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COMPARATIVE EVALUATION OF ENVIRONMENTAL TOXICANTS

- Health Effects of Environmental Toxicants Derived from Advanced Technologies -

Supplement

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Contents

Preface

Recent accelerated development of science and technology involves potential release of various artificial materials into the environment, which may increase health risks to humans. Radioactive release from nuclear facilities is a well-known example, in which the evaluation method of the health effects has been considerably well established because of due to the easiness of its dose definition. A variety of other materials associated with advanced technology, which do not exist in nature or have been extremely localized in the environment, are also possible causes of increased risks. The effect of one material on risk may sometimes be mutually modified by those of other materials. The influence from combined and competing effects among various materials should be taken into account for the assessment of risk on our life and health as well as all other living beings and the natural environment.

In view of this, the National Institute of Radiological Sciences (NIRS) organized the "International Workshop on Comparative Evaluation of Health Effects Of Environmental Toxicants derived from Advanced Technologies" to promote the exchange of knowledge and experience among the experts working in the field of environmental toxicants, such as radioactive materials and other chemicals, associated with energy production and other advanced technologies, and to provide us with a forum to illustrate a prospective for future research. The Workshop focused on 4 fields of studies on various aspects of environmental toxicants: 1) migration in the environment (environmental study), 2) the effect on humans and living forms (biological research), 3) the effect on the eco-system (ecological study) and 4) mathematical methodology.

This review is a supplement of the Proceedings of the Workshop, including 24 papers which were presented as poster presentation in the Workshop. For special lectures and oral presentations, we hope you refer to the Proceedings separately published.

On behalf of the organizing committee, we would like to express our sincere gratitude to the Japan International Science and Technology Exchange Center (JISTEC) for its co-sponsorship and also to the committees, chairpersons, panelists, invited speakers, and all other participants. We would also like to thank the members of the Division of Planing and Coordination of the NIRS for their eager assistance.

December 1998

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1. Behavior of Uranium in a Mesotrophic Lake : Lake Biwa in Japan

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ABSTRACT

Uranium concentrations were measured in the waters of Lake Biwa and 18 rivers inflowing into the lake to investigate the uranium behavior in drainage area and lake water. The ^{238}U concentrations in river waters were measured to be ranging from 0.03 to 1.3 mBq/l (mean : 0.34 mBq/l), and was higher in eastern shore rivers (from limestone area) and lower in northern and western shore rivers (from slate and granite area).

Vertical distribution of dissolved uranium in the lake water showed seasonal variation. Concentrations of dissolved ^{238}U in the water of northern basin have varied in the range from 0.1 to 0.3 mBq/l with seasonally and vertically. The concentration showed large difference between surface and deep layer during stratification and showed uniform distribution vertically during circulation period. Contributions of riverwaters inflow and release of uranium from shallow-sediment were considered as sources to increase uranium concentration in surface layer of the lake during stratification period. On the other hand, U/Al data of suspended particles in lake water suggests that uranium is scavenged from water column by sinking particles.

Apparent residence time of uranium (3-4 years) in lake water was found to be a little shorter than that of lake water itself (5.5 years), suggesting a non-conservative behavior of uranium in the lake water.

INTRODUCTION

Studies on behavior of trace elements and/or toxic materials in lake ecosystem become increasingly important not only from geochemical viewpoint but also from viewpoint for the long term impact of these materials on human and environment. Lakes are more dynamic compared with the open sea, therefore, generalization is difficult due to the effects such as horizontal and vertical boundaries as a result of its small water-capacity as a reserver and short-time movement occurring seasonally. Natural and man-made radionuclides have been used as useful tracers to study the geochemical cycle of elements in the ocean, because of their defined input function. Only a few investigations have been reported on limnological studies using radionuclides to get information on in-lake process such as residence times and transport pathway of trace materials in lake water.

Lake Biwa is largest lake in Japan and the water is supplied to 14 millions of peoples living in central area of Japan, especially in Kinki district. This lake has been gradually eutrophicated by deforestation, agricultural activities and so on. We have started in 1993 the

limnological studies using natural and artificial nuclides such as $^{238}U(^{234}U)$, ^{226}Ra, ^{210}Pb, ^{210}Po, ^{7}Be, ^{137}Cs, $^{239,240}Pu$, etc., to understand the cycling of some elements in the lake.

In this paper, we present the behavior of uranium in the lake as a fundamental study to construct geochemical cycling of this element. The naturally occurring uranium, ^{238}U, ^{235}U and ^{234}U are dissolved in water by chemical weathering and form stable uranyl carbonate species $UO_2(CO_3)_3^{4-}$ in oxidizing aqueous environments [1]. In suboxic to anoxic environments, hexavalent uranium is reduced to tetravalent state and removed easily from solution [1]. In the estuary, uranium shows both conservative and non-conservative behavior [2,3]. Thus, it is particular interest to investigate the behavior of uranium as redox sensitive element.

Here, seasonal variation of uranium distribution in the water of Lake Biwa is discussed together with uranium input from offshore water and atmosphere

Study area

Lake Biwa is a mesotrophic lake located in the central area of Japan as shown in Fig.1. A detailed limnological description of the study area is given elsewhere [4]. The lake is geographically divided into two parts, the northern and southern basins. The northern basin has a surface area of 616 km^2 and a volume of 2.73×10^9 m^3 with a mean depth of 44 (max. : 104 m). The southern basin has a surface area of 58 km^2 and a volume of 0.2×10^9 m^3 with a mean depth of only 3.5 m (max.:8m). The watershed surrounding the lake has a total area of 3174 km^2, which corresponds to about 5 times of lake surface. About 460 rivers inflow into the lake, but natural outlet is only one (Seta River). The lake is thermally stratified from May to mid-November, and mixed by convection from December to March. Bottom waters generally remain oxygenated state throughout the stratified period, but a reduced zone may be formed near the sediment-water interface. On the other hand, the southern basin is not stratified throughout the year.

Sampling and analytical methods

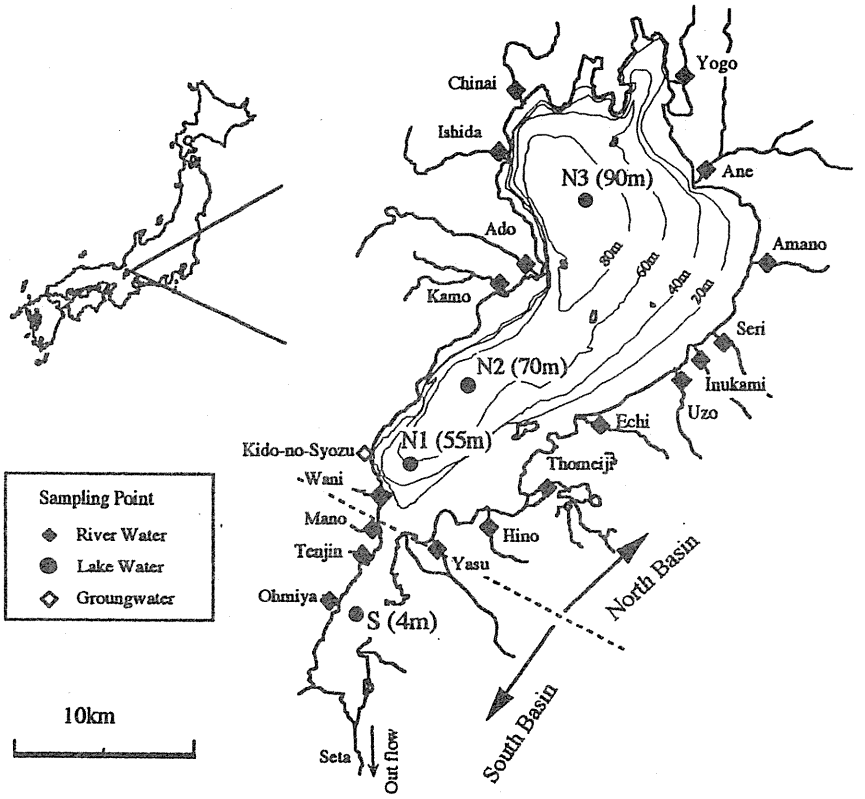
In order to evaluate the amount of ^{238}U derived from the rivers, water samples (15-20 liter) were collected two times in August 1993 and April 1994 from the downstream regions of 18 rivers, which contribute to 83% of the water inlet into the lake through rivers (Fig.1). Each sample was immediately filterated through a 0.45-μm millipore filter using a pressurized filtration apparatus. Enough amount concentrated HCl was added to the filtrate to bring the pH near 1.0.

Lake water samples (usually 20 liter) were also collected seasonally from several points at various depths (Fig.1). Sampling was conducted by sinking Niskin-bottle rosette (5 liter × 24) to appropriate depths. Sampling depths were checked automatically by using fine-scale-profiler (SBE-25 of Sea Bird Electronics Co.), along with the depth profiles of water temperature, conductivity, turbidity, chlorophyll-a fluorescence, etc. Besides these samples, another one liter of lake water was collected and filtrated through a 0.45-μm millipore filter. These filters were air-dried to measure the amount of suspended materials and stored for the neutron activation analysis of trace elements.

In the laboratory, both filtrate (15-20 liter) and suspended particles collected on filter were subjected to radiochemical analysis of uranium, with the addition of known amount of

^{232}U as a yield tracer.

The uranium in the filtrate was co-precipitated with $Fe(OH)_3$ after well heating and stirring. The precipitate was separated by centrifugation and re-dissolved in 10M HCl. After removing a large amount of Fe by solvent extraction using isopropyl ether, the aqueous phase was evaporated to dryness, and the residue was re-dissolved in 10 M HCl. The solution was passed through a Dowex 1-X8 anion exchange resin column. After the column was washed sufficiently with 8M HCl, and uranium was eluted from the column by 0.5M HCl. The uranium thus purified was electroplated from $(NH_4)_2SO_4$ solution onto a polished stainless steel disc. Suspended materials collected on filter paper were completely decomposed by HNO_3, H_2O_2 and $HClO_4$, and separated and purified in the same manner as



Physical Dimensions

Altitude	Northern basin	Southern basin	Total	
Surface area	-	-	85.6	m
Volume	27.3	0.2	27.5	$x10^9$ km^2
Maximum depth	104	8	104	m
Mean depth	44	3.5	41	m
Length of shoreline	-	-	235	km
Residence time	5.5	0.04	505	yr
Area of drainage basin	-	-	3,174	km^2

(Extract from Data Book of World Lake [4])

Fig. 1 Sampling locations and physical dimensions of Lake Biwa

the case of filtrate described above. The activity of uranium on the disc was measured by alpha-ray spectrometry using a Si(Au) surface barrier detector. The concentrations of trace elements, mainly Al, in the suspended materials were determined by INAA (instrumental neutron activation analysis) using the TRIGA type nuclear reactor at Rikkyo University.

RESULT AND DISCUSSION

Uranium from the river, groundwater and atmosphere

In order to evaluate the uranium inlet into the lake from offshore water, contributions of the river, groundwater and atmosphere were considered as follows.

River Water

The ^{238}U concentrations in river waters collected in 1993 and 1994 are summarized in Table 1 with some parameters such as the river basin, water flow, amounts of suspended materials, etc. The dissolved ^{238}U concentrations varied largely from river to river, ranging from 0.03-1.3 mBq/l by a factor of about 50, but the variation of ^{238}U concentrations in each river water was found to be less changeable within factor 2. Kametani et al. [5] measured ^{238}U and ^{234}U concentrations in representative 17 rivers in the eastern area of Japan. The ^{238}U concentrations ranged from 0.06 to 0.8 mBq/l with an average value of 0.27 mBq/l, depending on the difference in size of drainage basin. The average uranium concentration obtained in the present work appears to be consistent with Kametani's data. In general, the uranium concentrations were higher in rivers of eastern shore (from limestone area) of the lake and lower in rivers of northern and western shore (from slate and granite area) (see Fig. 2). As for the relationship between uranium concentration in river waters and geological trend, Palmer et al. [6] found that the higher content of uranium was mainly derived from the dissolution of limestone, based on the measurements of uranium concentration in over 250 river waters from Orinoco, Amazon basin etc. The higher uranium content in rivers flowing the eastern shore of Lake Biwa may be, therefore, caused by the dissolution of limestone. Morii et al. [7] measured the concentrations of inorganic components (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-} and SiO_2) in rivers surrounding Lake Biwa and found that inorganic components were high in eastern rivers and low in northern and western river waters. Distribution of uranium concentration in river waters found here

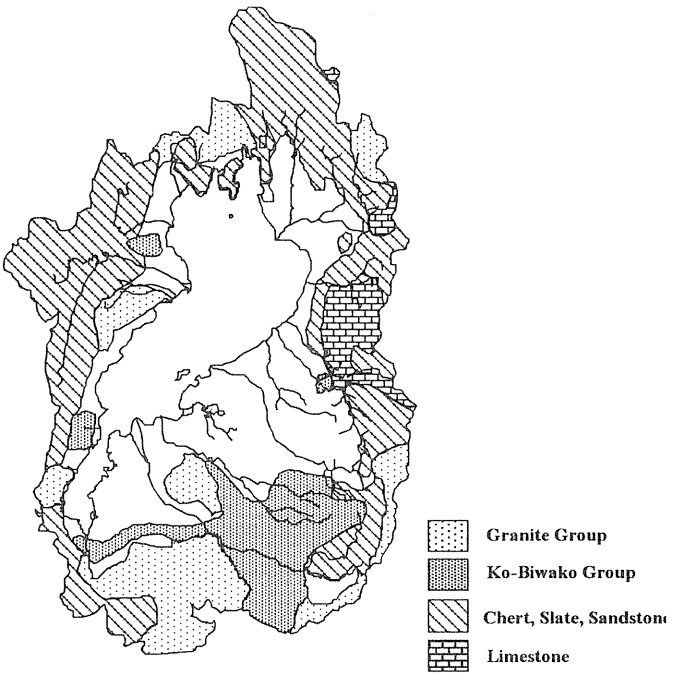


Fig. 2 Geological map of riverheads around Lake Biwa [7]

Table 1 Dissolved and particulate uranium concentration in river waters and some parameters of rivers flowing into Lake Bisa

Sampling Site	River** Basin (km^2)	Water** Flow (10^{10} m^3/y)	Sampling Date	SS Weight (mg/l)	Dissolved Uranium ^{238}U (mBq/l)	Dissolved Uranium $^{234}U/^{238}U$ ratio (Bq/Bq)	Particulate Uranium ^{238}U (mBq/l)	Particulate Uranium ^{238}U (mBq/mg)	Particulate Uranium $^{234}U/^{238}U$ ratio (Bq/Bq)
Yogo River	71	1.06	93/8/10	3.8	0.077 ± 0.004*	1.3 ± 0.1*	0.068 ± 0.006*	0.018 ± 0.002*	1.2 ± 0.1*
			94/4/25	3.3	0.077 ± 0.007	1.4 ± 0.2	0.153 ± 0.027	0.046 ± 0.008	1.2 ± 0.2
Ane River	369	6.07	93/8/10	2.7	0.571 ± 0.031	1.3 ± 0.1	0.103 ± 0.005	0.038 ± 0.002	1.0 ± 0.1
			94/4/25	7.6	0.248 ± 0.037	1.7 ± 0.3	0.268 ± 0.054	0.035 ± 0.007	1.2 ± 0.2
Amano River	112	1.16	93/8/10	6.1	0.598 ± 0.025	1.6 ± 0.1	0.093 ± 0.005	0.015 ± 0.001	1.1 ± 0.1
			94/4/25	5.5	0.758 ± 0.064	1.6 ± 0.1	0.179 ± 0.010	0.032 ± 0.002	1.1 ± 0.1
Seri River	64	0.69	93/8/10	3.8	0.574 ± 0.024	2.2 ± 0.1	0.054 ± 0.005	0.014 ± 0.001	1.4 ± 0.2
			94/4/25	3.5	1.272 ± 0.129	1.6 ± 0.1	0.057 ± 0.005	0.017 ± 0.001	1.3 ± 0.1
Inukami River	105	1.11	93/8/10	3.9	0.120 ± 0.007	1.2 ± 0.1	0.070 ± 0.006	0.018 ± 0.002	1.2 ± 0.1
Uzo River	96	0.82	93/8/10	6.2	0.228 ± 0.012	1.3 ± 0.1	0.145 ± 0.009	0.023 ± 0.002	1.1 ± 0.1
			94/4/25	70.3	0.440 ± 0.031	1.2 ± 0.1	2.780 ± 0.207	0.040 ± 0.003	1.1 ± 0.05
Echi River	202	2.62	93/8/10	2.1	0.383 ± 0.016	1.6 ± 0.1	0.052 ± 0.004	0.025 ± 0.002	1.1 ± 0.1
			94/4/25	4.6	0.105 ± 0.011	1.1 ± 0.2	0.114 ± 0.007	0.024 ± 0.001	1.2 ± 0.1
Thomeiji River	78	0.64	93/8/10	18.3	0.222 ± 0.011	1.1 ± 0.1	0.573 ± 0.025	0.031 ± 0.001	1.0 ± 0.1
Hino River	211	1.79	93/8/10	12.0	0.469 ± 0.022	1.1 ± 0.1	0.295 ± 0.015	0.025 ± 0.001	1.0 ± 0.1
			94/4/25	26.0	0.680 ± 0.044	1.0 ± 0.1	1.010 ± 0.049	0.039 ± 0.002	1.0 ± 0.03
Yasu River	384	3.69	93/8/13	15.4	0.247 ± 0.012	1.2 ± 0.1	0.595 ± 0.025	0.039 ± 0.002	1.0 ± 0.04
			94/4/19	7.1	0.233 ± 0.023	1.2 ± 0.1	0.213 ± 0.042	0.030 ± 0.006	1.2 ± 0.2
			94/4/25	5.2	0.289 ± 0.035	1.4 ± 0.2	0.125 ± 0.009	0.024 ± 0.002	1.0 ± 0.1
Chinai River	49	0.81	93/8/13	1.8	0.246 ± 0.014	1.4 ± 0.1	0.143 ± 0.013	0.079 ± 0.007	1.1 ± 0.1
			94/4/19	2.6	0.231 ± 0.015	1.3 ± 0.1	0.240 ± 0.008	0.094 ± 0.003	1.1 ± 0.04
Ishida River	54	0.95	93/8/13	2.0	0.037 ± 0.004	1.4 ± 0.2	0.062 ± 0.004	0.031 ± 0.002	1.1 ± 0.1
			94/4/19	4.0	0.026 ± 0.003	1.4 ± 0.2	0.111 ± 0.006	0.028 ± 0.001	1.0 ± 0.1
Ado River	307	4.94	93/8/13	5.3	0.064 ± 0.005	1.3 ± 0.1	0.176 ± 0.016	0.033 ± 0.003	1.0 ± 0.1
			94/4/19	3.9	0.061 ± 0.013	1.4 ± 0.4	0.074 ± 0.018	0.019 ± 0.005	1.4 ± 0.4
Kamo River	43	0.60	93/8/13	6.6	0.535 ± 0.026	1.3 ± 0.1	0.528 ± 0.043	0.080 ± 0.007	1.2 ± 0.1
			94/4/19	3.2	0.478 ± 0.022	1.4 ± 0.1	0.238 ± 0.021	0.075 ± 0.006	1.2 ± 0.1
Wani River	15	0.20	94/4/19	5.1	0.759 ± 0.056	1.2 ± 0.1	0.163 ± 0.007	0.032 ± 0.001	1.0 ± 0.05
Mano River	20	0.21	94/4/19	30.0	0.374 ± 0.032	1.3 ± 0.1	--------	--------	--------
Tenjin River	10	0.03	94/4/19	11.2	0.390 ± 0.043	1.2 ± 0.1	0.463 ± 0.018	0.041 ± 0.002	1.0 ± 0.03
Ohmiya River	6	0.06	94/4/19	3.9	0.969 ± 0.087	1.3 ± 0.1	0.164 ± 0.008	0.042 ± 0.002	1.2 ± 0.1

* : One sigma standard deviation from counting statistics
** : Extract from Lake Biwa catalog [8]

is similar to the distributions of these inorganic components.

The average concentration of dissolved ^{238}U in river waters was calculated to be 0.34 mBq/l by taking into account of water flux of each river [8]. This value is one order of magnitude lower than the average value of 3.8 mBq/l reported for worldwide river waters [6]. One possible reason of the difference may be explained that Japanese rivers have small drainage area and short length compared with the continental rivers. Thus, the low weathering in drainage area appears to control the uranium concentration in river water.

The concentrations of uranium in suspended materials were in the narrow range of 0.014-0.046 mBq/mg and lied within factor 2 relative the value of 0.022 mBq/mg which is expected from the natural abundance of uranium in crust. A little higher ^{238}U concentration found for Chinai River and Kamo River (riverhead based on granite) may be explained by the debris of granite.

Total amount of dissolved ^{238}U supplied by rivers was roughly estimated to be 0.95-1.1 GBq/y by using average ^{238}U concentration in river waters and annual water flow (28-32 $\times 10^8$ m^3/y [8]) into the lake through rivers.

Ground water

Around the lake, there are many inlets of groundwater. Although a quantitative evaluation on the water flux from groundwater into the lake is rather difficult, annual flux of groundwater into the lake is estimated to be 7-11$\times 10^8$ m^3/y [9]. This value corresponds to 22-39 % of the total water supply from rivers into the lake. No data has been reported about ^{238}U concentration in groundwater around the lake previously. The ^{238}U concentration in groundwater collected at one spring (Kido-no-Syozu: see Fig. 1) in the western shore of the lake in 1993 was measured to be 0.22 mBq/l. This value is not so largely different from the average ^{238}U concentration (0.34 mBq/l) in river waters estimated above. Therefore, as a first approximation, by assuming that ^{238}U concentration in groundwater is the same as the average ^{238}U concentration in riverwater (0.34 mBq/l), a total flux uranium from the groundwater was estimated to be 0.2-0.3 GBq/y. This needs further investigation.

Atmospheric Fallout

As one of the possibility of other ^{238}U sources into the lake, atmospheric contribution was examined. No data have been reported on the deposition of ^{238}U from the rain, snow and airborne dust. We have started since 1996 the measurements of ^{210}Pb and ^{7}Be using fallout samples collected monthly on the roof of the Lake Biwa Research Institute (35° 00'N,135° 53'E), Otsu City, located in the southern part of the lake. The same survey including U and Th isotopes have been performed since 1986 at our Laboratory (36° 26'N,136° 36'E) in Ishikawa. Comparison of ^{210}Pb and ^{7}Be data between two sampling sites showed that their deposits at the Lake Biwa Research Institute is about half of those at our Laboratory. The average value of 0.5 Bq/m^2/y for the deposition of ^{238}U have been obtained at our Laboratory during the period of 1986-1989 [10], and furthermore, about 20% of ^{238}U deposited was found to be existing in soluble form. Such findings being taken into account, total flux of ^{238}U from atmospheric fallout is roughly estimated to be about 0.03 GBq/y in the northern basin. This value seems negligibly small compared with the flux from river waters.

As a result, the riverwater and groundwater were considered to be dominant sources of

^{238}U into the lake.

Uranium in the lake

Vertical distributions of dissolved and particulate ^{238}U at station N1 (the northern basin) are shown in Fig. 3 with the data of water temperature. In circulation period (February), the concentration of dissolved ^{238}U was vertically uniform (about 0.15 mBq/l). On the other hand, in stratification period (May to November), the concentration of dissolved ^{238}U was higher in surface layer than that in deep layer. Similar vertical distributions were observed at station N2 and N3 in this period, therefore, this trend of vertical distribution of dissolved uranium seems to occur in the whole northern basin during stratification period. Variation of dissolved uranium concentration at surface and deep water measured at station N1 is shown in Fig. 4-1 together with the average concentration of uranium in water column as a function of time. The dissolved uranium concentration in surface layer increased during the stratification period, and maximum value was found in August. In deep layer, dissolved uranium concentration tended to show slight decrease, and minimum value was observed in November. Variation of dissolved uranium concentration in surface water at station S is also shown in Fig. 4-2. The uranium concentration of uranium in station S (the southern basin) showed seasonal variation similar to that in surface layer of northern basin. Uranium concentration on southern basin was higher than that in surface water of northern basin throughout all sampling period.

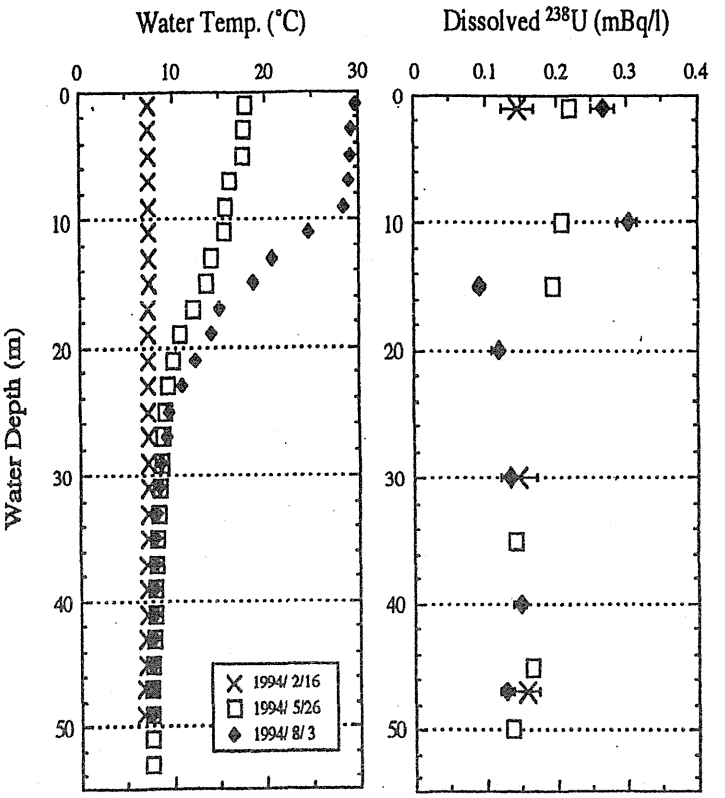


Fig. 3 Seasonal variation of vertical profile of dissolved ^{238}U concentration and water temperature at station N1 in Lake Biwa : Error bars mean one sigma standard deviation from counting statistics.

On the other hand, concentrations of particulate uranium were ranging from 0.007 to 0.034 mBq/l and were one order of magnitude lower than dissolved uranium. The vertical distribution of particulate uranium didn't show any seasonal variation such as the case of dissolved uranium. However, especially high uranium concentrations were observed in August 1994 at the depths of 15 and 20m (Fig. 5)

Similar seasonal variations have been previously observed for other several elements in Lake Biwa. A seasonal variation of dissolved aluminum was observed in northern basin by

Hori et al. [11]. Concentration of dissolved aluminum showed a large difference between surface and deep layer during stratification period and vertically uniform distribution during circulation period. They explained this phenomenon as a result of dissolution of aluminum from suspended particles, because the aluminum concentrations in suspended particles were two orders of magnitude higher than that in lake water. Similar seasonal variation of dissolved vanadium concentration was also found by Sugiyama [12]. He tried to explain the result by inflow/outflow of vanadium, biological activity and release of vanadium from sediment and suspended particles. However, he could not explain which path is more contribute to variation of vanadium because of the absence of data for river and atmospheric input.

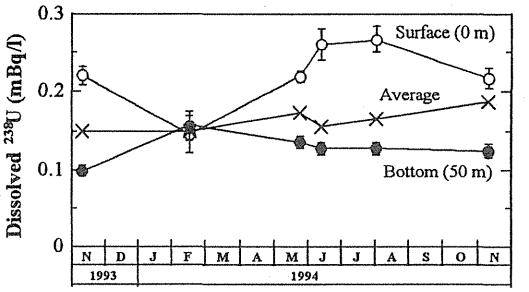


Fig. 4-1 Station N1

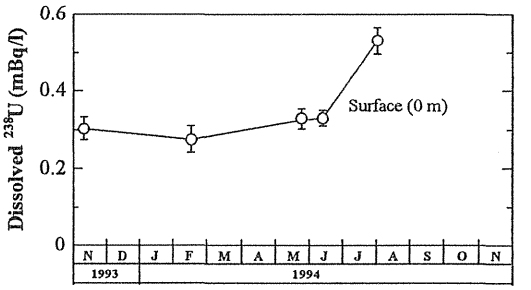


Fig. 4-2 Station S

Fig. 4 Seasonal variation of dissolved ^{238}U concentration in surface (0 m) and bottom (50 m) water at station N1 and S : Error bars mean one sigma standard deviation from counting statistics.

In the case of uranium, suspended particle is insufficient as uranium source to explain the increase of dissolved uranium concentration in surface layer of northern basin, because uranium concentrations in suspended particles were one order lower than dissolved uranium.

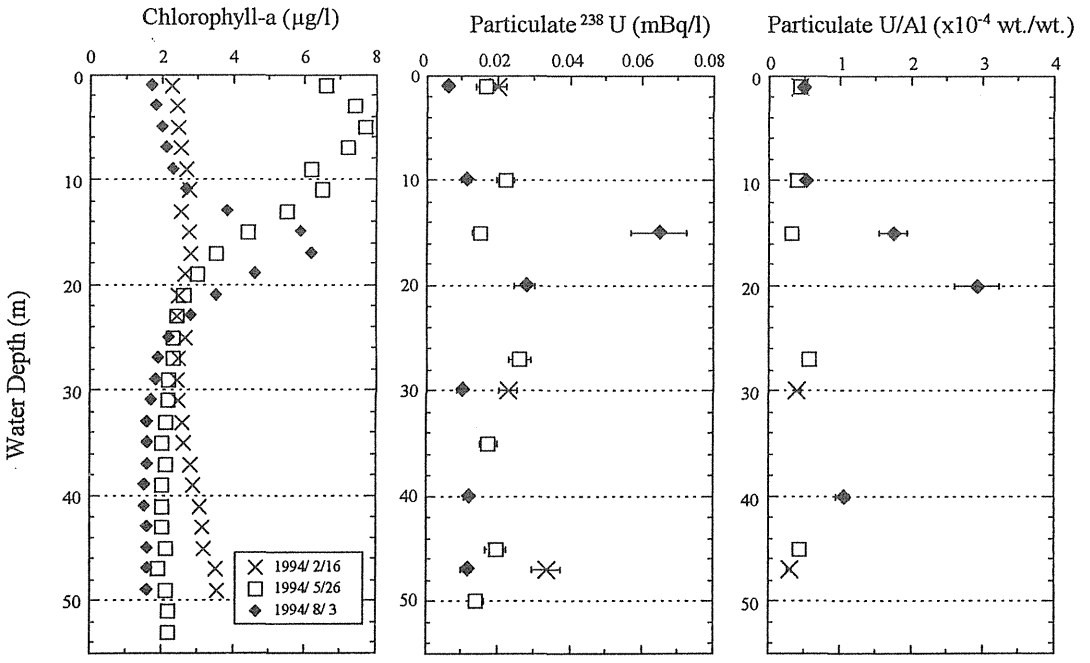


Fig. 5 Seasonal variation of vertical profile of chlorophyll-a fluorescence and particulate ^{238}U concentration and U/Al ratio in suspended particles and at station N1 in Lake Biwa : Error bars mean one sigma standard deviation from counting statistics.

River waters may be one of factors which cause the increase of uranium in surface layer during spring to summer. Because average concentration of uranium in rivers was higher than that in lake surface, the supply of river water to lake surface may bring the increase of uranium concentration in the surface layer of the lake.

We tried to calculate how much contribute the uranium inflow from offshore water (riverwater, groundwater and rainfall) to increase the uranium concentration in lake water during the period from March to August. According to the estimation discussed above, 1.2.-1.4 GBq/y of ^{238}U is supplied to the lake with 5.1×10^{9} m^3/y of water through rivers, groundwaters and rainfall. So, the amounts supplied during March to August were calculated to be 0.6-0.7 GBq and 2.6×10^{9} m^3, respectively. Even if these ^{238}U and water are assumed to be stagnated in upper 10m (a depth of thermocline at August 1994) of northern basin, uranium concentration in lake water increase from 0.15 mBq/l at February to only 0.22 mBq/l as maximum, because the water inflow during March to August is only half volume of lake water in upper 10 m (5.6×10^{9} m^3). Thus, another source of uranium into the lake water must be considered to explain the increase of uranium concentration in surface layer of the lake.

Sediment at shallow area may be anticipated as one of the uranium source into the lake water. In Amazon shelf, uranium was released from sediment to water by disruption from ferric oxyhydroxide as a result of the oxidation-hydrolysis of organic matter [13]. In Lake Biwa, temperature of surface water increases to near 30°C in August, therefore, oxidation-hydrolysis of organic matter in sediment also would occur. High uranium concentration in southern basin (Fig. 4-2) compared with that in northern basin suggests the possibility of dissolution of uranium from shallow-sediment consistent.

Average concentration of dissolved uranium in the lake water of northern basin was 0.15-0.18 mBq/l, which is lower than the average concentration of uranium (0.27-0.30 mBq/l) in all water inlets (riverwaters, goundwaters and rainfall). Low concentration of dissolved uranium in the water of northern basin suggests that uranium is removed from lake water to bottom sediment, thus, northern basin seems to be a sink for uranium. Higher U/Al ratio ($0.3\text{-}2.9\times10^{-4}$, mean: 0.6×10^{-4}) than natural abundance (0.22×10^{-4}) was observed in suspended particles collected from northern basin. This fact indicates that scavenge of uranium by sinking particles would be one of the removal processes in the lake. Especially high U/Al ratios in suspended particles were obtained in August 1994 at the depth 15 and 20 m (Fig. 5). Maximum of chlorophyll-a fluorescence as indicator for amount of phytoplankton was also observed at the same depth. This coincidence suggests a biological uptake of uranium by phytoplankton. The concentration factor 10^2 - 10^3 for phytoplankton and algae in seawater has been reported for uranium [14,15]. Mahon [16] reported high concentration factor 4420 by phytoplankton in high natural radioactivity area (Okanagan, Canada). In marine system, biological removal of uranium from seawater is reported to be less than 10% of total removal flux [2,3,6]. Since uranium concentration in Lake Biwa is extremely low compared with seawater (39mBq/l), the biological removal process may became relatively important.

Total amount of dissolved uranium in lake water of northern basin was estimated to be 4-5 GBq using average concentration of uranium in water column. Apparent residence time of uranium was calculated to be 3-4 years using total amount of uranium in the lake and annual uranium inflow discussed above. This value is a little shorter than apparent residence

time of water itself (5.5 years [4]). However, net residence time may be shorter than this value, because the presence of another significant sources of uranium into the lake (release from sediment) is predicted in the lake. These residence times indicates that uranium in the water of Lake Biwa may be rather non-conservative.

ACKNOWLEDGMENTS

We would like to thank to members of Lake Biwa Research Institute and Low Level Radioactivity Laboratory for their kind help in collection of sample and their collaboration. We are also indebted to the captain and crew of R/V *Hakken* of Lake Biwa Research Institute for shipboard support.

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2. Mercury Transport from Minamata Bay to the Surrounding Sea Area : Possible Ecological Impact

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INTRODUCTION

Discharge of total 150 tons of mercury with industrial wastewater to Minamata Bay, Kumamoto, Japan, started in 1932 and stopped in 1968. The discharged mercury accumulated in marine organisms of various trophic levels finally to cause acute or chronic methyl-mercury poisoning, known as Minamata disease (M. d.), to 2252 of inhabitants who ate polluted fish. So far extensive clinical, anatomical and epidemiological studies have been conducted concerning M. d. In contrast, site-specific, academic study of mercury transfer in ecological components such as sediment, sea water, microbes, plankton and fish of Minamata Bay and the surrounding sea area is scarce except for those by authors (reviewed by Kudo et al., 1998), although behavior of mercury in biota and abiota is the most important factor that triggered the M. d.

We have investigated the migration of mercury from Minamata Bay to the adjacent sea since 1975 by annual sampling of bed sediment at 26 stations in the Yatsusiro Sea (reviewed by Kudo, 1998). Adding to this, sediment cores were collected at two of the annual sampling stations in 1997 in order to clarify the history of mercury migration from Minamata Bay to the adjacent sea. The present study is about the measurement of mercury concentration in the core and the comparison of the obtained result with annual sampling data.

MATERIALS AND METHODS

Sediment has been collected annually since 1975 using an Ekman sampler at 26 stations in Yatsushiro sea (area 636 km^2) adjacent to Minamata Bay (3km^2), which is located at the southwestern coast of Japan on Kyushu Island (Fig. 1). The error in locating each sampling station was $\pm$100 m between 1975 and 1985, and was $\pm$10 m after 1986. The sampling was conducted during the summer months from a research ship "Kakusui" of the Nagasaki University.

In august 1997, we collected sediment cores at sampling stations No.1 (27m depth) and No.4 (35m depth). Station No.1 is 3.9 km from the wastewater effluent in the Minamata Bay, and is located beside Koiji-shima, which barriers the northern outlet of Minamata Bay together with Myojin Cape. Station 4 is 8.0 km from the effluent, facing the west outlet of Minamata Bay. To collect cores, we hired two divers who dived to the bottom with a sampling device and drove it into the sediment vertically. Acrylic resin was used for surfaces, which contact with the sediment sample in order to prevent cross contamination from metallic components of the sampling device. Contiguous 1 cm increments are taken from the top of the core to 20 cm, and 2 cm sections are taken thereafter.

Total mercury in the layers from sediment was measured by inductively coupled plasma-mass spectrometry (ICP-MS), after acid-decomposition of freeze-dried sediment samples. Analytical errors of mercury measurement by ICP-MS were less than 2 % of the certified values of standard sediment and soil samples, SRM-2709 (National Institute of Standards and Technology), and Pacs-1 and Mess-2 (National Research Council, Canada).

RESULTS AND DISCUSSION

Sediment depth (cumulative sediment deposition rate (g/cm^2)) vs. mercury concentration profile in the sediment core from station 1 is shown in Fig. 2 together with annual observation data at the same station. Some of annual sampling data are unavailable because sampling at the station No. 1 could not be conducted from 1978 to 1985. Because low mercury concentration of 0.1 to 0.14 ppm was observed in the old sediment layer (19-21 g/cm^2), the core contained historical record of mercury deposition before the deposition of mercury from Minamata Bay started at the station.

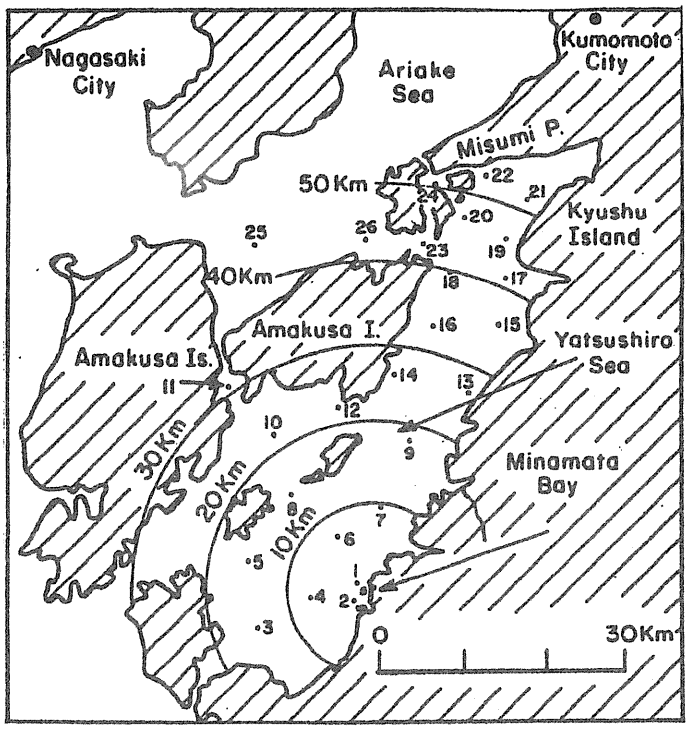

Fig. 1 Sampling locations

The mercury concentration profile in the core had two peaks, one at 5.4-5.6 g/cm^2, another at 9.9 g/cm^2 (Fig. 2a). Its comparison with annual sampling data suggests that one of the maximum deposition rates occurred in the first half, and the other in the latter half, of the 1980s. The latter probably is related with decontamination project conducted at Minamata Bay from 1984 to1989. The cause of the former peak is unknown at present.

The history of mercury deposition at station 1 also indicates that mercury had been confined in small Minamata Bay (3 km^2) relatively well before the 1970s. The mercury concentration in the core from station 1, only 4 km from the outlet of wastewater containing mercury, began to rise at 16 g/cm^2 (Fig. 2), which probably corresponds to early 1970s or late 1960s, namely more than 30 years after first discharge of mercury to the Minamata Bay (1932). The cause of M. d. may be partly attributable to this efficient containment of 150 tons of discharged mercury.

The mercury profile in the core collected from station 4 also had two peaks (Fig. 3), as the core from station 1 had. The mercury concentrations in the deep part of this core (deposition rate > 5 g/cm^2) were only 10 % of those observed by annual sampling in the past. The apparent loss of mercury in the buried sediment layer is explainable by (1) relatively large experimental error in locating each sampling station (±100 m) before 1985, (2) desorption of mercury to interstitial water and subsequently to overlying water (observed in

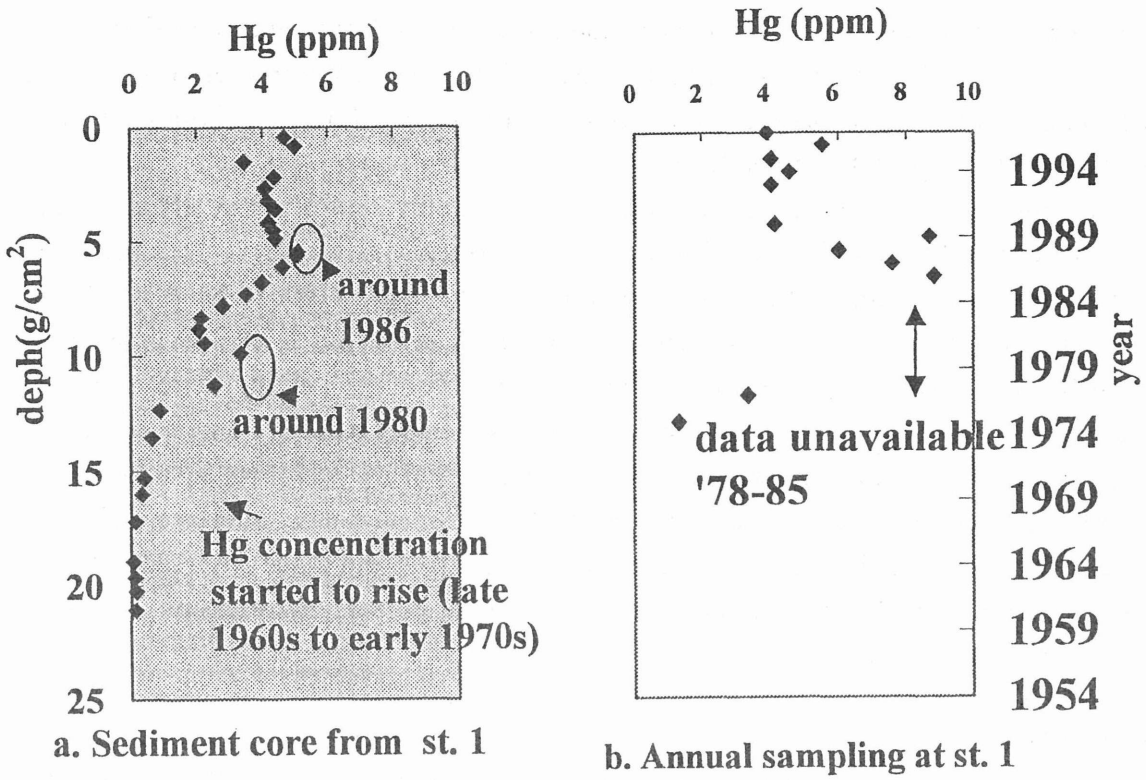

Fig. 2 Hg concentrations at station 1 (core and annual sampling)

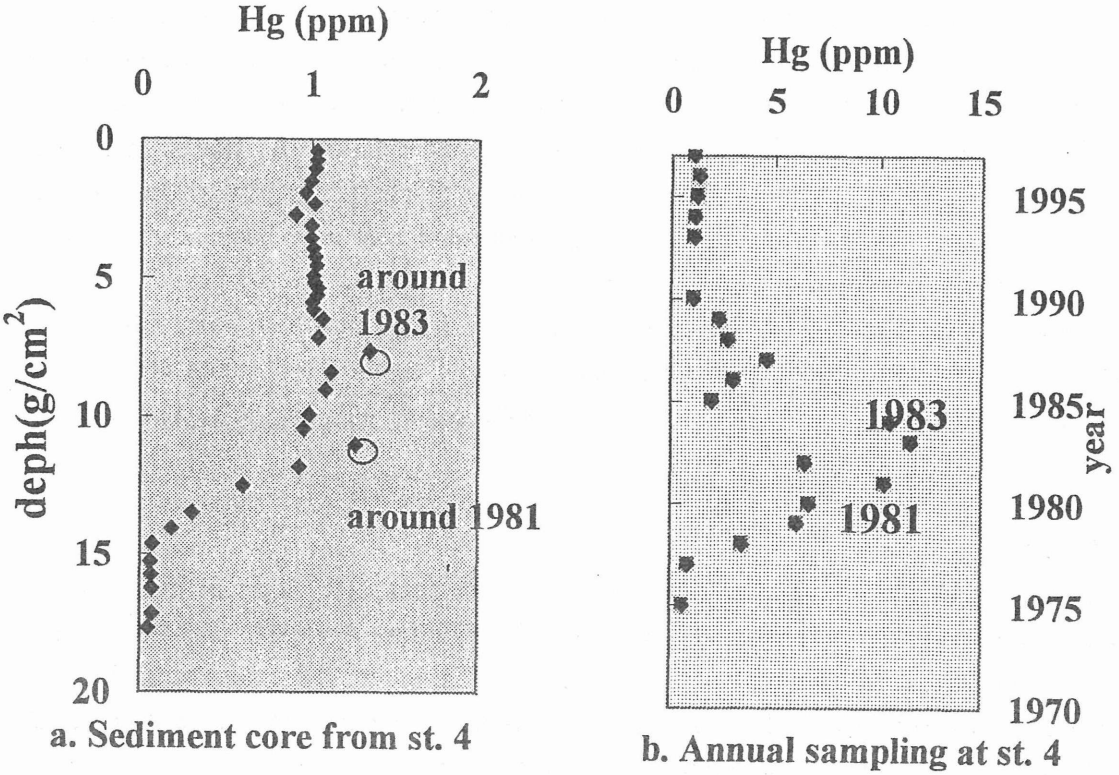

Fig. 3 Hg concentrations at station 4 (core and annual sampling)

reducing sediments from highly contaminated estuarine environment by Bothner et al. (1980) and Lindberg and Harris (1974)), and (3) vaporization of mercury as demethylmercury or Hg(0) mediated by bacterial activities (Zhang and Planas, 1994; Radosevich and Klein, 1993). Net loss of mercury from deep sediments may occur by the reconstitution of buried polluted sediments back to the sediment-water interface by bioturbation followed by bottom sediment-water column exchange (Officer and Lynch, 1989).

