	154.5	18.4	185.9
8	17.7	n.d.	43.3	11.0	64.0	7.7	35.9
9	86.0	n.d.	23.2	5.9	n.d.	5.9	14.4
10	13.1	n.d.	n.d.	9.8	49.7	7.1	24.0
11	41.9	75.3	198.4	35.5	472.3	17.0	265.8
12	14.6	n.d.	50.3	7.6	n.d.	5.4	76.7
13	n.d.	n.d.	19.0	3.4	n.d.	4.0	15.2

^a Not detected.

Mutagenic activity in roadside soils.

Table 3. Determination of heavy metals concentrations in soils.

Sample No.	$\mu\text{g/g}$ dry soil						
	Cu	Pb	Zn	Mn	Cr	V	Ca
1	47	28	213	339	57	53	3,616
2	21	19	99	393	45	40	3,279
3	6	8	117	323	12	24	887
4	8	2	96	371	11	37	2,206
5	2	4	51	297	2	6	913
6	47	41	662	254	58	42	4,612
7	57	51	1,219	259	63	41	4,612
8	21	11	109	350	25	23	2,116
9	4	4	47	467	2	6	279
10	9	15	106	311	17	21	809
11	18	42	255	304	24	20	1,844
12	27	21	216	253	19	17	1,909
13	17	2	39	334	17	37	2,568

Fig. 2. Comparison of B(a)P/B(ghi)P concentration ratios of soils.

strains can sensitively detect aromatic nitro compounds, aromatic hydroxylamines, aromatic amino compounds, some oxidative mutagens, and mutagenic alkylating agents in soil extracts. All samples showed mutagenic responses toward strain YG1041 with and without S9mix, and this was higher in strain YG1041 than in strain YG1042. This result indicates that the soil in Kurume City is polluted with the frame-shift type of mutagens rather than the base pair substitution types, and is consistent with the results in strains TA98 and TA100. The soil extracts showed a 4-90 fold higher mutagenicity in strain YG1041 than in strain TA98, and was 6-77 fold higher in strain YG1042 than in

strain TA100. The extracts from soils 1, 6, and 7 of the median strip and soil 11 of the intersection in particular showed higher mutagenic responses toward strains YG1041 and YG1042 than did other samples. Additionally, the extracts of these soils showed 14-90 fold higher mutagenicity in strains YG1041 and YG1042 than in TA98 and TA100. These results indicate that soils 1, 6, 7, and 11 were more polluted with nitroarenes and aromatic amines compared to others. No sample showed mutagenic responses toward strains YG3003 and YG7108 with or without S9mix, indicating that the extracts from soils in Kurume City were not or only weakly polluted by oxygen-activated mutagens and alkylating agents.

Density profile of mutagenicity and concentrations of PAHs and heavy metals

Fig. 3 shows the density profile of the mutagenicity and concentrations of PAHs and heavy metals of soil extracts from the median strip of Route 210, roadside of Route 210 and park neighboring the roadside of Route 210 (see Fig. 1 and 3; Nos. 1, 2, 3 and 4 sampling sites). The mutagenicity and concentrations of PAHs and heavy metals of the soil extracts decreased in the order of median strip, roadside, and park neighboring the roadside. Decrease of mutagenicity and concentrations of PAHs and heavy metals in soils from the median strip to the park was effected by longer distance from the median strip or the capability of plants to reduce air pollution (Suzuki *et al.*, 1992; Nakajima *et al.*, 1994).

Table 4. Mutagenic activity of soil extracts in strains YG1041, YG1042, YG3003, and YG7108 with and without S9mix.

Sample No.	Mutagenic activity (net revertants/g dry soil)							
	YG1041		YG1042		YG3003		YG7108	
	–S9mix	+S9mix	–S9mix	+S9mix	–S9mix	+S9mix	–S9mix	+S9mix
1	16,420	11,109	3,945	4,922	—	—	—	—
2	5,406	5,325	2,879	2,910	—	—	—	—
3	1,813	514	731	472	—	—	—	—
4	2,951	407	873	— ^a	—	—	—	—
5	2,928	1,784	1,472	1,209	—	—	—	—
6	48,791	38,945	3,980	8,505	—	—	—	—
7	12,605	53,217	8,347	5,802	—	—	—	—
8	12,872	6,470	5,586	3,989	—	—	—	—
9	4,671	1,953	1,852	1,167	—	—	—	—
10	10,825	6,165	7,477	4,153	—	—	—	—
11	27,598	29,772	18,001	5,269	—	—	—	—
12	13,104	8,968	3,393	3,657	—	—	—	—
13	11,907	1,244	1,117	851	—	—	—	—

^a The revertants were less than twice those of the control.

Spontaneous controls: YG1041 –S9mix, 16; YG1041 +S9mix, 31; YG1042 –S9mix, 104; YG1042 +S9mix, 100; YG3003 –S9mix, 280; YG3003 +S9mix, 300; YG7108 –S9mix, 10; YG7108 +S9mix, 12 revertants per plate.

