— **Minireview** —

Genotoxicity of Soil

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A review of the literature published on the genotoxicity of soil is presented in this report. Subheadings of the report include outlines of genotoxicity assays that have been used to examine the soil samples and methods commonly used to prepare soil samples for genotoxicity assay, and a review of the genotoxicity of soil. Soil has been grouped according to potential sources of pollution, *e.g.* industrial activity, agricultural practices and motor vehicles. The possible causes of the genotoxicity of the soil are also mentioned.

Key words —— genotoxicity, soil, *Salmonella* mutation assay, mutagen

INTRODUCTION

Thousands of chemicals are released and find their way into the environment, *i.e.* air, land, groundwater and surface water, by industrial activity, agricultural practices, domestic activity *etc*. Numerous genotoxic compounds have been detected in both the particulate and gas phases of outdoor air, particularly in densely populated urban regions.[1)] Combustion of fossil fuels for power generation or transportation in industrial facilities, power plants and motor vehicles is thought to be a major source of these genotoxic compounds. In addition to the genotoxic compounds released directly into the environment by combustion process, some of these compounds are thought to be formed from primary combustion products via chemical and photochemical reaction in the outdoor environment.[2)] Most of these atmospheric compounds eventually descend to the ground, and therefore the ground surface may be contaminated with these genotoxic compounds. It was reported that some industries, *e.g.* pulp and paper mills, steel foundries and organic chemical manufacturing facilities, discharge wastes of noteworthy genotoxic potency.[3)] When improperly handled and disposed of, these industrial wastes and effluents also contaminate the soil with their genotoxic compounds. For agricultural land, naturally occurring genotoxic compounds in cultivated plants may be ploughed into the soil by tillage. An abundance of chemicals are applied to agricultural land as fertilizers, pesticides and herbicides. Soil microflora also may convert nongenotoxic compounds to genotoxic derivatives.

Genotoxic compounds in soil may have an affect on human health in an exposed population through pathways such as inhalation of dust which contains these compounds, ingestion of plants that uptake the compounds from soil, and leaching of the compounds from soil to groundwater and surface water used as drinking water. Because of the complex chemical nature of soil, standard chemical analyses are limited in their ability to characterize the chemical composition of genotoxicants in soil to assess its potential genotoxicity. Bioassays, however, provide a means of assessing the toxicity of a complex mixture like soil without prior knowledge about its chemical composition. Many papers have been published on the genotoxicity of soil. In this mini-review, we summarize the genotoxicity assays applied to soil, the preparation methods of soil samples and genotoxicity of diverse soil.

Genotoxicity Assay for Soil

Although there are a large number of genotoxicity assays, a relatively small number have been used to examine soil genotoxicity, and most of these used the *Salmonella* mutation assay.[4–20)] Other DNA damage assays, chromosome assays and so

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forth using rat lung DNA,[21] bacteria,[8,12,14,19] cultured cells,[17] mice[17] and plants[21–24] have also been employed for this assessment. The coupling of the *Salmonella* mutation assay with other fractionation techniques, *i.e.* bioassay-directed chemical fractionation, enhanced the utility of this bioassay and permitted the isolation and identification of the chemical fraction that contains genotoxic activity and genotoxic compounds.

Preparation of Soil Samples for Genotoxic Assay

It is expected that the results of genotoxicity assays of complex mixtures like soil are strongly influenced by the method of sample preparations and extractions, because chemical and physical properties of constituents, including major genotoxic compounds, in the mixtures differ greatly. In most of the studies assessing this genotoxicity, leachates of soil samples were prepared prior to the assay. The leachates from soil samples were generally made by shaking soil samples with aqueous[4,19,21–25] and/or organic[9,11,18,21,23,25] solvents. An ultrasonic apparatus,[6,7,15,16,20] a Soxhlet extractor[5,12–14,17] and so forth[8,10] were also used to obtain extracts of soil. In the genotoxicity assay of soil samples using plants, aqueous extracts of the soil samples were often used to treat the roots of plants or plant cuttings. Solid phase adsorbents such as XAD-2 resin[4] or PAD-1 resin[19] were utilized to concentrate less hydrophilic compounds from aqueous soil extracts, and substances adsorbed on the resins were eluted with organic solvents. Organic solvent extracts were concentrated and redissolved in a solvent compatible with the genotoxicity assay.