If remobilization of mercury from sediment was actually significant at station 4, intake of mercury desorbed from polluted sediment by marine organisms may have been pertinent to mercury transfer from abiota to biota and finally to human beings in case of M. d. incident.

The significant loss of mercury from the sediment at station 4, if it ever happened, questions the documentation of mercury discharge history from the station-4 core. To clarify historical evolution of mercury deposition from the sedimentary record, we must assume that the rate of mercury remobilization and redistribution within the sediment column is small compared with the rate of Hg deposition at the sediment-water interface. The large discrepancy between the Hg concentration observed in the past and those observed in the core at station 4 may render the above assumption inapplicable. Further investigation is necessary to clarify this point.

CONCLUSION

We collected two sediment cores in the Yatsushiro Sea at annual sampling stations No.1 and 4 in order to clarify the history of mercury migration from Minamata Bay to the surrounding sea.

The concentration profiles of total mercury in the cores had two peaks. The background mercury concentration at the station 1 was approximately 0.1 ppm.

The mercury concentration profile from the core at station 1 showed that migration of mercury was most significant in the 1980s, partly because of sediment dredging in the Minamata Bay. Mercury had been confined within the Minamata Bay relatively well before the 1970s, because at station 1 (4 km from Minamara) mercury deposition started in the late 1960s or early 1970s (more than 30 years after mercury discharge to Minamata Bay had started). Such containment of mercury within the Minamata Bay may have contributed to subsequent accumulation of mercury in the biota there.

The mercury concentration in the deep part of core from station 4 was considerably smaller than those obtained by past 22 year observation of surface sediments at the same location. The clarification of the cause of observed discrepancy will be the topics of future investigations.

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3. Analysis of Metal Distribution in Annual Tree Rings as an Indicator for Deposition of Acidic Materials on a Forest Ecosystem

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INTRODUCTION

Chemical composition of soil solution is closely related to matrix of soil and is controlled by some chemical reactions such as cation exchange, precipitation occurring between/in soil matrix and soil solution. An exogenous perturbation like acid rain will move the present chemical equilibrium condition between soil solution and soil matrix to new one. At an early stage of acid rain deposition on a forest floor, movement of metals from soil matrix to soil solution will take place by buffer effect of soil system. That would result in chemical composition change in the soil solution. As shown in Fig. 1, trees take up metal nutrient from soil solution with water and use them in metabolic processes. Some of the metals in sap being taken up through root is fixed in annual tree rings[1]. If historical changes of soil solution chemistry by deposition of acidic materials are recorded in annual rings as a change of metal distribution in tree rings, we can use trees as a biomonitor for the historical impact of acid rain on a forest ecosystem. To use trees for the above purpose, we have to make clear some points about metal distribution in trees. Japanese cedar or sugi, *Cryptomeria japonica* D. Don, is one of the species showing decline in Japan and acid rain is suspected agent of the decline. In this report basic characteristics on cation distribution in annual tree ring of sugi are described and a possibility of tree ring analysis for assessment of acid rain was discussed.

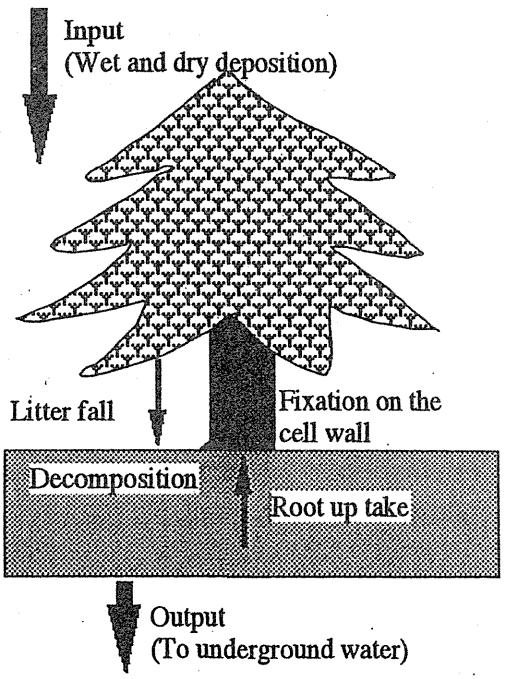

Fig. 1. Metal cycle in a forest ecosystem

MATERIAL AND METHODS

Sugi were obtained from the experimental sugi stands in Fukuoka, Japan. No significant local emission sources of S and N are found around the sugi stands. Stem sections at different vertical positions were cut and oven-dried. Each stem section was divided into appropriate year increments and about 1 g of each increment was dry-ashed. The ash was dissolved in 1M HNO_3 and cation concentrations were determined by flame emission spectroscopy (FES) and inductively coupled plasma-atomic emission

spectroscopy (ICP-AES). Concentrations of ^{90}Sr were analyzed for the 70 year old sugi tree by beta spectrometry after chemical separation[2]. A portion of the increment was ground in a Wiley mill to pass 20-mesh screen and part of the ground wood was washed repeatedly with 0.1 M HCl to displace exchangeable cations in binding sites of the wood with H^+. About 100 mg of the H^+ wood was put in 50 mM $CaCl_2$ solution, and displaced H^+ with Ca^{2+} was titrated with NaOH solution to the initial pH[3]. Calcium binding capacity (CBC) of the wood at pH 6 and its variation with pH was measured.

RESULTS AND DISCUSSION

Major cations in sugi stemwood were Ca, Mg, and K, together accounting for more than 95% of the total cations. The K concentrations were significantly different between heartwood and sapwood, while Ca showed a gradual decrease from the pith to the outermost ring. The distribution of Mg showed an intermediate distribution pattern between K and Ca. Radial distributions of 8 cations at different vertical positions were similar as shown in Fig. 2 and the cations were classified into three groups[2];

(I) constant radial concentrations (Ca^{2+}, Sr^{2+}, Na^+, probably Ba^{2+})

(II) high concentrations in the heartwood and low in the sapwood (Mg^{2+}, K^+, Rb^+, Cs^+)

(III) increasing concentrations in the sapwood (P, Mn^{2+}).

The similar radial distributions of cations in the bole of sugi, no significant difference at different vertical position, suggest that we can use any position of the stem as a sample[4]. Metabolic processes of tree would alter radial distribution of cation by transfer and redistribution. It is, therefore, important to find out cation that is fixed for a certain period on the annual tree rings and gives the information about the history of soil solution chemistry. One way to judge cation immobility in stemwood is the comparison of radial distribution of radioactive fallout in stemwood and cumulative deposition pattern on the forest floor. Because atmospheric nuclear testings were carried out in the early 1960s, radionuclides with long half-life are available to this purpose[5]. In Fig. 3 distribution of ^{90}Sr in the sugi stemwood is shown together with the cumulative deposition pattern in the northern hemisphere[2]. Trees take up water by sapwood, suggesting that present change in soil solution chemistry is introduced to older rings. This is seen in Fig. 3 as an occurrence of ^{90}Sr- in the annual rings formed in 1930s but small amounts. The similar increasing pattern of ^{90}Sr in the stemwood to that of the cumulative deposition suggests that the change of the specific activity of Sr in the soil was recorded in the sugi stemwood. This proves Sr is fairly immobile in sugi stemwood and heartwood formation did not contribute to mobility of Sr in xylem. Sr and Ca are alkali earth elements and clime up from base to top of red spruce stemwood interacting with cell walls by identical degrees[3]. The relation of Ca and Sr concentrations in sugi stemwood shown in Fig. 4 has a high correlation. These facts speculate us that Ca is probably immobile in sugi stemwood.

Calcium-bind capacity (CBC) measured at pH 6 for sugi stemwood but different tree ages are shown in Fig. 5. It is interesting that the CBCs are not so different in the sapwood of different trees, about 60 μeq/ g. However, the CBCs increase toward the pith and have similar values at the center of the bole, about 120 μeq/g. The reason for the increase in the heartwood is not clear but formation of additional CBC occurred in the heartwood. The pH dependency of CBC is shown in Fig. 6 and the

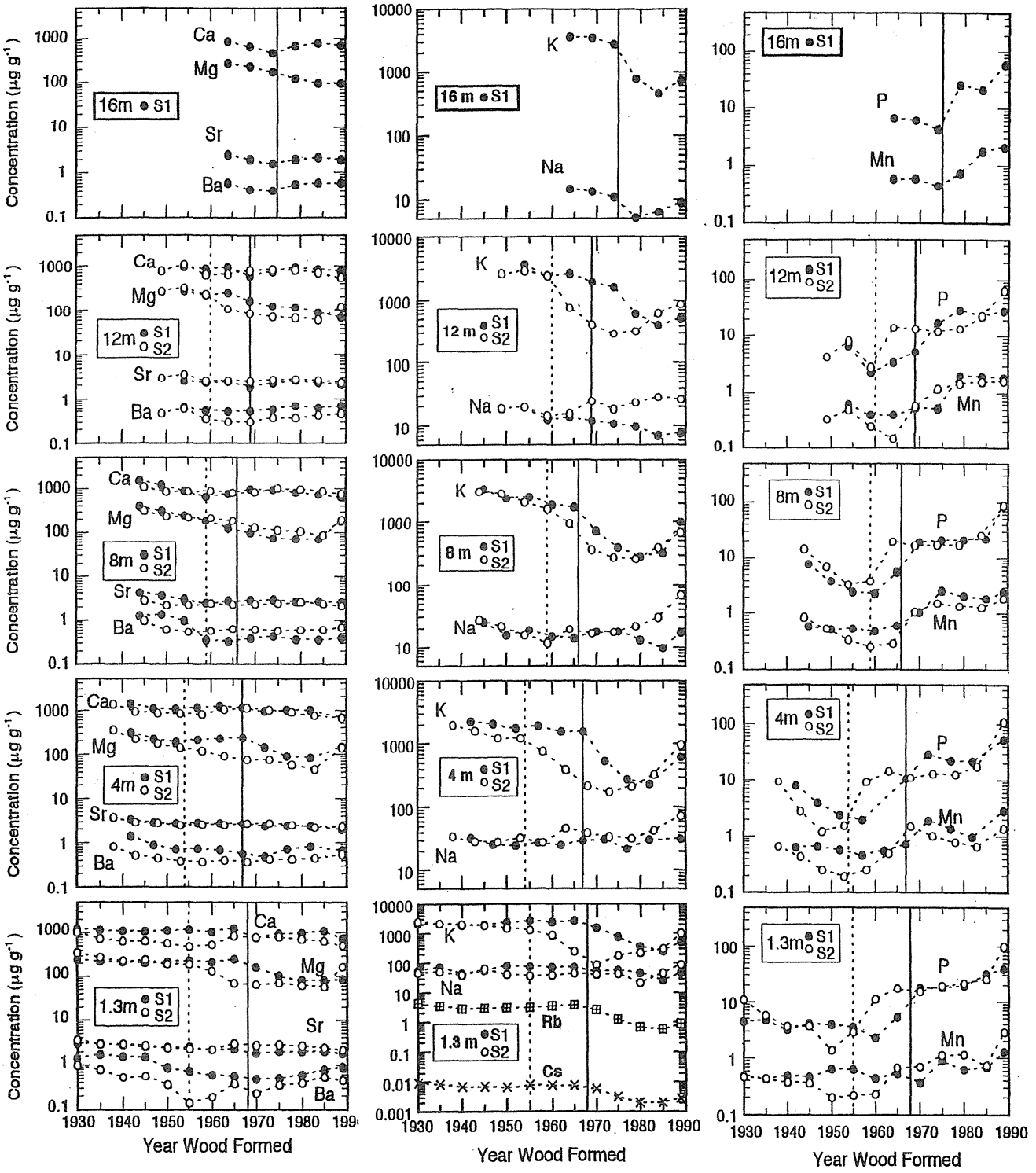

Fig. 2. Distribution of cations in annual ring at different vertical positions of sugi stemwood

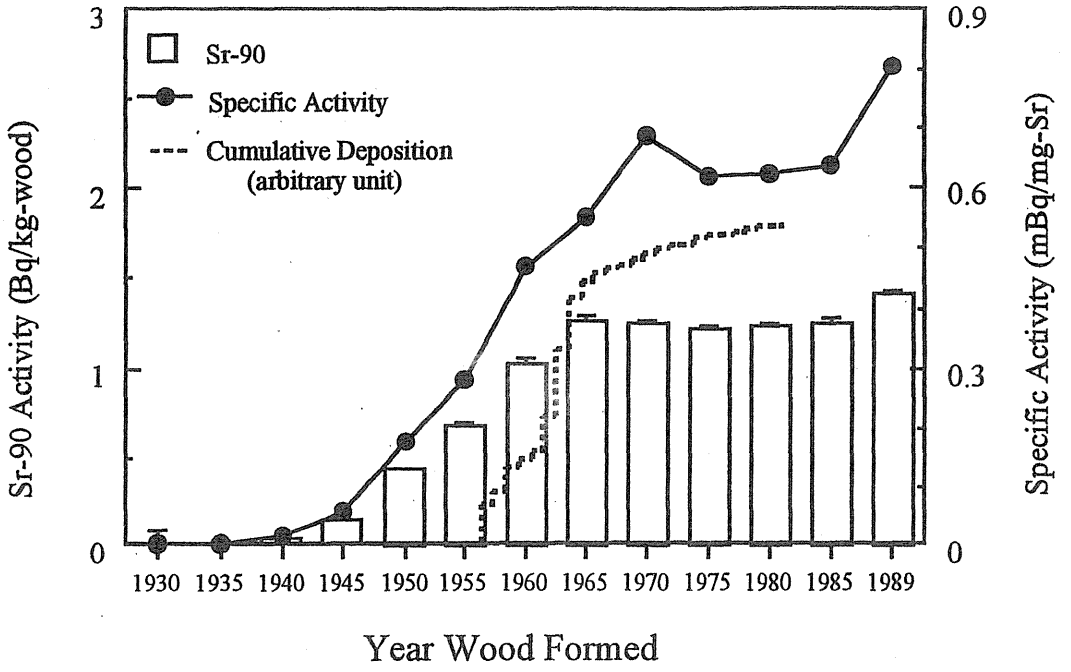

Fig. 3 Radial distribution of Sr-90 in sugi stemwood

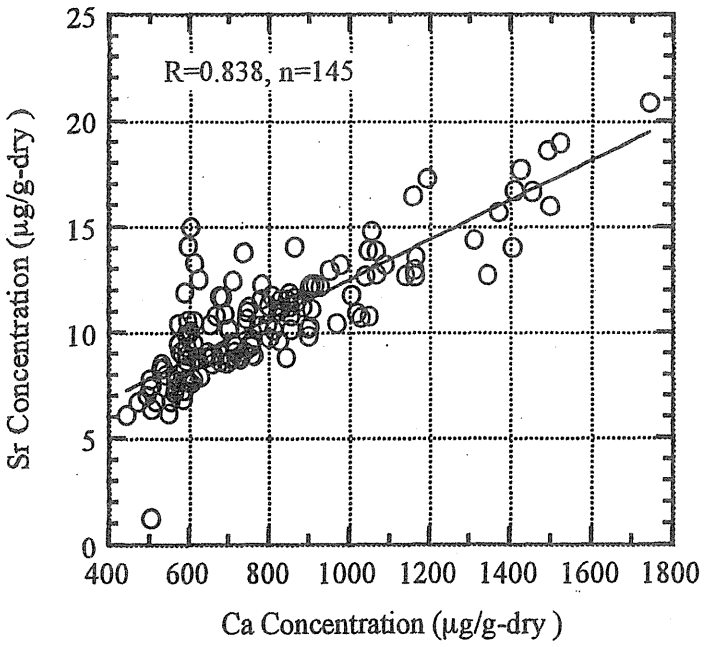

Fig. 4. Relation between Ca and Sr in sugi stemwood.

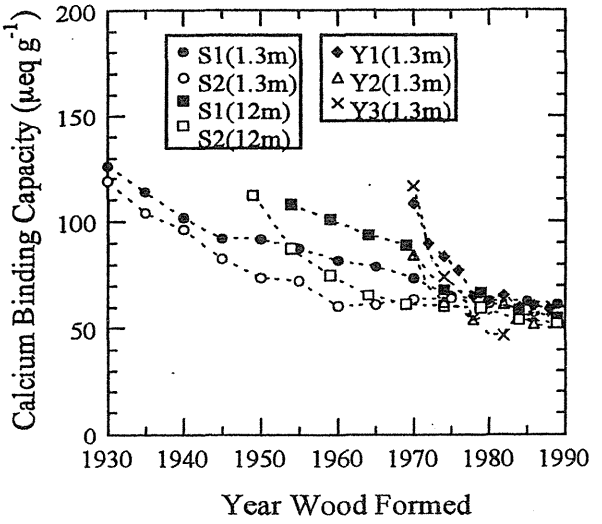

Fig. 5 Calcium-binding capacity in sugi stemwood as a function of the year the wood was formed.

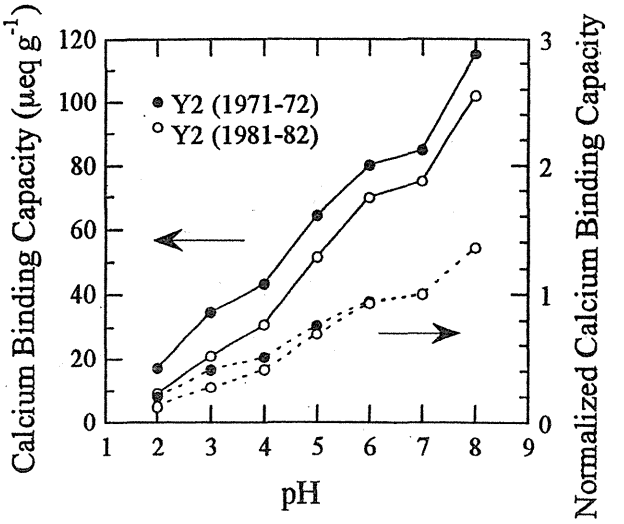

Fig. 6. Calcium-binding capacity of sugi stemwood as a function of pH.

normalized CBC shows some deviation at pH around 3. The deviation would be related to the formation of additional CBC in the heartwood.

If all of the cations in xylem exist in binding sites of the cell wall, total equivalents of cations should be equal to CBC at the sap pH. As shown in Fig. 7 the total equivalents exceed the CBC measured at pH 6 in the heartwood while almost comparable in the sapwood[2]. The actual sap pHs are shown by small figures in the each bar in Fig. 7 and ranged between 5.42 and 6.26. The sap pH higher than 6 suggests that somewhat larger actual CBC in the heartwood and the sap pH lower than 6 suggests smaller CBC in the sapwood compared to the CBC measured at pH 6. The pH dependency curve of CBC in Fig. 6 predicts that an amount of cations which can be fixed in the binding site at observed sap pHs is at most 80 μeq/g and can not explain the observed total cation equivalents. The relation between the CBC and the total cation equivalent suggests that a part of the cations in the heartwood exists as salt, not in the binding site. Washing of sugi xylem with water indicates most of the K is reachable from the wood and exists as the form of salt especially in the heartwood (Fig. 8). Most of the Ca and Mg are not reach out from the xylem with water, they are fixed in the binding site of the cell walls[6]. Total equivalent of cations is shown in Fig. 7 for the sugi xylem washed with water, indicating summation of Ca, Mg and K is apparently lower than the CBC at pH 6. CBC of sugi xylem untreated, not washed with HCl to convert the binding site to H^+-form, was measured as the same manner of the CBC measurement and the value obtained represents the size of binding site which was originally occupied with H^+ and is shown in Fig. 7 as titratable hydrogen (TH). The total equivalent including TH is well consistent with the CBC measured at pH 6. This also supports that Ca and Mg is fixed in the binding site of the cell wall.

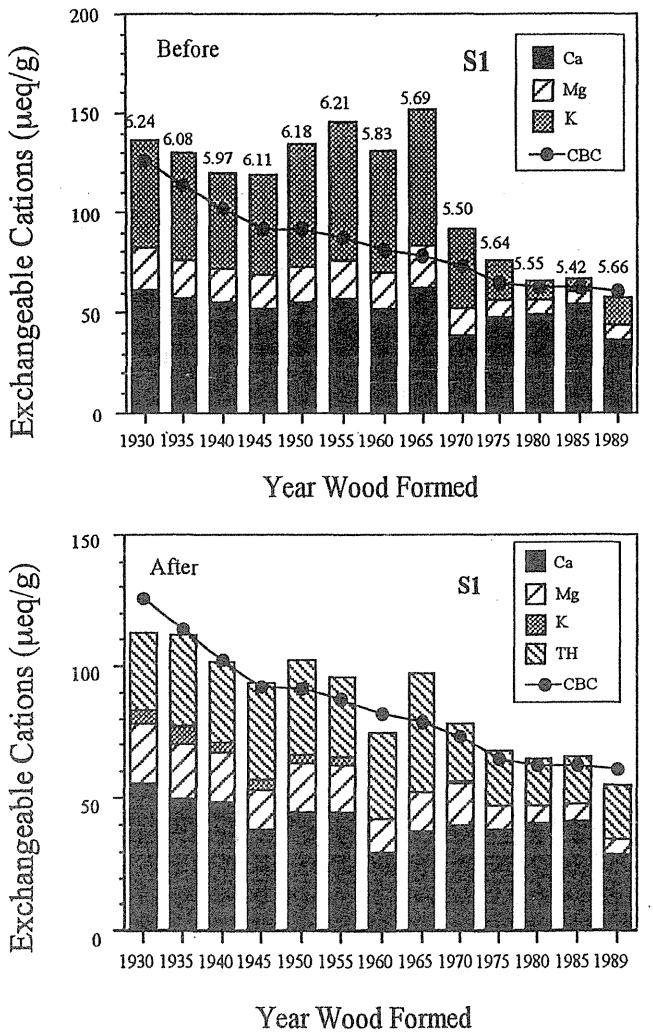

Fig. 7. Comparison between calcium-binding capacity and cation equivalents in sugi stemwood before and after washing with water.

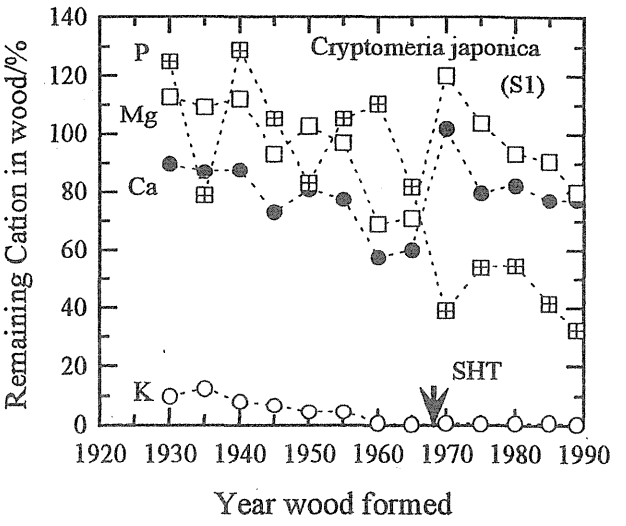

Fig. 8. Ca, Mg, K and P remaining in sugi stemwood after washing with water.

CONCLUSION

Influence of acid rain on forest ecosystem will appear at first as a cation concentration increase in soil solution. If the increase in soil solution is introduced to tree through root uptake, it would be recorded as the concentration increase in the annual tree rings of sugi for immobile element such as Sr. High level of cations in the soil solution continues as long as the soil has enough buffering capacity for acid rain and it allows a elevated level of cations in annual tree rings for successive periods. At a final stage of acid rain effect on sugi forest, a decrease of soil pH accompanying low cation concentrations in soil solution will occur and result in an decline of the cation concentrations in annual rings. The scenario is illustrated in Fig. 9. Metal contained at high concentrations in trees would be more suitable element than those at low concentrations for assessment of acid rain effect on soil environment. Availability of

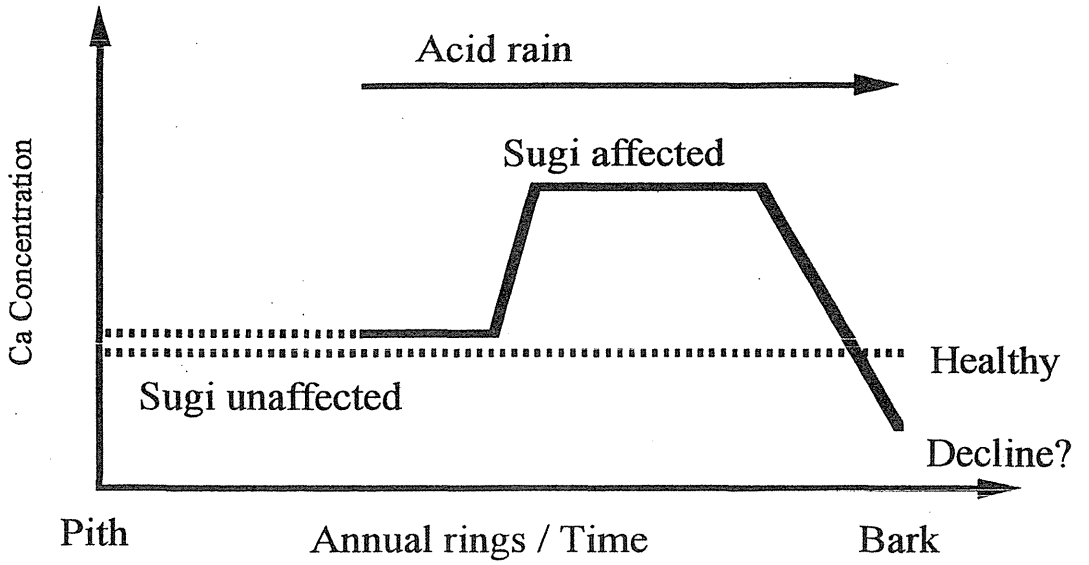

Fig. 9. Most probable scenario of Ca in sugi growing an area where acid rain influences on soil solution chemistry.

trace elements in soil is variable in each site and their concentrations are tend to vary with materials in the soil solution such as organic materials. Calcium would be suitable because it is the major element in sugi xylem and would be immobile as observed for Sr.

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4. Natural Analogue Studies on the Koongarra Uranium Deposit, Australia: Behavior of Uranium and Decay Products in the Environment

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ABSTRACT

Natural analogue studies were carried out on the Koongarra uranium deposit, Australia, under the international collaborative projects. In the projects, Environmental Geochemistry Laboratory of Japan Atomic Energy Research Institute obtained much information on behavior of uranium and decay products in geosphere. The most essential result is that uranium is more strongly fixed by several ways in nature than the simple adsorption on minerals observed in laboratories. Fixation mechanisms of uranium are coprecipitation with iron minerals and precipitation of uranium minerals through alteration of the host rock and uranium minerals. These are direct evidences of uranium migration and fixation in near surface condition. Natural analogue studies can provide us not only direct contribution to the performance assessment of scenario on the nuclear waste disposal, but also securities for various natural systems through general understanding of geological processes.

INTRODUCTION

For the accurate safety assessment of the nuclear waste disposal, we have to understand the long-term migration behavior of radionuclides in geosphere. Quantitative evaluation and modeling of processes that occur in nature enables us application for prediction of migration behavior of elements in nuclear waste repositories. For these purposes, studies on natural systems have been carried out as natural analogue studies on migration of radioactive nuclides.

The most important processes regarding to migration are dissolution, transportation and fixation of elements. In nature, these are common processes of ore deposit formation. Remobilization of elements in ore deposits can be considered good analogue to the migration phenomena of nuclides from radioactive waste repository. Especially, evolution of uranium ore deposits has unique and significant value because uranium is not only a direct analogue to trans uranium elements (TRU) in radioactive waste but also uranium and related elements have various geochemical and geochronological characters. We can extract key processes for migration and fixation of elements from studies on the uranium ore deposits. Based on the observation on nature, we can build models that represent the natural processes, and evaluate how accurate it is. In this paper, the natural analogue studies on the Koongarra uranium deposit, Northern Territory, Australia, carried out by our laboratory are introduced briefly.

Koongarra uranium deposit, Australia

Koongarra deposit is located 220 km east from Darwin, and it is in the Kakadu national park (Figure 1). In the region, several uranium deposits were found and some of them are under mining operation. Among them, Koongarra deposit has quite characteristic features on uranium migration. Formation of the Koongarra deposit is estimated to be approximately 1.6 billion years ago in quartz chlorite schist, an iron-rich low-grade metamorphic rock [1]. After that, the ore body was under relatively stable geological setting. However, gradual erosion of ground surface let the depth of the ore body getting shallow. At approximately 2 million years ago, oxidized surface water began to affect to the ore body.

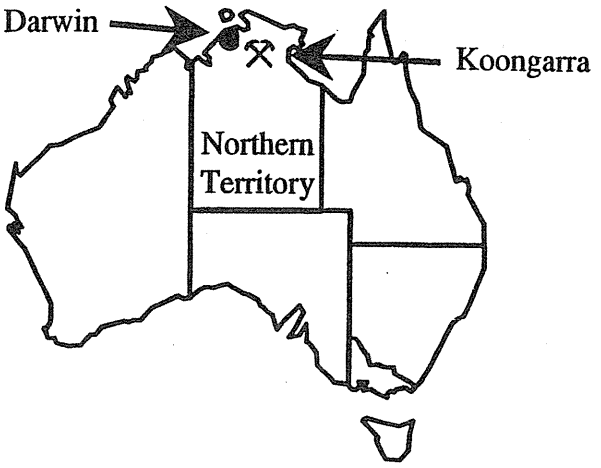

Fig. 1 Location of the Koongarra deposit.

The current Koongarra deposit has three distinct sections (Figure 2). The deeper zone is the unweathered, primary ore region where uraninite (uranium oxide) and uranyl silicate minerals are

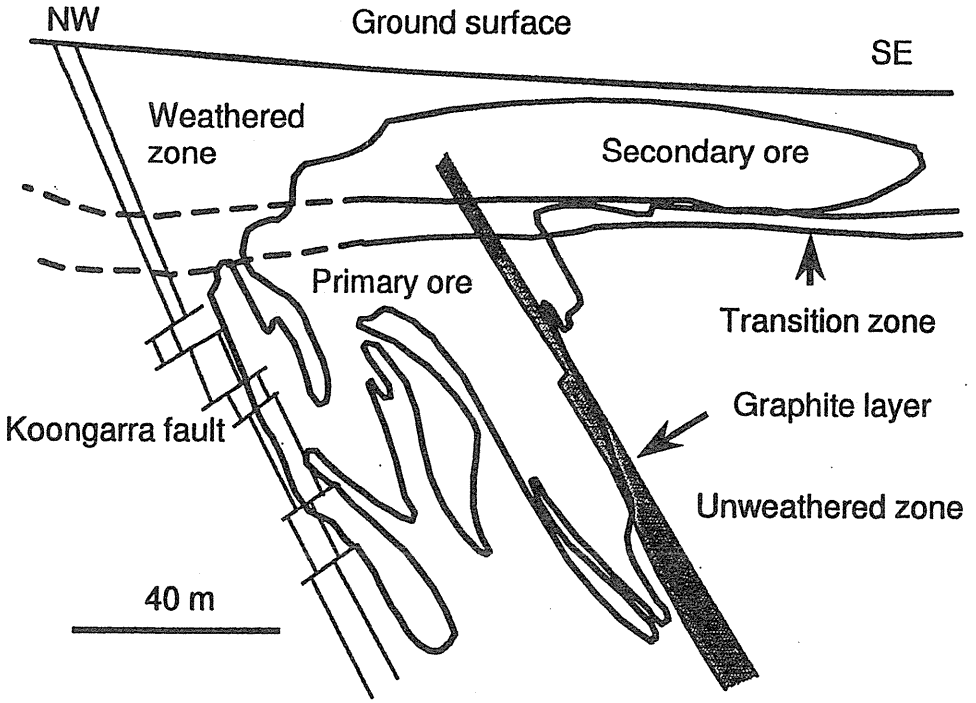

Figure 2 Schematic vertical cross section of the Koongarra deposit (NW-SE) modified after Snelling (1980). Ground water flow from NW to SE formed the secondary ore as a dispersion fan.

dominant. The shallower zone is the weathered, secondary ore region where uranium occurs as uranyl phosphate minerals or absorbant on iron and clay minerals [2]. Border of the weathered and unweathered zones is the transition zone where redox condition changes drastically.

Main oxidized alteration products of the host rock are kaolinite and iron minerals. Iron minerals have high sorption capacity of elements from ground water. Flow of the oxidized surface water dissolved the upper part of the ore body and moved uranium downstream, then precipitated the secondary ore body. This feature makes the Koongarra deposit to be significant and unique natural analogue site for migration of nuclides in surface condition. We can observe migration and fixation mechanisms of uranium occurred in nature.

To study the above features of Koongarra, the deposit is subject to international natural analogue studies for more than ten years. In 1987, Alligator Rivers Analogue Project (ARAP) was organized as a multilateral project sponsored by OECD/NEA. Japan Atomic Energy Research Institute (JAERI) joined the project and kept contributing to the next project, Analogue Studies in the Alligator Rivers Region (ASARR).

RESULTS OF NATURAL ANALOGUE STUDIES

Distribution and disequilibrium of uranium

Distribution of uranium in the ore body was determined by measurement of radioactivity coupled with sequential selective extraction techniques [3,4]. Uranium in the primary ore body is attributed to uranyl minerals or uranium oxide minerals. In the secondary ore body, majority of uranium coexists with crystalline iron minerals (Figure 3). The residual phases like quartz and kaolinite in the secondary ore body have extraordinary higher activity ratio of $^{234}U/^{238}U$ than unity [4]. This disequilibrium on

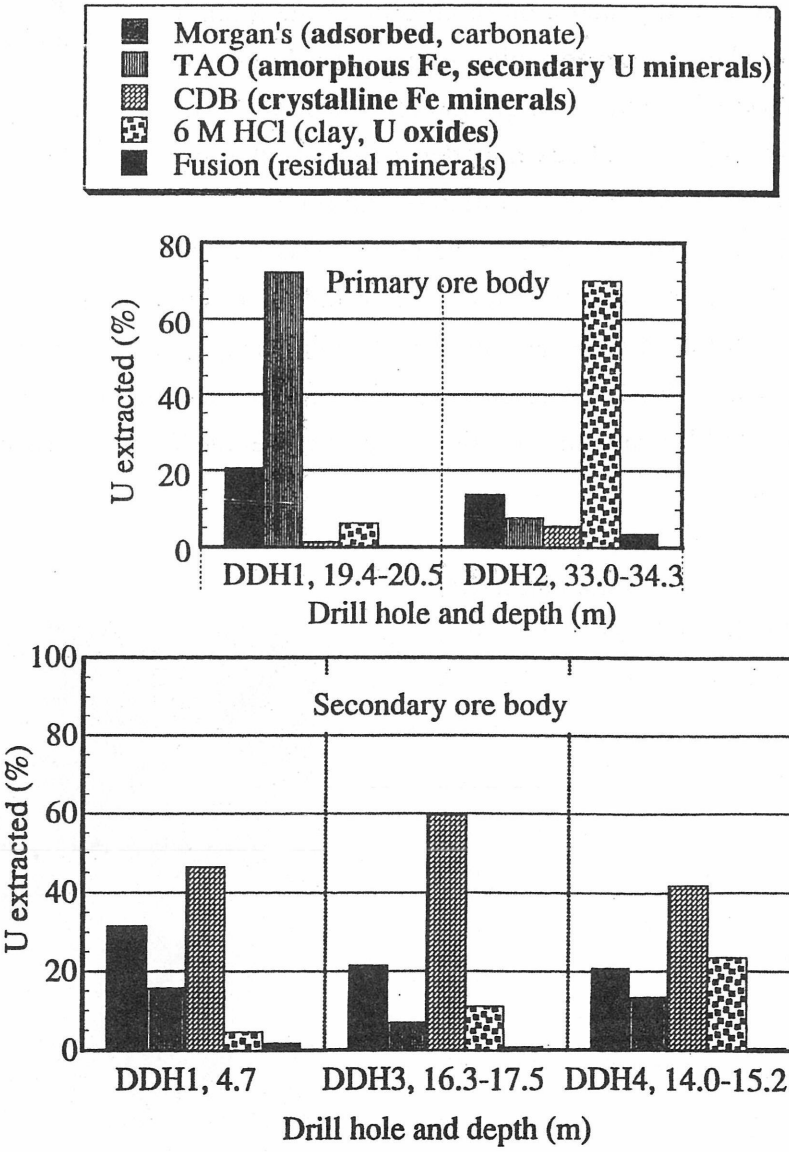

Figure 3 Fraction of uranium extracted by sequential extraction technique

uranium series nuclides also means that uranium in the weathered zone is related to iron minerals coating quartz. Disequilibrium of uranium series nuclides can provide us information concerning migration rates of uranium in several hundred thousand years.

Weathering of host rock and fixation of uranium by iron minerals

Detailed mineralogical observation on weathering process of the host rock was carried out [5-9]. Main mineral of the host rock, chlorite, altered to kaolinite through vermiculite by oxidized ground water (Figure 4). In this process, iron in the chlorite is oxidized and released, then iron minerals such as amorphous ferrihydrite and goethite precipitated. Magnesium and silicon also released during alteration of chlorite to the ground water. These elements play important role on the fixation of uranium. Migration rate of uranium to the secondary ore body may depend on weathering rate controlled by the alteration of chlorite.

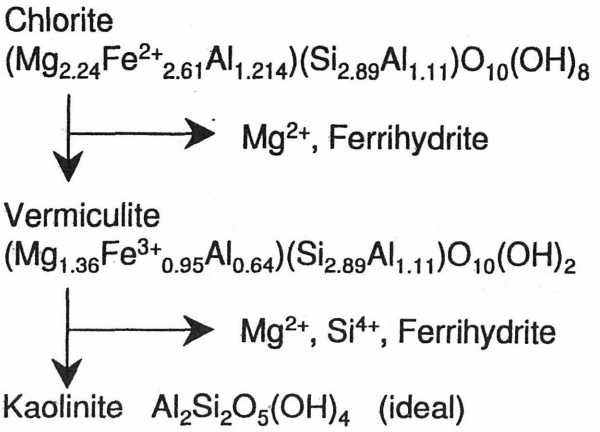

Figure 4 Schematic alteration path of chlorite weathering.

In the central region of the current secondary ore body, iron minerals play the most important role to fix and keep uranium by their high sorption capacity on dissolved species. Observations by high-resolution transmission electron microscopy, nanocrystals of uranyl phosphate minerals were observed [10-12]. Precipitation of uranium minerals from low concentration ground water is conducted by catalytic reaction by iron minerals. This process may have a key role on fixation of uranium.

Alteration of uranium minerals

In the primary ore body of the Koongarra deposit, alteration of uranium minerals determines distribution and migration of uranium [13]. The initial uranium mineral of the deposit is uraninite (UO_{2+x}) precipitated just below the graphite layer of the host rock. Uraninite is still found in the limited area of the primary ore body (Figure 5). The most dominant uranium mineral in the current primary ore body is sklodowskite, uranyl Mg

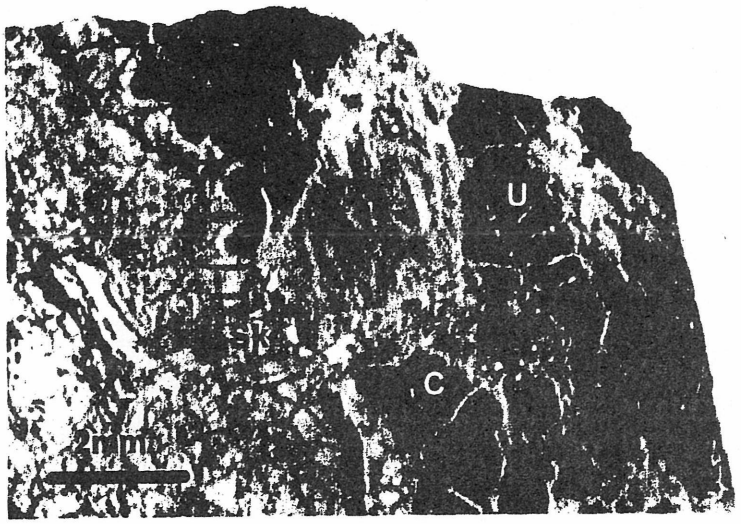

Figure 5 Sample of the primary ore body. Uraninite (U) is surrounded by curite (C), and sklodowskite (Sk) veins.

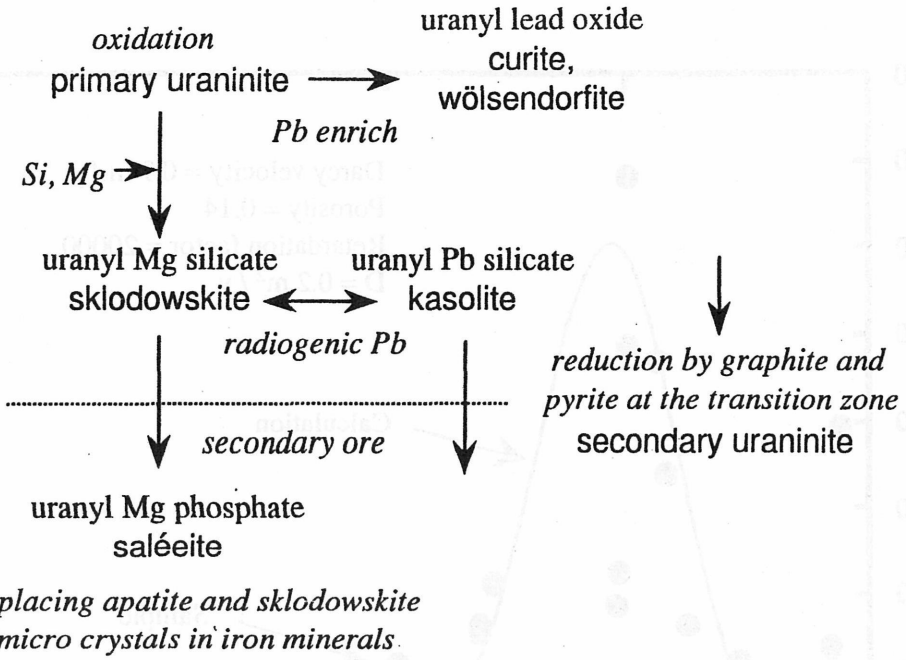

Figure 6 Schematic alteration path of uranium minerals in the Koongarra deposit. uranium and lead are retained in the system.

silicate minerals. Alteration processes of uraninite to uranyl silicate minerals through uranyl lead oxide minerals (mainly curite) are controlled by migration of lead and uranium (Figure 6).