Fig. 3. Mutagenicity and concentrations of PAHs and heavy metals of soil extracts.

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REFERENCES

- Arashidani, K., Someya, T., Yoshikawa, M. and Kodama, Y. (1992): Polynuclear aromatic hydrocarbon concentration and mutagenic activity in soils sampled at the roadsides. *J. Japan Soc. Air Pollut.*, **27**, 190-197.
- Hagiwara, Y., Watanabe, M., Oda, Y., Sofuni, T. and Nohmi, T. (1993): Specificity and sensitivity of *Salmonella typhimurium* YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity. *Mutat. Res.*, **291**, 171-180.
- Kitamori, S., Horikawa, K., Kataoka, K. and Tokiwa, H. (1982): Mutagenic activities of urban air pollutants in recent times. *Jpn. J. Public Health*, **29**, 37-42.
- Knize, M.G., Takemoto, B.T., Lewis, P.R. and Felton, J.S. (1987): The characterization of the mutagenic activity of soil. *Mutat. Res.*, **192**, 23-30.
- Maron, D.M. and Ames, B.N. (1983): Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, **113**, 173-215.
- Matsumoto, M., Ando, M. and Tamura, K. (1993): Differences of mutagenic activity of airborne particulates by particle size. Assay by the *Salmonella* microsuspension procedure. *Jpn. J. Toxicol. Environ. Health*, **39**, 139-147.
- Matsumoto, Y., Nakajima, T., Sakai, S., Noguchi, I. and Akiyama, M. (1991): Seasonal variation of the mutagenic activity of airborne particulate matter in the city of cold district. *Nippon Kagaku Kaishi*, **6**, 837-844.
- Nakagawa, R., Kitamori, S., Horikawa, K., Nakashima, K. and Tokiwa, H. (1983): Identification of dinitropyrenes in diesel-exhaust particles. Their probable presence as the major mutagens. *Mutat. Res.*, **124**, 201-211.
- Nakajima, D., Teshima, T., Ochiai, M., Tabata, M., Suzuki, J. and Suzuki, S. (1994): Determination of 1-nitropyrene retained in leaves of roadside trees. *Bull. Environ. Contam. Toxicol.*, **53**, 888-894.
- Nasrullah, N., Tatsumoto, H. and Misawa, A. (1995): Effect of road dust originated from vehicular movement on heavy metal content of roadside soil (1). Effect of road structure and roadside planting. *J. Jpn. Soc. Atmos. Environ.*, **30**, 157-168.
- Nishimura, T., Goto, S., Kato, Y., Okunishi, M. and Matsushita, H. (1984): Mutagenicity and benzo(a)pyrene contents in soils in Tokyo. *J. Japan Soc. Air Pollut.*, **19**, 228-238.
- Ono, Y., Okamura, H., Kawara, O., Aoyama, I. and Oda, Y. (1999): Genotoxicity and ecotoxicity of sediment in road runoff. *J. Jpn. Soc. Water Environ.*, **22**, 561-567.
- Sato, Y. and Kanoh, T. (1991): Heavy metal contents in suspended particulate matters of atmosphere. Density profile versus distance from road site. *Ann. Rep. Tokyo Metr. Res. Lab. P. H.*, **42**, 221-228.
- Sawicki, E., Hauser, T.R., Elbert, W.C., Fox, F.T. and Meeker, J.E. (1962): Polynuclear aromatic hydrocarbon composition of the atmosphere in some large American cities. *Am. ind. Hyg. Assoc. J.*, **23**, 137-144.
- Sera, N., Tokiwa, H. and Miyata, N. (1996): Mutagenicity of the fullerene C60-generated singlet oxygen-dependent formation of lipid peroxides. *Carcinogenesis*, **17**, 2163-2169.
- Suzuki, J., Kuwayama, K. and Suzuki, S. (1992): Mutagenicity assay for nitroarenes of air pollutants held in leaves of woody plants. *Mutat. Res.*, **271**, 89-96.
- Tokiwa, H., Kitamori, S., Takahashi, K. and Ohnishi, Y. (1980): Mutagenic and chemical assay of extracts of airborne particulates. *Mutat. Res.*, **77**, 99-108.
- Watanabe, T., Ishida, S., Minami, H., Kasai, T., Ogawa, S., Wakabayashi, K. and Hirayama, T. (1998): Identification of 1,6- and 1,8-dinitropyrene isomers as major mutagens in organic extracts of soil from Osaka, Japan. *Chem. Res. Toxicol.*, **11**, 1501-1507.
- Yamada, M., Matsui, K., Sofuni, T. and Nohmi, T. (1995): New tester strains of *Salmonella typhimurium* lacking O6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents. *Mutat. Res.*, **381**, 15-24.
- Yamaguchi, T., Yamazaki, H., Yamauchi, A. and Kakiuchi, Y. (1994): Mutagenicity of airborne particulates in a roadside atmosphere. *Jpn. J. Toxicol. Environ. Health*, **40**, 542-549.