Genotoxicity of Soil Contaminated with Industry Related Chemicals

There are several reports on the genotoxicity of soil contaminated with chemicals originating from industrial sources. The contaminants of these soil samples varied widely, *e.g.* polychlorinated biphenyl (PCBs),[10,12,24] polycyclic aromatic hydrocarbons (PAHs),[19] heavy metals,[19,22,24] solvents,[24] munition wastes,[19] wood preserving wastes[14,21] *etc.*[24,25] Donnelly *et al.*[10] evaluated the genotoxicity of soil samples collected from the vicinity of a PCB disposal area, using the *Salmonella* mutation assay. They reported that sequential extracts of the soil samples with methylene chloride and methanol were mutagenic toward strain TA98 in the presence of the mammalian metabolic activation system (S9 mix), however, none of the samples induced a positive response in the absence of S9 mix. Cotelle *et al.*[24] used three plant bioassays, *i.e.* the *Vicica faba* (broad bean), the *Allium cepa* (white onion) and the *Tradescantia* (spiderwort) micronucleus tests, to evaluate aqueous extracts of two soil samples for genotoxicity. One of these soil samples was collected from an industrial waste site and the aqueous extracts were mainly contaminated by metals, PCBs and organic solvents. The other one was collected from a cokeworks waste site and the extract contained metals and PAHs. Plant cuttings of *Tradescantia* and roots of *Vicia* and *Allium* were treated with aqueous extracts of these soil samples and both extracts induced micronuclei in each assay system. Ehrlichmann *et al.*[19] evaluated genotoxicity of concentrated and nonconcentrated aqueous soil extracts from various soil samples using three bacterial assays: the *umu* test with *Salmonella typhimurium* TA1535/pSK1002, the NM2009 test with *S. typhimurium* NM2009 and the SOS Chromotest with *Escherichia coli* PQ37. The soil samples included sandy samples contaminated with mineral oil hydrocarbons, soil contaminated with explosives, *e.g.* 2,4,6-trinitrotoluene and other nitroaromatic compounds, a sandy soil sample contaminated with heavy metals, and soil taken from a coal mine and coking plant. Each sample was extracted with distilled water and less hydrophilic compounds in the aqueous extracts were concentrated with PAD-1 resin. The concentrated and nonconcentrated aqueous extracts from the samples contaminated with nitroaromatic compounds exhibited an extremely high genotoxic potential in all of the genotoxicity tests.

Genotoxicity of Agricultural Soil

Agricultural soil was reported to be mutagenic in the *Salmonella* mutation assay both in the presence and absence of S9 mix.[4,5,8,18] Goggleman and Spitzauer[5] examined *n*-hexane/acetone extracts of soil from several agricultural fields on which crops such as hops, asparagus, rye, oat pasture and meadow grew, and showed that all soil samples were mutagenic toward *S. typhimurium* TA98 and TA100 with some differences in potency. Brown *et al.*[8] demonstrated that dichloromethane extracts of three types of agricultural soil exerted mutagenicity in a eukaryotic test using *Aspergillus nidulans* as well as *Salmonella* assay, and suggested that the activity was related to past agricultural practices, including biocide application, fertilization and cultivation. Inconsistent results were reported by Edenharder *et al.*,

however.[18] They examined *n*-hexane/acetone extracts from 1) agricultural and forest soil collected in the environment of Mainz, a region highly charged by anthropogenic air pollution, 2) near Bayreuth, a rural low charged region of Germany, and 3) in a remote region of western Corsica without anthropogenic air pollution for the presence of mutagenicity in *S. typhimurium* TA98 and TA100. Most soil from Mainz and Bayreuth exhibited mutagenic activities in TA98, but not that soils from Corsica. No correlation could be detected between the levels of mutagenic activities and agricultural practice (rye growing, viniculture, fruit growing, meadow and fallow), texture of soils (% composition of clay, slit and sand), or the contents of organic matter. Moreover, they monitored soil mutagenicity in 10 rye fields near Mainz for one year and demonstrated that low levels of mutagenic activities in late summer increased during autumn, reached a peak in late winter and subsequently decreased during spring and summer. In conclusion, they offered a hypothesis of an airborne origin of soil mutagens, deposition and an adjacent transformation to non-mutagenic compounds by soil microorganisms.

Genotoxicity of Roadside Soil and Others

Soil samples from roadsides and some points where there is no apparent industrial or agricultural pollution source have also been reported to be positive in the *Salmonella* mutation assay[6,7,9,11,13,15–17,20] and plant assays.[23] Arashidani *et al*.[13] reported that mutagenic activities of ethanol/benzene extracts of soil samples from roadsides in Kyushu and Chugoku Districts in Japan were correlated with the amount of benzo[*a*]pyrene (B[*a*]P), which is a representative PAH mutagenic toward both strains TA98 and TA100, in the extracts. However, the contribution of B[*a*]P to the mutagenic activity of the soil extracts was less than 2%. A similar low contribution of B[*a*]P to the mutagenicity of soil extracts was reported for the samples collected from roadsides in other sampling areas such as Tokyo[6] and Sendai[7] in Japan. To test the assumption that automobile exhausts contribute to soil mutagenicity, Wesp *et al*.[17] exposed two soils with low levels of mutagenic activities to traffic exhausts at a heavily charged junction of German motorways (Autobahnen) for 3, 7, 10, 13, 17, 21 and 26 weeks. They found that average increases of mutagenic activities toward strain TA98 (TA100) were 8 and 9 (4 and 12) revertants per gram per week in the presence of S9 mix, supporting the hypothesis that automobile exhausts contribute to soil mutagenicity. On the other hand, they quantified several PAHs in the soil extracts, but could not detect any correlation between the increase of mutagenicity and the PAHs content.