At the bottom of the secondary ore body, replacement by a uranyl phosphate mineral (saleeite) with a uranyl silicate mineral or a calcium phosphate mineral (apatite) occurred [14,15]. These processes are also evidences of precipitation of uranium minerals from unsaturated ground water.

Fixation of uranium at the redox front

In the transition zone, the redox front between weathered and unweathered zones, growing grains and layers of uranium minerals are observed [16] (Figure 7). These minerals are reduced products of dissolved uranyl ion in ground water by reducing minerals such as graphite (C) and pyrite (FeS_2). This is the direct evidence of fixation of uranium from ground water by reducing reaction in the natural system.

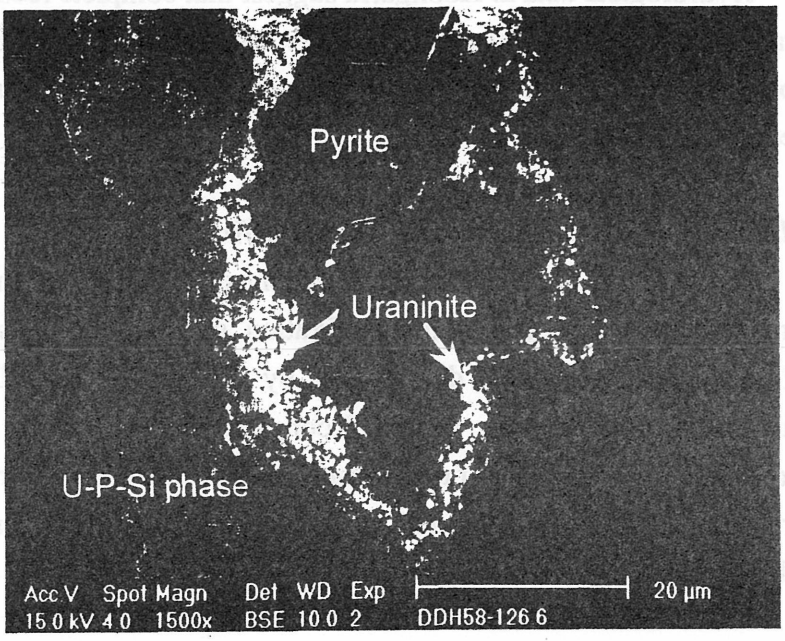

Figure 7 SEM image of pyrite in the transition zone. Layer of U-P-Si mineral with submicron uraninite grains occurs around pyrite.

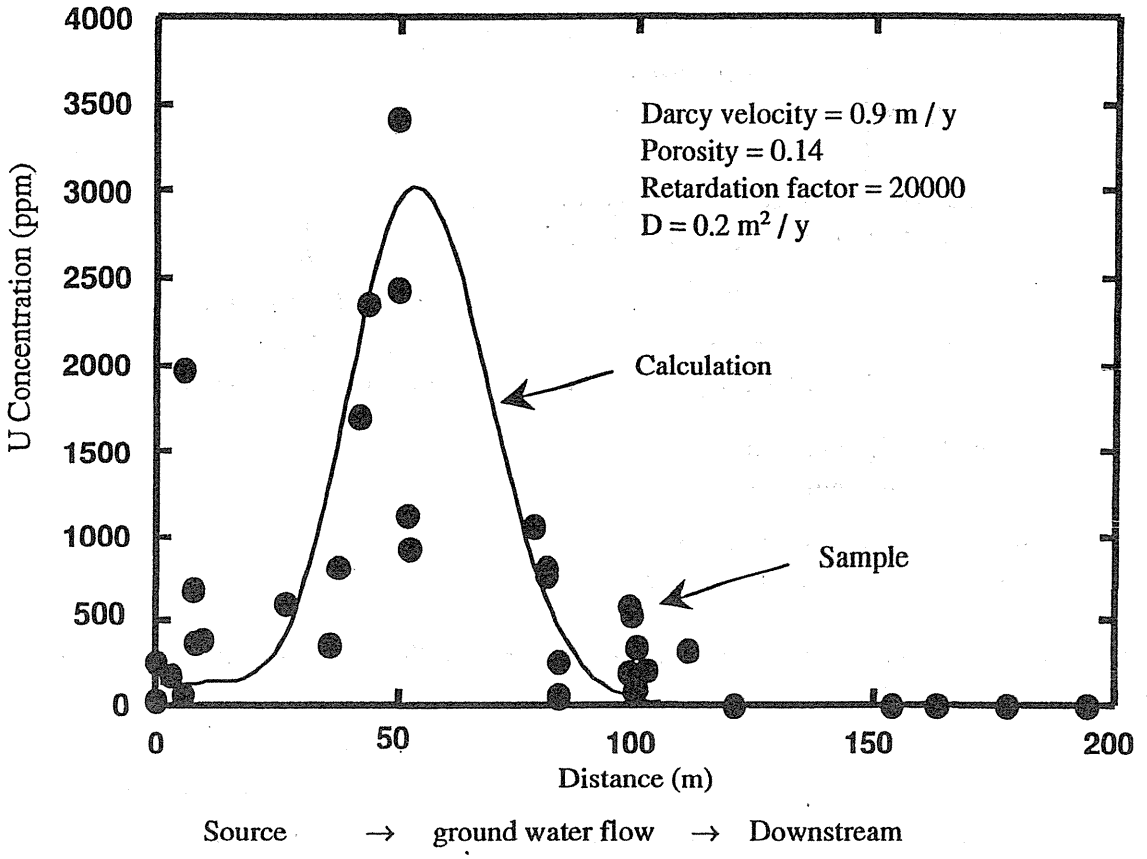

Figure 8 Comparison of calculated uranium concentration with observed ones. The most fitted calculation (shown here) needs retardation factor more than 20 times higher than that is obtained by laboratory experiments on the media.

Hydrological data at Koongarra suggest that complete reduction and fixation of uranyl ion at the transition zone dose not conflict with weathering rate assumed by alteration processes of the host rock. Reduction of uranyl ion by graphite and sulfide minerals may be universal process at the redox front.

Modeling of uranium migration regarding fixation

Based on the observations of alterations of the host rock and distribution of uranium, a model of uranium migration that contains fixation of uranium by secondary minerals was constructed [17,18]. The calculated distribution by the model is well concordant with one-dimensional distribution of uranium in the Koongarra deposit, while conventional sorption model with experimental retardation factors can not reproduce the distribution of uranium (Figure 8). The uranium migration is governed by fixation mechanisms rather than simple adsorption observed in laboratories.

CONCLUSION

The results of the natural analogue studies on the Koongarra deposit revealed important information on radionuclide migration, especially distribution and fixation mechanisms of uranium

in nature. We observed evidences of uranium migration and fixation in near surface condition. Coupling of mineralogical observation with measurement of uranium series disequilibrium has possibility to provide us constraint on migration rates of uranium in nature. Observation of natural analogue sites can provide us not only direct contribution to the performance assessment of the particular scenario on the nuclear waste disposal, but also securities for various natural systems through general understanding of geological processes.

ACKNOWLEDGEMENTS

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5. Water Solubility of Rare Metals in Soils as Estimated by High Resolution Inductively Coupled Plasma Mass Spectrometry

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INTRODUCTION

Water solubility of trace elements such as rare metals in soil - water systems is one of the most important parameters needed for environmental assessment. Most data on such water solubility has been obtained under experimental conditions in which relatively large amounts of elements have been added[1, 2)]. It is highly probable, however, that the values obtained under such conditions would be quite different from those of natural conditions, where concentrations would be much lower.

In recent years high resolution inductively coupled plasma mass spectrometers (HR-ICP-MS) combined with an ultrasonic nebulizer (USN) are developed to analyze trace elements in water samples without any pre-concentration[3, 4)]. This method has made it possible to perform simple yet highly accurate analyses of such long half-lived nuclides as uranium and thorium as compared with the conventional methods based on the measurements of radioactivity[5, 6)]. As it has become possible to measure stable nuclides at or below the ng/L (ppt) level[7)], studies on the absorption / desorption behavior of trace elements at natural condition levels have also become feasible[8)].

In this study, distribution coefficients (Kd) of rare metals for representative soils of Japan were estimated by batch experiments, with much smaller amounts of element addition and / or no element addition at all.

MATERIALS AND METHODS

Physical and chemical properties of soils used in this experiment are shown in Table 1. Joetsu soil is Gley soils high in clay (mostly montmorillonite) and very low in coarse sand contents. The soils from Morioka, Mito and Tsukuba were all derived from volcanic ash. The Morioka soil is classified as wet Andosols (Kuroboku) containing high amounts of humus. The Mito and Tsukuba soils are also Andosols, but Tsukuba soil is characteristic in its high content of silt and clay. These four soil types are typical for arable soils in Japan.

The total contents of trace elements in the soil samples were determined after decomposition with nitric, perchloric, and hydrofluoric acids. The standard solutions were prepared from commercially available mixed standard solutions (SPEX, Inc., USA). Double distilled water and ultra pure grade nitric acid (Tamapure AA-100, Tama Chemicals Co., Ltd., Japan) were used to prepare the standard solutions. The standard solutions were stored in Teflon (PFA) containers, which had already been soaked in 2*M*-HNO_3 for at least 10 days and washed with double distilled

water.

Table 1 Physical and chemical properties of sample soils

Location		Joetsu	Morioka	Mito	Tsukuba
Classification		Gley soils	Wet Andosols	Andosols	Andosols
pH(H_2O)		5.43	6.33	6.20	6.58
EC(μS/cm)		223	98	223	258
Total-C(%)		3.16	9.45	5.26	3.52
C/N		9.7	13.6	19.5	11.1
Cation Exchange Capacity (meq/100g)		28.3	45.1	17.8	16.9
Exchangeable Ca		9.0	7.5	7.6	8.3
(meq/100g)	Mg	2.1	1.6	2.0	3.1
	K	0.29	0.34	1.0	0.85
	Na	0.66	1.0	0.10	0.48
Coarse sand (%)		0.7	14.9	24.5	8.8
Fine sand (%)		25.1	25.5	22.3	21.8
Silt (%)		35.9	28.0	27.5	36.1
Clay (%)		38.3	31.6	25.7	33.3

There have been two types of experiments for obtaining Kds in laboratory conditions: batch and column methods[2]. The former method was employed in this experiments due to its ease of use under the present laboratory conditions. Five grams of the soils (finely powdered and air-dried) were put into 50 mL polypropylene centrifugal tubes, then a 50 mL of standard solution containing the trace elements listed in Table 2 either in double distilled water (pH 5.9) or 0.001*M* HCl (to simulate acid rain) was added. Cerium, Pr and Nd, Gd and Tb, and Yb and Lu are respectively termed in this study as light rare earth elements (LREEs), middle rare earth elements (MREEs), and heavy rare earth elements (HREEs). Each trace elements were added in levels considered to be the equivalent of the natural abundance in the soil. After this addition, or spiking, the centrifugal tubes were shaken vigorously for 18 hours at 25°C. The liquid phase was first separated by centrifugation at 3500 rpm, and then passed through a 0.45 μm membrane filter to obtain the solution for measurement.

Table. 2 Amount of spiked elements (μg/50 mL = μg/5g soil)

Elements	Non	Low	Middle	High
Ag, Cd, Pr, Cd, Tb, Yb, Lu, Tl, Bi, U	0	5	10	15
Ce, Nd, Pb	0	50	100	150

Preliminary experiments were conducted to clarify the effects of the equilibrium time and the number of extraction on Kds. It was found that a steady state was established by 18 hours of equilibration. The concentrations of the liquid phase decreased with the increase of the number of extraction for such high natural abundant elements as Na, Mg, Al, K, Ca and Fe. However,

since there were no significant decrease for the elements listed in Table 2, only the results obtained from the first extraction were used in the following discussion.

Twelve elements (Na, Mg, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu and Zn) that existed at relatively high concentration levels in the solution were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES / Maxim-III, Applied Research Laboratories, Switzerland). Twenty-nine elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Y, Ag, Cd, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Tl, Pb, Bi and U) were determined by HR-ICP-MS (PlasmaTrace, VG Elemental, UK) with an ultrasonic nebulizer (USN, Applied Research Laboratories, Switzerland) using indium (100 pg/mL) as an internal standard. Further details of the ICP-MS analytical conditions has been presented in Tsumura & Yamasaki[3].

RESULTS AND DISCUSSION

1) Distribution Coefficient of Unspiked Samples

The distribution coefficient (Kd: mL g^{-1}) has been defined as the ratio of exchangeable concentrations in a solid phase to that in a solution at equilibrium[9]. However, since there are several different definitions for exchangeable forms, total contents in the soils are used as concentrations in solid phases in this study rather than the ambiguous exchangeable forms, and the distribution coefficient is defined as follows:

Kd is defined here as

$Kd = q / C = ((qs + qa) / W) / C$, where

Kd : distribution coefficients (mL g^{-1})

q : concentrations in solid phase ($\mu g\ g^{-1}$)

qs : total amount in soil (natural abundance) (μ g)

qa : amount in spiked solution (μg)

C : concentrations in extracted solution ($\mu g\ mL^{-1}$)

W : weight of solid phase (g)

Figure 1 shows the distribution coefficients (Kd) derived from unspiked samples either with water or dilute HCl. Elements with lower Kd (that is, with a higher rate of leaching), were Na, Mg, K and Ca, each having a value of 10^0 - 10^2. The major soil components, Al and Fe, along with U and rare earth elements (REEs), had similar higher level of Kd. Among the heavy elements, Cu, Cd and Tl dissolved most easily (Kd = 10^1 - 10^3), similar to the results of the spiked experiments which will be discussed later. The Kd of U and REEs ranged from 10^2-10^5. HREEs (Yb, Lu) had lower Kd values than LREEs (Ce, Pr), especially in Tsukuba soil. Easily dissolved alkaline metals and alkaline earth elements (Na, Mg, K, Ca), as well as Mn and Cd showed lower Kd with dilute HCl than with water. These results agreed well with previous reports[2]. However, the elements with relatively higher Kd, *i.e.*, Al, V, Cr, Fe, Zn, Ag, REEs, Pb, Bi and U, resulted in a lower Kd with water as compared with that with dilute HCl. This was

especially noticeable in Joetsu soil. Although extractability of trace elements in the soil is usually higher in acids than in water, the opposite was the case for many of the trace elements in this study.

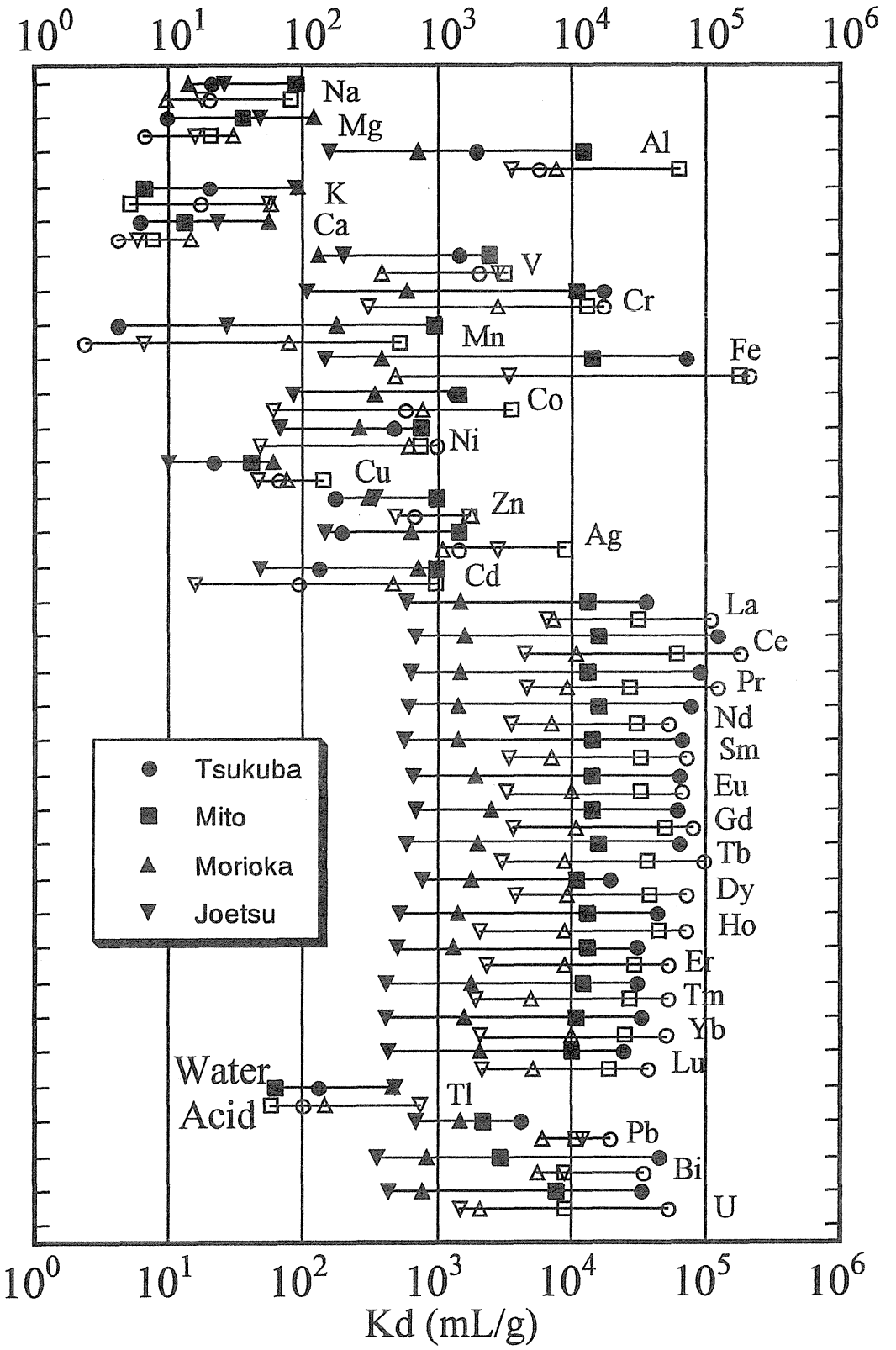

Figure 1 Estimated distribution coefficient (Kd) without spiked elements

Because the hydrogen ion (H^+) is strongly adsorbed to soils, the pH values of the liquid

phases with dilute HCl were only 0.2 to 0.5 pH unit lower than those with water in this study. On the other hand, experiments with water resulted in the dispersion of colloidal particles, and some of which apparently passed through a 0.45 μm filter. The existence of soil colloids in the filtrate could be confirmed by the increase in concentrations of the major components of these colloids, *i.e.*, Si, Al, Fe, *etc.*, in the filtrates. The most noticeable colloid dispersion was observed in the Joetsu soil, which also showed the greatest difference between the Kd values obtained by the water and dilute HCl. This was apparently because soil colloids were coagulated and the number of colloid particles in the filtrate was decreased by the addition of the acid. Higher electrolyte concentrations (including H^+) and lower pH values of the equilibrium solution due to the addition of HCl are considered to be responsible for the coagulation of colloidal particles.

In the Mito and Tsukuba soils, the dispersion of colloidal particles was negligibly small both with water and dilute HCl, and therefore, Kd values were similar in both methods. For such elements as Na, K, Ca and Mg, the concentrations in the liquid phase were relatively higher, and hence, contribution due to colloidal particles was smaller. Accordingly there were little discrepancy between Kd values obtained by water and HCl. On the other hand, as the concentration levels of trace elements in the colloidal particles were extremely higher than those in liquid phase, even a small amount of admixture of colloidal particles may result in noticeably higher Kd values. These above results also suggested that operationally defined specification of "water soluble form", *i.e.* smaller than 0.45 μm, should be revised or reconsidered.

The application of lime is one of the common practice to suppress the absorption of trace elements by plants. However, the rise of pH can sometimes encourage the dispersion of colloids for certain types of soils. Therefore, trace elements contained in the colloids can be washed out into streams and groundwater, and might resulted in the causes of water pollution. McCarthy & Zachara reported that radionuclides contained in organic and inorganic soil colloids can infiltrate groundwater[10].

2) Distribution Coefficient with Spiked Elements

Figure 2 shows the effect of the spiked amounts on Kd for 13 trace elements. In Morioka, Mito and Tsukuba soils, Kds of unspiked REEs, Bi and U were nearly one order of magnitude higher than those of their spiked counterparts. However, Kd values were not affected by the spiking in Joetsu soil for all elements with the exception of Tl and Cd. The Kd values of these elements tended to decrease with the increase of the spiked amounts. Similar exceptions were also observed for Pb in Mito and Tsukuba soils. In case of Ag in Tsukuba soil, however, Kd value increased with the increase of spiking.

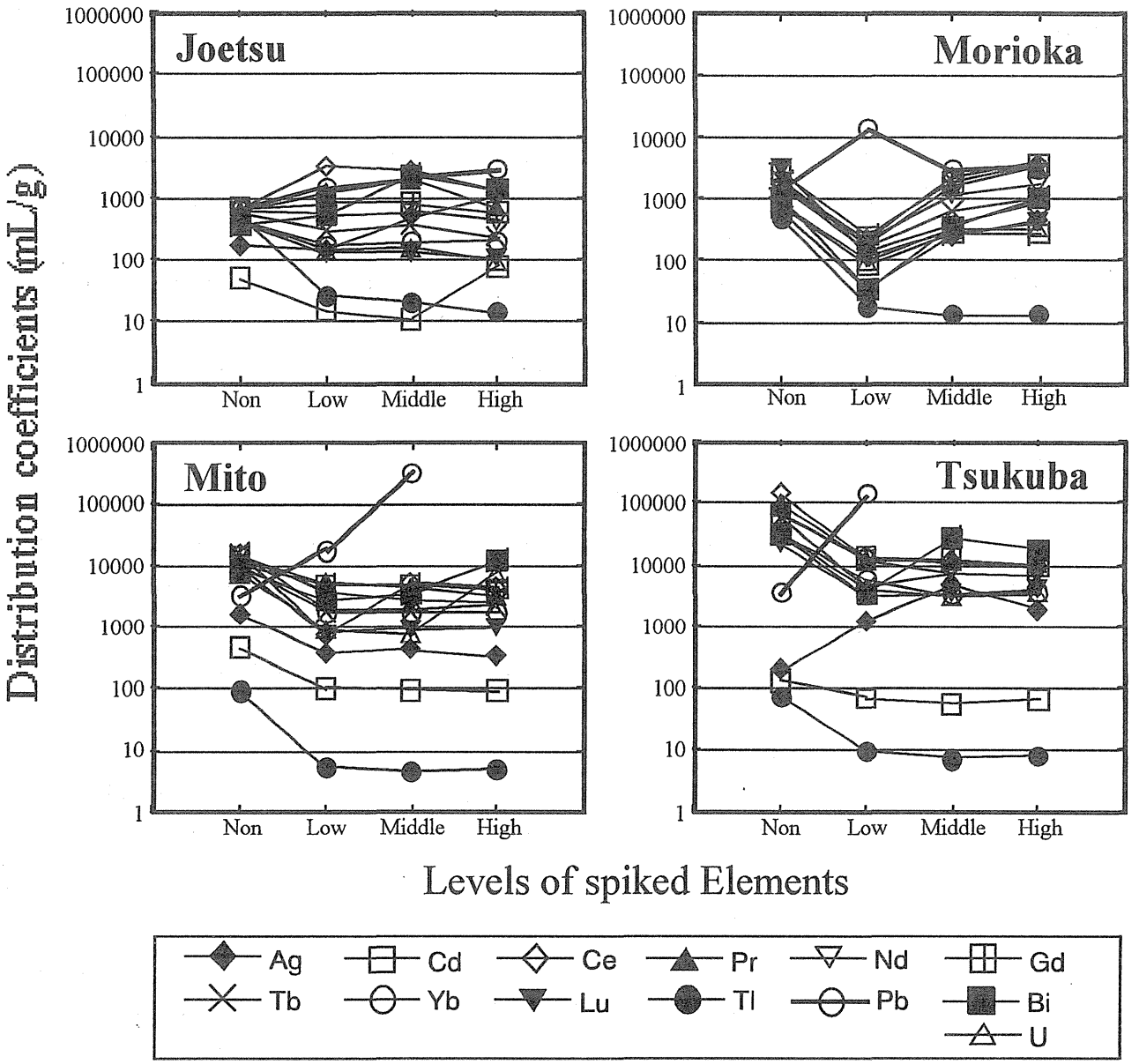

Figure 2 Distribution coefficients (Kd) in terms of the amount of spiked elements

The reason why Kd was generally higher in the spiked experiments than in the unspiked ones, that is, increased solubility in the former, may have been as follows: The chemical forms of trace elements in soils can be divided into two groups: relatively water-soluble fraction (such as inorganic oxides, carbonates, and phosphates), organic complexes, and elements adsorbed by the electric charges of the clay; and fraction that are difficult to dissolve like crystalline components. Zhu & Xing reported that 70-80% of naturally occurring REEs in soils were found in nearly insoluble crystalline fraction[11]. In contrast, large proportions of artificially spiked REEs were found either as exchangeable form that can be extracted by water, dilute HCl, ammonium acetate, *etc.* or as carbonates, but were not incorporated into the crystalline structure of the clay minerals. It can be concluded, therefore, the discrepancy between the Kd values of spiked and unspiked experiment is due to the differences of chemical forms of REEs.

3) Comparison with Previously Reported Values

Figure 3 shows the Kd values of Ag, Cd, Ce and U previously obtained[1)] together with the results of the present study. As Kd values are known to differ depending on measurement method, experimental conditions, and types of soils used, it is difficult at times to compare directly. Nevertheless, all elements in the present study resulted in a higher Kd values than the previous studies. Furthermore, comparison of spiked and unspiked results showed that, with the exceptions of Ag and Pb (Figure 2), spiking tended to reduce Kd values. Such suppressed Kd values might be due to the different chemical forms of these elements. However, with the exception of the Morioka soil, nearly constant Kd values were obtained regardless of the level of the spiking (Figure 2). These results can be attributed to the fact that the amount of spiked elements was around 1% of the cation exchange capacity in this experiment.

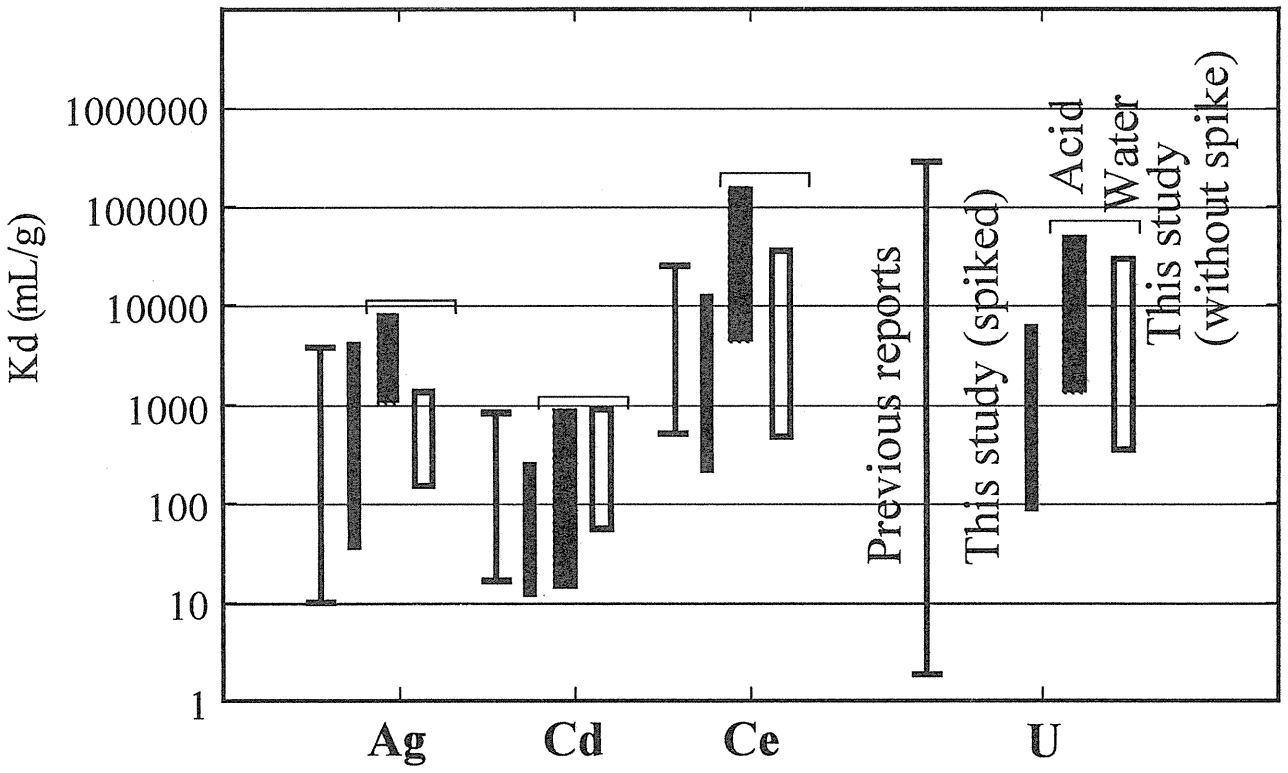

Figure 3 Comparison with previously reported Kd (Okabayashi & Uchida, 1990)

CONCLUSION

The distribution coefficients obtained without the addition of elements were much higher than those obtained with the addition of elements. This can be attributed to the differences in the amounts and/or chemical forms of elements employed in the experiments. As there are apparently other factors affecting Kd values, extreme care should be exercised in adopting these values for modeling.

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6. On the Environmental Behavior of Radioactive and Stable Iodine

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ABSTRACT

In order to understand the behaviour of radioactive and stable iodine in the environment, we have carried out radiotracer experiments and analyses of the nuclides in several environmental and geochemical materials. Parameters important for the assessment of radioiodine movement from the environment to man were obtained, e.g. soil-solution distribution coefficient and soil-plant transfer factor for various agricultural crops (including rice). Mechanisms of iodine sorption and desorption on soil were also studied. Microorganisms were found to play an important role in the fixation of iodine on soil. Levels of the long-lived ^{129}I in environmental samples collected around Tokai-mura, where several nuclear installations are located, were studied. Concentrations of iodine in several materials forming the Earth's crust, such as igneous rocks, sedimentary rocks, metamorphic rocks and ocean sediments have been determined. Distribution of iodine in the Earth's crust and its geochemical cycling were also investigated.

INTRODUCTION

Radioiodine is one of the most important radionuclides released from nuclear industries into the environment, while from a nutritional viewpoint, stable iodine is an important nutrient element. When iodine is ingested into the human body it is concentrated in the thyroid gland. Therefore, it is necessary to obtain information on the behavior of radioiodine in the environment for safety assessment. Two radioisotopes of iodine, ^{129}I (half-life: 1.6×10^7y) and ^{131}I (half-life: 8 d), are specifically important in terms of radioecology. Since the amount of ^{131}I produced in a reactor is much higher than that of ^{129}I, the former isotope is more important at the time of a reactor accident. However, ^{131}I decays away within several months of contamination. The long radiological half-life of ^{129}I, on the other hand, prevents this nuclide from disappearing, causing it to enter the geochemical and ecological cycles of stable iodine. Interest in ^{129}I is increasing, particularly regarding the operation of nuclear fuel reprocessing plants. NCRP (1983) estimated that about 3700 Ci of ^{129}I will have been accumulated in spent fuel from nuclear plants worldwide by the year 2000 and from that, about 7.4 Ci of ^{129}I will have been released into the atmosphere during fuel reprocessing. Due to the long half life of ^{129}I, it is important to study biogeochemical cycling of both

stable and radioactive iodine in the environment for a reliable assessment.

In this paper, we summarize our recent investigations on (1) behavior of iodine in the soil-plant-atmosphere system, (2) concentrations of ^{129}I in environmental samples, and (3) distribution and cycling of stable iodine in the environment.

MATERIALS AND METHODS

1. Sorption of iodine on soil

Sorption of iodide (I^-) and iodate (IO_3^-) on soils was examined by the batch method (Muramatsu et al. 1990). Samples (3 g) were mixed with deionized water (30 ml) in polyethylene bottles (50 ml), and radioiodine tracers ($^{125}I^-$ and $^{125}IO_3^-$) were added. The bottles were stoppered shaken at 23℃. Finally the samples were centrifuged and aliquots of the supernatant solution were counted with an NaI scintillation counter. The soil-solution distribution coefficient (K_d) was calculated from the activities remaining in the solution.

2. Transfer of iodine from soil to plants

Radiotracer (^{125}I) was thoroughly mixed with the soil (3 kg) in a Wagner pot (3 *l*). Andosol, one of the most common soils in Japanese agricultural fields, was mainly used in the experiments. A plant growth chamber, installed in a hot laboratory, was used for the cultivation (Muramatsu et al. 1989, Muramatsu et al. 1995). Light intensity of the chamber was about 70000 lux. During cultivation, temperature and moisture in the chamber were controlled in accordance with the outdoor conditions. After the plants were harvested, they were separated into organ parts (e.g. leaves, grains) and ^{125}I concentrations were determined with an NaI scintillation counter. The soil-plant transfer factor (or concentration ratio) is defined as "the ^{125}I concentration in the plant part (Bq g^{-1}, fresh)" divided by "the ^{125}I concentration in soil (Bq g^{-1}, dry)."

3. Volatilization of methyl iodide from the soil-plant system

Radioiodine volatilized from plants was collected with two traps. The first trap contained silver wool for collecting inorganic iodine and the second trap contained activated charcoal (supplemented with triethylendiamine) for collecting organic iodine (Muramatsu and Yoshida 1995a). Activities were measured with an NaI scintillation counter. Gas chromatographic mass spectrometry (GC-MS) and gas chromatography with an electron capture detector (ECD-GC) were also used to identify the iodine species (Muramatsu and Yoshida 1995a).

4. Determination of ^{129}I and ^{127}I in environmental samples

Neutron activation analysis was applied for the determination of ^{129}I and ^{127}I. Details of the analytical procedures were described in our previous papers (Muramatsu and Ohmomo 1986, Muramatsu and Yoshida 1995b). An outline of the method is as follows. A sample (30-180 g) was

mixed with ^{125}I (half-life: 60 d) yield tracer and then placed in a quartz tube and heated at 1000℃ under oxygen gas flow. The evaporated iodine was collected in trap solution containing KOH and K_2SO_3. Iodine was extracted into CCl_4, then back-extracted into the aqueous phase as I^-. The I^- containing aqueous phase was transferred into a quartz ampule and irradiated in a research reactor. The following reactions were considered for quantification of ^{129}I and ^{127}I; $^{129}I(n, \gamma)^{130}I$ (half-life: 12.3 h) and $^{127}I(n, 2n)^{126}I$ (half-life: 13 d). After cooling the sample, the iodine fraction was purified by solvent extraction. Finally the iodine was precipitated as PdI_2 and counted on a Ge-detector to quantify ^{130}I and ^{126}I peaks. The detection limits for ^{129}I and ^{127}I in soil by this method were about 0.1 mBq kg^{-1} and 0.1 mg kg^{-1}, respectively.

5. Estimated distribution of stable iodine in the Earth's crust

A reasonably large series of samples representing about 300 rocks of major units and subunits of the Earth's crust were provided by K.H. Wedepohl, Göttingen University, Germany. Some other materials of environmental importance such as soil, rain water etc. were also measured for iodine.

An outline of the analytical method is as follows. About 100 to 2500 mg of powdered sample were placed in a ceramic boat (details: see Muramatsu and Wedepohl 1998; Schnetger and Muramatsu 1996). Radiotracer (^{125}I) was added to the sample to determine the chemical yield during the separation. In order to accelerate sample combustion about the same weight of V_2O_5 was mixed with it. The sample was placed in a quartz combustion tube. The end of the quartz tube was connected to a trap containing 7 ml of H_2O, 0.4 ml of TMAH (25%) and 0.1 ml of 5000 ppm Na_2SO_3 solution. A wet oxygen flow was passed through the tube during the heating. The sample tube was heated gradually at the edge of the furnace for about 3 min, then the whole tube was moved manually into the furnace to be heated in the hot zone (1100°C) for about 15 min. Finally the down stream part of the tube was heated for about 3 min to remove any iodine deposited near the connection to the trap. The evaporated iodine was collected quantitatively in the trap solution. Iodine concentrations were determined with ICP-MS (Yokogawa PMS 2000). Practical detection limit in this method was about 0.2 ppb in sample solutions, which is about 1 ppb I in solid materials, when a 2 g sample is diluted in 10 ml.

RESULTS AND DISCUSSION

1. Behavior of iodine in the soil-plant-atmosphere system

1.1. Sorption of iodine on soil

The soil-solution distribution coefficient (K_d) is the most important parameter for assessing the migration of radionuclides in soil. The results obtained for soils (Muramatsu et al. 1990) and minerals are shown in **Table 1**. High K_d values (high sorption) were found in soils having high concentrations of total organic carbon, active-Al and active-Fe (Al and Fe extracted by a mixture of oxalic acid and ammonium oxalate) (Whitehead 1984). Andosol, one of the most typical Japanese

soils derived from deposits of volcanic ash, showed specifically high K_d values. These results indicated that added radioiodine was associated on the surface of sesquioxides of Fe and Al, noncrystalline silicates such as allophene, organic materials and complexes of metals with humus.

Table 1 Distribution coefficient (K_d, ml g^{-1}) for soil and related materials.

	I^-	IO_3^-
Andosol (wheat field)	7500	7000
Gray lowland soil (paddy field)	560	430
Sandy soil	35	32
Kaolinite	0.5	0.7
Al_2O_3	0.2	<0.1
Fe_2O_3	47	520

For examining the microbial participations in the sorption, soils sterilized by autoclaving have been used together with fresh soils (untreated wet soils) for the batch experiments. Results obtained for the influence of autoclaving on the sorption are shown in **Fig. 1**. Both iodide and iodate were readily sorbed on the fresh Andosol and Gray lowland soils. In the case of autoclaved soils, drastical decreases in the iodide sorption were found. More than 80% of the iodide sorptions (at the shaking time of 1 day) was lost due to autoclaving. In our previous study we found that the sorption of iodide markedly dropped through heat treatment of the soil (in a dry oven) at 150°C and above (Muramatsu et al., 1990). The effect of autoclaving on the sorption observed in the present study tended to be larger than that by heating. The following three possibilities can be expected to explain the decrease of iodine sorption on soil by autoclaving (or heating); (a) living microorganisms which acted in the iodine fixation were killed, (b) products of microorganisms (e.g. enzymes) which affect the iodine fixation were decomposed and (c) soil fractions which related to the iodine sorption were destroyed. In our previous study we observed that the sterilization by gamma-irradiation at 27 kGy also affected the sorption of iodide and iodate (Muramatsu et al., 1990). However, the decrease was obviously smaller than that of the autoclaved (or heated) soils. Most of the microorganisms such as microfauna, fungi and bacteria would not survive a radiation of 25 kGy (Cawse 1969). If the iodide adsorption was mainly due to the living microorganisms, there would be no large differences in the effect between gamma-irradiation and autoclaving. The smaller effect observed in the gamma-irradiation may suggest that the direct involvement by such living microorganisms was not the main process in the fixation of iodine by soil. It is possible that the products of microorganisms such as enzymes and/or other organic substances may play an important role in the fixation. Enzymes are known to be unstable to heating.

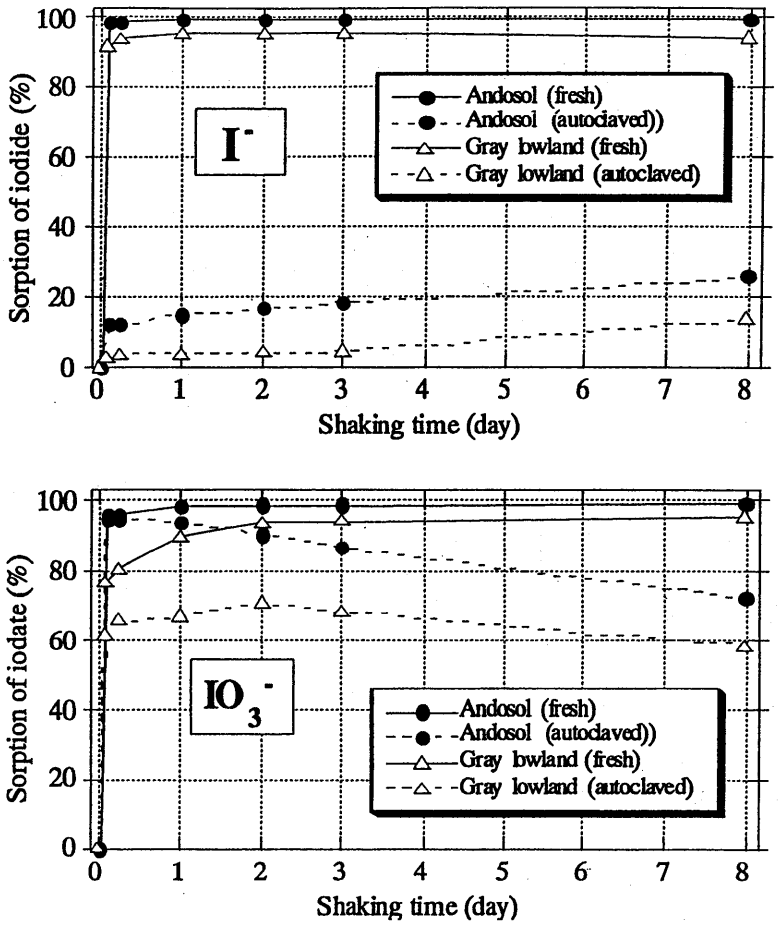

Fig. 1 Effects of autoclaving on the sorption of iodide (I^-) and iodate (IO_3^-) on Andosol and Gray lowland soil.

In contrast to the iodide sorption, the decrease of the iodate sorption by autoclaving was markedly smaller. This indicated that the sorption sites for iodate were not so sensitive to heating and the sorption mechanisms differed between the two species. We reported the different sorption phenomena between iodide and iodate (Yoshida et al. 1992; Muramatsu et al. 1990). We found that the adsorption isotherm of iodate on Andosol was similar to that of Kanuma soil (allophane rich soil) which suggested that the high iodate sorption on Andosol was caused by the high adsorbability of iodate on allophene and/or sequioxides of Fe and Al. However, the high iodide sorption on Andosol could not be explained analogously.

In order to examine whether the decreased sorption of iodide by autoclaving could be recovered through an increase of microbial activities, we have carried out the following radiotracer experiment. To the autoclaved soil (Andosol), a certain amount (1 or 10%) of fresh soil was mixed in and the mixture was incubated for 6 weeks. During the incubation period microbial biomass is expected to increase. We compared the iodide sorption for the incubated soil mixtures together with the unincubated ones. The results are shown in **Fig. 2**. The iodide sorption for the incubated samples increased clearly in comparison to the freshly prepared samples (without incubation). During the

incubation period of the mixed soil, microorganisms might grow and recolonize in the autoclaved samples. This suggested that microorganisms and/or their products (e.g. enzymes) participating in the iodide sorption might increase during incubation, and subsequently the sorption increased in the incubated samples.