To clarify the mutagenic potential of surface soil in Japan, we collected a total of 110 nonagricultural soil samples from parks, roadsides, banks *etc*. in five geographically different areas: Hokkaido, Kanto, Chubu, Kinki and Kyushu areas, and examined methanol extracts of these samples using *Salmonella* mutation assay.[16] Most of the soil extracts showed mutagenicity toward strains TA98 and TA100, and the potencies of soil samples collected at Hekinan, Kobe and Osaka toward TA98 were extremely high, while samples from Muroran showed strong mutagenicity toward TA100 with S9 mix. These results suggest that surface soil is largely contaminated with environmental mutagens, and that there are some sites where the surface soil is heavily contaminated with mutagenic compounds even in regions where no apparent industrial or agricultural pollution is suspected. It further implies that major mutagens in the soil vary with the site.

We recently identified 1,6- and 1,8-dinitropyrene isomers (DNP) as major mutagenic compounds in the organic extracts of soil samples collected from the ground of parks in Osaka, Japan.[15] Furthermore, we developed a highly sensitive quantification method of 1,3-, 1,6- and 1,8-DNP isomers in soil[26] and applied soil samples collected at 10 sites in the three areas, Kanto, Chubu and Kinki, which are districts of a megalopolis (Fig. 1, Table 1).[20] The highest contribution ratios were observed for the sample collected at Sumiyoshi-ku in Osaka, and the total of the contribution ratios of three DNP isomers was about 50%. 1,3-, 1,6- and 1,8-DNP isomers are among the most potent mutagens in the *Salmonella* mutation assay identified to date in the literature.[27] Moreover, all isomers showed distinct carcinogenicity in experimental animals, and the International Agency for Research on Cancer (IARC) listed 1,6-

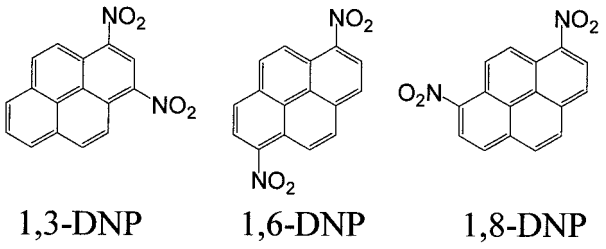

Fig. 1. Structures of 1,3-, 1,6- and 1,8-DNP Isomers

Table 1. Amount of 1,3-, 1,6- and 1,8-DNP Isomers in Soil and Contribution of DNP Isomers to the Mutagenicities of Organic Extracts from Soil toward *Salmonella typhimurium* TA98 without S9 mix[20)]

Sampling point	Sampling date	Mutagenicity	Amount of DNP (pg/g of soil)			Contribution ratio of DNP (%)		
		(revertants/g of soil)	1,3-	1,6-	1,8-	1,3-	1,6-	1,8-
Kanto area								
Tokyo Shinagawa-ku	1998 Feb. 2	319	25	34	125	3	4	30
Higashimurayama	1997 Dec. 29	438	17	14	17	2	1	3
Hachioji	1998 Apr. 1	380	21	22	30	2	2	6
Chubu area								
Nagoya	1998 Jan. 29	180	12	16	13	3	3	6
Gifu	1998 Jan. 29	260	51	82	77	8	12	22
Hekinan	1997 Jan. 15	34300	2437	4209	4369	3	5	11
Kinki area								
Uji	1998 May 23	3300	318	633	863	4	7	20
Osaka Sumiyoshi-ku	1997 Apr. 19	9780	2683	3069	3646	11	12	28
Higashiosaka	1997 Apr. 19	248	29	25	61	4	4	19
Kobe	1997 Jan. 30	10200	1120	1849	2573	4	7	20

DNP and 1,8-DNP as possible human carcinogens (group 2B) in *IARC Monographs*.[28)] These results suggest that DNP isomers are one class of major mutagenic and carcinogens contaminating surface soil. These DNP isomers were detected in airborne particulate matters collected in several cities,[29–33)] and motor vehicles[29,34–36)] and other combustion systems such as municipal incinerators[37)] are thought to be major sources. Innumerable motor vehicles in metropolises are suspected to be one source of DNP isomers in the soil, and DNPs could be accumulated on the ground surface. In addition, other human activities such as combustion at industrial power plants and municipal incinerators might be causes of the high levels of DNP isomers detected in some sites.

CONCLUSION

To examine the genotoxicity of soil, the *Salmonella* mutation assay has been most commonly used. Literature published on the mutagenicity of soil suggests that there are some sites where soil is heavily contaminated with genotoxic chemicals originating from industrial sources. Agricultural soil and nonagricultural surface soil, particularly in heavy traffic areas in urban regions, are also commonly polluted with mutagenic compounds. There are several reports describing attempts to identify chemicals causing this mutagenicity, however, the structures of the major mutagenic compounds remain unclear with a few exceptions.

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