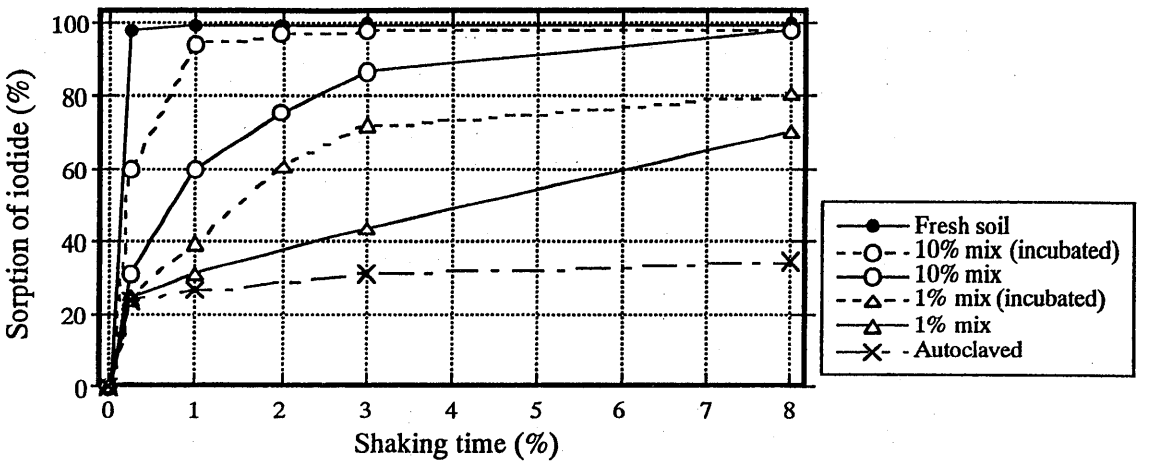

Fig. 2 Effects of mixing fresh soil (1% or 10%) with autoclaved soil on iodide (I^-) sorption (Incubation time: 6 weeks)

The sorption sites for iodide in soil are not well known yet. The sorptions of iodide on common inorganic constituents of soil are very low. Organic matters may contribute to the sorption. However, organic matter (i.e. humic acids extracted from soil) alone showed a low sorption capacity for iodide (Muramatsu et al., 1990). From the above mentioned results we can say that the sorption of iodide relates to the microbial activities (including the products of microorganisms) in the soil.

1.2. Transfer of iodine from soil to plants

The soil-to-plant transfer factors (or concentration ratio of radionuclide between plants and soil) in the edible parts of crops obtained in this study were in the range 0.0002-0.016 (**Table 2**) (Muramatsu et al. 1989, Muramatsu et al. 1995) The values for the common Japanese leaf vegetable komatsuna was comparable to the IAEA (1982) recommended value (0.02) for edible parts of common crops, which include several different crops. However, the values for tomato, sweet potato, carrot, soybeans and rice were significantly lower than their leaf values. The transfer factor for polished rice (0.0019) was less than 1/1000 of that of the rice plant leaves. Since the transfer factor of iodine for agricultural crops varies widely, we note that only one representative value for the transfer factor is insufficient. Different values of transfer factors should be established for plant groups as categorized by the type of their edible part. In addition to the type of crops, the transfer factors are also expected to be influenced by the sorption characteristics of soils. High K_d values in common Japanese field soil (e.g. Andosol) (Yoshida et al. 1992) may explain the relatively low transfer factors observed in our experiments using Andosol.

Table 2 Soil-to-plant transfer factors (TF) of radioiodine for edible parts of agricultural crops

Crops	TF
Rice (polished)	0.002
Spinach	0.0031
Komatsuna	0.016
Tomato	0.0003
Soybeans	0.0029
Carrot	0.001
Sweet potato	0.0002
IAEA recommended (1982)	0.02

1.3. Desorption of iodine from soil

Desorption of iodine from the flooded soil during the cultivation of rice plants in pots was studied by radiotracer experiments using ^{125}I. The changes of the redox potential (Eh) in soil and ^{125}I activities in soil solution collected with porous-cups were measured as a function of time (Muramatsu et al 1996). The results obtained are shown in **Fig. 3**. In the first 6 weeks after planting, the activity of ^{125}I in the solution of the cultivated pots was very low. This was explained by the high Kd value (more than 1000) for the soil used in the experiments. However, the activity in the solution collected from the cultivated soils markedly increased from about 40 days after the planting. It was suggested that the increase was caused by the effects of roots and/or microorganisms in the soil. Eh decreased considerably after soil was waterlogged. The decrease of Eh was larger in the pots with rice plants in comparison to the uncultivated pot. When soil is flooded with water, molecular oxygen disappears and nitrates diminish due to the activities of aerobes and facultative anaerobes, consequently Eh drops. With the drop of Eh, a succession of microorganisms occurs, and sulfides and a little later, methane, are formed by strict anaerobes (Takai 1984). In our experiment, we observed that the Eh value dropped to about -240 mV in the pots with plants. At the end of the cultivation experiment Eh value of one of the cultivated pots increased. This was due to the soil having dried out in this pot, so that the Eh increased due to the oxidation of the soil with air. The decrease of Eh in the pot without plants (uncultivated soil) was smaller (down to about 0 mV) which resulted in the low iodine desorption. According to the other relevant data reported in our previous study (Muramatsu et al., 1996), high desorption was frequently observed when the Eh dropped to about -100 mV or below. From these results we could conclude that the desorption of iodine from soil was also controlled by the effects of microorganisms. Due to the reducing conditions (low Eh) created by the microorganisms in the flooded soils, iodine once adsorbed on the soils was leached

into the soil solution; consequently total iodine concentration in paddy soil was considerably lower than forest and upland field soils with time.

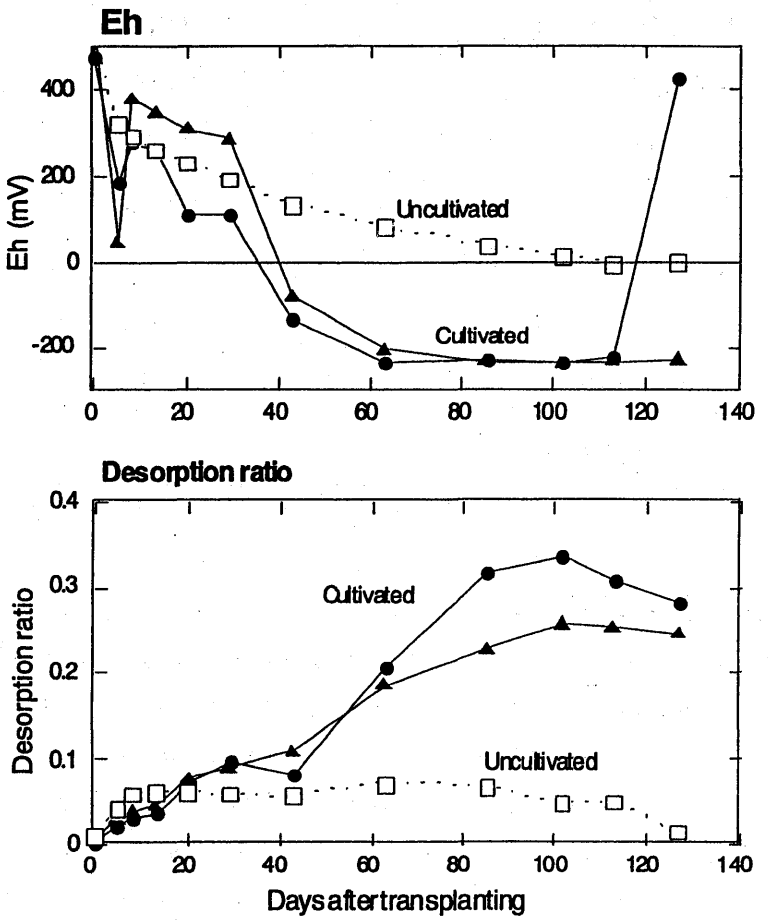

Fig. 3 Temporal changes of Eh and desorption ratio of iodine in Gray lowland soil after flooding.

1.4 Volatilization of iodine from plants.

In addition to the iodine decrease from soil due to the desorption, iodine was found to be evaporated from the soil-plant system as methyl iodide. **Fig. 4** shows the volatilization of iodine from the soil-plant system (rice and oat plants). The iodine emission was highly stimulated by the presence of plants. The emission of gaseous iodine from rice plants grown on flooded soil was much higher than that of plants grown on unflooded soil, such as oat plants. Seasonal patterns in the iodine emission were observed for rice and oat plants. The emission rate increased with time from planting and the maximum value was observed in the late tillering stages (shortly before heading) of the plants. The chemical species of volatilized iodine was identified as methyl iodide (CH_3I) from gas chromatography (Muramatsu and Yoshida 1995a).

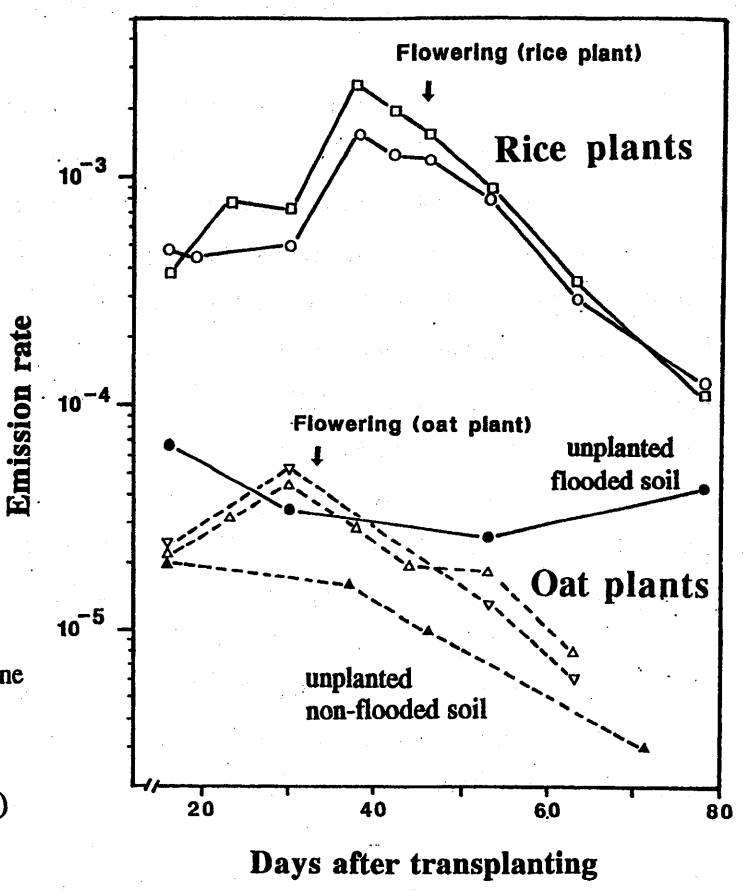

Fig. 4 Volatilization of organic iodine from rice (○,□) and oat (△,▽) plants, unplanted flooded soil (●) and unplanted non-flooded soil (▲)

The following mechanism for the high production of methyl iodide in the soil-rice plant system seems reasonable. Microorganism activities in the soil (particularly the rhizosphere) are increased by flooding and the effects of root exudates and/or root autolysis, so that eventually an anaerobic condition (low Eh) is generated. Under this condition iodine is dissolved from the soil into the soil solution as iodide (I^-). We presume that the iodide dissolved in the soil solution in the rhizosphere is biomethylated, possibly by the effect of enzymes produced by microorganisms or roots. For example, the enzyme methyl halide transferase might be a candidate for methylating iodide. The biogenic methyl iodide produced in the soil might be transported through the intercellular gas space and aerenchym system in the plants into the atmosphere, presumably by a mechanism similar to methane emission from rice fields. The volatilization phenomena should also be important in understanding the biogeochemical cycle of iodine (and possibly also of bromine) in the environment.

Iodine-129 was still detected in the air collected around a reprocessing plant several years after its closing as reported by Brauer and Strebin (1982). This might be explained by the volatilization of the nuclide from the contaminated soil-plant system. Volatilization of iodine from the soil-plant system is also thought to be important to understanding the behavior of the long-lived ^{129}I in the environment and this pathway should be considered in establishing a transfer model for ^{129}I in the environment.

2. Concentrations of ^{129}I in environmental samples

Fig. 5 summarizes analytical results for ^{129}I concentrations and $^{129}I/^{127}I$ ratios obtained in our previous studies (Muramatsu and Ohmomo 1986; Muramatsu and Yoshida 1995b) and also in the present study for samples collected around Tokai-mura, Ibaraki Prefecture, where several nuclear installations (including a nuclear fuel reprocessing plant) are located. Wide variations were found in all the sample groups. The highest concentration of ^{129}I was observed in a forest soil sample (0.18 Bq kg^{-1}). However, this value was much smaller than the ^{137}Cs concentration found in soils in the same area (ca. 50 Bq kg^{-1}). The higher ^{129}I concentrations in soil samples collected in small coniferous forests (or woods) suggests that the nuclide released into the atmosphere may be trapped by leaves (or needles) and transferred to the ground through wash-out by rain and/or falling leaves and accumulated in the surface soil. Lower ^{129}I concentrations in field soils compared to forest soils might be explained by the lower deposition rate and tilling procedures used in the fields. The $^{129}I/^{127}I$ ratios were higher in rice paddy soil than in wheat field soil. This was due to the lower stable iodine concentrations in the former soil. Vertical distributions of ^{129}I in soil collected from a small forest in Tokai-mura, Ibaraki, were measured. The results indicated that most of the ^{129}I has been retained in the first 10 cm of the surface soil.

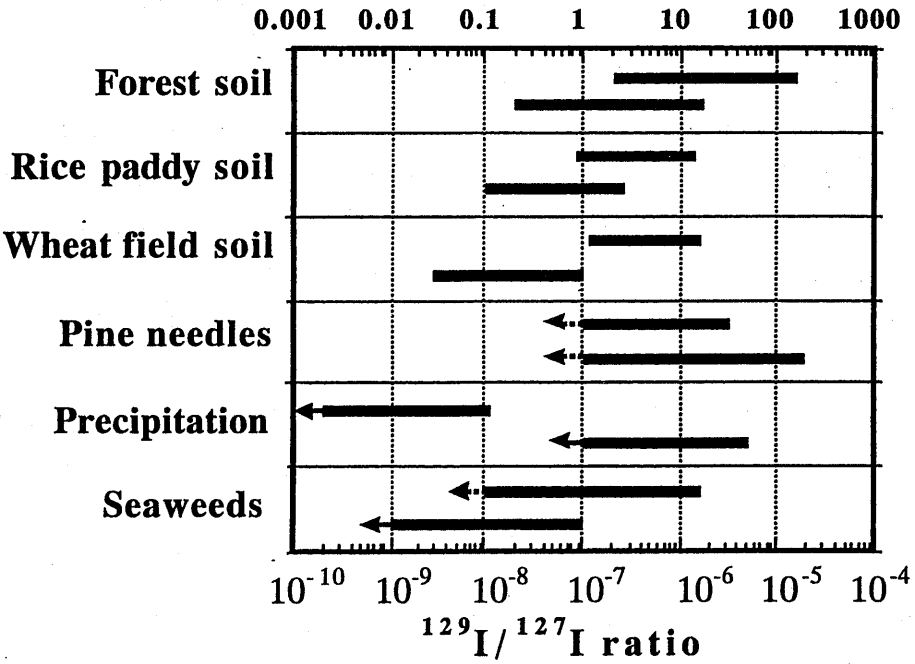

Fig.5 Levels of ^{129}I in environmental samples collected around Tokai-mura

Higher $^{129}I/^{127}I$ ratios found in pine needles and precipitation should reflect the atmospheric levels of the nuclide because ^{129}I is expected to be released into the atmosphere, although the level is very low. The measured $^{129}I/^{127}I$ ratios in seaweeds varied between $<10^{-9}$ and 10^{-7} contrary to the ratio in sea water which was estimated to be much lower than $1x10^{-9}$. However, we found that the

ratio was related to the vertical distribution of seaweed species. Funori algae (Gloiopeltis furcata) showed the highest $^{129}I/^{127}I$ ratio ($9.9x10^{-8}$) and they grow in the upper littoral belt where they are exposed to the atmosphere for relatively long periods of time at low tide. Therefore, this species may be highly influenced by the ^{129}I concentration in the atmosphere.

We tried to analyze ^{129}I in rice. However, difficulties were encountered during the sample combustion procedure, i.e. a large amount of undecomposed (un-oxidized) gas was released on heating which condensed in the tube and trap. Therefore, we could not directly analyze this nuclide in rice. The ^{129}I concentration in rice was estimated from concentrations in the soil (highest value for rice paddy soil: 13 mBq kg^{-1}) obtained in this study and the transfer factor (0.002) from soil to rice obtained in our previous study using radioiodine tracer. The ^{129}I concentration in rice was calculated as about 0.023 mBq kg^{-1}.

3. Distribution and cycling of stable iodine in the environment

3.1. Levels of stable iodine in soil

Analytical results on the iodine concentrations (mean values of 2 - 3 determinations) in typical soils collected from different areas in Japan are shown in **Table 3** together with data on rocks typically found here.

Table 3 Iodine concentration in major soil types in Japan and in the possible parent material

Sample types*	Code	Sampling location	ppm I (dry)
Upland soils			
Andosols	F-007	Mito/Ibaraki	32.6
(Andosols)	F-015	Shiojiri/Nagano	26.0
	F-025	Kimotukigun/Kagoshima	24.2
	F-045	Rokkasho/Aomori	44.9
	F-064	Kawasaki/Kanagawa	33.0
		Mean (5)	32.1
Yellow soils	F-017	Toyohashi/Aichi	11.3
(Orthic Acrisols)	F-021	Fukuyama/Hiroshima	2.8
	F-068	Takayama/Gifu	11.8
		Mean (3)	8.6
Rice paddy soils			
Gray lowland soils	P-013	Kumagaya/Saitama	0.63
(Dystric Fluvisols)	P-015	Mito/Ibaraki	1.93
	P-028	Oomagari/Akita	1.64
	P-038	Koriyama/Fukushima	2.76
	P-050	Kashihara/Nara	0.92
		Mean (5)	1.58
Gley soils	P-012	Kumagaya/Saitama	1.32
(Dystric Gleysols)	P-042	Gamougun/Shiga	1.46
	P-068	Nakakannbaragun/Niigata	2.08
		Mean (5)	1.62
Possible parent materials for soils			
Basalt		JB-1 (GSJ)**	0.028
Andesite		JA-2 (GSJ)**	0.004
Granodirite		JG-1 (GSJ)**	0.005

* Soil names recommended by FAO/UNESCO are described in brackets.

** Rock samples are standard rocks of Geological Survey of Japan (GSJ).

Iodine concentrations in soils range from 0.63 to 44.9 ppm (on a dry weight basis). Average iodine concentrations are 32 ppm for Andosol (upland fields), 8.6 ppm for Yellow soils (upland fields), and 1.6 ppm for Gray lowland soils (lowland fields) and Gley soils (lowland fields).

These data also indicate that the iodine levels in upland soils are much higher than those in common crustal rocks in Japan. It is interesting to note that the concentrations of iodine in Andosols of upland fields are very high, even through the samples are collected from different places in Japan. The levels are about 3 orders of magnitude higher than those in the parent materials (i.e. basalt and andesite), suggesting that Andosols have specifically higher ability for iodine accumulation than the other soils analyzed in this study. Since the sorptions of iodide (I^-) and iodate (IO_3^-) on common clay minerals are not very high, it is expected that organic materials and/or microorganisms may play important roles in the accumulation of iodine in soil from water, e.g. rainwater, irrigation water. (Some details on the participation of microorganisms in the iodine sorption on soil are discussed in the next section.) Entering of fallen plant materials, on which iodine is deposited from the air, into the soil also seems to be important in the accumulation pathway.

There is a marked difference in the iodine concentrations between lowland soils (rice paddy soils) and upland soils, as shown in Table 1. Iodine concentration in the 8 rice paddy soils studied was on average 1.6 ppm. This value was significantly lower than that in the 8 upland soils (average: 23 ppm) we analyzed. Yuita et al. (1982a and 1982b) also found that the iodine levels in paddy soils were much lower than those in forest and upland soils in Niigata and other places in Japan.

We mentioned that Andosols contain high levels of iodine. However, iodine levels in Andosols collected from paddy fields were markedly lower than those in upland fields. **Table 4** shows a clear example of the decreased iodine concentration in paddy soil found in our study. The iodine concentration in soil sample collected from a rice field in Imaichi (Tochigi Prefecture) was only one tenth of that in a sample collected from a neighboring forest. The rice fields in this area were reclaimed from the forests some hundred years ago. Both soils were Andosol and of the same origin, but they had a big difference in their iodine concentration. This suggested that iodine could be expected to be eluviated from rice fields through reclamation and rice cultivation under flooded conditions. The decrease of iodine from the flooded soil due to microbial activities is discussed later.

Table 4 Iodine concentrations in Andosols collected from the same area

	Number of determinations	Iodine concentration ppm (dry)
Andosols*		
Forest soil (Imaichi)	3	47.9
Paddy soil (Imaichi)	2	8.3

* Forest and paddy soils were collected in a neighboring place (separation distance: about 30 m) in Imaichi, Tochigi Prefecture.

3.2. Geochemical cycling of iodine

Concentrations of iodine in several materials forming the Earth's crust, such as igneous rocks, sedimentary rocks, metamorphic rocks and ocean sediments have been determined. Igneous and metamorphic rocks contained very low iodine levels, i.e. 0.005-0.05 ppm. High iodine concentrations up to 30 ppm were found in oceanic sediments and sedimentary rocks. The iodine inventory in the Earth's crust was calculated by using the analytical results. It was found that nearly 70% of the iodine is present in oceanic sediments, followed by continental sedimentary rocks (about 27%). The abundances of iodine in igneous and metamorphic rocks are very low, although their masses in the Earth's crust are very large. These results indicate that iodine is biogenically accumulated, e.g. by planktons from seawater, then precipitated into the ocean sediments and recycled into the ocean at the subduction zone due to the spreading of the oceanic plate. Distribution of iodine in the Earth's crust is illustrated in **Fig. 6** (Muramatsu and Wedepohl 1998). Residence time of iodine in the ocean was estimated to be on the order of 10^5 years, which is much shorter than the radiological half-life of ^{129}I.

The results for stable iodine gained in this study could be used in predicting the fate of anthropogenic ^{129}I in the global environment for a long time scale.

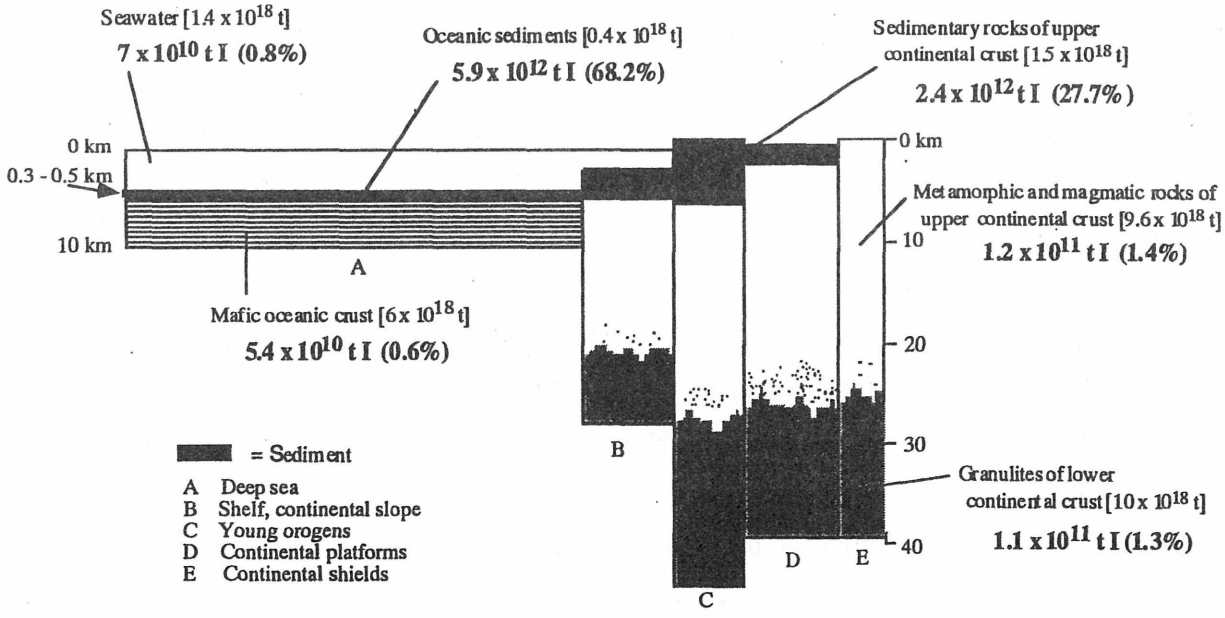

Fig. 6. Distribution of iodine in the Earth's crust and major flows of iodine from and to the ocean (from Muramatsu and Wedepohl 1998)

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7. A Dosimetric Determination of ^{137}Cs Ingestion from Global Fallout and the Related Risks to Japanese

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INTRODUCTION

Hazardous materials released by our industrial activities have been globally dispersed in the environment and have been a cause of chronic adverse health effects through various pathways. For the human health risk evaluation, the reliable mathematical model is essential, and, for the model validation global scale monitoring data are required together with clear initial/boundary conditions for the model. These three conditions are fulfilled more by fallout radionuclide like ^{137}Cs than by non-radioactive materials: radioactive fallout has been monitored worldwide and its dietary data has been monitored in Japan since the late 1950's, no artificial radionuclide were in the environment before 1945 and the time, the location, and the scale of each nuclear detonation test were recorded. The aim of this research, therefore, is to quantitatively evaluate the risk to human health caused by chronic global radioactive food contamination. Fig.1 shows the model development flow chart used in this research. Predictions by the proposed model were compared with the monitoring data of ^{137}Cs in Japanese total diet as an attempt at validation. By using the previously published global model[1], the contribution of the imported food to the dietary intake of ^{137}Cs can be estimated without requiring excessive amounts of data. This approach is also useful for other contaminants in other regions of the world where portions of the food supply are imported.

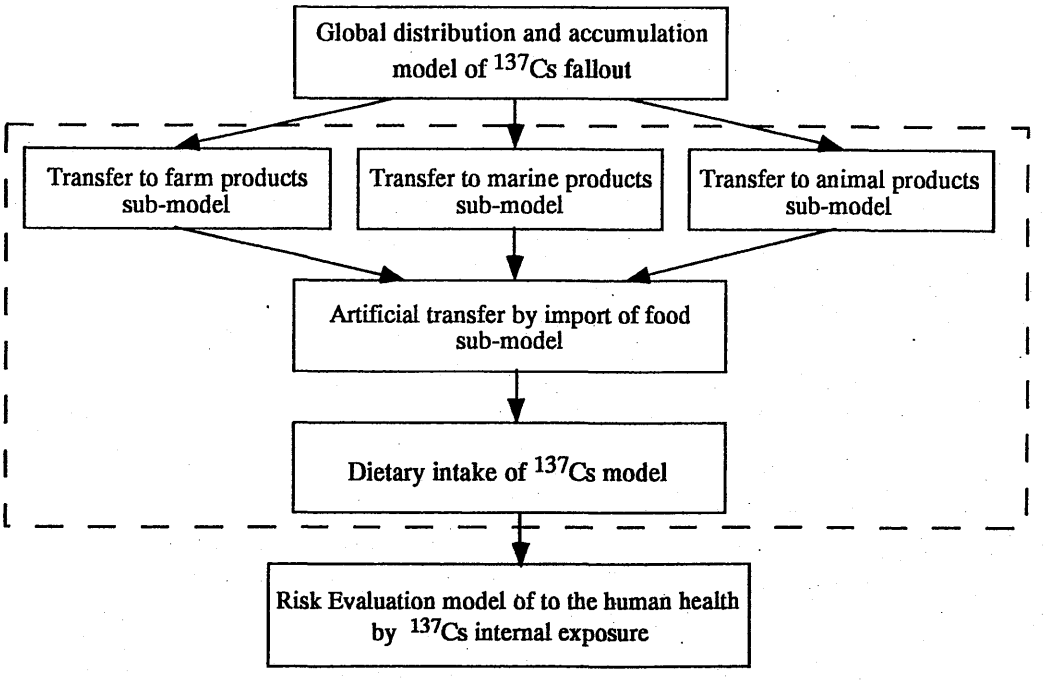

Fig.1 Flowchart for the model development used in this study

MATHEMATICAL MODEL AND NUMERICAL SIMULATION

The global distribution of ^{137}Cs was estimated by using the mathematical model for evaluating the dynamic performance of ^{137}Cs in the global atmospheric environment and its deposition on the land and ocean surface[1]. According to the wind and pressure system, the atmosphere and the land surface was divided by the latitude in this study as shown in Fig. 2. However, separation of ocean area must be different, since the fallout ^{137}Cs is transferred by ocean currents where not only east-west but also north-south movements are dominant. In this study, the ocean was divided

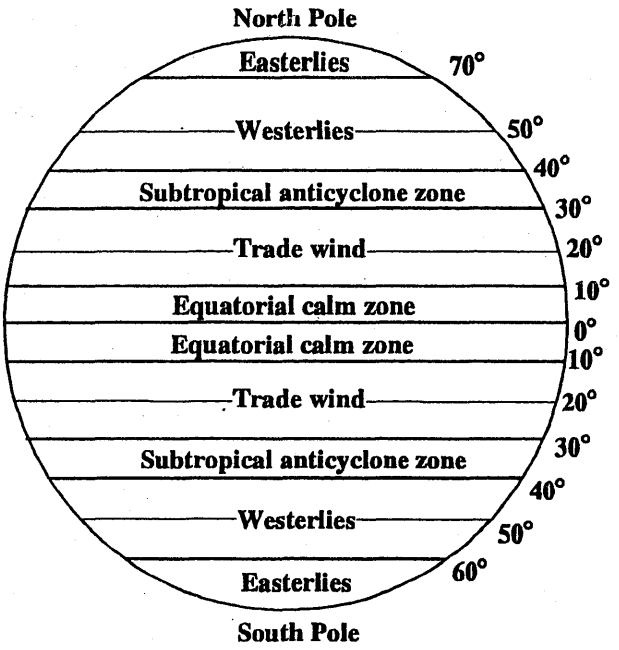

Fig.2 Zone separation of the atmosphere used in this study

laterally into six blocks: the Arctic Ocean, the South Atlantic Ocean, the North Atlantic Ocean, the Indian Ocean, the South Pacific Ocean, and the North Pacific Ocean. Each ocean block was also divided vertically in two layers: the surface and the deep layers. The model consists of a total of 54 compartments: stratosphere, tropo-sphere and land are respectively, divided into 14 compartments; surface and deep ocean are, respectively, divided into 6 compartments (see Fig.3 and Fig.4). By coupling this model, the mathematical model for the evaluation of the dietary intake of ^{137}Cs and the related risks to Japanese are proposed. The details of the model will be published in Health Physics Journal[2]. Table 1 shows the parameters used in the model. The risk of inducing cancer to the Japanese is estimated as the annual excess fatal rate of each cancer induced by ^{137}Cs internal exposure to each age cohort by using the age-dependent committed dose equivalents in target organs or tissues as a result of unit intake of ^{137}Cs, as is reported by ICRP[3] and the risk coefficient defined by ICRP[4].

The dietary intake of ^{137}Cs by Japanese and its risk were calculated by the above-mentioned model. The numerical simulation was executed on a monthly basis from 1945 to 1990. Since the nuclear detonation test started on July 1945, the ^{137}Cs inventory in each compartment of the global environment was set to zero before July 1945 as the initial condition.

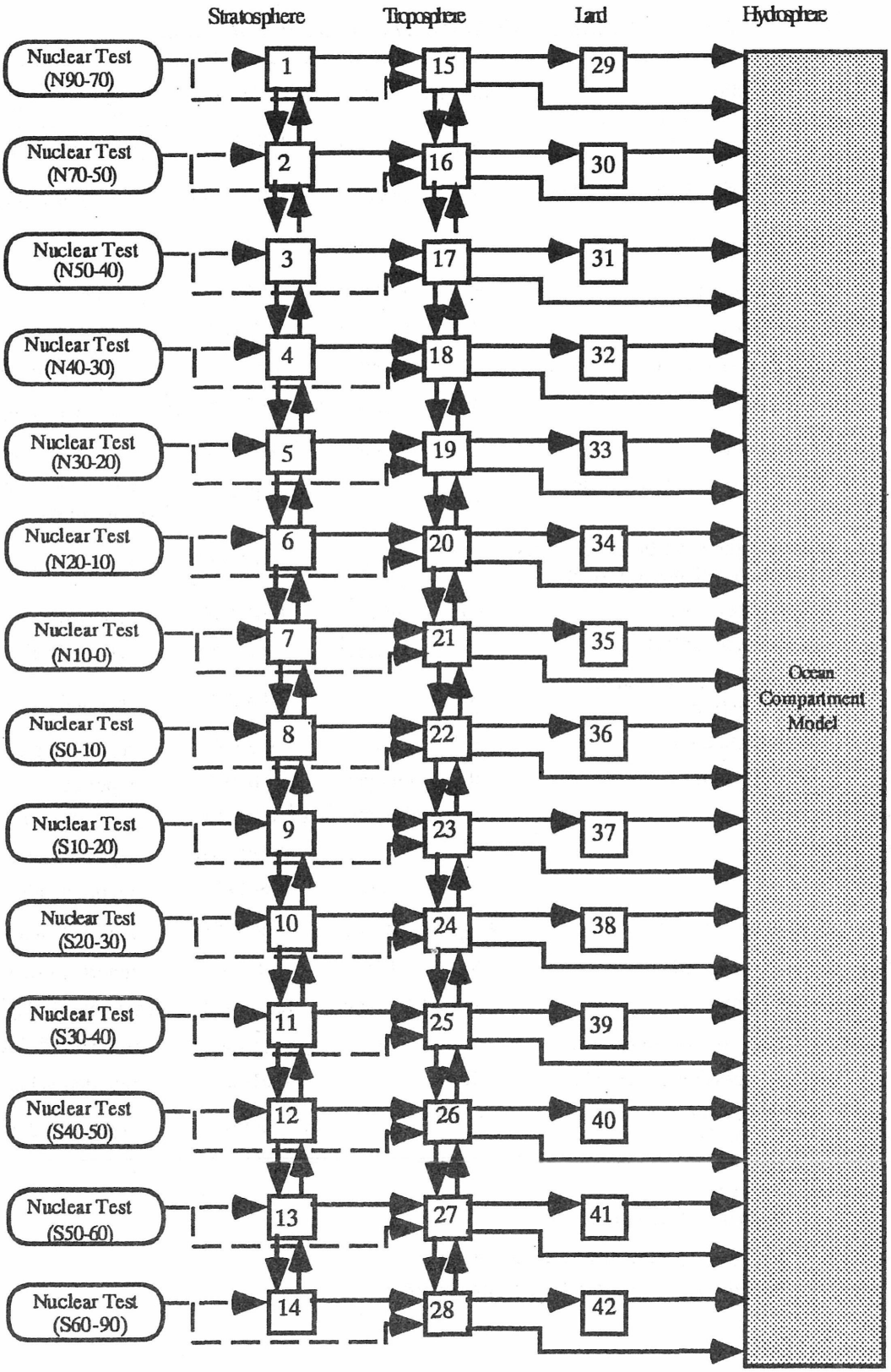

Fig.3 Compartment model for evaluating dynamic performance of the ^{137}Cs in global atmospheric environment and its deposition on land and ocean surfaces

Table 1 List of information about parameters used in the model

Description		Parameter	Units	Range	Method of value determination
^{137}Cs transfer coefficient in stratosphere		k_{ss}	month^{-1}	0.042-0.25	parameter fitting**
^{137}Cs transfer coefficient from stratosphere down to troposphere		k_{st}	month^{-1}	0.021-0.17	parameter fitting**
^{137}Cs transfer coefficient in troposphere		k_{tt}	month^{-1}	1.7-7.5	parameter fitting**
^{137}Cs deposition rate on surface land		d_L	month^{-1}	0.03-2.3	parameter fitting**
^{137}Cs deposition rate on surface ocean		d_o	month^{-1}	0.83-0.42	parameter fitting**
^{137}Cs runoff rate from surface land to surface ocean		r	month^{-1}	0.052-0.79	parameter fitting**
^{137}Cs transfer coefficient in ocean		w	month^{-1}	8.3×10^{-8}-8.3×10^{-3}	parameter fitting**
Sedimentation rate of ^{137}Cs in ocean		s	month^{-1}	0.0023-0.066	reported value
Fraction of ^{137}Cs locally deposited at the site near the nuclear detonation test		P_{res}	-	0.01-0.1	considering the mass balance of the model
Environmental decay constant λ^{*}_{env}	Distribution coefficient of ^{137}Cs between soil and soil water	k_d	mL/g	200-17000	reported value
	Soil bulk density	ρ_a	g/cm^3	0.84-1.96	reported value
	Soil water content	θ	mL/cm^3	0.2-0.8	reported value
	Fraction of the effective precipitation	β	-	0.6-0.9	reported value
	Evaporation rate	e	-	0.2-0.9	reported value
	Precipitation	R	mm/year	400-1900	reported value
Direct foliar absorption factor		K	cm^2•month^{-1}•g^{-1}	0.01-0.1	parameter fitting
Root uptake transfer factor		TF	-	0.0003-0.8	reported value
Biological half-life		$T_{0.5}$	day	8-200	reported value
Transfer factor from feed to egg contents		F_{egg}	day/kg	0.34-0.53	reported value
^{137}Cs concentration factor for seaweed		CF_{sw}	L/g	5-70	reported value
Gill respiration rate of marine animals		v	L/g•month	860-67000	parameter fitting**
^{137}Cs absorption rate by muscle of marine animals		a	-	5×10^{-7}-6×10^{-2}	parameter fitting
^{137}Cs residual rate by food processing and cooking		PR	-	0.01-0.93	reported value
Fraction of import of each production from each food exporting country***		PI	-	0-1.0	reported value

* $\lambda_{env} = \beta(1\text{-}e)\,R / \{L(\theta + \rho_a k_d)\}$

** based on the results of the meteorological study

** based on the results of the physiological study of marine animal

*** Japan is hypothetically treated as one of the food exporting countries

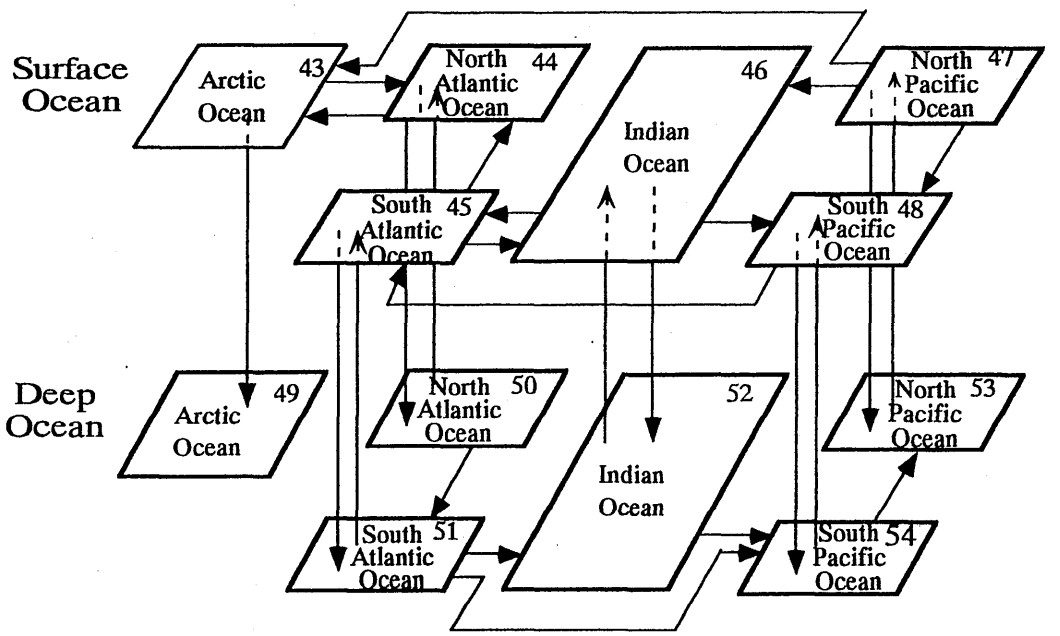

Fig. 4 Ocean compartment model

RESULTS AND DISCUSSION

(a) Examination of the model

The dietary intake of ^{137}Cs by Japanese was estimated with the model, which was compared with the observed data[5]. Fig.5 shows the results. The calculated ^{137}Cs concentration in the rice, the leafy vegetable (spinach), the root vegetable (Chinese radish) and the milk, which are produced in Japan, were compared with the data observed in Japan[5]. Fig.6 (a)-(c) and Fig.7 respectively show each result. The calculated values agree in general with the observed data.

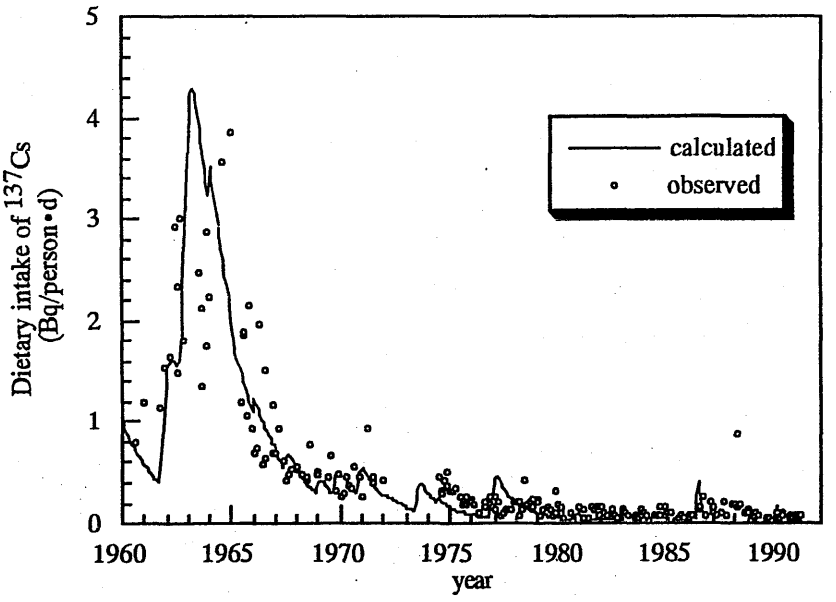

Fig. 5 Comparison between the calculated and observed dietary intake of ^{137}Cs by Japanese

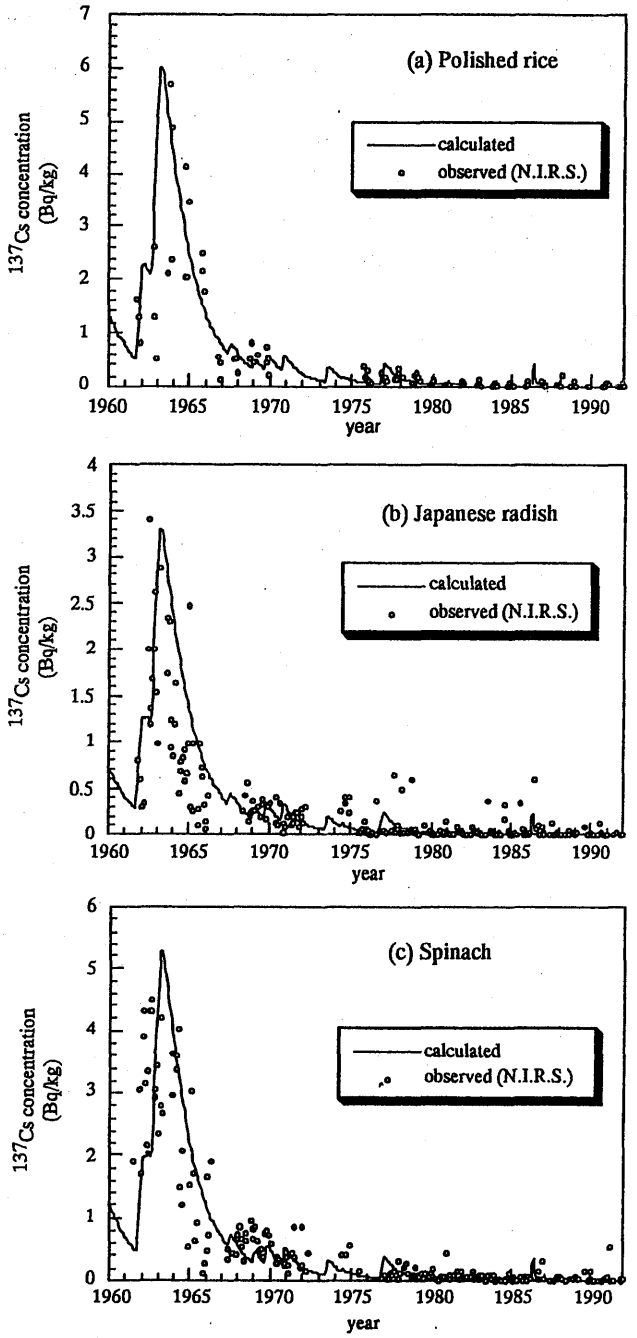

Fig.6 Comparison between the calculated and the observed ^{137}Cs concentration in each farm product in N40-30 zone (Japan)

(b) ^{137}Cs ingestion by Japanese

The percentage of the predicted value of the dietary intake of ^{137}Cs through each kind of foods is shown in Fig.8 (a)-(d). For reference, the percentage of the Japanese daily food intake is shown in Fig.9 (a)-(d). Most of ^{137}Cs is taken through farm products. The percentage of the ^{137}Cs ingestion through farm products has been gradually decreasing and that through animal products increasing. These results reflect the transition of the daily intake of foods by Japanese. The ^{137}Cs ingestion through marine products has been gradually increasing after 1980's; this evaluation may be consistent with the previous report indicated that ^{137}Cs is distributed more to the ocean after the 1980's[6]. Among farm products, rice and vegetables mainly influenced on the ^{137}Cs ingestion in the 1960's, but after the 1970's the fraction of

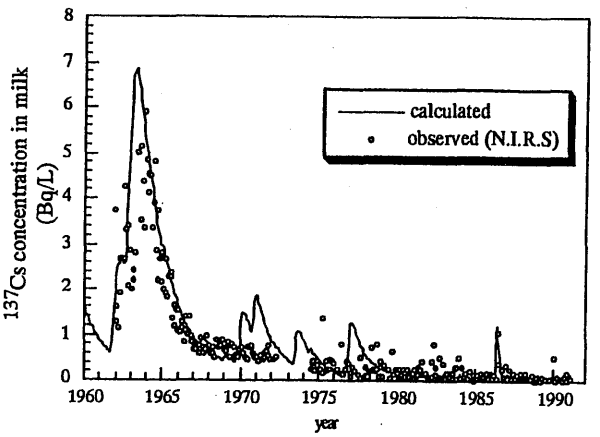

Fig.7 Comparison between the calculated and the observed ^{137}Cs concentration in milk in N40-30 latitude zone (Japan)

the contribution to rice has been decreasing and that to fruits increasing. Among animal products, milk and eggs have mainly influenced. In particular, The ^{137}Cs is more than 70% taken through milk after the 1970's; this may reflect the fact that more than 80 % of the feed for the cow cattle is the roughage in which the ^{137}Cs is less removed by milling than the concentrated feed[7]. The ^{137}Cs ingestion through marine products is more than 90% contributed by shellfish and the seaweed, though the daily intake of marine products is more than 50% contributed by raw fishes and processed fishes (see Fig.9). This may reflect the experimental results indicated that ^{137}Cs is less removed from shellfish and seaweed by cooking or processing than the others[7].

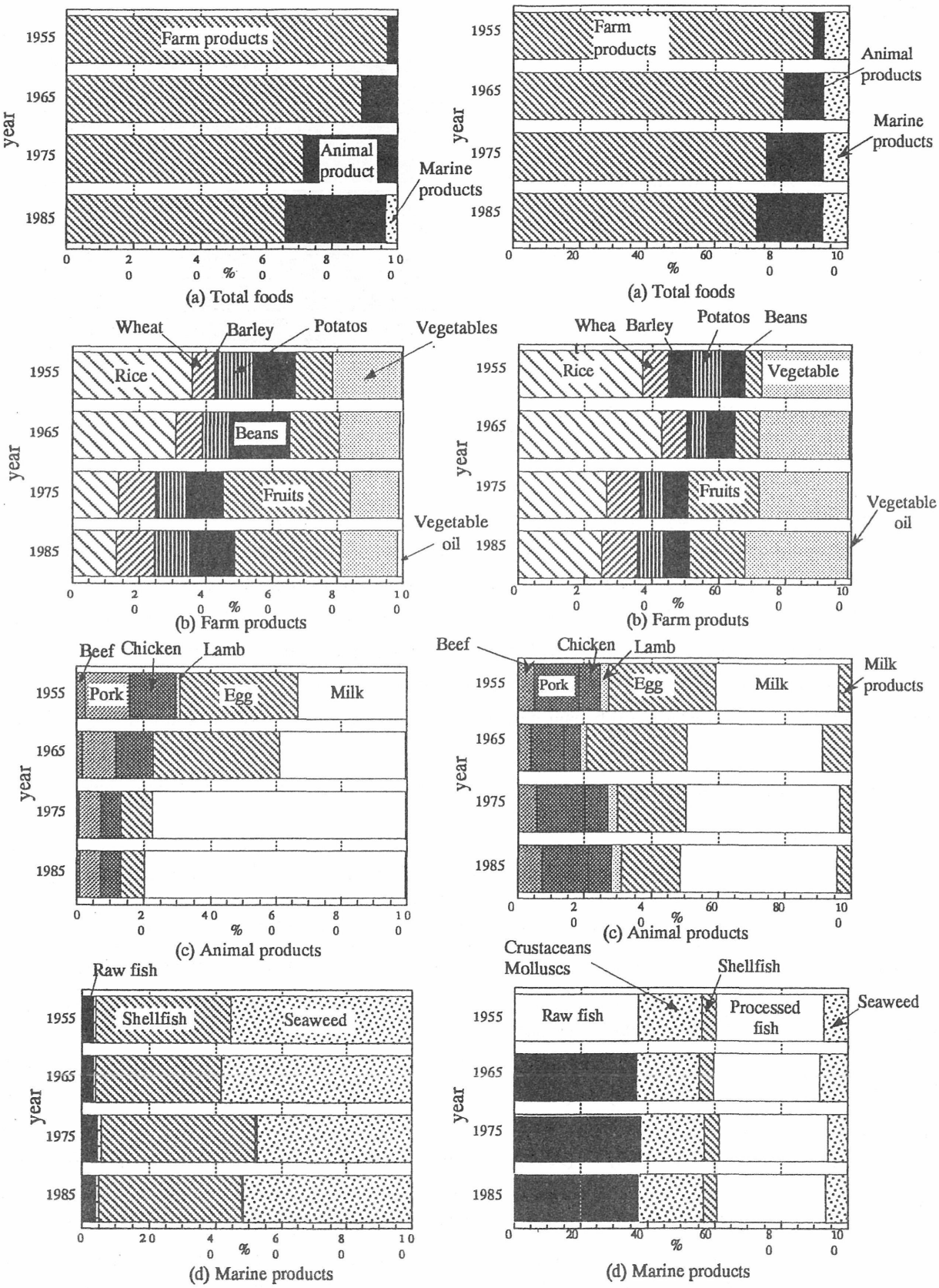

Fig.8 Percentage of dietary intake of ^{137}Cs through each kind of food

Fig.9 Percentage of dialy intake of each kind of food by Japanese

The percentage of the dietary intake of ^{137}Cs through imported foods was also calculated; it was found that the contribution by imported foods has been gradually increasing according to the decrease of the self-support rate of Japan. In particular, the ^{137}Cs ingestion through the imported farm products has been increasing: the rate of the contribution of imported farm products has increased from about 25% in the 1950's to about 40% in the 1990's as shown in Fig.10. It is found that ^{137}Cs is mainly taken up through imported farm product from U.S.A., Canada, China, Philippines, Brazil, Australia and New Zealand.

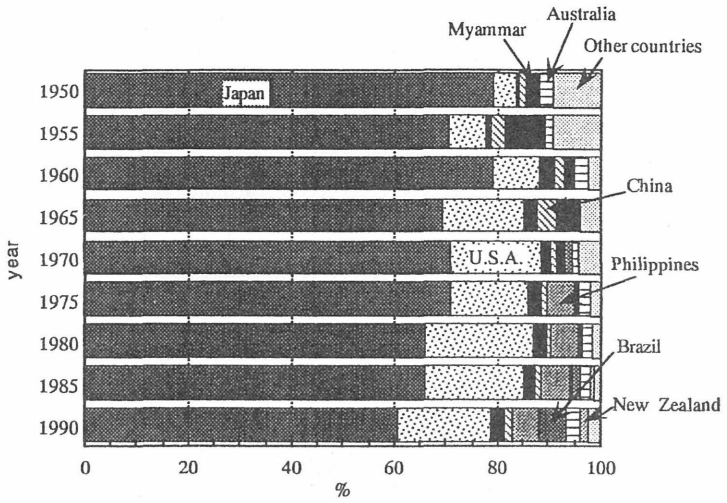

Fig. 10 Percentage of dietary intake of ^{137}Cs in farm products from various foreign countries

c) Risk to human health by internal exposure

The risk to the Japanese health by the ^{137}Cs internal exposure for each age cohort was calculated. Among the risk to each age cohort, the risk to infant health is the highest. The results for the adults are shown in Table 2, compared with the data of the annual fatal rate according to the vital statistics of Japan[8]. The annual fatal rate of each cancer induced by the ^{137}Cs internal exposure through the food ingestion was at its maximum in 1963; for 100 million people about 4 persons were at risk of death from breast cancer, leukemia or lung cancer, and about 1 person was at

Table. 2 Risk of inducing cancer to the reference Japanese by ^{137}Cs internal exposure through food ingestion

	Annual excess fatal rate of each cancer induced by ^{137}Cs internal exposure for 100 million people (Annual fatal data for 100 million people according to the vital statistics of Japan[a])									
Cancer	1950	1955	1960	1963	1965	1970	1975	1980	1985	1990
Breast cancer	0.0004 (1700)	1.3 (1800)	0.80 (2000)	4.4 (1900)	1.5 (2000)	0.44 (2400)	0.12 (3000)	0.050 (3600)	0.0027 (4100)	0.0001 (4800)
Leukemia	3×10^{-5} (1500)	1.1 (2300)	0.72 (2800)	3.8 (3000)	1.3 (3200)	0.38 (3500)	0.10 (3700)	0.043 (3900)	0.0024 (4300)	0.0001 (4600)
Lung cancer	3×10^{-5} (3300)	1.1 (4700)	0.72 (7400)	3.8 (9000)	1.3 (9900)	0.38 (12800)	0.10 (15600)	0.043 (20200)	0.0024 (25500)	0.0001 (31500)
Thyroid cancer	9×10^{-6} (900)	0.28 (1300)	0.18 (1600)	0.94 (1900)	0.33 (2100)	0.096 (2600)	0.026 (3500)	0.011 (4200)	0.0006 (5000)	3×10^{-5} (6000)
Bone cancer	9×10^{-6} (500)	0.28 (900)	0.18 (1100)	0.94 (1100)	0.33 (1100)	0.096 (900)	0.026 (600)	0.011 (400)	0.0006 (400)	3×10^{-5} (400)

[a] Taken from the Ministry of Health and Welfare, Japan 1992.

risk of death from thyroid cancer or bone cancer. With reference to leukemia, about 0.12% (=3.8 / 3000) of the fatalities in 1963 are estimated to have died by the ^{137}Cs internal exposure through food ingestion. After 1980's, the risk decreased; in 1990 the maximum annual excess fatal rate might have been about 1 person for 1 trillion people. These results show that the risk by ^{137}Cs internal exposure has been very small, which means that the contamination by global radioactive fallout has had little effect on our society. However, this research made it possible to propose a method for the quantitative evaluation of the risk to the human health caused by chronic global radioactive food contamination: this might be useful for other environmental contaminants which have potential risks to the human health.

(d) Analysis for determination of critical pathway to the health risk

The influence of each food pathway on the Japanese health risk caused by the dietary intake of ^{137}Cs was analyzed. The risk is in proportion to the dietary intake of ^{137}Cs; the influence was evaluated by the comparison of the simulation results of four different cases as follows: the reference case in this research, the case of the twice intake of farm products, the case of the twice intake of animal products and the case of the twice intake of marine products. Fig.11 shows the results. It was found that the pathway of the ingestion through farm products contributed the most to the dietary intake of ^{137}Cs; this means the ingestion of farm products was the main influence on the risk to the Japanese health. This appears to be substantiated by the result of the estimation of the dietary intake of ^{137}Cs by each food shown in Fig.8 (a).

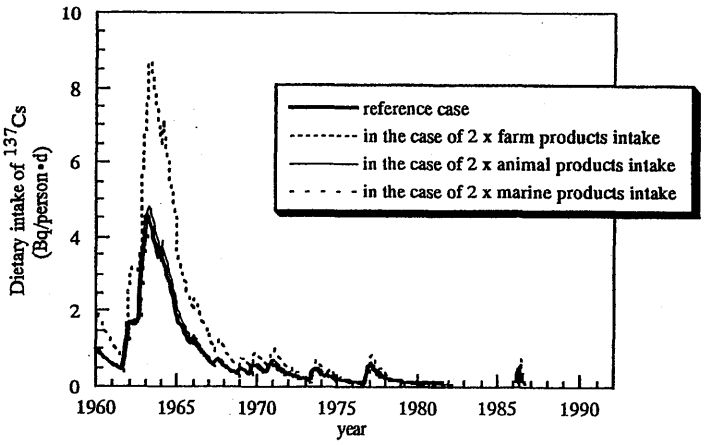

Fig. 11 Analysis for determination of the main pathway of Japanese dietary intake of ^{137}Cs

VALIDATION OF MODEL ESTIMATION

Most of the parameters incorporated in the model were set by the best-fit adjustment to observed data within the reported variation of the parameter value. The predicted values of the dietary intake of ^{137}Cs consequently include some unreliability associated with the parameter uncertainty in the model. Therefore the accuracy of the model should be analyzed. In this research, sensitivity analysis, the percentile estimate and robustness analysis was carried out.

(a) Sensitivity analysis

The sensitivity of the parameters incorporated in the model was evaluated with the comparison of the simulation results of three different parameter sets: the best-fitted parameters, 2 (best-fitted parameter) and 0.5 (best-fitted parameter). It was found that the transfer coefficients from the stratosphere vertically down to the troposphere (k) and the direct foliar absorption factor to the leaf of farm or feed product (K) significantly affected the dietary intake of ^{137}Cs. In particular the k value had much effect on the peak value and the decrease in rate after the peak time of the predicted dietary intake of ^{137}Cs.

(b) Variation analysis

Each parameter in the model includes its value variation, which is uncertainty of the parameter. The range of variation of the predicted value of the dietary intake of ^{137}Cs was analyzed by varying each parameter independently within its value variation. The percentile estimates of dietary intake of ^{137}Cs were calculated by the Monte Carlo simulation with a super computer (FACOM M1800). The results of the calculation are shown in Fig.12. The variation of the predicted value is remarkable in the 1960's. The observed data are within the 5 and 95 percentile estimates; this means that the possibility of the predicted value being discrepant from the observed data is less than 10%.

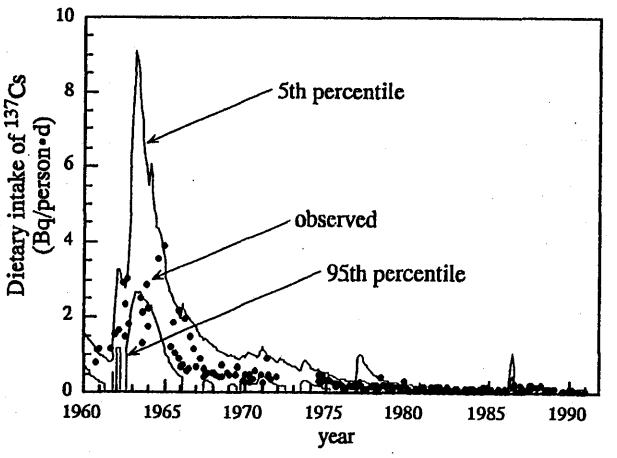

Fig. 12 5th and 95th percentile estimates of dietary intake by Japanese

(c) Robustness analysis

The Robustness Index[9] was used to analyze the extent of the variation of the predicted value due to the uncertainty of the parameter values in the model. The Robustness Index is defined as follows[9]:

$$R(\mathbf{p}) = \mathrm{Min}\{D_{std}, D(\mathbf{p})\} / \mathrm{Max}\{D_{std}, D(\mathbf{p})\} \qquad (1)$$

In eqn (1), **p** is the number of sets of the parameters of the model, D_{std} is the predicted value in this research and D(**p**) is the value calculated by the model incorporating one of the sets, **p**. The values of R(**p**) ranges between zero to 1.0. The smaller the variation of the predicted value is, the nearer 1.0 the value of R(**p**) should be set. This research utilized the calculated values by the Monte Carlo simulation as R(**p**). The results are shown in Fig.13. It was found that the predicted value is less robust down to the troposphere and the direct foliar absorption factor to the leaf of farm or feed products.

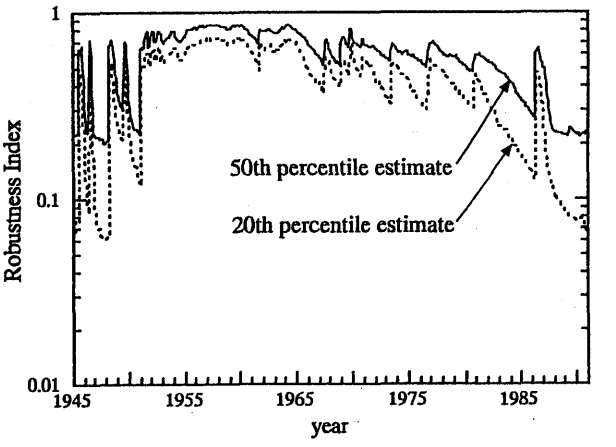

Fig. 13 Robustness Index of calculated dietary ^{137}Cs intake by Japanese

CONCLUSIONS AND REMARKS

The major findings obtained in this research under the limits considered are summarized as follows:

1) The proposed mathematical model is promising for quantitatively evaluating the Japanese health risk caused by the dietary intake of global radioactive fallout ^{137}Cs.
2) The ^{137}Cs is taken up by Japanese mostly through farm products. The ingestion of ^{137}Cs through animal and marine product has been gradually increasing in recent years.

3) The ingestion of ^{137}Cs through farm product is due to rice, vegetables and fruits; through animal products, by milk and eggs; through marine products by shellfish and seaweed.
4) The ingestion of ^{137}Cs through imported foods is increasing.
5) The risk to the Japanese health of inducing cancer by ^{137}Cs internal exposure reached a maximum of about 4 annual excess deaths for 100 million in 1963.
6) The ingestion pathway through farm products has the greatest affect on the Japanese health risk.
7) The dietary intake of ^{137}Cs is influenced by the transfer coefficients from the stratosphere vertically down to the troposphere and the direct foliar absorption factor to the leaf of farm or feed products.

As the next stage of our study, we attempt to apply the proposed method to the quantitative evaluation of the risk to the human health caused by other non-radioactive contaminants: heavy metals and pesticides. The food contaminants have been monitored worldwide by UNEP and FAO as 'GEMS Food Contamination Monitoring Program' established in 1976[10]. We try to develop and evaluate a mathematical model that can quantitatively evaluate the risk to the human health by long-term global non-radioactive contamination by using this monitoring data.

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8. Multi-element Analysis of Foodstuffs and Diet Samples in Relation to Comparative Evaluation in Public Hygiene

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INTRODUCTION

Information on dietary metal intakes is important from the viewpoints of radiation protection and public hygiene. Recently, inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have become valuable instrumental methods for trace element analysis because of their wide dynamic range and capability for multi-element analysis. These methods have generally equal to, and/or higher, sensitivity compared to conventional analytical methods, e.g. alpha-spectrometry, beta-counter, gamma-spectrometry, radioactivation analysis (NAA), atomic absorption spectrometry, and so on. For some radioactive and non-radioactive nuclides, dietary nuclide intakes in Japanese have been previously estimated by using ICP-MS and ICP-AES[1-5]. It is becoming more important to know both micro and macro environmental relationships among many kinds of nuclides and chemical compounds (radioactive nuclides, heavy or rare metals and organic substances) due to the recent accelerated development of science and technology. The more information on dietary nuclide intakes that is collected, the better is the understanding of the behavior of nuclides from the environment to humans. Typically, ICP-methods have two modes, one is the quantitative analytical mode and the other is the semi-quantitative mode. The latter is often used for preliminary studies prior to intensive quantitation[6-7]. However, the semi-quantitative analysis has resulted in effective and reliable results, which are sometimes equal to those from quantitative analysis. In this paper, a combination of both modes is demonstrated to be a powerful methodology for dietary intake studies.

MATERIAL AND METHODS

Foodstuffs were purchased from markets in the vicinity of Mito City during 1994-1995. Statistical consumption data were used for collection of the food samples. The collected samples were divided into eighteen groups and freeze-dried. Diet samples were also collected by duplicate portion studies from Japan and Ukraine. Freshwater samples were collected from the Ukraine, Russia, and Belorussia. Standard reference materials (Orchard Leaves SRM1571, Bovine Liver SRM1577a, Total Diet SRM1548, and Water SRM1643b) were obtained from the National Institute of Standards and Technology and used for quality control. Multi-element solutions, XSTC-1 (including 16 rare earth elements) and XSTC-13 (including 31 elements), and single standard solutions of Sr, Rh, Cs, Bi, Th and U were obtained from Spex Industries (Edison, NJ, USA). High purity mineral acids, TAMAPURE-AA were purchased from Tama Chemical Co. Ltd (Kawasaki, Japan). Freshly purified water was prepared using a Barnstead D-2764 four-module system (Boston, MA, USA).

The analytical procedure employed has been described in detail elsewhere[8] ; only a summary is given for foodstuff and diet samples. The samples were incinerated in a furnace below 400℃. An aliquot of approximately 0.25 g ash was repeatedly digested with a mixture of concentrated nitric acid, perchloric acid, and hydrofluoric acid until a white residue remained. The residue was dissolved in 10% nitric acid and kept in a Teflon bottle.

Numbers of nuclides including Sr, Cs, Th-232, and U-238 were determined in the quantitative analytical mode and the semi-quantitative mode by ICP-MS. The ICP-MS instruments used were a Yokogawa PMS2000 Model (Yokogawa Electric Co. Ltd., Tokyo, Japan) and a Hewlett Packard 4500 Model (Hewlett Packard Co Ltd., USA). Some specifications and operating conditions for the quantitative and semi-quantitative modes are summarized in Table 1. Strontium was determined by a Shimadzu ICPQ-1012W inductively coupled plasma emission spectrometer (Shimadzu Co Ltd., Kyoto, Japan).

Table 1. Operating conditions of ICP-MS

Mode: Quantitative analysis	Semi-quantitative analysis
Mass analyzer	
Scan mass range	103-238 a.m.u. / 4-240 a.m.u.
Number of points per peak	3 / 6
Number of scan sweeps	100 / 100
Dwell time per points	33 ms / 1 ms
Integration time per points	3.3 s / 0.1 s
Analysis time	153 s / 181 s
Plasma torch	
Frequency	27.12 MHz
R.f. power	1.3 kW
Position for ion extraction	6 mm
Nebulizer	Barmington
Carrier gas flow	0.83 ml/min
Auxiliary gas flow	1.0 ml/min
Plasma gas flow	15 l/min
Sample uptake rate	0.4 ml/min
Instrument :	Yokogawa ICP-MS Model HP4500

RESULTS AND DISCUSSION

Accuracy

The multi-element solutions (XSTC-1 and XSTC-13) were used for editing semi-quantitative response factors in the semi-quantitative mode. Standard reference materials were used to check accuracy. For Sr, Cs, Th-232, and U-238, analytical results of the Orchard Leaves SRM1571 in both the quantitative mode and semi-quantitative mode ICP-MS are shown in Table 2. Only strontium was determined by quantitative mode ICP-AES and the semi-quantitative mode for ICP-

MS. In the quantitative modes, the concentrations of 34.6 μg/g for Sr, 0.044 μg/g for Cs, 0.0597 μg/g for Th-232, and 0.0287 μg/g for U-238, were in good agreement with their respective certified values 37, 0.04, 0.064, and 0.029 μg/g. In the semi-quantitative mode, a relatively good agreement was obtained; ratios of the analytical result and certified value ranged from 0.78 to 0.88. Ratios of the semi-quantitative and quantitative modes were from 0.79 to 0.86. Therefore, the present analyses by the semi-quantitative mode are done with approximately 20% lower calibration curves than those by the quantitative mode.

Table 2. Analytical results for NIST SRM 1571 Orchard leaves by quantitative mode and semi-quantitative mode ICP-MS

Nuclide	Concentration, μ g/g-dry weight			Ratio of semi-quantitative mode & quantitative mode
	Present result*		Certified value	
	Quantitative mode	Semi-quantitative mode		
Sr	34.6 ± 0.5 †	30.0 ± 1.2	37 ± 1	0.86
Cs	0.044 ± 0.002	0.035 ± 0.002	0.04	0.79
^{232}Th	0.0597 ± 0.0010	0.0503 ± 0.003	0.064 ± 0.006	0.84
^{238}U	0.0287 ± 0.0010	0.0245 ± 0.001	0.029 ± 0.006	0.86

*Mean and SD of triplicate determinations.
† Determined by ICP-AES.

Comparison of the results in food samples between the quantitative and semi-quantitative mode

For the foodstuff and diet samples, approximately 70 elements above 50 cps (adjusted as minimum peak counts) were determined and printed out in the semi-quantitative mode program. However, not all analytical results were acceptable due to isobaric interference. Analytical results of Cs, Th-232, and U-238 in the semi-quantitative mode were compared with those of the quantitative analytical mode in the 18 food groups. Average ratios of the semi-quantitative mode and the quantitative analytical mode for Cs, Th-232, and U-238 were 0.70, 1.11, and 0.95, respectively. The result for Th-232 is shown in Fig. 1. Analytical data of 54 samples are shown; i.e. each sample for the 18 food groups was determined in triplicate analyses. The slope of the line for the two modes was 0.64 and a good correlation factor (r =0.961) was found. Strontium concentrations for sample solutions of the 18 food groups ranged from 20 to 1500 ppb. The Sr results of the semi-quantitative mode were compared with those of ICP-AES. The Sr results of the semi-quantitative mode and ICP-AES was 0.83±0.21 (nT). T he relationship for the two sets of results for 17 food groups is shown in Fig. 2. The slope and correlation factor were 0.88 and 0.993, respectively. Data for a seaweed group were excluded from this calculation because the concentrations of this group were much higher (ca. 20,000 ppb) than those of the other group (20-1500 ppb). For Sr, Th-232, and U-238, the semi-quantitative mode (ICP-MS) and quantitative modes (ICP-MS and ICP-AES) were found to be in a good agreement, i.e. within± 10%.

Ten other ten elements (Li, V, Mn, Cr, Co, Ni, Cu, Rb, Mo, and Cd) were simultaneously determined with fairly good accuracy, although there were different matrix conditions for all 18-food groups. Most of the elements in the sample solutions could not be determined by the semi-quantitative mode due to their lower concentrations. More elements could be measured if

concentrations were at least at the sub-ppb (ng/ml) level.

In the total diet samples, concentrations within 20% for the two modes could be determined at the sub-ppb level for Cs, Th-232, and U-238.

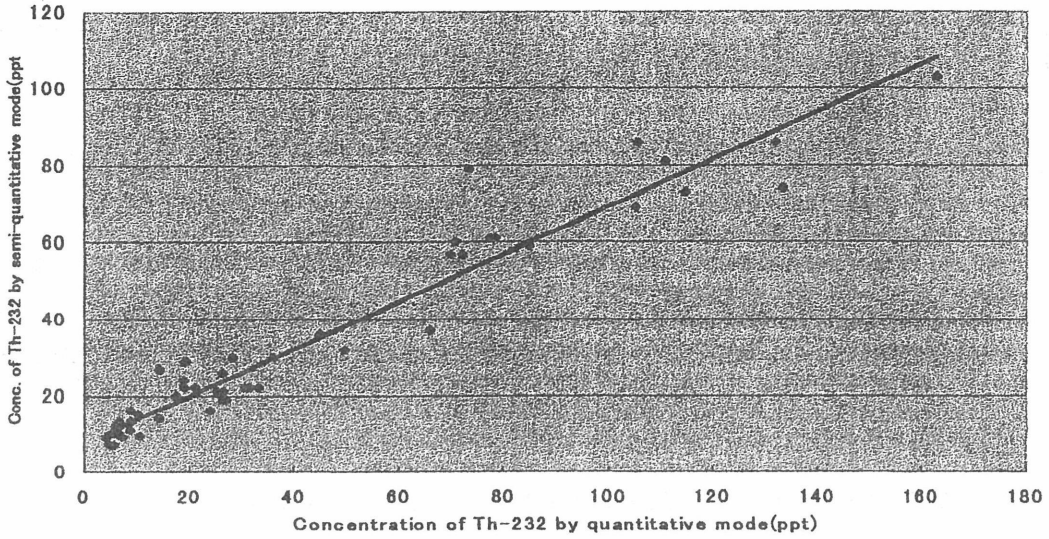

Fig. 1 Comparison of Th-232 concentration analyzed by quantitative mode with semi-quantitative mode in 18 food groups

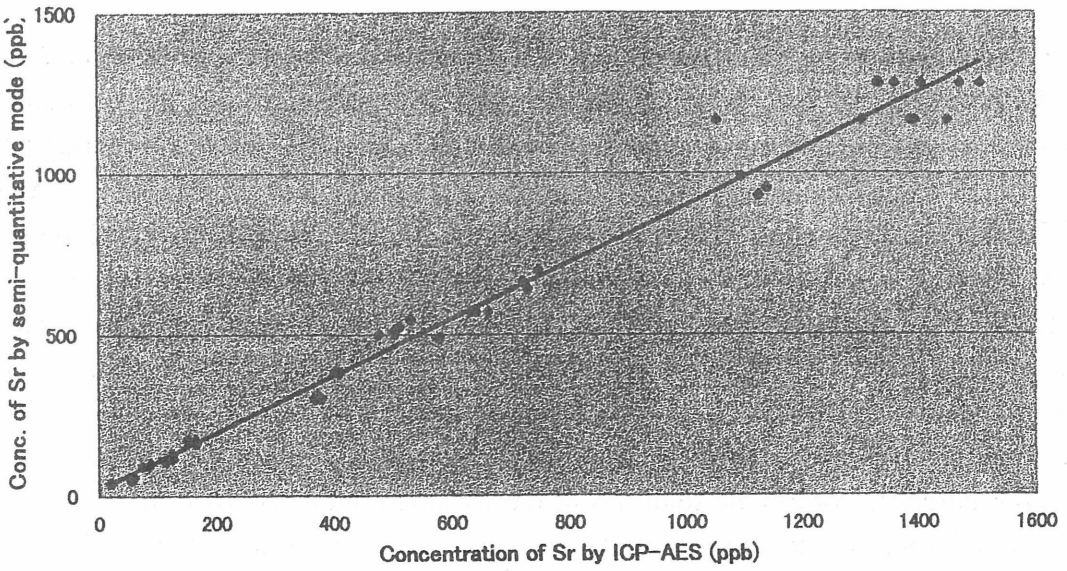

Fig. 2 Comparison of Sr concentration from ICP-AES and semi-quantitative mode ICP-MS in 17 food groups

Correlation of nuclides in 18 food groups

The semi-quantitative mode ICP-MS is typically used for simultaneous multi-element analyses. Correlations of nine nuclides (Li, Mn, Ni, Cu, Sr, Mo, Ba, Th, and U) analyzed in the 18 food groups are shown in Fig. 3. Higher correlation factors (above r=0.7) were found between the following pairs of : Li-Ni, Li-Ba, Mn-Cu, Mn-Mo, Ni-Ba, Cu-Mo, and Sr-U. Collecting those relationships in foodstuffs should provide a basis for comparison in future work on dietary studies and also allow comparative evaluations in the area of public hygiene.

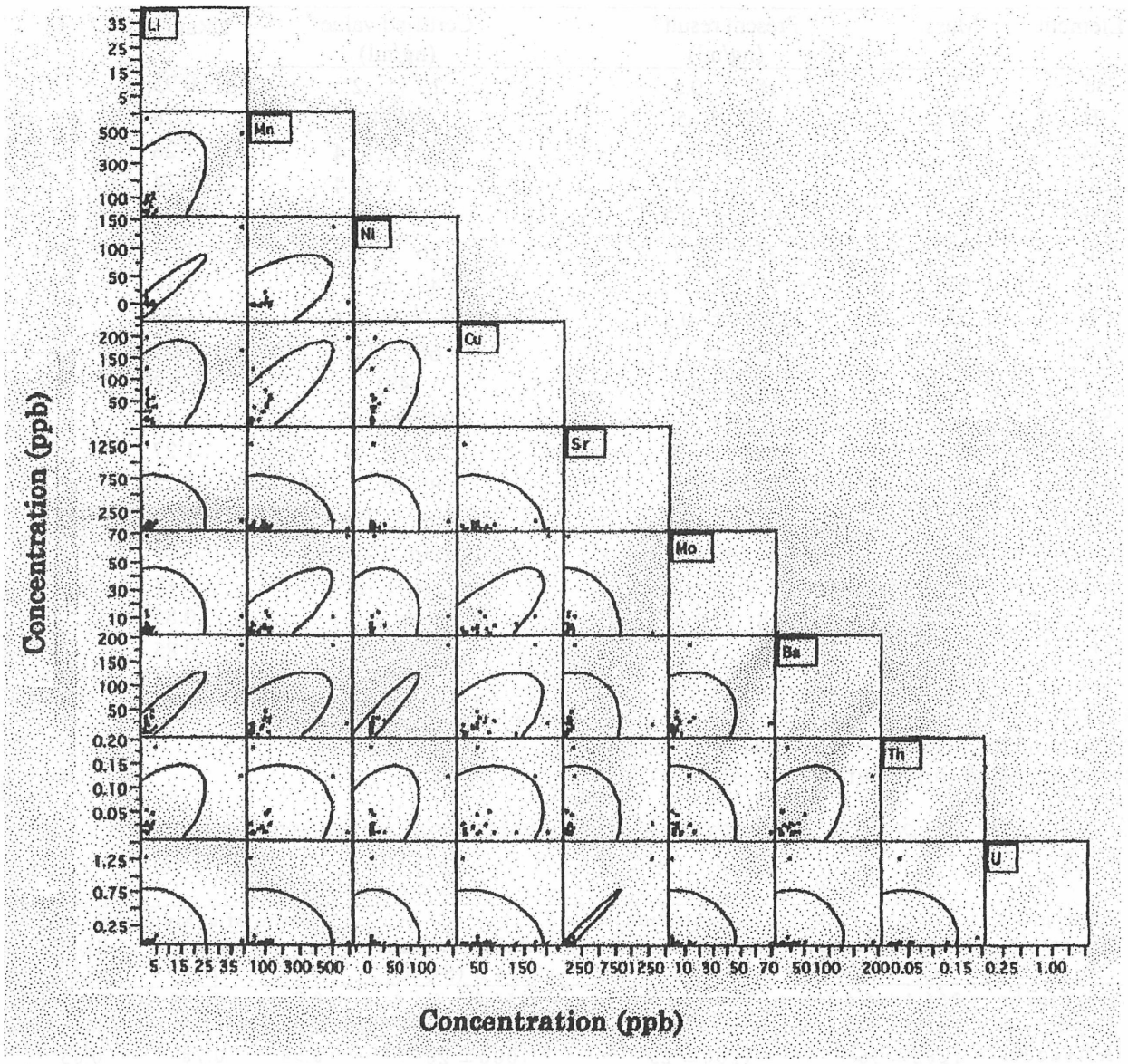

Fig. 3 Correlation of nine element concentrations in 18 food groups obtained simultaneously by semi-quantitative mode ICP-MS

Analysis of freshwater samples

A Knowing concentration of elements in drinking water sources is important in mineral intake studies[9]. The standard reference water 1643b was also measured three times in the semi-quantitative mode and these results were compared with its certified values. A summary is shown in Table 3. For nineteen elements, analytical data and certified values were in agreement within 30%. Furthermore, for Th-232 and U-238, the water samples were not diluted and measured another day and another machine. The nuclides were determined within 30% errors in the semi-quantitative mode in a concentration level of at least 10 ppb (ng/ml). The results of only U-238 are shown in Table 4. Uranium-238 could be analyzed better than Th-232. When the water samples were diluted 500-fold and measured by both modes under the same instruments conditions for a few hours, ratios of the semi-quantitative mode and the quantitative analytical mode were found to be in a narrow range of 0.96-1.01[10].

Table 3. Analytical results for NIST Standard Reference Material 1643b by semi-quantitative mode ICP-MS

Element	Mass	Present result* (ng/ml)	Certified value (ng/ml)	Ratio †
Be	9	18 ± 0.5	19 ± 2	0.95
B	11	120 ± 5	(94) ‡	1.28
V	51	58 ± 0.6	45.2 ± 0.4	1.28
Cr	54	25 ± 0.4	18.6 ± 0.4	1.34
Mn	55	35.2 ± 0.3	28 ± 2	1.26
Co	59	35 ± 0.7	26 ± 1	1.34
Ni	60	57 ± 1	49 ± 3	1.16
Cu	63	28.5 ± 0.3	21.9 ± 0.4	1.30
Zn	66	81 ± 1	66 ± 2	1.23
As	75	58 ± 0.9	(49)	1.18
Se	77	10.8 ± 0.3	9.7 ± 0.5	1.11
Sr	88	289 ± 3	227 ± 6	1.27
Mo	95	104 ± 1	85 ± 3	1.22
Ag	107	12.3 ± 0.2	9.8 ± 0.8	1.26
Cd	111	21 ± 0.8	20 ± 1	1.05
Ba	137	52 ± 1	44 ± 2	1.18
Tl	205	6.8 ± 0.06	8 ± 0.2	0.85
Pb	208	20.1 ± 0.4	23.7 ± 0.7	0.85
Bi	209	9.2 ± 0.1	(11)	0.84

* Mean ± standard deviation for three measurements.
† Ratio of the present results and certified values.
‡ Not certified (informative values).

Table 4. Comparison of 238U concentrations from quantitative mode and semi-quantitative mode ICP-MS

Sampling location	Concentration, ng/ml			Ratio of analytical results		
	Quantitative mode (Qt)*	Semi-quantitative mode A (SQ-A)*	Semi-quantitative mode B (SQ-B) †	SQ-A /Qt	SQ-B /Qt	SQ-B /SQ-A
Zaporozhe	266.4 ± 5.5 ‡	248.8 ± 11.2 ‡	256.8 ± 5.1 ‡	0.96	0.97	1.03
Zaporozhe	251.9 ± 7.1	253.8 ± 11.3	244.3 ± 4.9	1.01	0.97	0.96
Krasnodar	1282 ± 36	1200 ± 25	1000 ± 20	0.94	0.78	0.83
Krasnodar	948.2 ± 26.5	850.0 ± 25.0	891.5 ± 17.8	0.90	0.94	1.05
Krasnodar	284.6 ± 8.4	282.0 ± 20.0	339.6 ± 6.8	0.99	1.19	1.20
100 pg/ml Std. Soln.	0.101 ± 0.001	0.0997 ± 0.0100	–	0.99	–	–
NIST 1571 Orchard Leaves.	3.01 ± 0.03	2.80 ± 0.17	–	0.93	–	–

*Each water sample was diluted 500-fold and measured by both modes under the same instrument conditions for a few hours (Shiraishi et al 1994b).
† The samples were not diluted and measured another day. The optimum instrument conditions were slightly different from the above ones*.
‡ Variation of error in the semi-quantitative mode was given as ± 2% of the analytical values. In the quantitative mode, it was shown as the standard deviation of triplicate measurements.

CONCLUSION

It was concluded that the analytical difference between the quantitative and semi-quantitative analytical modes was mainly due to concentration levels of nuclides in sample solution, matrix conditions, and instrumental conditions. Furthermore, ICP spectrometry produces a wide calibration curve, which is linear over several orders of magnitude. The semi-quantitative mode

is equivalent to a method of one-point standardization and it provides the merit of high sample throughput. Therefore, multi-element analysis by the semi-quantitative mode should contribute to comparative studies in environmental science by optimizing analytical procedures.

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9. Uptake of ^{137}Cs and ^{90}Sr by Cucumber as Affected by Soil and Microbial Parameters of Three Soil-Types

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INTRODUCTION

Transfer of radionuclides from soil to plant varies depending on plant species (1) and the soil types. Soil characteristics are the most discussed parameters in bioavailability of radionuclides. Because of the sorption and fixation processes in soil, some radionuclides possibly remain in the rooting zone of soil for a long time and contribute to artificial radioactivity in the plants. This sorption is often governed by the content of clay, organic matter, soil pH, soil fertility, and microbial activity (2,3). Many workers reported that low soil pH conditions promoted plant uptake of radionuclides (4,5). Plants also are reported to show some preference in absorbing calcium and potassium (K) relative to strontium (Sr) and caesium (Cs) depending on the fertility conditions of the soil (6,7). Rhizosphere microbes are also often known to effect transfer of radionuclides from soil to plants (8).

Aiming at phytoremediation of soils polluted with radionuclides and optimizing the soil conditions that cause increased plant uptake, we studied uptake of ^{137}Cs and ^{90}Sr by cucumber plants from three different soils and found extremely higher uptake values from a particular soil. An attempt was made to relate this extremely high plant uptake to relevant soil parameters. Individual influence of soil pH, soil fertilization, and root-associated fungi, on the uptake of Cs and Sr by plants was studied and comparative degree of influence exerted by each parameter was observed.

MATERIALS AND METHODS

Soils. Three soil-types were used in this study. Soil-1 is a horticulture soil (Kureha engei baido, Kureha Chemical Industry Co., Tokyo), Soil-2 is collected from a cucumber field of Fukaya, Saitama, and Soil-3 is an unmanaged soil collected from a thickest in RIKEN campus (Wako, Saitama). For sterilization, each soil was autoclaved at 121 °C for 40 min. Texture of each soil has not been determined yet.

Radionuclide treatment and cultivation of plants. Multitracer containing radionuclides of many elements was used as the source of ^{90}Sr. A disk target of Au was irradiated with a 135-MeV/ nucleon ^{12}C, ^{14}N, or ^{16}O beam accelerated by the RIKEN Ring Cyclotron. The Au target containing various kinds of radionuclides produced mainly by target fragmentation was dissolved in aqua regia. The solution was evaporated to dryness and the residue was dissolved in 0.3 M HCl. Gold ions were removed by extraction with ethyl acetate from this solution, leaving the radioactive nuclides as a multitracer solution. Commercially available ^{137}Cs (Amersham International plc, Buckinghamshire, UK) was added to this in appropriate amount and the resulting solution was diluted with distilled water and was used for treating soil.

Cucumber (*Cucumis sativus* L. cv. Suyo, Yamato Seeds Co., Yokohama) plants were grown in plastic pots containing 200 g of each soil treated with ^{137}Cs and ^{90}Sr under the greenhouse conditions. After 30 days of growth, aerial part of each plant was collected and dried at 60 °C. Then the activity in the samples was measured using Ge-detector and the results are expressed as uptake percentage per gram dry weight of plant.

Soil pH measurement. Ten grams of each dried soil was suspended in 20 ml of distilled water and was kept stirring occasionally for 1 hr, and then the pH value of the supernatant was examined by pH meter with a glass electrode.

Fertilizer and potassium treatment. Artificial fertilization of Soil-3 was done by adding 2 g of Hyponex® (Hyponex Co., OH, USA) containing 15% of nitrogen (N), 18% of phosphorus (P), 12% of K, and 0.04% of manganese (Mn) to each pot. For treating soils with only K source, 1 g of potassium chloride was added to each pot.

Isolation and inoculation of soil microbes. For isolation of root associated fungal species, root of cucumber plants grown in each soil was collected. The root surface was briefly sterilized with 70% alcohol for 15 sec, washed with sterilized distilled water, and was placed on potato dextrose agar (PDA) medium containing chloramphenicol (100 μg/ ml) to eliminate bacteria and incubated at 25 °C. After ca. 10 days incubation, colonies of microorganisms grown from the root tissue were separated and maintained on PDA plate medium.

The isolates obtained from the root of cucumber were incubated on potato dextrose broth (PDB) at 25 °C for 10 days and poured onto the soil before seeding cucumber.

Soil pH alteration. For testing the effect of different soil pH conditions and addition of potassium on the plant uptake, an unfertilized sandy soil (Fuji-zuna) collected near Mt. Fuji, Yamanashi was used and uptake of Cs was studied.

To alter pH conditions of Soil-1 as well as Fuji-zuna, 1 M solutions of HCl and NaOH were used. Pots with a hole at the bottom were filled with the soils and the respective solutions were

added slowly. The percolated solution from the bottom of the pot was collected every time and the pH was measured. The process was repeated three to four times until the soil pH was stabilized at 5.3 and 7.4.

RESULTS AND DISCUSSION

The uptake value of Cs and Sr in three soils varied widely, with plants grown in Soil-3 showing the highest uptake (Fig. 1). Cs uptake was about four times higher compared to that of Soil-1 and several times higher to Soil-2. Uptake of Sr also was two to three times higher from Soil-3 compared to those of Soil-1 and 2. This suggests the influence of soil factors on the bioavailability of deposited radionuclides. Soil-1 which was a commercial soil is expected to be moderately fertilized and Soil-2 collected from a cucumber field was expected to be heavily fertilized, whereas, Soil-3 was an unmanaged forest soil which was largely depleted of nutrients but high in organic matter content with lots of decomposing plant material. The presence of organic matter could potentially inhibit Cs sorption onto soil particles by blocking ion-exchange sites and make the nuclides more bioavailable (2) and poor fertility of the soil also has been reported as a factor increasing the plant uptake (6,7).

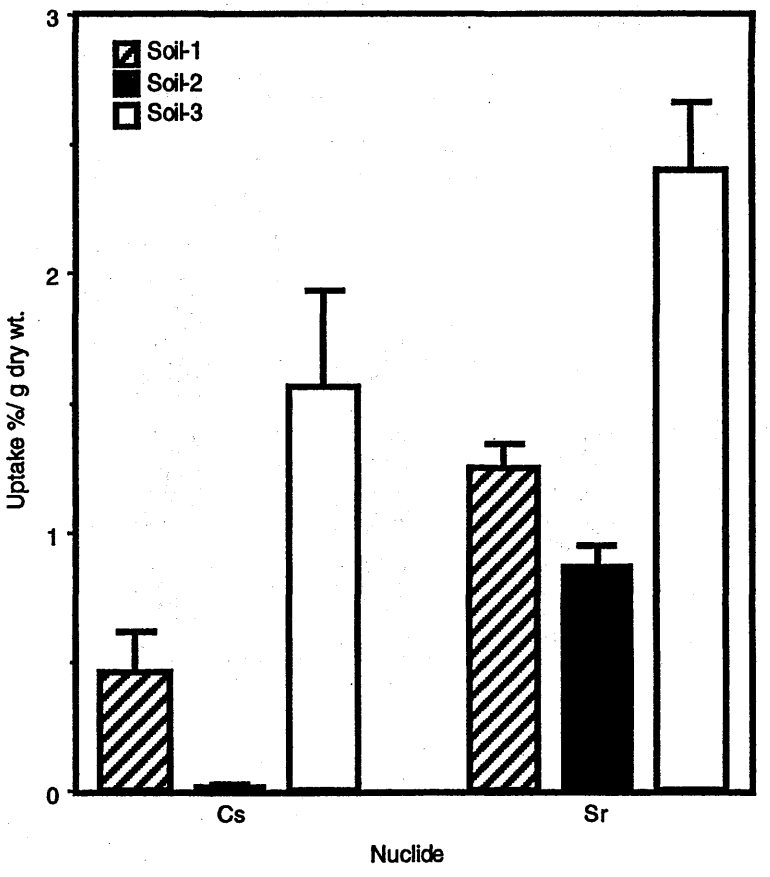

Fig. 1 Uptake of Cs and Sr in three soil-types

Plant uptake is reported to increase with decreasing pH in the case of both Cs and Sr (9). The measured pH for the three soils was 6.29, 5.44, and 6.44 for Soils-1, 2, and 3 respectively. In case of Soil-2, the plant uptake was not high following the lowest pH of the soil, while in case of Soil-3 with higher pH the plant uptake was also higher and thus the high plant uptake observed from Soil-3 can not be explained basing on soil pH factor. We assume that in Soil-2 and 3 whatever effect the soil pH exerted was largely negated by soil fertility factor.

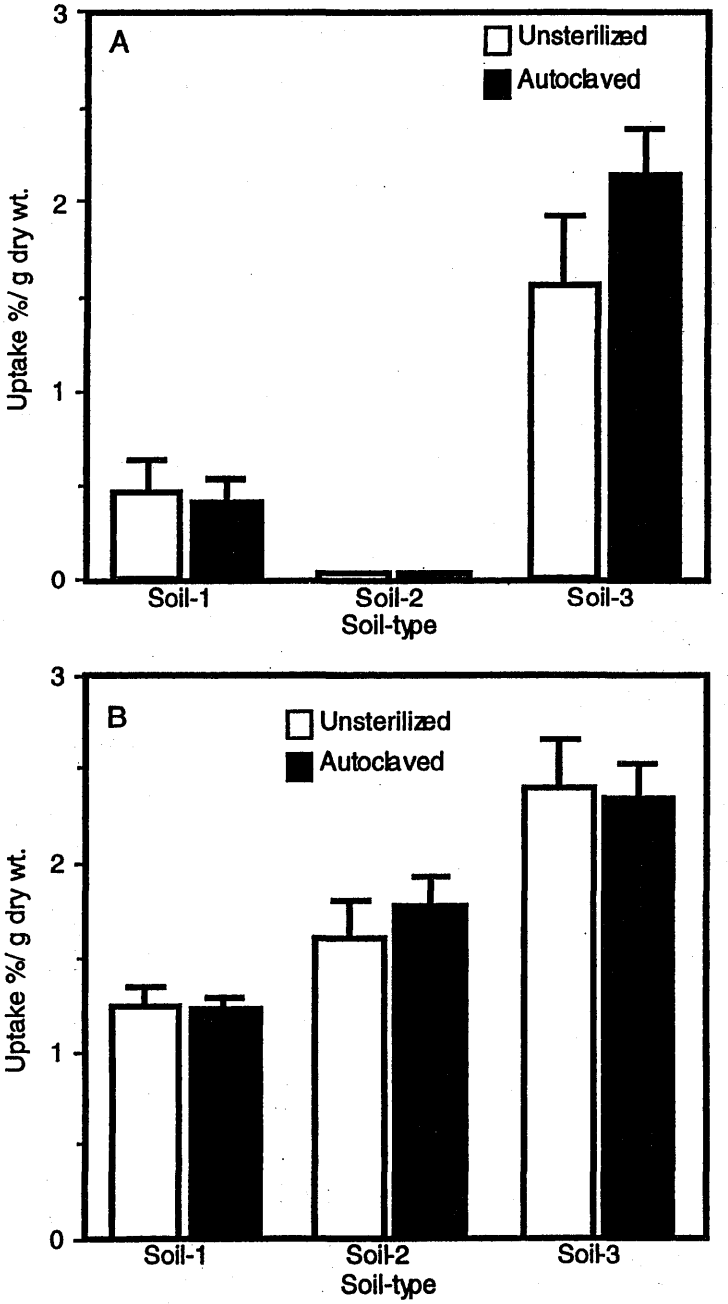

Fig. 2 Effect of soil sterilization on the uptake of Cs (A) and Sr (B) in three soil-types

Another possible factor examined was the influence of soil microbial activity. Primarily sterilization of the soil by autoclaving did not suggest any significant role of microbial activity on

plant uptake (Fig. 2A and 2B). Further, two fungal isolates, FCS2 from the root of cucumber grown in Soil-2 and RCS from Soil-3 were obtained. Occurrence of these species in the root shows a particular affinity to the plant, and thus might have baring on the plant uptake. However, reinoculation of the isolates to cucumber in horticulture soil showed little influence on the uptake of Cs and Sr (Fig. 3). Though the isolate RCS from Soil-3 caused an increased uptake of Cs and Sr, this small increase could not explain the extreme uptake values observed in Soil-3.

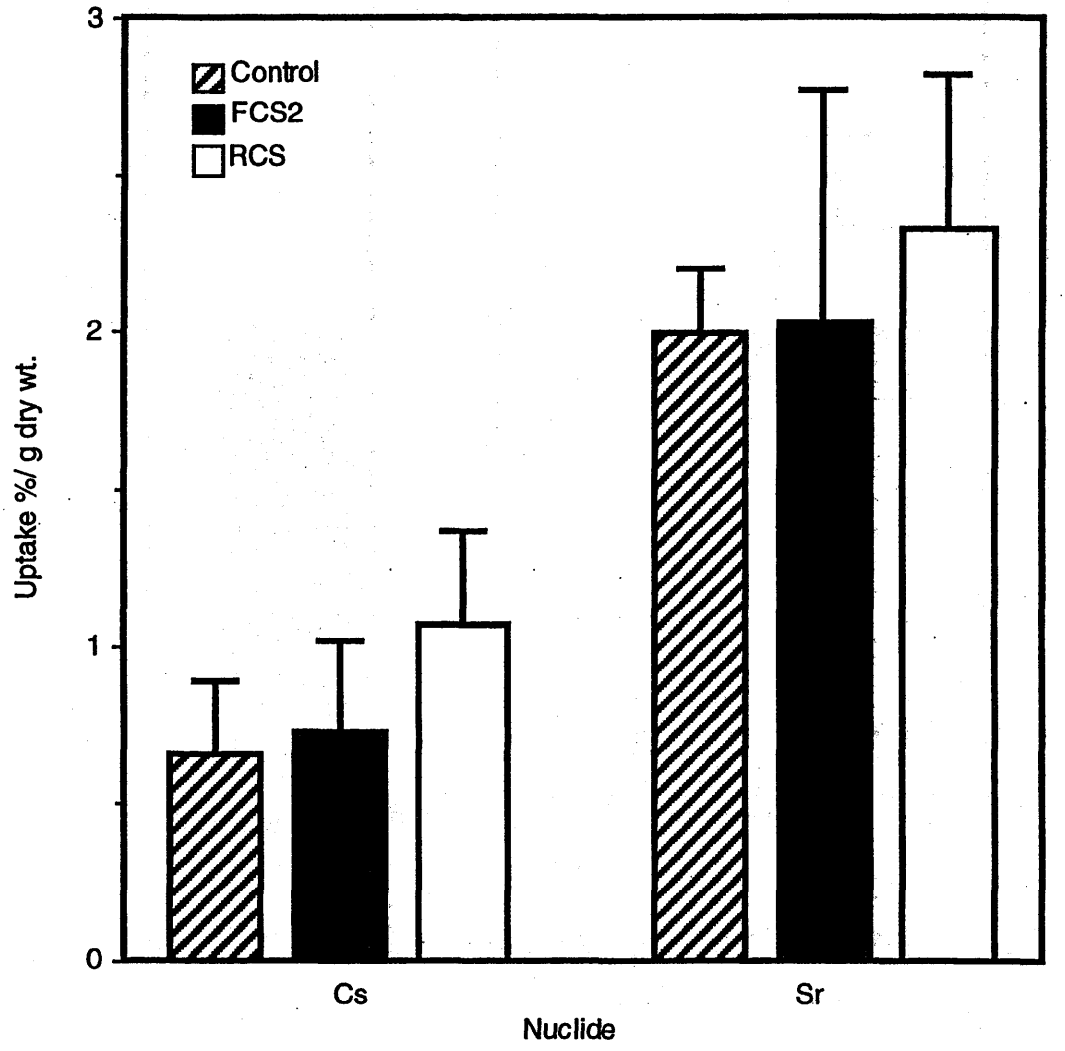

Fig. 3 Uptake of Cs and Sr by cucumber inoculated with soil-microbes (Soil-1)

Addition of fertilizer was examined to explain the high uptake from Soil-3 since the comparative poor growth of plants in this soil also suggested the lack of inorganic nutrient elements. Soil-3 was treated with N-P-K fertilizer and the uptake was examined. Addition of the fertilizer caused the loss of high plant uptake from Soil-3 and the uptake values of plants after fertilization of soil are comparable to those of Soil-1 and 2 (Fig. 4). Effectiveness of common N-P-K fertilizer in decreasing the plant uptake was reported by Jackson and Nisbet (1990) (10).

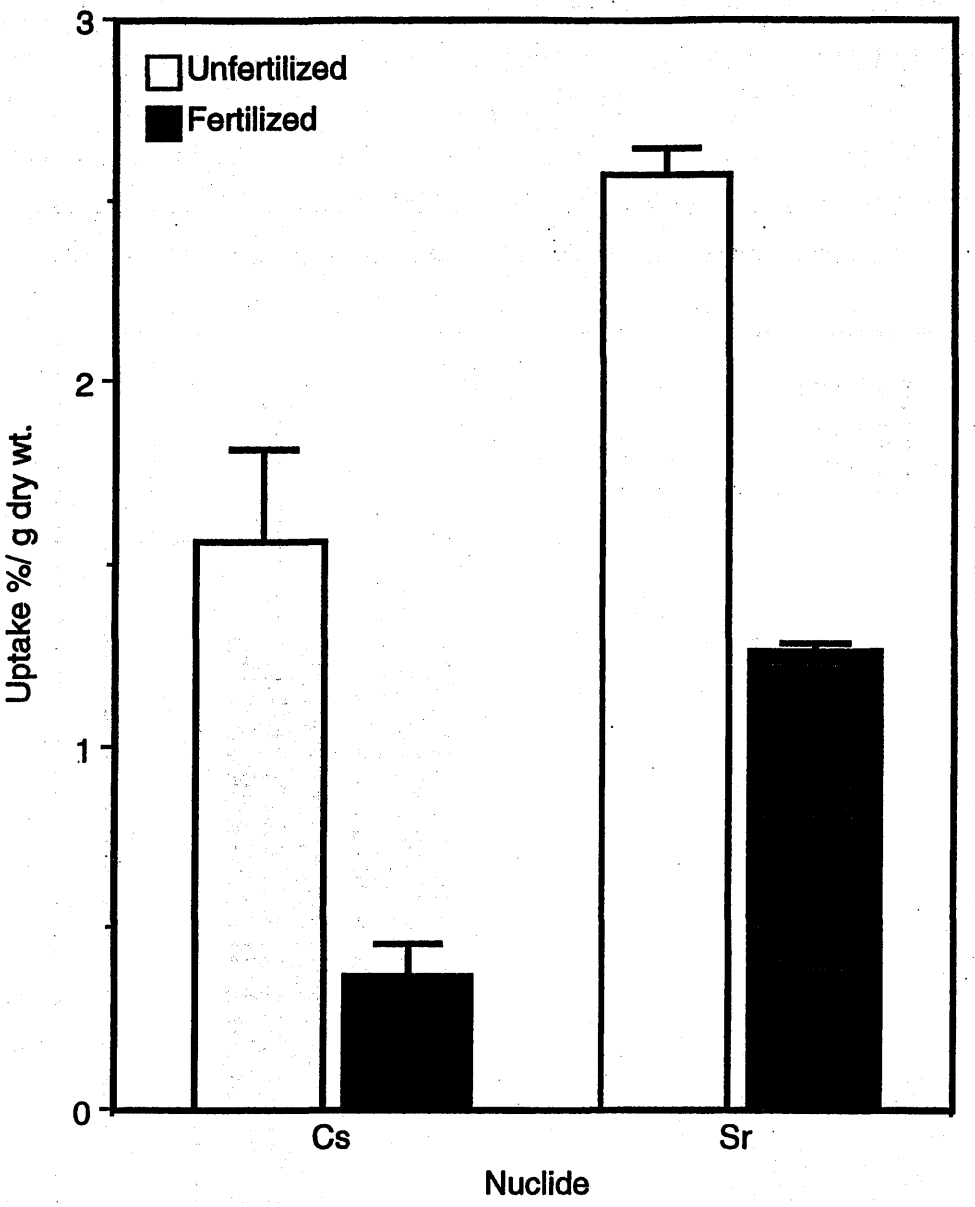

Fig. 4 Effect of fertilization on plant uptake from Soil-3

To assess the comparative degree of influence exerted by each soil factor, we studied the uptake of Cs by cucumber in horticulture soil and an unmanaged soil (Fuji-zuna) under lower pH, higher pH, and K-enriched conditions (Fig. 5). In both the soils the initial pH was close to 6.5. From the figure, it can be said that lowering the pH in both the soils resulted in a slight increase in plant uptake. While the addition of K to the soil had extreme effect on uptake, especially in the unmanaged soil. High Cs uptake from nutrient deficient unmanaged soil might be because in K-deficient soils, Cs acts as replacement for K to a limited degree (11) and when K is added, plants selectively absorb K and discriminate against Cs. It is also clear from Fig. 5 that the influence exerted by soil nutrient status is far more greater than that of pH.

Investigation to quantitatively relate reduced Cs and Sr uptake by plants to the presence of specific nutrient element added to soil is under progress.

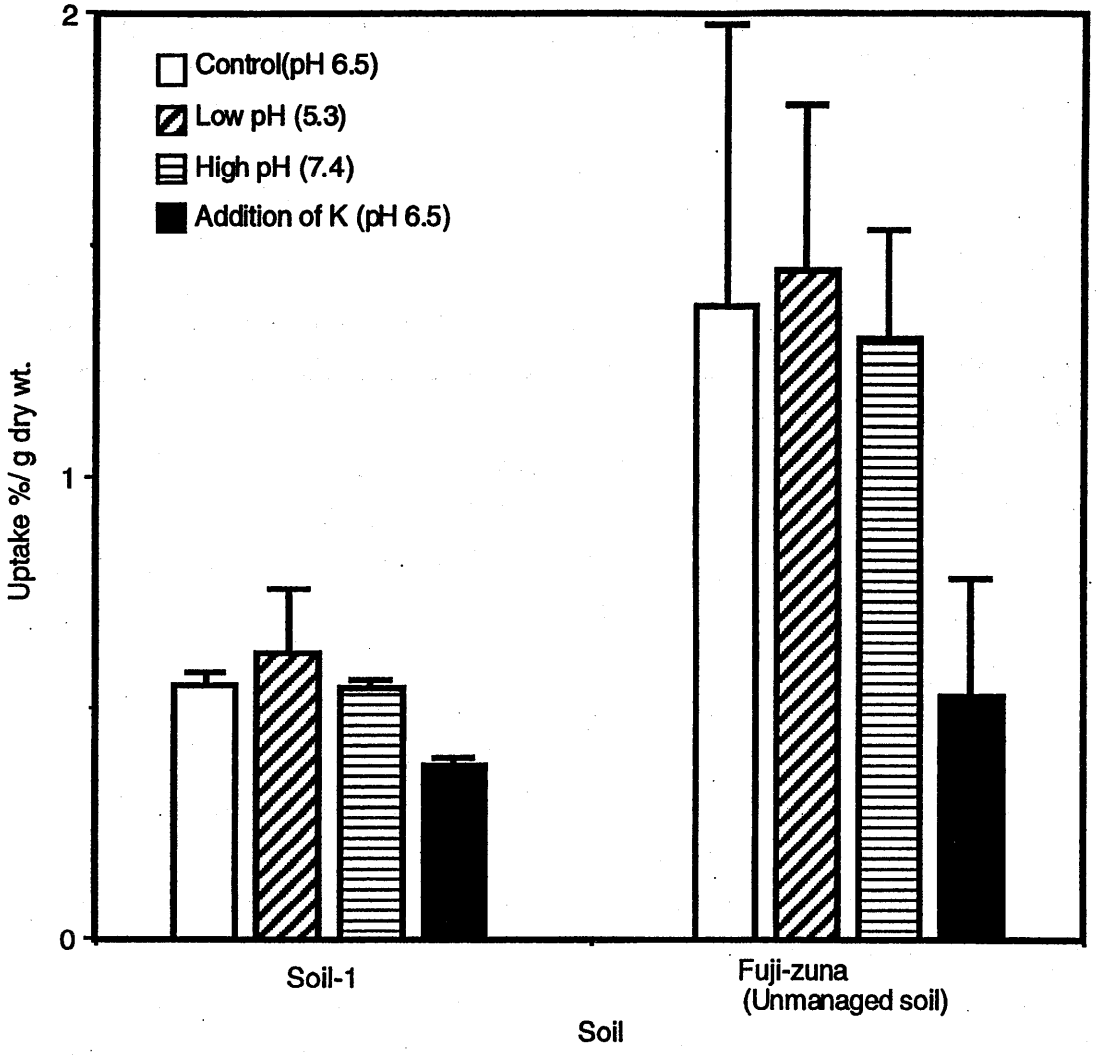

Fig. 5 Influence of soil pH and addition of K on the uptake of Cs by cucumber

CONCLUSIONS

Plant uptake of ^{137}Cs and ^{90}Sr is very high in the unmanaged soil (Soil-3), but treating the soil with common N-P-K fertilizer has reducing effect on the plant uptake. Significant influence of soil pH and microbial activity on plant uptake was not observed. Enrichment of unmanaged soils with K greatly reduced the uptake of Cs by plants.

ACKNOWLEDGEMENTS

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10. Transfer of Essential and Trace Elements from Soil to Agricultural Plants

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ABSTRACT

Essential and trace elements in agricultural plants and soils collected throughout Aomori Prefecture were determined by neutron activation analysis. Mean concentrations of elements in soils had similar values, whereas almost all those in plants had their own distinctive levels for each plant. The concentrations of major elements such as Ca, Cl and K in each species plant were relatively constant independent of levels in their soils. A weak correlation existed between concentrations of K and Cs in plants, while they were strongly correlated in soils. On the other hand, concentrations of Sr were strongly correlated with those of Ca in leaf vegetables. A strong correlation was also observed between mean concentrations of Ca and Sr in several plants. These findings may suggest that the behavior of Sr is closely related with that of Ca in semi-natural ecosystem similarly to previous reported in natural ecosystem.

INTRODUCTION

Radionuclides were released into the environment during past atmospheric nuclear weapons tests and the Chernobyl reactor accident. In addition, nuclear facilities, especially nuclear fuel reprocessing plants, have routinely released small amounts of radionuclides. Therefore, the characteristics of site-specific transfer parameters should be taken into account for precise and realistic dose assessment, because most of the transfer parameters were varied over several orders of magnitude[1]. In order to predict the long-term transfer of radionuclides in the environment, the knowledge of the geochemical and ecological cycle is needed concerning the behavior of not only radionuclides but also related stable elements. Transfer factor, which is the ratio of the concentration of element in plant or mushroom against that in soil, is one of the important parameters used to estimate the internal radiation dose from radionuclides through food ingestion. Komamura and Tsumura reported that transfer factors for ^{90}Sr and ^{137}Cs in polished rice were 3-5 times higher than those for stable Sr and Cs, respectively[2]. Transfer factors for radiocesium (^{134}Cs and ^{137}Cs) in mushrooms in the forest were higher than stable Cs but showed a strong correlation with it as shown in Fig. 1, although the origins of three Cs isotopes were different[3]. Several researchers observed a good correlation in a forest between K and ^{137}Cs in throughfall water[4], K and Cs in spruce needles[5], K and Cs, and Ca and Sr in plants[6]. These findings suggested that the behavior of stable elements related to radionuclides in a forest might be useful to predict the long-term changes of transfer parameters and as an indicator of the behavior of radionuclides in natural ecosystem.

However, there were insufficient data between both essential and trace elements in plants and soils, especially derived from field studies. Although farm fields are semi-natural

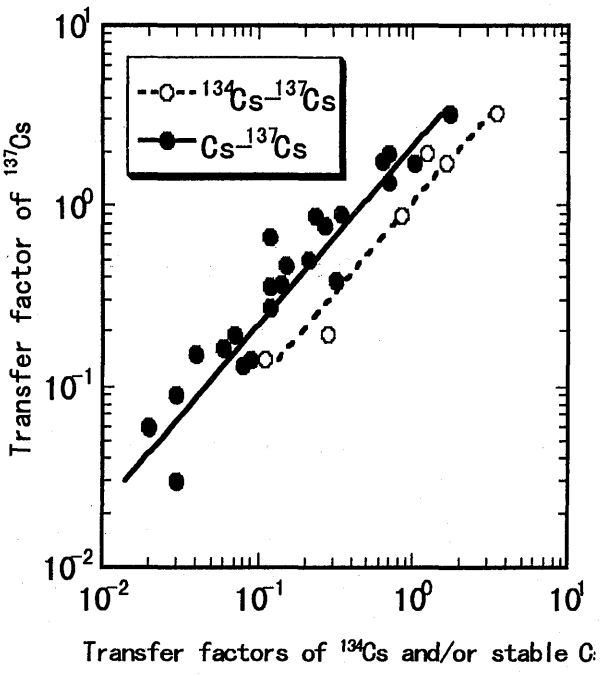

Fig. 1 Comparison among transfer factors of ^{137}Cs, ^{134}Cs and stable Cs in mushrooms

ecosystems, each field has different characteristics as determined by the application of fertilizers (inorganic and organic), pesticides and herbicides, different soil types, cultivation for each plant species and so on. Therefore, it seems that the characteristics of site-specific transfer parameters should be dependent upon the environmental conditions present in each field. In the present study, concentrations of elements in agricultural plants and their respective soils collected from Aomori Prefecture, in which a nuclear fuel reprocessing plant is currently under construction in Rokkasho-mura (40.6°N, 141.2°E), were determined by neutron activation analysis. The distributions and relationships for the essential and trace elements were discussed in each plant species. Agriculture is a very important industry and the fields account for approximately 1700 km^2, which is 18% of the total area of Aomori Prefecture (Fig. 2).

MATERIALS AND METHODS

Agricultural plants such as 62 samples of root crops (26 potato, 14 garlic 13 Japanese radish and 9 carrot samples), 44 samples of fruit vegetables (19 Tomato, 11 cucumber, 8 melon and 6 pumpkin samples) and 14 samples of leaf vegetables (8 cabbage and 6 Chinese cabbage samples), and their respective soils were collected from 120 farm fields throughout Aomori Prefecture (Fig. 2). Approximately 20 kg for each plant sample collected from the farm fields were washed and peeled. The edible parts (500 g for each) were cut into small pieces and then dried at 70°C for analysis. In each field, soil samples were collected from 8 points using a plastic core sampler 15 cm in diameter and 5 cm in depth. The respective soil samples were dried at 60°C after being passed through a 2 mm sieve and then the samples from the 8 soil cores in each field were mixed and pulverized with an agate ball laboratory planetary mill. Approximately 10-100 mg of both plant and soil samples were sealed separately

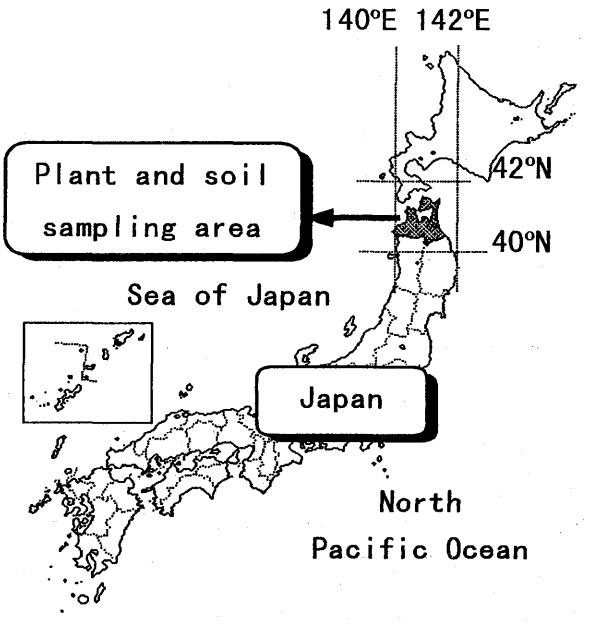

Fig. 2 Sampling areas of agricultural plants and soil

in small polyethylene bags and irradiated in the Rikkyo University TRIGA II and/or Japan Atomic Energy Research Institute JRR-4 reactor. The samples cooled after irradiation and then were counted by means of a Ge detector and the concentrations of elements were determined.

RESULTS AND DISCUSSION

The mean concentrations of elements in the soils for each plant had similar values, even though the cultivated land soils were fertilized with synthetic chemical compounds and natural materials[7]. On the other hand, mean concentrations of most elements in each plant species were respective values indicated in Fig. 3 and Fig. 4. The concentrations of several elements (Ca, Na, Sr, Zn, etc.) in each plant

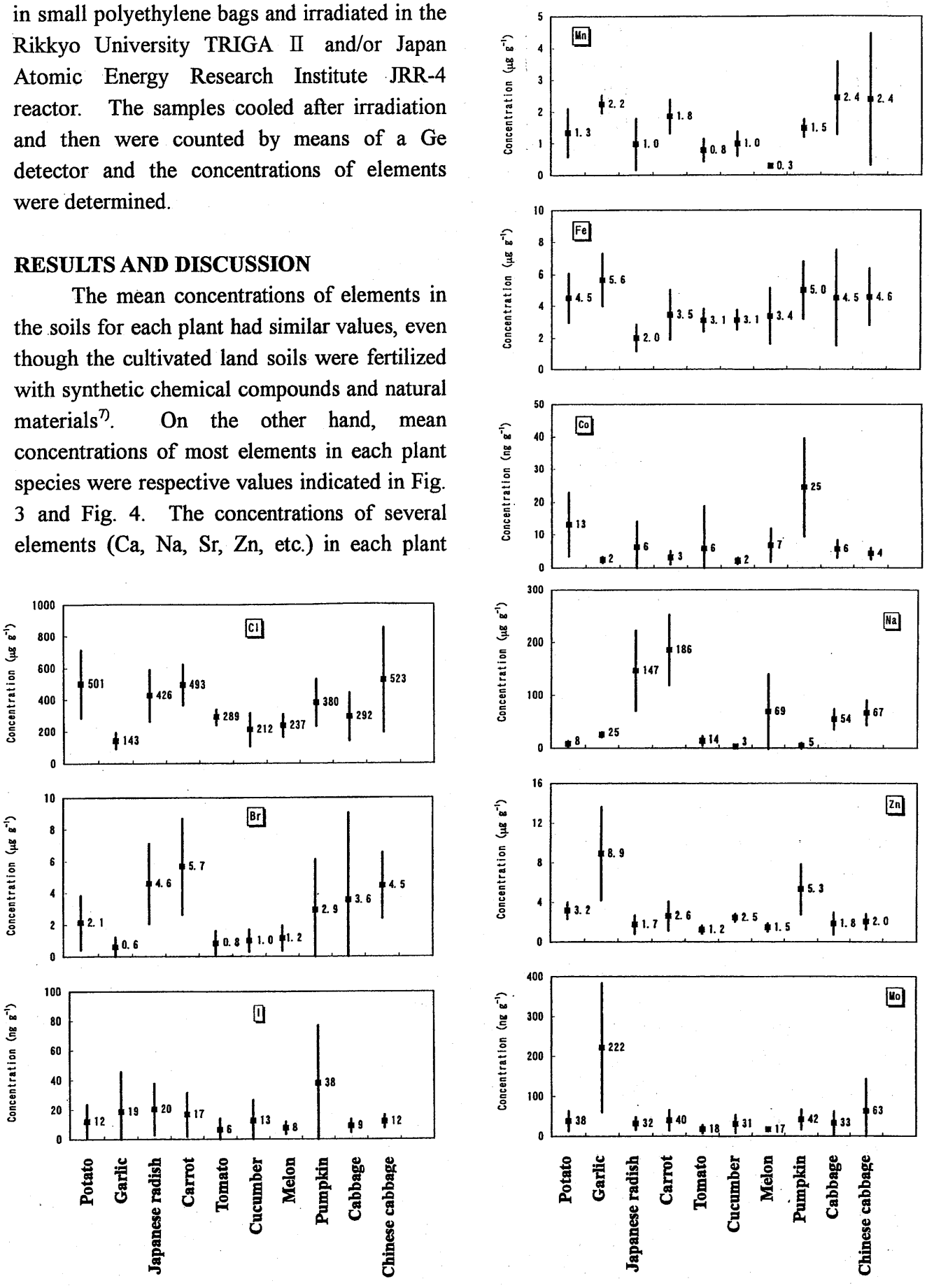

Fig. 3 Means concentration of elements in agricultural plants
Bars indicate one standard deviation

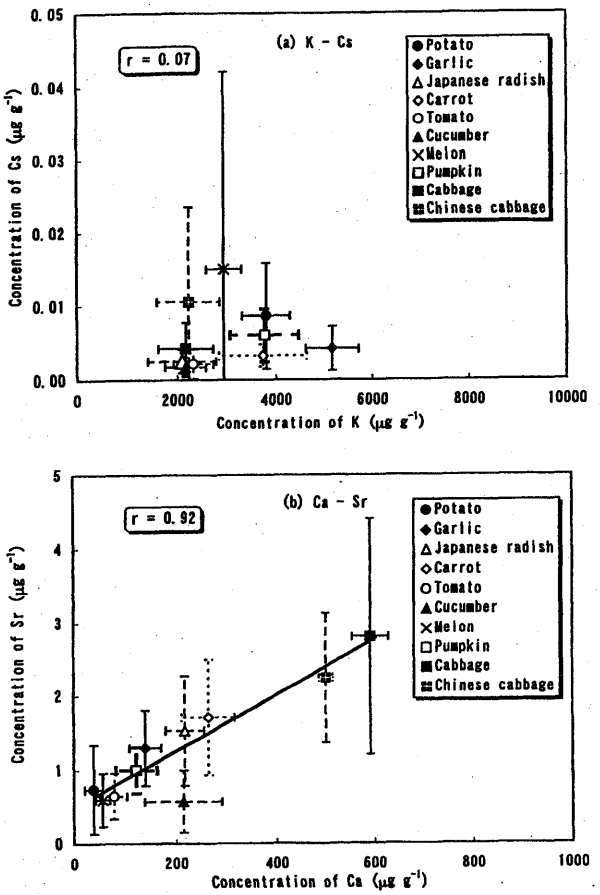

Fig. 4 Relationship among mean concentrations of elements for each plant species Bars indicate one standard deviation

The relationships among mean concentra-tions of essential and trace elements for alkali (K and Cs) and alkali earth elements (Ca and Sr) in several plant species are displayed in Fig. 4 (a) and Fig. 4 (b), respectively. The correlation between mean concentrations of K and Cs in plants was found to be weak, while concentrations of Ca were strongly correlated with those of Sr. In addition, relationships among the relative concentrations (normalized values divided by the concentration of arithmetic mean) of these elements in potatoes, tomatoes and cabbages are plotted in Fig. 5. The correlation between Cs and K in agricultural plants was weak, even though there was a strong correlation between K species had their own distinctive levels and/or those of most major elements (Ca, Cl, K, etc.) in plants were relatively constant, independent of levels in their soils. Almost all these elements were essential elements which are used by plants for growth, maintenance of cells, enzyme activities, photosynthesis, control of osmotic pressure, pH, and so on. Mean concentrations among halogens in plants are in the order of Cl>Br>I. Those of Cl in garlic, Br in garlic, tomato, cucumber and melon were lower than in other plants. Mean concentrations of Fe were relatively constant within a factor of 2. Moreover, concentrations of Co in pumpkin, Na in Japanese radish and carrot, Zn and Mo in garlic, and Ca and Sr in both cabbage and Chinese cabbage were higher than in other plants. These findings suggest that not only essential but also trace elements in plants have their own characteristic concentration.

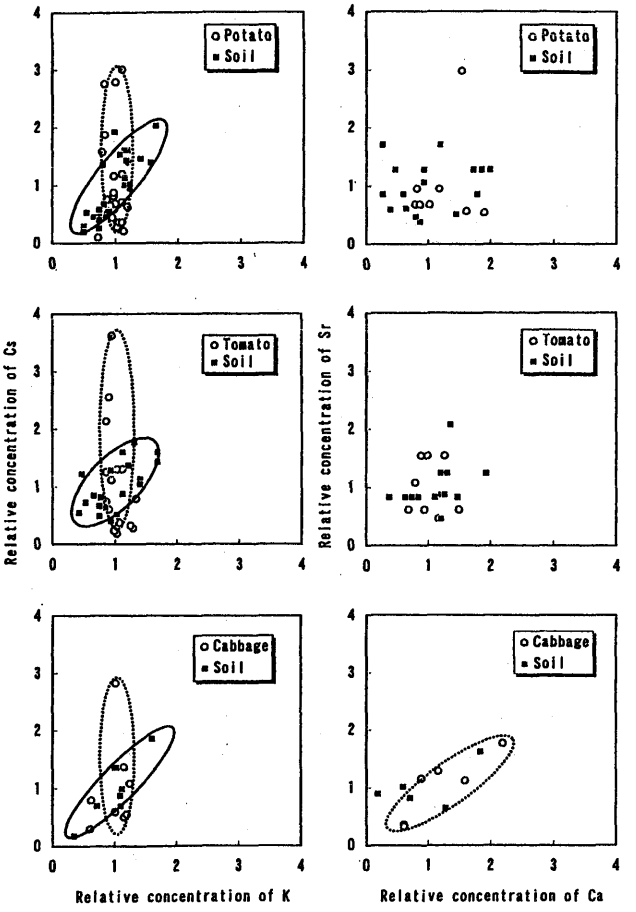

Fig. 5 Relationship among elements in agricultural plants and their soils

and Cs in the soil. There was a strong relationship between K and Cs in natural ecosystems as described previously[5,6], whereas this relationship was not observed in the semi-natural ecosystem. This difference may be due to the presence of adequate nutrients for plant growth in the agricultural soils and/or the fractions of transferable components of K and Cs in the soil. These findings suggest that the behavior of Cs does not follow the transfer of K in agricultural plants. As shown in Fig. 4 (b), the concentrations of Sr in plants are strongly correlated with those of Ca, which is similar to observations in natural ecosystem. Consequently, the behavior of Ca may be used as the indicator of the transfer of Sr in the semi-natural ecosystem.

The relationship between concentrations of elements in soils and the transfer factors was indicated in Fig. 6. The relationships between the concentrations of several elements in the soil and their transfer factors for plants were divided into two groups[8]. The first group of elements, which included Cl, K and Ca, had an inverse correlation between the transfer factor and the elemental concentration in the soil. This suggested that the transfer factors of these elements decreased with increasing concentrations of these elements in the soil. Similar observations for ^{137}Cs and natural radionuclides, which showed a non-linear (inverse) correlation between the transfer factors and concentrations in soils, were reported by Konshin[9], Martínez-Aguirre and García-León[10] and Martínez-Aguirre *et al.*[11]. In the second group, which included Sc and Co, the transfer factors were independent of the elemental concentrations in the soil. Those different transfer factor characteristics were observed for several plants including root crops, fruit vegetables and green vegetables reported in a previous paper[7].

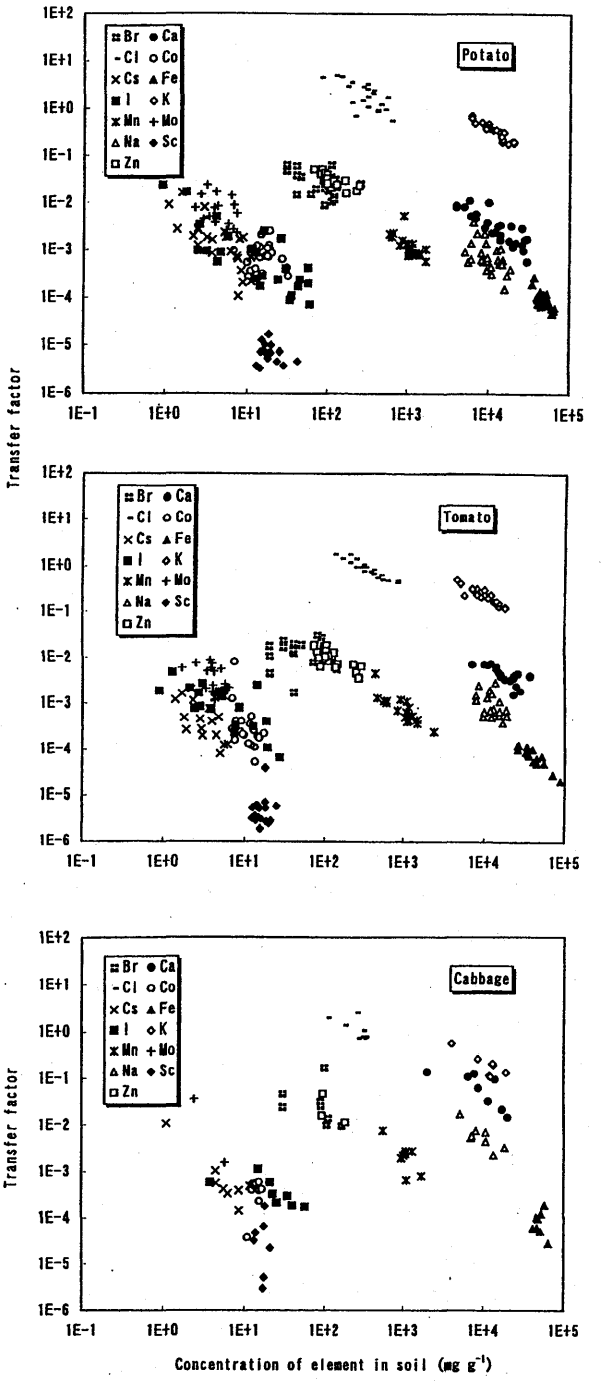

Fig 6. Relationship between concentration of elements in soil and the transfer factor

In order to be able to predict the transfer of radionuclides, using their relationship to related stable elements in the soil-plant system, further studies will be required on their migration and physical-chemical form in soil, their distribution in plant components, the controlling factors of the soil-plant ecosystem and the mechanism of plant physiology.

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11. Studies on the Transfer of Radionuclides from Soils to Vegetables

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ABSTRACT

Transfer factors (TFs) of radionuclides from soil to leaf and root vegetables (cabbage, Chinese cabbage, komatsuna, spinach, lettuce, radish and carrot) have been studied by radiotracer experiments using Andosol as a representative of Japanese soils. The TFs of radioactive Cs, Sr, Co, Mn, Zn and Ce for edible parts of vegetables (average of seven vegetables) were 0.085, 0.20, 0.034, 0.47, 0.40 and 0.0007, respectively. The TFs of Cs for different organs of the leaf vegetables were rather homogeneous. The TFs of Sr and Mn were higher for older (outer) leaves than younger (inner) ones. In contrast to Sr and Mn, transfer factors of Zn for younger leaves were higher than those for older ones. The TFs of Sr, Co, Mn, Zn and Ce for root vegetables were markedly lower than those for leaf vegetables. The TFs for leaf parts of root vegetables were higher than those for root parts, except for TF of Co for radish. The TFs for Cs in Andosol were markedly higher than those in the other soil types. These higher values of Cs in Andosol might be due to its soil property, which is characterized by high organic matter and alophen contents.

INTRODUCTION

For environmental safety assessments in areas where nuclear and other industries are sited, it is necessary to obtain transfer factors (TFs) of radionuclides and other toxic substances from soil to agricultural crops. Cesium-137 and ^{90}Sr are regarded as important fission products which may be released from nuclear industries in an accident. Cobalt-60, ^{54}Mn and ^{65}Zn are also important, because they are produced in nuclear facilities as activation products. Industrial use of rare earth elements is expected to increase in the near future. Many kinds of rare earth elements are separated from the same ore. For example, when 1000 t of Sm, which is used for magnetic materials, are produced, about 40000 t of Ce are produced, too[5]. Because these is little demand of Ce at present, a large amount of Ce has to be managed as industrial discharge. Therefore, for risk assessment it is important to obtain TFs on Ce, which is one of the rare earth elements as well as being able to serve as an analogue of Pu.

Recently, we have performed radiotracer experiments on the uptake of radioactive I and Tc by rice plants[1, 2] and vegetables[3, 4]. The soil-to-plant transfer factors (TFs) of these nuclides in rice grains (hulled) were 0.006 for I and 0.00005 for Tc. However, there is a lack of data on the transfer of other radionuclides such as radioactive Cs, Sr, Mn, Co and Zn from soil to agricultural crops in Japan. Information on TFs for Ce is also not sufficient.

In this study, we performed radiotracer (^{137}Cs, ^{85}Sr; ^{60}Co, ^{54}Mn ^{65}Zn and ^{141}Ce) experiments on (1) transfer of radionuclides to leaf vegetables, (2) transfer of radionuclides to root vegetables and (3) effects of soil types on the transfer factor.

MATERIALS AND METHODS

Preparation of soil and plants

(1) Transfer of radionuclides to leaf vegetables and (2) transfer of radionuclides to root vegetables

Uncultivated Andosol collected in Tokai-mura, Japan, was used in this experiment. The chemical properties of the soil are as follows: pH, 5.4; CEC, 20meq.100 g^{-1} (dry soil); humus content, 7.6%; N content, 0.3%. Radiotracers (^{137}CsCl: half-life = 30 y, 490 kBq pot^{-1}; $^{85}SrCl_2$: half-life = 64.8 d, 390 kBq pot^{-1}; $^{60}CoCl_2$: half-life = 5.27 y, 470 kBq pot^{-1}; $^{54}MnCl_2$: half-life = 312 d, 380 kBq pot^{-1}; $^{65}ZnCl_2$: half-life = 244 d, 860 kBq pot^{-1}; $^{141}CeCl_2$: half-life = 32.5 d, 14 - 40 MBq pot^{-1}) and a mixed chemical fertilizer (5 g/pot, N : P : K = 14 : 10 : 13) were thoroughly mixed with 3.0 kg of the soil, and placed in Wagner pots (surface area: 200 cm^2; volume: 3 *l*). Plant samples chosen for the experiments are common vegetables such as cabbage, Chinese cabbage, komatsuna, spinach, lettuce, radish and carrot. Some details on the cultivation conditions are given in Table 1. Young seedlings (or seeds) were planted in the pots with spiked soil. Two pots were used for each vegetable.

(3) Effects of soil types on the transfer factor

Several different soil types commonly used in the cultivation of vegetables in Japan were used in this experiment. The chemical properties of the soil (10 soil with 6 soil types) are shown in Table 2. Radiotracers (^{137}CsCl: 250 - 430 kBq pot^{-1}; $^{85}SrCl_2$: 100 - 140 kBq pot^{-1}; $^{60}CoCl_2$:1 - 14 MBq pot^{-1}; $^{54}MnCl_2$: 1 - 10 kBq pot^{-1}; $^{65}ZnCl_2$: 1.1 - 10 kBq pot^{-1}) and a mixed chemical fertilizer (1 g pot^{-1}, N : P : K = 14 : 10 : 13) were thoroughly mixed with 0.8 kg of the soil, and placed in pots (volume: 1 *l*). Some details on the cultivation conditions are given in Table 1. Young seedlings of radish were planted in the pots with spiked soil. Two pots were used for each vegetable.

Cultivation and harvest

The pots were placed in growth chambers (Puffer-Hubbard CEC38-15HLE or Koito Koitotoron) and cultivated until harvest. The chamber was operated at 24 - 28°C during the daytime

period (12h) and at 20 - 25°C during the night period (12h). The light intensity at the plant level in the chamber was about 50000 - 70000 lux. Cultivation periods of each vegetable are also given in Table 1.

Table 1. Vegetables chosen for the experiments

Common name	Botanical name	Precultivation period	Cultivation period
Cabbage	*Brassica olracea* L. *var. capitata* L.	25d	60d
Chinese cabbage	*Brassica campestris* L. *var. pekinensis* RUPER.	25d	50d
Komatsuna	*Brassica rapa* L. *var. perviridis* BAIL.	none (seed)	25d
Spinach	*Spinacia oleracea* L.	none (seed)	40-50d
Lettuce	*Lactuca sativa* L.	none (seed)	60d
Radish	*Raphanus sativus* L.	none(seed)	25d
Carrot	*Daucus carota* L.	none(seed)	80d

Table 2. The chemical properties of the soil

Soil type	Collected place	pH	CEC (meq/100g)	organic carbon (%)
Andosols-1	Rokkasho, Aomori	5.82	16.7	3.20
Andosols-2*	Tokai, Ibaraki	5.27	16.6	4.27
Gray Upland soils	Monbetsu, Hokkaido	5.72	18.4	2.43
Yellow soils	Takayama, Gifu	6.51	7.6	1.06
Dark Red soils	Niimi, Okayama	6.29	8.0	1.32
Gray Lowland soils-1	Ureshino, Mie	6.55	7.2	1.64
Andosols-3	Tokai, Ibaraki	6.27	13.6	3.98
Cumulic Non-allophanic Andosols	Kawasaki, Kanagwa	6.71	16.6	3.40
Gley soils	Yamagata, Yamagata	5.98	22.6	2.84
Gray Lowland soils-2	Toyama, Toyama	6.15	6.9	1.36

* This soil was used in experiments (1) and (2).

After the plants were harvested, they were divided into organ parts (e.g. leaf, stem, root). In order to examine the differences in the transfer factors (TFs) by leaf age, leaves of various positions (from inner to outer) were also taken. Root vegetables were peeled. Roots and hypocotyls were considered to be "edible parts", because it was difficult to separate them.

Calculation of transfer factors (TFs)

Counting samples was placed in polyethylene vials to measure radioactivities with a Ge-detector. (For counting samples of Ce, an automatic well type NaI scintillation counter was used.) Errors due to counting statistics (1σ) were mainly less than 10%. The radionuclide concentrations in the soil samples used in the experiments were also measured. Decay corrections were made to the beginning of the experiment. The soil-to-plant transfer factor (TF) of a radionuclide is defined as "concentration of the nuclide per unit weight of the plant or plant organ at the time of harvest (Bq g^{-1})" divided by "concentration of the nuclide per unit weight of dry soil (Bq g^{-1}) at the time of planting".

The weighted mean values of TFs of radionuclides for edible parts of each vegetable were calculated from the individual values for leaves. In the TF calculation for edible parts, old leaves (Chinese cabbage: leaf positions lower than 13, cabbage: leaf position lower than 11, spinach and lettuce: a cotyledon) were not taken into account, because these leaves are generally not eaten.

RESULTS

(1) Transfer of radionuclides to leaf vegetables

The transfer factors (TFs) of the radionuclides for edible parts of the leaf vegetables are given in Table 3. In the calculation for the edible parts, weight of the leaf parts was considered. The standard deviations of the values were, in many cases, less than 30%.

Table 3. Transfer factors of radionuclides from soil to leaf vegetables (edible parts) grown on Andosols

Vegetables	Transfer factor (wet basis)					
	Cs	Sr	Co	Mn	Zn	Ce
Cabbage	0.13	0.13	0.013	0.23	0.11	0.0001
Chinese cabbage	0.15	0.15	0.018	0.27	0.34	
Komatsuna	0.057	0.44	0.016	0.30	0.17	
Spinach	0.17	0.30	0.18	1.5	1.7	0.0015
Lettuce	0.055	0.18	0.0048	0.72	0.24	0.0009

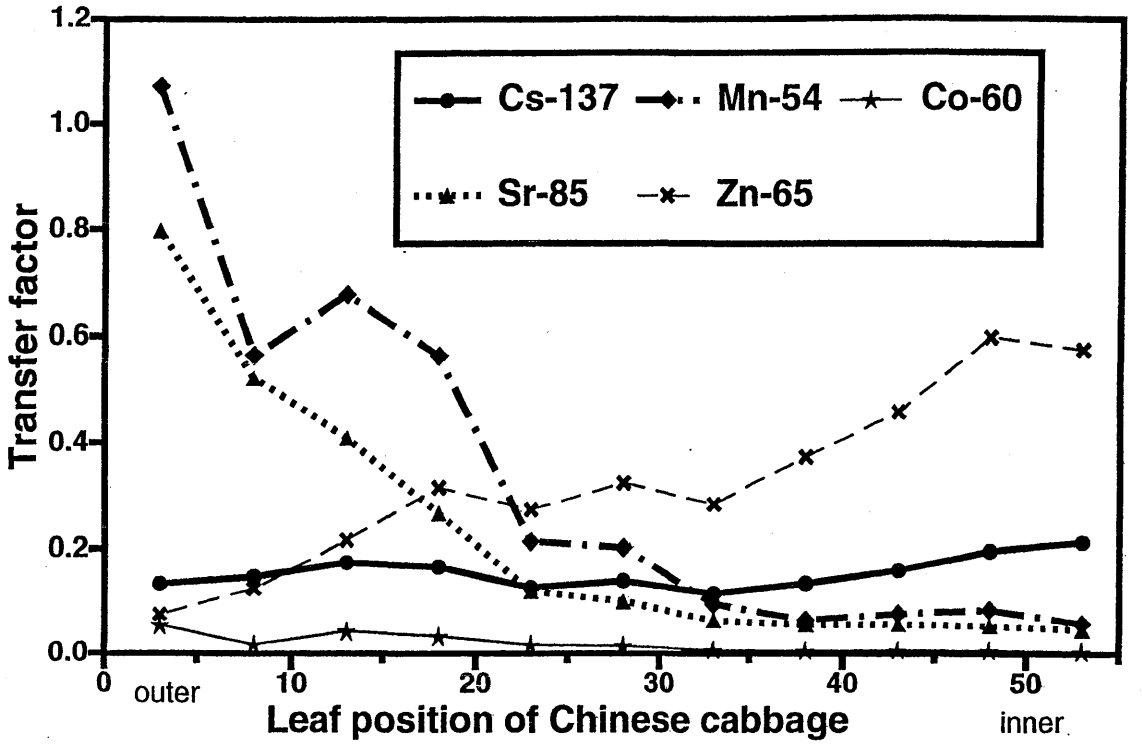

Fig. 1. Transfer factors of radionuclides for different leaf positions of Chinese cabbage

The TFs (on a wet weight basis) were obtained for leaf vegetables (edible parts) grown on Andosol: 0.11 for Cs, 0.24 for Sr, 0.61 for Mn, 0.05 for Co, 0.52 for Zn, Ce for 0.0008. The TFs of Co, Mn, Zn and Ce for spinach were higher than those for other vegetables.

Fig. 1 shows the relationship between TFs of the radionuclides and the leaf position (i.e. age of leaf) for Chinese cabbage.

(2) Transfer of radionuclides to root vegetables

The transfer factors (TFs) of the radionuclides for root and leaf parts of the root vegetables are given in Table 4. The average TFs (on a wet weight basis) of Cs, Sr, Co, Mn and Zn for edible parts of root vegetables (radish and carrot) were 0.17, 0.09, 0.005, 0.14 and 0.13, respectively. The TFs of Mn and Zn for carrot were higher than those for radish, although the TFs of Cs, Co for leaf parts of radish were higher than those for carrot. A very low TF was found for Ce in carrot (0.0002). The TFs of Sr, Co, Mn, Zn and Ce for root vegetables were markedly lower than those for leaf vegetables.

(3) Effects of soil types on the transfer factor

Results obtained for TFs of radish (edible part) in 10 different soils (6 soil types) are shown in Table 5. Geometric mean values of Cs, Sr, Co, Mn and Zn for edible parts of radish 0.008, 0.029, 0.0009, 0.0034 and 0.064, respectively.

It was found that TFs for Cs in Andosols were markedly higher than those in the other soil types.

Table 4. Transfer factors of radionuclides from soil to root vegetables grown on Andosols

	Organ	Transfer factor (wet basis)					
		Cs	Sr	Co	Mn	Zn	Ce
Radish	edible parts	0.020	0.05	0.004	0.02	0.04	
	leaf	0.097	0.78	0.011	0.42	0.12	
Carrot	edible parts	0.014	0.12	0.005	0.23	0.21	0.0002
	leaf	0.039	0.96	0.004	3.0	0.36	0.0045

Table 5 . Transfer factors of radionuclides from various kinds of soils to edible part of radish

	Soils	Transfer factor (wet basis)				
		Cs-137	Sr-85	Co-60	Mn-54	Zn-65
Run-1	Andosols-1	0.032	0.010	0.0015	0.0088	0.020
	Andosols-2	0.033	0.10	0.0026	0.020	0.086
	Gray Upland soils	0.002	0.009	0.0009	0.0021	0.055
	Yellow soils	0.005	0.003	0.0002	0.0007	0.013
	Dark Red soils	0.001	0.011	0.0002	0.0005	0.038
	Gray Lowland soils-1	0.008	0.008	0.0002	0.0008	0.035
Run-2	Andosols-3	0.016	0.065	0.0008	0.0037	0.18
	Cumulic Non-allophanic Andosols	0.003	0.082	0.0025	0.0050	0.086
	Gley soils	0.007	0.18	0.0032	0.0038	0.35
	Gray Lowland soils-2	0.064	0.16	0.0030	0.032	0.15
	Mean value	0.017	0.063	0.0015	0.0076	0.10
	Geometric mean value	0.008	0.029	0.0009	0.0034	0.064

DISCUSSION

1. Transfer factors of radionuclides for leaf vegetables and root vegetables

Transfer factors of Cs

Transfer factors (TFs) of Cs for edible parts of the leaf vegetables were in the range of 0.055 - 0.17. The TFs of Cs for edible parts of the radish and carrot were 0.02 and 0.014, respectively. Mocanu *et al.*[6] reported that the TFs (on a dry weight basis) for white cabbage, lettuce and spinach were 0.029, 0.16 and 0.18, respectively. These values were lower than our values (as calculated on a dry weight basis) which were in the range of 0.5 - 1.1.

The concentrations of Cs in the inner parts (young leaves) of the Chinese cabbage are comparable to those in the outer leaves (old leaves). Nisbet and Shaw[7] reported on the basis of results for peat soil that there appeared to be no discrimination in the distribution of Cs between edible parts (inner hearts) and unpalatable parts (outer leaves) of cabbage in agreement with our results.

The TFs of Cs for root vegetables were lower than TFs for edible parts of the leaf vegetables and TFs for leaf parts of the root vegetables. Mocanu *et al.*[6] reported that the TFs (on a dry weight basis) for leaf vegetables were higher than those for root vegetables (average of TFs for carrot, celery and parsley). The difference in distribution of Cs in the root vegetables might be caused by the dilution of Cs in the root by accumulation of nutrition such as carbohydrates. In contrast to root vegetables, it was not thought that the root of Chinese cabbage has the role of storing nutrition. In our previous report[8], it was shown that the TF of Cs for leaves of Chinese cabbage was comparable to TFs for the stem and root.

Transfer factors of Sr

The transfer factors (TFs) of Sr for edible parts of the leaf vegetables were in the range of 0.13 - 0.44. TFs of Sr for edible parts of the radish and carrot were 0.05 and 0.12, respectively. The TF of Sr for the radish was lower than those for carrot. These values were lower than TFs for leaf parts of the root vegetables.

The concentrations of Sr in younger leaves (inner position) were markedly lower than those in the old leaves (outer position). These trends suggest that Sr taken up through the roots was transported into the leaves and accumulated there, whereas water was transpired from the leaves. The concentrations of Sr in leaves should increase with the age of leaves, because translocation of Sr from the leaves is known to be very small[8]. The lower concentration factors obtained in the inner parts of Chinese cabbage can be explained by the fact that the amount of transpiration from the inner part of the plants forming a tightly packed head should be very small. Our findings agree with Nisbet and Shaw's results[7]. They reported that the Sr distribution for outer leaves was also higher than that for inner hearts of cabbage. And they mentioned that outer leaves are high transpiring tissues and

responsible for most of the xylem transport of Ca ions and their analogues such as Sr. Therefore, accumulation of Sr in outer leaves was higher than in the inner ones.

Transfer factors of Co, Mn and Zn

The transfer factors (TFs) of Co, Mn and Zn for edible parts of the leaf vegetables were in the ranges of 0.005 - 0.2, 0.2 - 1.5 and 0.1 - 1.7, respectively. Average of TFs of Co, Mn and Zn for edible parts of the root vegetables were 0.005, 0.13 and 0.13, respectively. The TFs of Co were lower than those of other nuclides, except for Ce. The TFs of Mn and Zn for radish were lower than those for carrot.

Distribution patterns of Mn in the different parts of leaf vegetables showed a similar tendency to those of Sr. The higher TFs observed for the old leaves indicated that Mn and Sr were transported into the leaves through xylem systems with water and accumulated there, whereas water was evaporated from the leaves. In contrast to Mn and Sr, the TFs of Zn for the Chinese cabbage were higher for younger leaves (inner parts) than for older ones (outer ones). It has also been reported[10] that the concentration of Zn in extracellular fluid from sieve tubes of rice plant was higher than that of Mn, although the concentration of Mn in the whole leaf was higher than that of Zn. It was suggested that Zn was apt to move to the growing young parts in comparison to Mn.

The TFs of Mn, Co and Zn for spinach were higher than those for cabbages, komatsuna and lettuce. Tanaka *et al.*[11] also reported that the Mn concentrations in spinach were higher than cabbage based on their cultivation experiments using culture solution (Mn concentration : < 0.1ppm). These values were comparable to our data.

Transfer factors of Ce

The transfer factors (TFs) of Ce for edible parts of the leaf vegetables were in the range of 0.0001 - 0.0015. The TFs of Ce for edible parts of the carrot were 0.0002. The TFs of Ce were markedly lower than those of other nuclides in this report.

Mitsui and Tensho[12] carried out an experiment on uptake of ^{134}Cs, ^{90}Sr, ^{141}Ce and gross fission products by roots of young wheat. Absorption rates of the shoot were in the order: Sr > Cs > gross fission products >> Ce. Absorption rates of the root part had no difference among these elements. They also reported that Ce taken up by the root is only slightly translocated to shoots. In our data, the TF of Ce for edible parts (root) of carrot was lower than those for leaf parts. This might be caused by peeling off the outer layer and by the dilution of Ce in the root by accumulating of nutrition such as carbohydrate.

Use of rare earth elements in industry is expected to increase. Results obtained for Ce can be used in the assessment of rare earth elements from soil to agricultural crops. In addition to this the TF values for Ce might be used as an analogue of those for Pu, because of the chemical similarity. Nisbet

and Shaw[13] reported equilibrium soil-to-plant concentration ratio for Pu. The values for carrot were estimated by using different soil types; 1.5×10^{-4} for Loam, 1.1×10^{-5} for Peat, 6.3×10^{-5} for Sand. Our value for Ce (0.0002) was comparable to their value for loam.

Comparison with values recommended by IAEA

In Table 6 average values for the transfer factors (TFs) in edible parts of vegetables obtained in this study and wheat[14] are summarized. For comparison, the transfer values recommended by IAEA[15] are also listed.

The TFs for leaf and root vegetables were in the order Mn > Zn > Sr > Cs > Co > Ce. Except for Co, the TFs of the elements for wheat (on dry weight basis) were higher than those for vegetables, This might be due to the high water contents in vegetables (commonly about 80% H_2O) compared to wheat (almost completely dried). TFs for wheat were in the order Zn > Mn > Sr > Cs > Co > Ce. The high Zn value in wheat suggested that this element is necessary in grain.

The mean values of the TFs for vegetables of Sr, Co, Mn and Zn obtained in this study are comparable to the IAEA recommended values. However, our Cs value for leaf vegetables is higher than the IAEA one. This difference might have a relation to the soil properties (see discussion below).

2. Effects of soil types on the transfer factor

It was found through the experiments using 6 soil types that TFs for Cs in Andosols were markedly higher than those in the other soil types (Table 5). The higher values of Cs in Andosol might be due to its soil property, which is characterized by high organic matter and alophen contents[16]. Because these substances adsorb Cs less strongly as compared to clay minerals, the uptake of Cs by plants might be higher.

The TFs for Yellow soils of ^{85}Sr and ^{65}Zn were lower than other soil types. The TFs for Dark Red soils of ^{137}Cs and ^{54}Mn were lower than the other soil types.

CONCLUSIONS

The following statements summarize our radiotracer experiments.

(1) The soil-to-plant transfer factors (TFs) of some selected radionuclides were obtained for leaf vegetables (edible parts) grown on Andosols (as a representative of Japanese soils): 0.11 for Cs, 0.24 for Sr, 0.61 for Mn, 0.05 for Co, 0.52 for Zn. These values should be used as parameters in safety assessment for Japanese agricultural environments. The TFs obtained in this study were comparable to IAEA recommended values, except for Cs.

Table 6. Transfer factors (average) of radionuclides from Andosols to root vegetables, leaf vegetables and wheat

Crops	Transfer factor (wet)					
	Cs	Sr	Co	Mn	Zn	Ce
Root vegetables	0.017	0.09	0.005	0.14	0.13	0.0002
Leaf vegetables*	0.11	0.24	0.05	0.61	0.52	0.0008
Vegetables**	0.085	0.20	0.034	0.47	0.40	0.0007
Wheat (grain)***	0.10	0.24	0.019	2.4	2.9	<0.01
IAEA (1982)	0.03	0.3	0.03	0.5	0.4	

* : Average of TFs for Ce is from the values of cabbage, spinach and lettuce.
** : Average of TFs for Ce is from the values of cabbage, spinach, lettuce and carrot.
*** : Grains were dried in the air.

(2) The distributions of Cs in different organs of the leaf vegetables were rather homogeneous. Strontium and Mn were higher in older (outer) leaves than younger (inner) ones. This indicated that Sr and Mn were transported in fluid into the leaves through the xylem systems. The elements accumulated there, whereas water was transpired from the leaves. In contrast to Sr and Mn, concentrations of Zn in younger leaves were higher than those in older ones.

(3) The TFs of Sr, Co, Mn, Zn and Ce for root vegetables were markedly lower than those for leaf vegetables. The TFs for leaf parts of root vegetables were higher than those for root parts, except for TF of Co for radish.

(4) It was found that TFs for Cs in Andosols were markedly higher than those in the other soil types. The higher values of Cs in Andosols might be due to its soil property, which is characterized by high organic matter and alophen contents. Because these substances adsorb Cs less strongly as compared to clay minerals, the uptake of Cs by plants might be higher.

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12. Transfer of Technetium from Paddy Soil to Rice Seedling

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INTRODUCTION

Technetium-99 has a long half-life (2.1 x 10^5 y) and relatively high fission yield. For nuclear safety assessment, it is necessary to obtain information on the behavior of this nuclide in the soil-plant system. Using common soils in Japan, we have carried out radiotracer experiments on the transfer of Tc from soil to edible parts of crops[1, 2]. In paddy rice, we found the transfer factor of technetium was 0.00005 for Andosol and 0.0006 for Gray lowland soil, respectively[3]. Compared with other cereal crops, these transfer factors were markedly lower than those for wheat (0.03)[4] or upland rice (0.02)[5] grown in a non-flooded condition. Concerning to other aquatic plants, the transfer factor of seri (Oenanthe stolonifera DC.) leaves was 0.4 for Andosol and 0.07 for Gray lowland soil, respectively[6]. The values were also lower than that of other leaf vegetables such as spinach (2.4) or lettuce (5). These results indicated that $^{95m}TcO_4^-$ added to soil was rapidly transformed to an insoluble form due to the reducing condition created by flooding. In the flooded soil, the concentration of ^{95m}Tc in the soil solution decreased with time. During the cultivation of paddy rice, ^{95m}Tc concentration in soil solution collected from Gray lowland soil was consistently higher than in Andosol. This explained the higher transfer factors observed in Gray lowland soil than in Andosol. In the seri cultivation, the ^{95m}Tc concentration in soil solution of Gray lowland soil was also kept higher than that of Andosol. However, the concentration of ^{95m}Tc in the surface water of seri collected from the pot of Andosol was higher than that of Gray lowland soil. The higher concentration of soluble Tc in the surface water of Andosol might cause the higher transfer factor of Tc in seri leaves. This phenomenon cannot be explained by the chemical transformation of $^{95m}TcO_4^-$ observed in the two soils. To understand the cause of these experimental results, soil incubation and uptake experiments by plant were carried out.

MATERIALS AND METHODS

Andosol and Gray lowland soil were used. Andosol was collected in Tokai Village, Ibaraki and Gray lowland soil was collected in Mito City, Ibaraki. Chemical properties of these soils are shown in Table 1. Before use, these soils were air dried and sieved to pass a 1mm mesh. Soil (10 g), deionized water (20 ml), 1800 kBq of $^{95m}TcO_4^-$ and glucose (100 mg) were mixed thoroughly and place in 50 ml vial. The vial was filled with the mixture to the half of

Table 1. Chemical properties of soil used in the experiments

	Gray lowland soil	Andosol
CEC*(me/100g of soil)	10.2	16.6
Total carbon (percent)	2.4	4.3
PH	5.7	5.3

*: Cation exchange capacity

their volume.

To keep similar condition with the surface water of the pots, the surface of the mixture in vials was exposed to the air and allowed to oxygen diffusion. The soil mixture was incubated at room temperature (about 20℃) for 40 days. Water soluble Tc was extracted as follows. After mixing thoroughly by shaking, the samples were centrifuged at 5000 rpm for 20 minutes. The supernatant was filtered with a 0.4 μm filter. The chemical form of soluble ^{95m}Tc was analyzed by gel filtration chromatography (Sephadex G-25; column diameter=1 cm; column length=10 cm; eluents= 0.1N NaCl; flow rate=12ml/h; injection volume=2 ml; fraction size = 1 ml). To detect soluble organic matter in the solution, optical density of the effluent was measured at 254 nm (Tokyo Rikakikai UV-9000). Samples of the supernatant and the fractionated effluent were placed in polyethylene vials and ^{95m}Tc concentrations were measured with an automatic well type sodium iodine scintillation counter (Aloka ARC-300). Decay corrections were made to the beginning of the experiment.

Determination of availability by rice plant

Supernatants obtained from incubation experiments using two soils (about 1 or 2 ml) were diluted with nutrient solution (Kasugai solution[7]) to a volume of 200 ml. As a control, 200 ml of Kasugai solution containing 2 kBq of $^{95m}TcO_4^-$ were prepared. These solutions were placed in glass beakers and shaded with black polyethylene film. Rice plant seedlings (Oryza sativa L. C.V. Kosihikari) were precultivated on a nylon mesh (2mm) using Kasugai solution for 20 days and exposed to the three nutrient solutions mentioned above. During the precultivation and the exposure experiment, temperature in the growth chamber was controlled at 30℃ during the daylight period (14h) and 25℃ during the night. The light intensity at the plant level in the chamber was about 20000 lux. Five plants were used for determination of the availability of Tc for each solution. After 24h exposure, the concentrations of ^{95m}Tc in the leaves and roots of each rice seedling were measured. At harvest, each plant was separated into leaves and roots. Samples of the plant parts and the nutrient solution were placed in polyethylene vials and measured with an automatic well type sodium iodide scintillation counter. Values of the concentration ratio between plant parts and the nutrient solution were calculated as "the activity per unit weight of plant (on a fresh weight basis)" divided by "the activity per unit weight of the nutrient solution". The ^{95m}Tc concentration in the nutrient solution decreased during exposure due to its uptake by plant. Therefore, in calculation of the concentration ratio the average of the initial and final ^{95m}Tc concentrations in nutrient solution have been used.

RESULTS AND DISCUSSION

Chemical form of ^{95m}Tc in soluble fractions

The concentration and the distribution of ^{95m}Tc in the soluble phase of both soils were shown in Table 2. More than 97% of the ^{95m}Tc in both soils were distributed in the soil solid phase.

Table 2. Concentration and distribution of ^{95m}Tc in the water phases

	Gray lowland soil	Andosol
Concentration (Bq/ml)	15000	1200
*Distribution (%)	2.4	0.2

*: In percent of total.

This can be explained by the transformation of $^{95m}TcO_4^-$ to the insoluble form due to the reducing condition generated by soil microorganisms in the flooded condition. The concentration of the soluble ^{95m}Tc in the Gray lowland soil was higher than that of the Andosol. This tendency agreed with the results obtained in our previous experiments. The chemical forms of 95mTc in these solutions were analyzed by the gel filtration chromatography. The chromatogram of Gray lowland soil and Andosol are shown in Fig. 1 and Fig. 2. In the Gray lowland soil, the ^{95m}Tc peak did not appear at the TcO_4^- position. It was found that the most of the TcO_4^- was changed during the incubation. The peak of ^{95m}Tc corresponded to that of the optical density, which re-presented the amount of soluble organic matter, eluted. These results indicates that most of the soluble ^{95m}Tc in the Gray lowland soil was associated with soluble organic matter. In the soluble fraction of Andosol, $^{95m}TcO_4^-$ was observed at the TcO_4^- position, and the amount of ^{95m}Tc correspond to the change of the optical density were smaller than that of TcO_4^-.

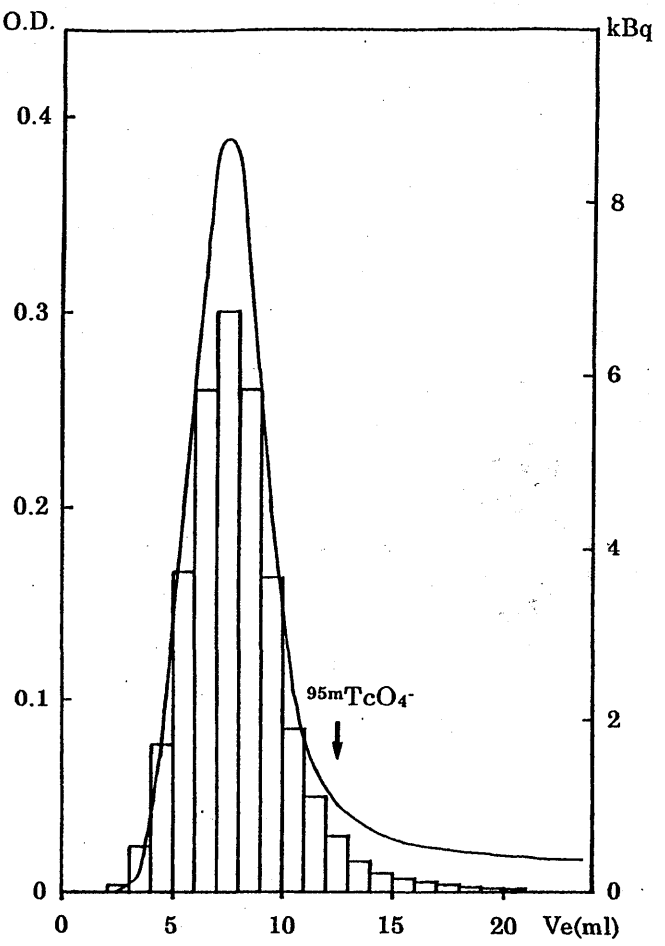

Fig. 1 Gel chromatograms of supernatant isolated from Gray lowland soil

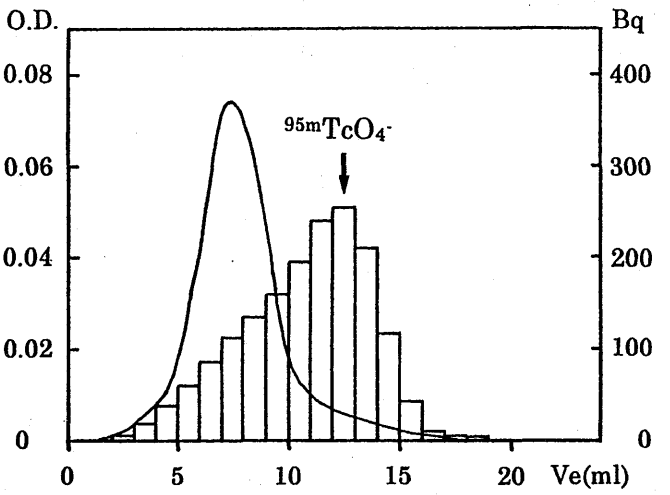

Fig. 2 Gel chromatograms of supernatant isolated from Andosol

In contrast to the soluble fraction of Gray lowland soil, part of the soluble ^{95m}Tc in Andosol was a TcO_4^-. Peaks of the optical density of Andosol (Fig. 2) were significantly lower than those of Gray lowland soil (Fig. 1), indicating the lower concentrations of soluble organic matter in the solution of Andosol. Our previous incubation experiments with these soils showed that the lower production of soluble organic matter in Andosol result in the lower concentration of Tc-organic matter complex than that of the Gray lowland soil. Although this results were obtained under the isolated condition from oxygen diffusion, Tc was not in the form of TcO_4^- in both soils. The result of this experiments observed in Andosol suggested that in the surface water of the pots, the chemical transformation from TcO_4^- to Tc-organic matter complex was lower than that of Gray lowland soil. Production of the Tc-organic matter complex in the surface water of Andosol would be

reduced by the oxygen diffusion and connected to the soil nature. However, production mechanism for the soluble Tc-organic matter complex in the soil solution system remain to be investigated.

Availability of soluble Tc to rice seedling

Plant availability of soluble Tc was examined by the nutrient solution containing soluble fraction of the two soils. In addition to the solutions, a nutrient solution containing $^{95m}TcO_4^-$ (10 Bq/ml) was used as a control. The concentrations of ^{95m}Tc in the nutrient solutions containing soluble fraction of the two soils were 150 Bq/ml for Gray lowland soil and 24 Bq/ml for Andosol, respectively. Table 3 shows the ^{95m}Tc concentration ratios observed in the leaves and the roots of rice seedlings. In the leaves, the concentration ratio were as follows: 3.3 for Gray lowland soil, 12 for Andosol and 15 for control pertechnetate.

Table 3. Concentration ratios for rice plant seedling and nutrient solution after a 24h exposure

Plant parts	Gray lowland soil	Andosol	TcO_4^- (Control)
Leaves	3.3 ± 0.2	12 ± 0.9	15 ± 1.0
Root	11 ± 0.9	8.3 ± 0.2	5.7 ± 0.5

±: Standard deviation for 5 samples

The concentration ratios obtained in the soluble fraction of the Gray lowland soil was lower than that of the Andosol. Since the fraction of Gray lowland soil contains mainly Tc-organic matter complex, the Tc-organic matter complex is less available than pertechnetate. The higher value observed in the Andosol and $^{95m}TcO_4^-$ (control) suggested that the pertechnetate is well available by plant. The order of concentration ratio observed in the root was Gray lowland soil > Andosol > control. This may be caused by the lower transfer of Tc-organic matter complex from root to leaves than that of TcO_4^-. The low concentration ratio in leaves observed in the soluble fraction of Gray lowland soil was caused by the formation of Tc-organic matter complex and the low availability of this form. The above mentioned concentration ratio in rice seedlings may explain the higher transfer factor of ^{95m}Tc in seri leaves grown on Andosol than in Gray lowland soil.

CONCLUSIONS

Experiments on the chemical transformation of TcO_4^- in the surface water of flooded soil system and the availability to plant were conducted using $^{95m}TcO_4^-$ as a tracer. Andosol and Gray lowland soil have been used. The chemical form and availability of soluble Tc formed in the soils were different between the two soils. The chemical form of Tc in the soluble fraction of Gray lowland soil was mainly Tc-organic matter complex, whereas the soluble fraction of Andosol was a mixture of TcO_4^- and Tc-organic matter complex. It was suggested that the soluble Tc-organic matter complex was less available by plant than TcO_4^-.

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13. Some Considerations on the Fate of ^{99}Tc in Paddy Fields

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ABSTRACT

Field observations and radiotracer experiments were carried out to clarify the fate of ^{99}Tc in paddy fields. Seven paddy field soil samples were collected throughout Japan and their global fallout ^{99}Tc concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS). Also, the Tc behaviour under rice paddy field conditions was simulated by radiotracer experiments.

The results of the field observations of global fallout ^{99}Tc indicated that the radionuclide had been accumulating in rice paddy fields. The mechanisms can be explained by the immobilization of Tc in soil under anaerobic conditions. From the radiotracer experiments, it was clear that under waterlogged conditions, the highly mobile TcO_4^- in soil was readily changed to other insoluble physicochemical forms, such as TcO_2, TcS_2 and organically bound forms. When the soil, which was once kept in anaerobic conditions, was air-dried again and kept in aerobic conditions, the chemical forms of immobilized Tc did not change remarkably.

INTRODUCTION

Nuclear energy generates many radioactive wastes containing fission products and management of these wastes is inseparable from the peaceful use of nuclear energy. Technetium-99 is one of the most important long-lived fission products because the nuclide has a long half-life (2.1×10^5 y) and it is produced by fissions of ^{235}U and ^{239}Pu giving about a 6% yield. At present, the ^{99}Tc from nuclear weapons testing in the atmosphere (1945 - 1963) is a main source of the nuclide in the environment. The global inventory of ^{99}Tc has been estimated to be 140 - 160 TBq.[1, 2] When the total fission explosion yield was assumed to be 155 Mt, which was obtained from measured ^{90}Sr depositions,[3] the amount of ^{99}Tc released into the atmosphere could be 130 TBq. In the future, the quantity of ^{99}Tc in the environment may increase due to releases from nuclear power plants, nuclear facilities or nuclear fuel waste disposal vaults. For these reasons, ^{99}Tc in the environment requires special consideration for the environmental dose assessment to humans.

A soil-to-plant system is one of the important paths between released ^{99}Tc and humans. The most stable chemical form of Tc is pertechnetate, TcO_4^-, in oxidizing aqueous solution. The TcO_4^- is considered to be both mobile and bioavailable. In the surface soil environments, however, the fate of the element is influenced by combinations of chemical, physical and biological factors. For example, under reducing conditions, it could be insoluble forms.[4, 5] In rice paddy fields, the reducing conditions are established by waterlogging in the soil during the cultivation period. The soil-rice plant system is an important path for transfer of ^{99}Tc to humans in Japan and other Asian countries because rice is the main crop in these areas.

In this study, we focused on the behavior of ^{99}Tc in paddy fields. Seven paddy soil samples, which had been collected throughout Japan, were subjected to ICP-MS to determine their global fallout ^{99}Tc concentrations. Analysis data of global fallout ^{99}Tc in environmental samples should give useful information for predicting the nuclide behaviour. Cesium-137 concentration in the sample was also measured and, then, the activity ratio of ^{99}Tc/^{137}Cs was calculated to understand Tc mobility in the paddy soil environment. We also have carried out radiotracer experiments to clarify the ^{99}Tc fate in paddy fields.

EXPERIMENTAL

Determination of ^{99}Tc in Japanese paddy field soils

Soil preparation

Seven surface soil samples (0 - 20 cm) were collected in paddy fields. Figure 1 shows the sampling sites. Each sample was air-dried and passed through a 2 mm mesh sieve. Before separating Tc from the soil sample, it was incinerated for 8 h at 450°C to decompose organic matter the presence of which would interfere with Tc separation. For the determination, 300 to 500 g of the incinerated soil samples were used.

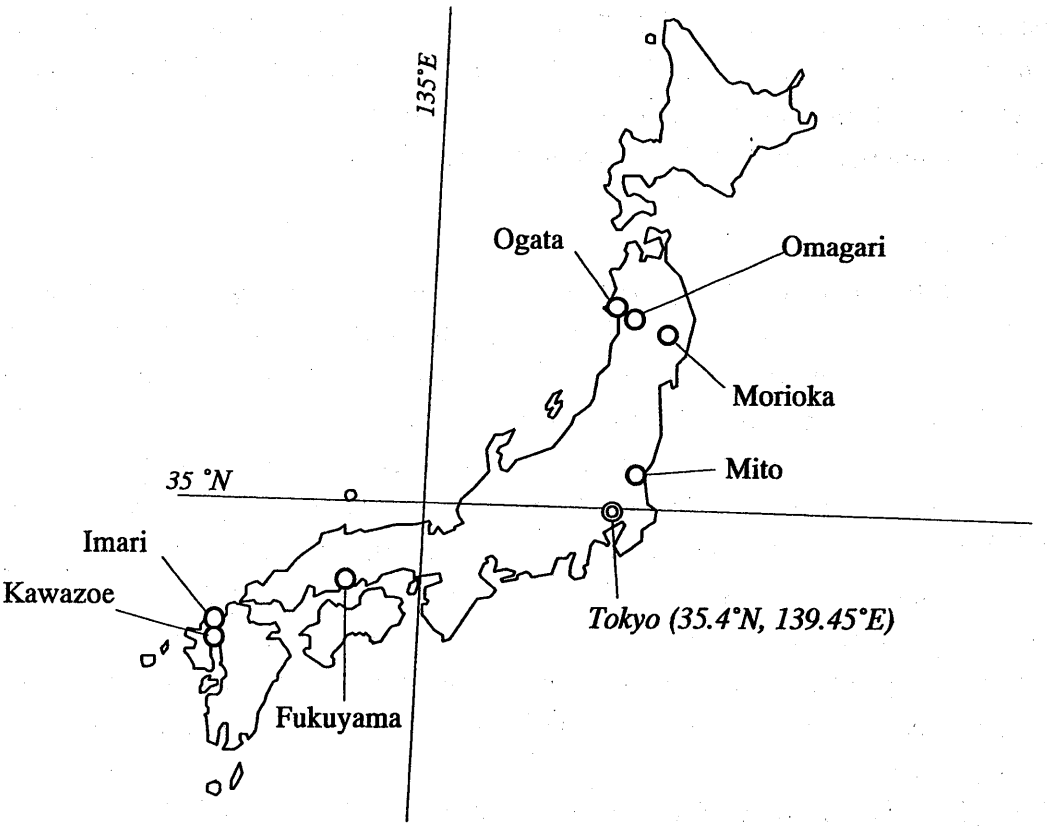

Fig. 1 Soil collection sites in Japan.

Analytical method

A simple chemical separation method was used for ^{99}Tc purification from the soil, and the concentration was measured by ICP-MS. A brief explanation of the method is given here, since it was reported previously.[6, 7]

(1) A carrier-free ^{95m}Tc solution was added to the soil sample as a tracer, then Tc was separated from the sample in a combustion apparatus at 1000°C and the gaseous element was trapped in deionized water (>16MΩ).

(2) After adjusting the solution to 0.1M HNO_3 (Tama Chemicals, AA-100), the solution was passed through a TEVA Spec resin column (Eichrom Ind., Inc). Tc was sorbed onto the resin.

(3) The resin was washed with 2M HNO_3, then Tc was stripped with 5 mL of 8M HNO_3. The eluate was evaporated to dryness at <70°C on a hot plate. The residue was dissolved with 2% HNO_3. Activity of ^{95m}Tc in the solution was measured with a NaI (Tl) scintillation counter (Aloka, ARC-380). After that, the ^{99}Tc concentration was measured by ICP-MS (Yokogawa, PMS-2000). The detection limit for the ICP-MS was 0.05 ppt (0.03 mBq/mL).

The activities of ^{137}Cs in the soil samples were measured by a Ge detector (Seiko EG&G Ortec) coupled with a multi channel analyzer (Seiko EG&G, Model 7800). 100 mL of each soil sample were transferred into a plastic vessel and measured for 80000 s.

Speciation of Tc in a paddy field condition using selective extraction methods

Soil pretreatment

Soil used in this experiment was collected from a paddy field in Omagari City, Akita Prefecture. The soil was classified as Gray lowland soil (GLS), a typical Japanese paddy soil. Some of the characteristics of the soil were: CEC (meq/100 g dry), 18.4; AEC (meq/100 g dry), <0.05; Moisture (%), 3.5; Total C (% dry), 1.87; Total N (% dry), 0.16. The sample was kept at 5°C without air-drying. After passing through a 2 mm mesh sieve, a 120 g aliquot of the soil was weighed into a plastic vessel. 10 mL of $^{95m}TcO_4^-$ solution (Dupont, 300 kBq) were added and the soil was mixed uniformly. After mixing, the soil was saturated with 20 mL of deionized water and kept in a dark place at room temperature for 52 days.

Reagents

The individual use of the four extractants, which were proposed by McLanren and Crawford,[8] was adopted for the Tc speciation in soil to extract the Tc in the following four fractions:

(F1) soil solution and exchangeable fraction - 0.05M $CaCl_2$ (CA);

(F2) fraction specifically adsorbed onto inorganic sites - 0.5M CH_3COOH (AA);

(F3) fraction bounded on organic sites - 0.1M $Na_4P_2O_7$ (PY); and

(F4) fraction occluded by oxide - 0.175M $(COONH_4)_2$ / 0.1M $(COOH)_2$, acid oxalate (AO).

All extractants were prepared from analytical grade reagents (Wako Pure Chemical Industries Ltd.). Deionized water (>16.5 MΩ) was used throughout.

Methods

Following sequential radiotracer experiments, Test-1 and Test-2 were carried out.

Test-1. At 1, 5, 14, 30 and 52 days, ca. 20 g (wet basis) of the saturated soil was transferred into a glass vial and mixed uniformly. Sub-samples, ca. 2 g (air-dried basis) each, were subjected to individual extractions with 40 mL of CA and 40 mL of AA, and sub samples, ca. 1 g each were extracted with 100 mL of PY and 75 mL AO from the 20 g wet basis soil sample.

Test-2. After the 52-day saturation period, the soil sample, ca. 40 g on a wet basis, was air-dried

and kept at room temperature. Single extractions with CA, AA and neutralized 1M CH_3COONH_4, (NH_4Ac) were studied at 1, 6 and 43 days after the air drying of the soil samples. At each sampling time, ca. 2 g (air-dried basis) of each were extracted with 40 mL of each extractant. Conventionally, neutralized ammonium acetate, or NH_4Ac is used for the extraction of plant-available metals in soil.

The solid and solution were mixed at 20°C for 18 h in an end-over-end shaker at 150 rpm and separated by centrifugation at 2000 rpm for 10 min. All extractions were carried out in duplicate. To determine the water content of each sample, 5 to 10 g of soil from each batch test were also transferred into a beaker and oven-dried at 60°C until the weight became constant.

Activities of ^{95m}Tc in each supernatant were measured with the NaI scintillation counter. 5 mL of the supernatant were put into a polyethylene vessel and counted for 20 min.

RESULTS AND DISCUSSION

Concentration of global fallout ^{99}Tc in Japanese paddy field soils

Cataldo *et al.*[9] hypothesized that if ^{99}Tc, that occurred by weapons testing fallout, was uniformly present on the surface of the northern hemisphere, it contributed to a ^{99}Tc soil concentration (to a 25-cm depth) of 0.01 pg/g, that is, 0.0063 Bq/kg. Table 1 lists the results of ^{99}Tc and ^{137}Cs measurements. The ranges of ^{99}Tc and ^{137}Cs concentrations were 0.006 - 0.11 Bq/kg dry and 1.7 - 28.2 Bq/kg dry, respectively. The obtained ^{99}Tc values were usually higher than expected, up to 17 times. Because global fallout radionuclides would not deposit uniformly on the earth's surface,[3] it is difficult to explain the fate of ^{99}Tc in the paddy field environment from only the data of ^{99}Tc concentrations.

Table 1 Concentration of ^{99}Tc and ^{137}Cs in paddy field soils in Japan on a day weight basis and activity ratios of ^{99}Tc to ^{137}Cs.

Collection place	Year	^{99}Tc (mBq/kg)	^{137}Cs (Bq/kg)	$^{99}Tc/^{137}Cs$ ($x10^{-3}$)
Ogata Village, Akita Pref.	1991	110 +/- 30	28.2 +/- 0.8	3.9 +/- 1.0
Omagari City, Akita Pref.	1992	34 +/- 5	16.9 +/- 0.6	2.0 +/- 0.3
Morioka City, Iwate Pref.	1992	52 +/- 10	10.1 +/- 0.6	5.1 +/- 0.1
Mito City, Ibaraki Pref.	1991	8.4 +/- 1.3	3.2 +/- 0.4	2.6 +/- 0.6
Fukuyama City, Hiroshima Pref.	1991	6.1 +/- 0.5	1.7 +/- 0.9	3.6 +/- 1.9
Kawazoe Town, Saga Pref.	1991	22 +/- 3	4.9 +/- 0.5	4.5 +/- 0.8
Imari City, Saga Pref.	1991	88 +/- 15	16.8 +/- 0.7	5.2 +/- 0.9

(Note) +/- : Counting errors in the measurements or statistical errors in calculation.

Then fallout ^{137}Cs was used as an indicator. The activity ratios of $^{99}Tc/^{137}Cs$ in the last column of the table are (2.0 - 5.2) x 10^{-3}. The activity ratio of $^{99}Tc/^{137}Cs$ could be calculated

theoretically from fission as 1.4 x 10^{-4} at the time of nuclear weapons' detonations, and the ratio would now be estimated to be about 3.0 x 10^{-4} with correction of decay out. The measured ratios were one order of magnitude higher than the theoretical one from fission. However, this ratio in soil presumably depends on not only both radionuclides' characteristics in the soil, but also their distributions on the earth's surface. It is difficult to understand the ^{99}Tc behaviour in the terrestrial environment because of the limited numbers of ^{99}Tc data in deposition samples. Our results, at least, lead to a tentative conclusion that more Tc might be fixed on the soil than we had expected before.

Chemical transformation of Tc in the paddy field environment

Previously, we carried out a radiotracer experiment using several soil samples.[10] When the soils were waterlogged with $^{95m}TcO_4^-$ solution and kept at room temperature, relative activity of Tc in the surface solutions of the soils decreased during the experiment. The Tc was thought to be transformed to other insoluble chemical forms in soil under waterlogged conditions. The speciation of Tc in the soil should be helpful in prediction of the environmental distribution of the radionuclide. Thus, the changes of Tc chemical forms in paddy field soil under typical water management were investigated.

Test-1. Fixation patterns of added Tc in soil

Figure 2 shows the Tc fixation pattern in the soil sample under water-logged conditions. The relative activity (Q_{ra}) was defined as "the total activity in extracted solution from unit soil sample at each sampling time" divided by "the total activity of the subjected unit soil sample". The Q_{ra} of CA-Tc was subtracted from each Q_{ra} of PY (PY-Tc) and AO (AO-Tc). The Q_{ra} of CA decreased over time. On the other hand, the Q_{ra} s of PY and AO increased to 0.4 - 0.5 within 30 days and the values were the same until day-52. The same trend in the Tc fixation patterns during waterlogging was also observed by using air-dried soil samples as reported elsewhere.[11] The fixations observed in the present study seemed to have occurred in the organic matter and the sesquioxide surface of the soil.

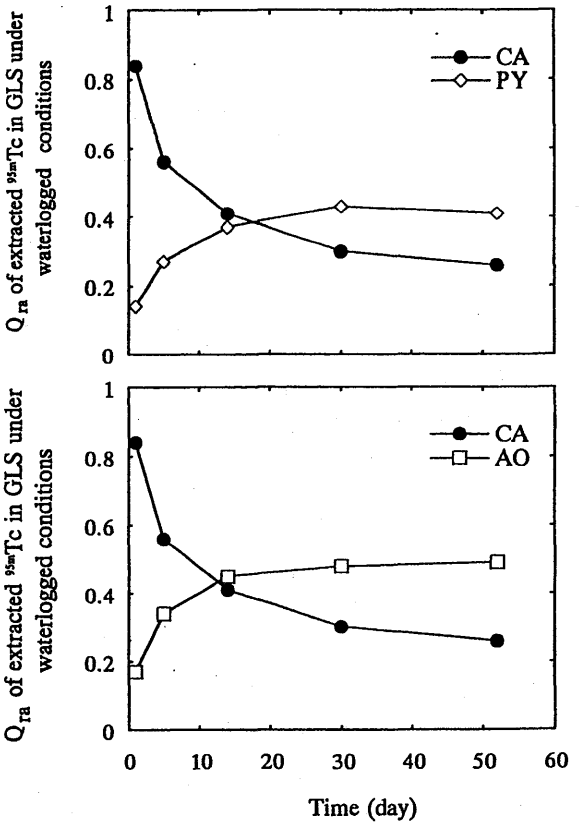

Fig. 2 Time dependence on Q_{ra} of CA-Tc, PY-Tc and AO-Tc in GLS under waterlogged conditions. The Q_{ra} of CA-Tc was subtracted from each Q_{ra} of PY-Tc and AO-Tc.

Test-2. Possibility of change in Tc extractability after air-drying

Paddy fields are not waterlogged all the time; from just before the harvest to the next transplanting, they are air-dried. Thus, in the experiment, air-drying was carried out following 52 days of waterlogging. Figure 3 shows Q_{ra} values for extractions by CA, AA and NH_4Ac as a

function of time. Even though the soil was air-dried and kept in aerobic conditions, Tc extractability was almost the same as the initial value, that is, the value at just the end of the waterlogging period.

The results of the previous[10, 11] and present radiotracer experiments showed the TcO_4^- would change to other insoluble physicochemical forms, such as TcO_2, TcS_2 and organically bound forms. The sulfides might occur because H_2S gas can be produced in rice paddy fields under the waterlogged condition. If all the Tc that adsorbed onto the soil was TcO_2, it could be transformed to TcO_4^- though its rate would be slow.[12] Thus, other chemical forms of Tc which could be stable, even in aerobic conditions, would probably be present. It was reported that in the lower oxidation states, Tc has a strong tendency to form complexes with various ligands.[13] Furthermore, the oxidation state of Tc becomes considerably stabilized on complex formation. This suggested that there was a possibility for stable Tc compounds to exist in waterlogged conditions.

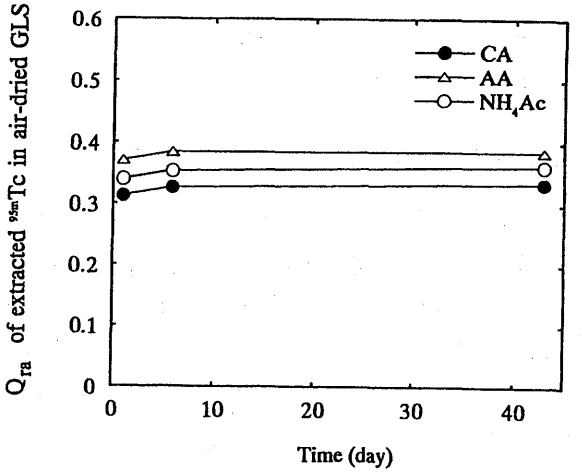

Fig. 3 Time dependence on the amounts of 'readily available Tc' in the air-dried GLS followed by waterlogging.

Note: The soil samples were waterlogged for 52 days then air-dried for 43 days. Each extraction was carried out in duplicate.

From these results, it was assumed that Tc had been accumulating in the surface layer of paddy fields, although the fields were kept under upland conditions from the harvest to the transplanting season.

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14. Comparative Evaluation of Ecological Effects of γ-Radiation and UVC-radiation using an Aquatic Microcosm

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INTRODUCTION

With an increase of energy consumption and an advancement of science and technology, environmental release of various toxic agents is increasing. For an environmental conservation and human sustainable development, it is needed to comparatively evaluate the effect of these toxic agents on natural ecosystem. However, the method to evaluate an ecological effect has not been established yet, because natural ecosystems are extremely complex and many variable factors affect the evaluation. So we have started comparative studies on ecological effects of various toxic agents by using microcosms as an experimental model ecosystem.

Microcosms have some parts of the physical, chemical and biological elements of natural ecosystems. Microcosms also contain interactions among those elements, as do natural ecosystems. Microcosms provide biotic or abiotic simplicity, controllability and replicability[1)]. It has been demonstrated that microcosm can act as fairly realistic models of natural ecosystems[2-4)]. Microcosms have therefore been used for studies of basic ecology or the evaluation of ecological effects of toxic agents at the community level[1)]. In the present study, using an aquatic microcosm we tried to evaluate and compare the ecological effects of γ-radiation and UVC- radiation.

MATERIALS AND METHODS

The microcosm used in this study was synthesized by Kawabata *et al*[5)]. It consisted of flagellate algae *Euglena gracilis* Z as a producer which has chloroplast to do photosynthesis, *Tetrahymena thermophila* B as a consumer which grazes bacteria, and *Escherichia coli* DH5α as a decomposer which decomposes metabolite and breakdown products of the other two species. The culture medium was a half-strength modified #36 Taub and Dollar s salt solution[6)] containing proteose peptone of 500 mgL. The microcosm is cultured in an incubator with fluorescent lamps under a 2500 lux (12-12 hours light and dark light regime) at 25°C.

In the microcosm the change in the population density of each organism reached a steady state about 40 days after inoculation as a result of interactions among the organisms. All organisms can co-exist in the microcosm for as long as one year. In the microcosm at early stage, protease peptone mainly contributes to the growth of *Eu.gracilis* and *E.coli*.

After exhaustion of protease peptone, the microcosm is maintained with energy which *Eu.gracilis* fixes by photosynthesis. Each organism is supported with metabolites or breakdown products of the other two organisms. *T.thermophila* cannot exist without *E.coli* because *T.thermophila* grazes *E.coli* as its staple food[7].

The microcosm in a steady state in which the population density of individual species have been almost on constant level was exposed to γ-radiation or UVC-radiation at various doses. At various time points before and after the exposure, the population density of each organism was measured. The population of *Eu.gracilis* and *T.thermophila* was counted in a 1 mm deep counting chamber of 50×20 mm^2 having a 1 mm^2 grid. The number of viable *E.coli* was measured by counting colonies formed in the broth-agar medium (extract bonito : 3.0g, polypeptone : 3.0g, Nacl : 5.0g, agar powder : 15.0g, distilled water : 1 L, pH adjusted to 7.0) after incubation at 25°C for 5 days.

RESULTS

The results obtained in the present study were graphically summalized in Fig. 1 for γ-radiation and in Fig. 2 for UVC-radiation. In these figures, the changes in the population density of three organisms in the microcosms after the exposure at different doses are shown. In the case of γ-radiation, irradiation at 50 Gy and 100 Gy did not affect the microcosm except temporary decrease of the population of *E.coli* just after exposure. At 500 or 1000 Gy irradiation, *E.coli* died out just after irradiation and the populations of *Eu.gracilis* and *T.thermophila* decreased, though at 1000 Gy *T.thermophila* temporarily increased. Irradiation at 5000 Gy extinguished all species in the microcosm. In the case of UVC-radiation, at 1 kerg/mm^2 *Eu.gracilis* and *T.thermophila* decreased temporarily. At 10 kerg/mm^2 all species decreased temporarily, and later only *Eu.gracilis* decreased again after the temporal decrease. At 50 kerg/mm^2 *Eu.gracilis* and *T.thermophila* died out, while the populations of *E.coli* temporarily decrease and increased, and again deceased. At 100 kerg/mm^2 all species died out.

Thus, the effects observed on the microcosms after exposure to γ-radiation or UVC-radiation were dose-dependent. The results obtained were thought to be caused by not only direct effect but also secondary effect. The decease in the population density of *Eu.gracilis* or *T.thermophila* observed after irradiation at 500 Gy or 1000 Gy should be a result of the extinction of *E.coli* and also a temporary increase of *E.coli* after UVC-irradiation at 50 kerg/mm2 should be a result of extinction of *T.thermophila*.

The results on the microcosm test also suggested that there were some differences in the aspect of the effects between γ-radiation and UVC-radiation. To clarify the differences of the effects between γ-radiation and UVC-radiation, the effects on individual organisms were distinguished into three levels as described in Table 1. That is, the first is the level found the temporal decrease or increase in the population density, the second is the level showed the persistent decease of the population and the third is the level observed the extinction of the organism. When the effect between γ-radiation and UVC-radiation is compared by using these indexes, it can be concluded that *E.coli* is more sensitive to γ-radiation than the other organisms and *Eu.gracilis* is more sensitive to UVC-radiation than others.

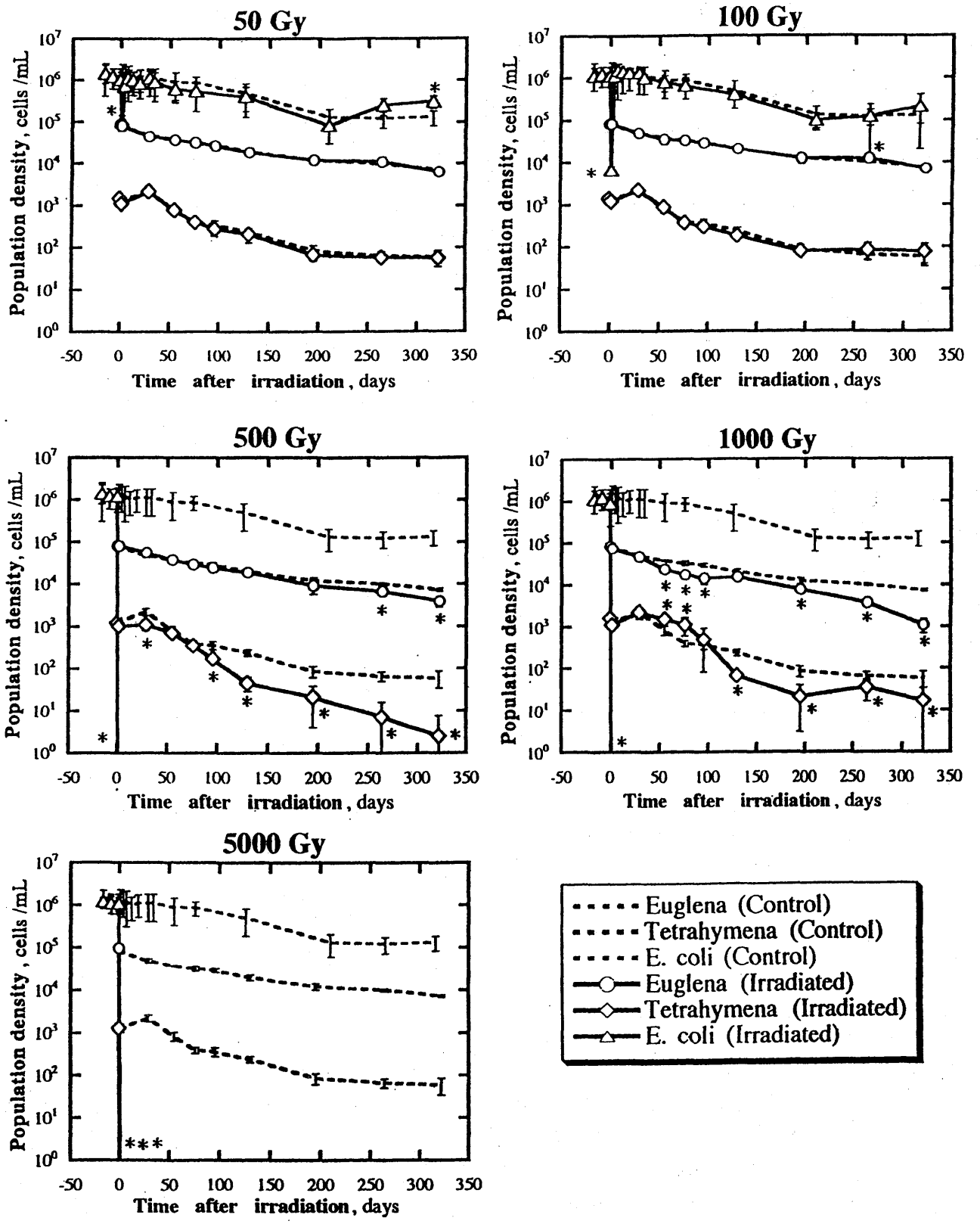

Figure 1. Effects of γ-radiation on microcosm

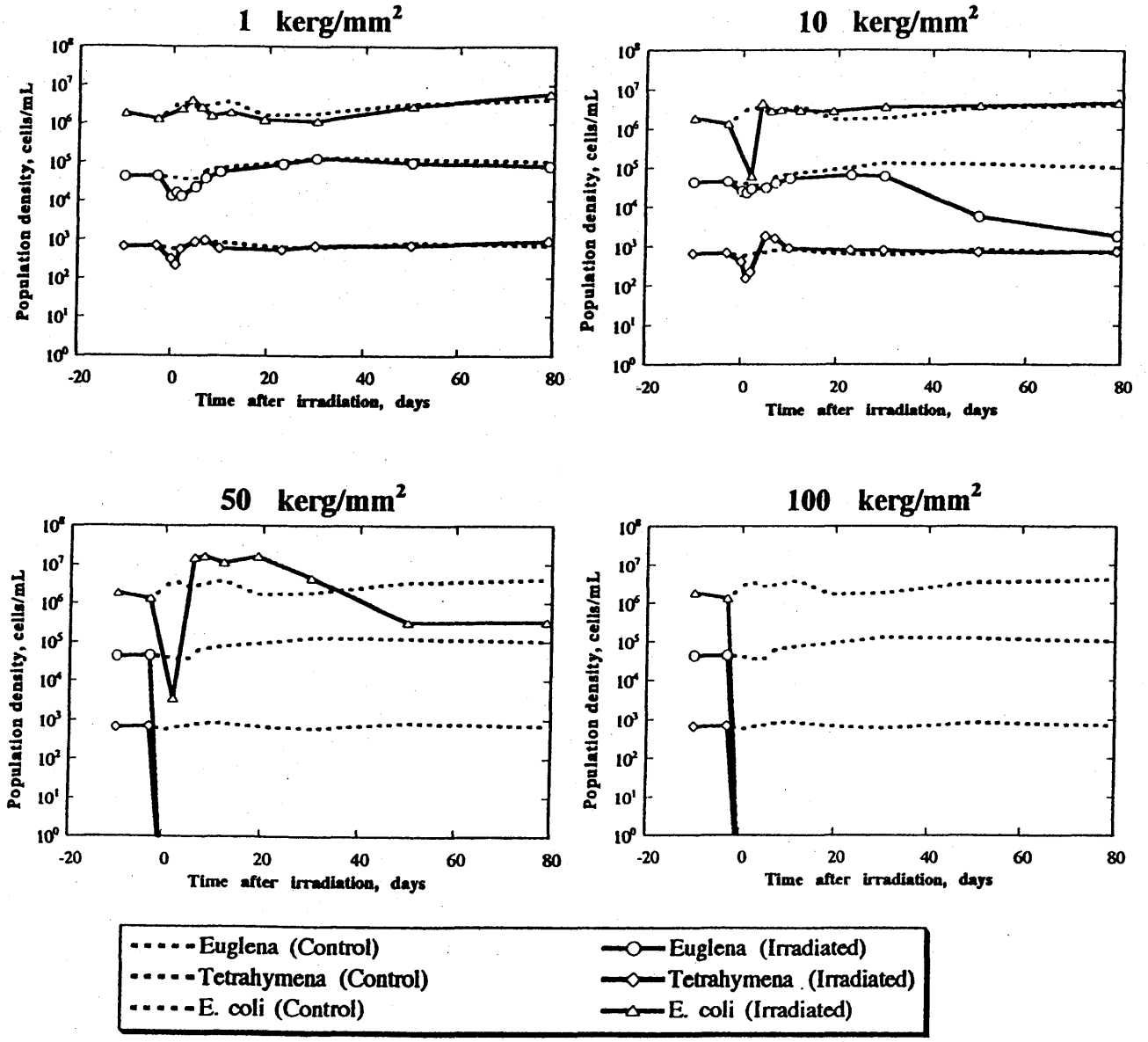

Figure 2. Effects of UVC-radiation on microcosm

DISCUSSION

In the present study, an aquatic microcosm was used as a tool to evaluate and compare ecological effects of γ-radiation and UVC-radiation. The results showed that the effects observed on the microcosm were dependent on the dose of γ-radiation or UVC-radiation, respectively. The aspect of the observed effects was, however, different between γ-radiation and UVC-radiation. The change of population with lower doses of γ-radiation was found in E.coli, while that with lower doses of UVC-radiation was observed in Eu.gracilis. The changes seemed to be caused by the direct effects of γ-radiation or UVC-radiation and should be concluded that among three organisms in the microcosm, *E.coli* was most sensitive to γ-radiation, while *Eu.gracilis* was most sensitive to UVC-radiation.

After the direct effects, the secondary effects were also observed in this microcosm, such as the decrease of *T.thermophila* caused by the extinction of *E.coli* after γ-irradiation at 500 or 1000 Gy and the temporal increase of *E.coli* caused by the extinction of *T.thermophila*

Table 1. Comparison of ecological effects caused by γ-radiation and UVC-radiation

Effect of γ-radiation on microcosm

Constituent species of microcosm	Dose of γ-radiation (Gy)				
	50	100	500	1,000	5,000
Eu. Gracilis Z	○	○	○	▲	×
T.thermophila B	○	○	▲	▲	×
E.coli DH5α	△	△	×	×	×

○ : no effect △ : temporal effect ▲ : persistent effect × : extinction

Effect of UV-radiation on microcosm

Constituent species of microcosm	Dose of UV-radiation (kerg/mm^2)			
	1	10	50	100
Eu.gracilis Z	△	▲	×	×
T.thermophila B	○	△	×	×
E.coli DH5α	○	△	▲	×

○ : no effect △ : temporal effect ▲ : persistent effect × : extinction

after UVC-irradiation at 50 kerg/mm^2. The reason of the secondary effects is considered to be due to the presence of predator-prey interactions between *E.coli* and *T.thermophila*[7].

The results obtained in the present study indicate that the microcosm test is expected to make it possible to examine not only direct effects but also secondary effects, which should be taken into account for an actual evaluation of ecological effects. If we use the result of present study as criteria to compare the ecological effects, it can be concluded that the effects of γ-radiation at 500 Gy or 1000 Gy is equivalent to those of UVC-irradiation at 50 kerg/mm^2.

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15. Effect of Acidification on the Population of Growth Stage Aquatic Microcosm

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INTRODUCTION

Acidification phenomenon of natural ecosystem caused by human activity such as industrial energy production or motorization is recognized as one of the most serious ecological stress. It is necessary to establish a reasonable method to evaluate the ecological effect of acidification comparing with the other environmental toxicants, if human beings want to maintain a sustainable development. As the natural ecosystem consists of various biological species and includes the complex mutual interaction among them, it is not easy to assess any effects on ecosystem from accumulated knowledge of the effect on single species. Experimental studies on model ecosystem might have an ideal possibility, by which we can get valuable information that cannot be induced from either experiments for single species in a laboratory, or observation of complicated phenomena in natural ecosystem. In this study an aquatic microcosm system was adopted as a model ecosystem for ecological assessment of acidification effect. This system is a biological community, which has capability to demonstrate an indirect effect by any ecological stress on the species composing the ecosystem.

MATERIAL AND METHODS

The aquatic microcosm system consists of three species of microorganism in a small container like a test tube or a small plastic bottle. This system was composed by Kawabata *et al.*[1)], and the mutual interaction of materials among the three species incubated in a regular condition has been investigated. This microcosm consists of flagellate algae *Euglena gracilis Z* as a producer which has chloroplast to do photosynthesis, ciliated protozoa *Tetrahymena thermophila* B as a consumer which grazes bacteria, and bacteria *Escherichia coli* DH5 α as a decomposer which decomposes metabolite and dead bodies of the other two species. They can survive by exchanging materials each other in a closed container with limited nutrients at the start of incubation, and their population densities are kept in a steady-state for a long time, usually for more than a year.

An experiment of acidification was carried out as follows: Each microorganism was preincubated following the method of Kawabata *et al.*[1)] Then three species of microorganisms were inoculated into a culture medium (0.05 % protease peptone in half strength of modified Taub and Dollar's solution) in test tubes. The culture medium was in advance acidified to pH 4.0 by adding the volume equivalently mixed solution of 1N nitric acid and 1N sulfuric acid, while the control medium was originally pH 7-8. Population densities of each organism were determined at various time intervals after starting incubation under a 2500 lx and 12-12 hrs. LD light regime at 25 ℃. The population density of *Eu. gracilis* and *T. thermophila* were counted microscopically, and that of *E. coli* was measured by counting colonies formed in the broth-agar medium.

RESULTS AND DISCUSSION

Figs. 1-3 show the time dependent variations in the population densities of three

microorganisms after inoculation in the control microcosm and in the microcosm acidified to pH 4.0, which are compared with the changes in the population densities of each microorganism incubated in the single pure culture.

I. Acidification effect on each three microorganism in the single pure culture

Eu. gracilis (Fig. 1): *Eu. gracilis* in the pH 4.0 single pure culture increased more than that in the control. The population density of *Eu. gracilis* in the steady-state from the twentieth day after inoculated into the pH 4.0 medium reached to 10^5 cells/ml order, while that in the control is 10^4 cells/ml order. This is because *Eu. gracilis* is not stressed by acidification but promoted to increase by addition of nitric acid and sulfuric acid.

T. thermophila (Fig. 2): The population density of *T. thermophila* in the pH 4.0 single pure culture was not so much different from that in the control. Although *T. thermophila* is not stressed by acidification to pH 4.0, it was extinguished after thirty days passed from inoculation, in the same way as the control. Because it cannot survive alone with neither organic matters which were consumed during the thirty days, nor *E. coli* which plays a role of feed for *T. thermophila* .

E. coli (Fig. 3): The population density of *E. coli* in the pH 4.0 single pure culture kept the approximate same order of that at the time of inoculation, which means 10^{-3} times lower than the control in the steady-state. *E. coli* was not extinguished but could not increase in the stressed condition by acidification.

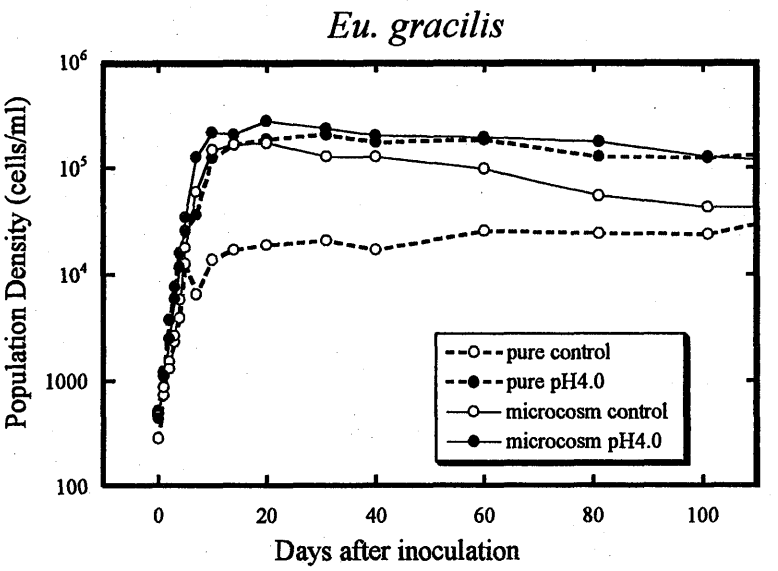

Fig. 1 The changes in the population density of Eu. gracilis in the control and the pH 4.0 single pure culture, and also in the control microcosm and in the pH 4.0 microcosm

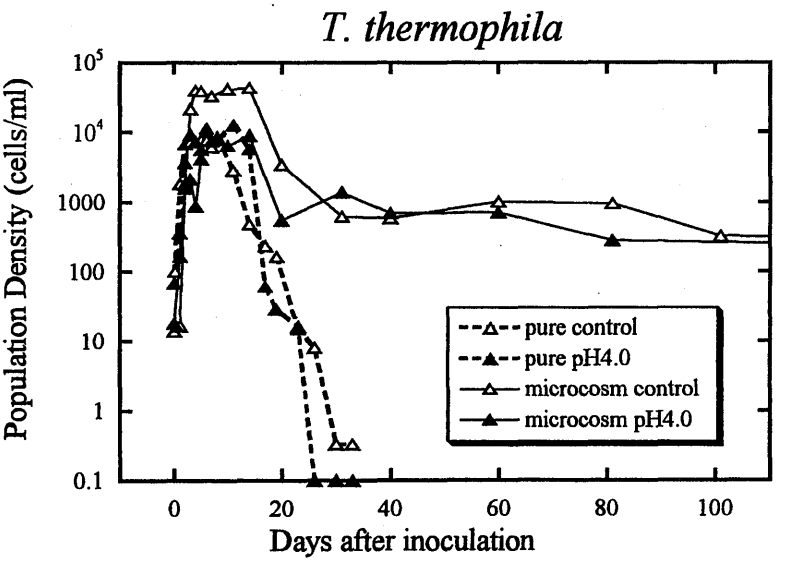

Fig. 2 The changes in the population density of T. thermophila in the control and the pH 4.0 single pure culture, and also in the control microcosm and in the pH 4.0 microcosm.

II. Acidification effect on each three microorganism in the microcosm

Eu. gracilis (Fig. 1): The population density of *Eu. gracilis* in the pH 4.0 microcosm is higher than that in the control microcosm. Consequently, the population density of *Eu. gracilis* in the steady-state was highest in both cases of single pure culture and microcosm system, when the medium was acidified to pH 4.0. It is concluded that *Eu. gracilis* is not stressed by acidification but promoted to increase. It is interesting that the control microcosm system is better condition for

Eu. gracilis to increase and to keep the high population density, compared with the control single pure culture.

T. thermophila (Fig. 2): It is clearly recognized that the population density of *T. thermophila* in the microcosm increased and kept a steady-state of 10^3 cells/ml even after thirty days from the inoculation. This is because *T. thermophila* can survive without protease pepton by grazing *E. coli*. Although the population density of *T. thermophila* was not affected by the acidification to pH 4.0 in the case of single pure culture, the population density of *T. thermophila* in the pH 4.0 microcosm is one order lower than that in the control microcosm in the growth stage until twenty days passed after inoculation. It is considered that the population density of *E. coli*, which plays a role of feed for T. thermophila was not enough during this period, compared with the control, as is described later.

E. coli (Fig. 3): The population density of *E. coli* in the pH 4.0 microcosm decreased and did not increase until seven days passed after inoculation, while in the control microcosm it increased and reached a steady-state of 10^6 cells/ml. Then the population density of *E. coli* in the pH 4.0 microcosm had increased till the tenth day and reached the same order as that of the control. As *E. coli* cannot increase in the pH 4.0 medium in the case of single pure culture, it is considered that the condition in the acidified microcosm changed to be suitable for increasing of *E. coli* until ten days passed, as is described later.

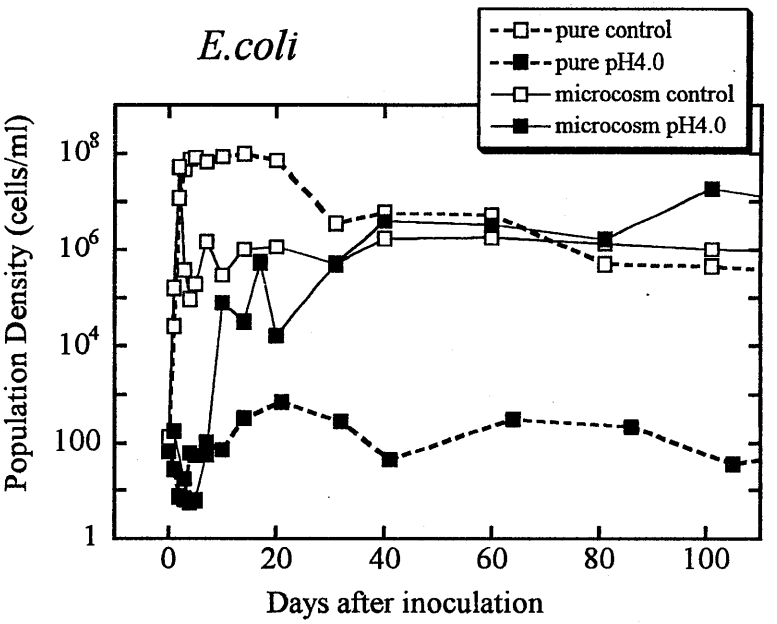

Fig. 3 The changes in the population density of E.coli in the control and the pH 4.0 single pure culture, and also in the control microcosm and in the pH 4.0 microcosm

On the other hand, as mentioned above, the population density of *T. thermophila* , the predator for *E. coli*, in the pH 4.0 microcosm was lower than that in the control in the growth stage. As *E. coli*, the feed for *T. thermophila* , did not increase during this period, *T. thermophila* was inhibited to increase by the low population density of *E. coli*. This phenomenon is recognized to be an indirect effect resulted from interspecific interaction occurred in a model ecosystem, microcosm. *T. thermophila* was affected secondarily by low population density of *E. coli*, which was directly affected by acidification.

III. The change of pH of microcosm

Fig. 4 shows the time dependent variation of pH of microcosm. The pH of the control microcosm increased from 7.8 to 8.2 until ten days passed after inoculation. The pH of the acidified microcosm also increased from 4.0 to 7.1 until ten days passed after inoculation. As the pH was changed to be suitable for increasing of *E. coli*, the population density of *E. coli* reached to the same 10^5 cells/ml order as that of the control on the tenth day after inoculation in the pH 4.0 microcosm. What worked for elevating pH of the microcosm?

Fig. 5 shows the time dependent variation of pH of single pure culture of *Eu. gracilis*. It

was also elevated from 4.0 to 6.5 until ten days passed after inoculation. It is, therefore, concluded that in the pH 4.0 microcosm the pH was elevated by *Eu. gracilis*, and as a result, *E. coli* started increasing. This is thought to be one kind of demonstration of an interspecific interaction occurred in a model ecosystem, microcosm.

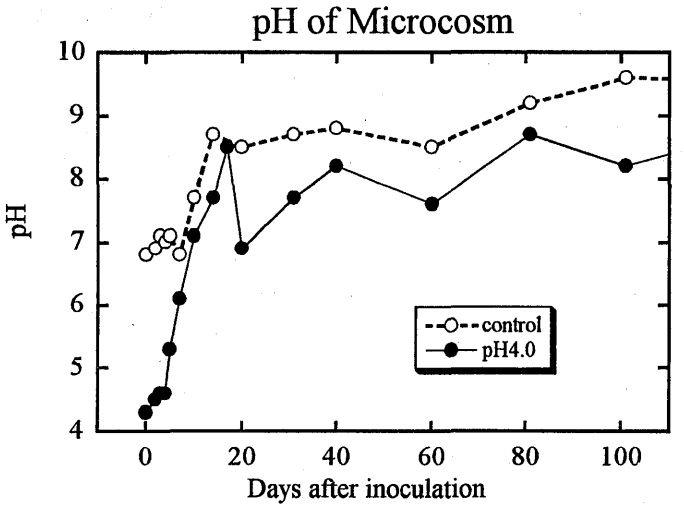

Fig. 4 The change of the pH of microcosm

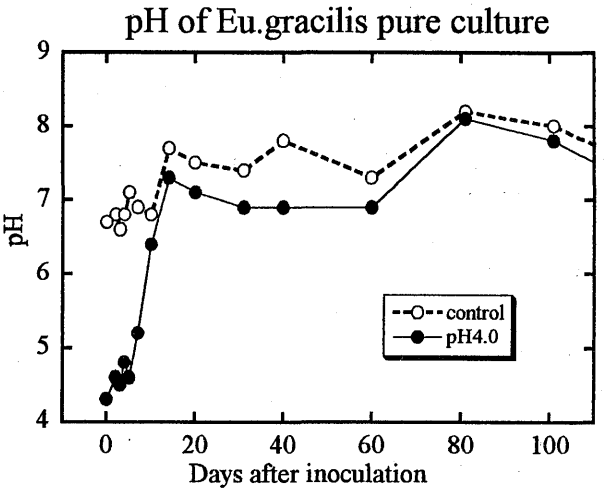

Fig. 5 The change of the pH of single pure culture of Eu. gracilis

CONCLUSION

The present study showed that acidification did not affect on the microcosm, consequently. Although one of the species was directly affected, the other contributed to remediation of the condition of microcosm system. This microcosm system could be expected to be applied to examine other various ecological toxicants, by its advantage of demonstrating indirect effects originated from mutual interaction among the organisms.

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16. Ecotoxicity Assessment of Surfactant on Aquatic Ecosystem Using Microcosm

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ABSTRACT

Microcosm system was applied to assess effect of anionic surfactant (LAS) on aquatic ecosystem. Anionic surfactant such as LAS was added to a flask microcosm consisting of four species of bacteria as decomposer, one species of ciliate protozoa (*Cyclidium glaucoma*), two rotifers (*Philodina* sp. and *Lepadella* sp.) and one aquatic oligochaete (*Aeolosoma hemprichi*) as predator, and a green alga (*Chlorella* sp.) and a filamentous blue-green alga (*Tolypothrix* sp.) as producer, comparing with that of a natural lake model ecosystem derived from natural lake water. In the flask microcosm system and the natural lake model ecosystem, biodegradation rates of LAS were almost same and NOECs (no observed effect concentration) of LAS were also below 1.5 mg · l^{-1}. It was found that flask microcosm test could provide precise ecological effect assessment of LAS on number of microorganisms because the system showed higher reproducibility and stability than natural lake model ecosystem. It was suggested that flask microcosm test was useful ecological effect assessment method which can reflect natural aquatic ecosystem.

INTRODUCTION

Recognition of the importance of toxicity testing at community and ecosystem levels has increased[1-6]. But there is no clear answer to the question of how to predict the effects of chemical agents on an ecosystem. On the other hand, over the last several years, a variety of single-species assays (acute, chronic toxicity test etc.) have developed - using vertebrates, invertebrates, algae, and microorganisms - that are useful in determining the effects of xenobiotics on individual species. Single-species assays were relatively rapid and simple. They can be replicated, and thus statistical measures of certainty are available[7]. Although these assays have been proved to be very useful in protecting a variety of species, no information is available on the impacts of toxicants on many aspects of the dynamics and metabolism of an ecosystem. The goal of a multispecies assay is to model the characteristics of naturally occurring ecosystem that can be reproduced on a smaller scale[8]. Instead of natural ecosystems it is convenient to test microcosm systems consisting of biotic and non-biotic factors originated from a natural ecosystem, because they provide biotic simplicity and replication[9-12]. The microcosm system is comparable with natural aquatic ecosystem at basic functions such as material cycle, energy flow and interaction of microorganisms, etc.[13, 14]. Therefore, the microcosm system is considered as a useful

environmental assessment method with these respects. In this study, the effects of LAS on aquatic ecosystem were examined using a flask microcosm system and a natural lake model ecosystem derived from natural lake water.

MATERIAL AND METHODS

Microcosm description

Microcosm tested in this study is an aquatic stable ecological model derived from natural selection of pond water, and preserved for more than 20 years. It consisted of four species of bacteria *Bacillus cereus*, *Pseudomonas putida*, *Acinetobacter* sp. and Coryneform bacteria as decomposer, ciliate protozoa *Cyclidium glaucoma*, two rotifers *Philodina* sp. and *Lepadella* sp., and aquatic oligochaete *Aeolosoma hemprichi* as predator, a green alga *Chlorella* sp. and a filamentous blue-green alga *Tolypothrix* sp. as producer. The microcosm shows very high reproducibility and reflectivity of natural ecosystem[14, 18]. It had been suggested that this microcosm could be used for screening tests on generic ecosystem-level toxicity[13, 19]. Natural lake model ecosystem used in this study derived from lake Kasumigaura water.

Surfactant

Surfactant tested in this study was LAS, a major anionic surfactant used worldwide. LAS tested was the sodium liner-dodecyl benzene sulfonate standard (C12), above 99% purity (Wako Pure Chemical Industries, Ltd.). Initial concentration series of LAS was adjusted to 0.5, 1.5, 2.5, 5.0 and 10.0mg $\cdot$ l^{-1}.

Cultivation

Flask microcosm system and natural lake model ecosystem were cultivated at 2 5℃ under static condition with illumination of 2,800 lux controlling in a regime of 12 hours light and 12 hours dark. At flask microcosm system, a 300 ml conical flask containing 200 ml of Taub's basal medium[20] was used and the polypeptone was added to the medium with initial concentration of 50 mg $\cdot$ l-1, and the system was sterilized with autoclave. After sterilization, 5 ml of a stock culture microcosm grown for two months was inoculated to the medium. LAS was added to microcosm after 16 days from the start of cultivation, when all microorganisms in the microcosm system had reached the stationary phase. At natural lake model ecosystem, LAS was added to this system at start of cultivation.

Monitoring and sampling

The number of living bacteria was counted by the plate culture method (containing 0.5% polypeptone, 0.3% yeast extract, 0.4% NaCl, and 1.5% agar in distilled water). Other microorganisms were counted with a Fuchs-Rosenthal counting chamber with a microscope. Concentration of LAS was analyzed with HPLC after solid phase extraction by sep-pac C18 (Waters co.).

Evaluation

Evaluation of effect of LAS on bases of population densities was carried out by classify the assessment level into four classes such as assessment A, B, C and D (Fig.1). Assessment A represents no observed effect concentration (NOEC), assessment B, concentration at which system recovers as same with the control, assessment C, concentration at which one or more species of predators, producers and decomposers can survive and assessment D, concentra-tion at which all species of predators, producers or decomposers can not survive.

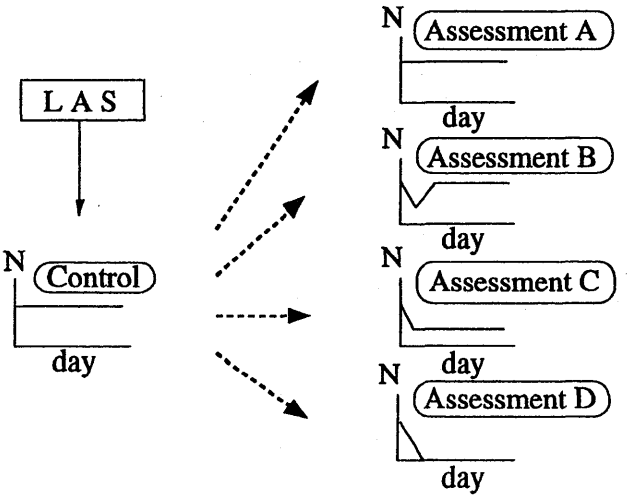

Fig.1 Outline of evaluation method

RESULTS AND DISCUSSION

Characteristics of the two microcosms

Flask microcosm system

When the subculture solution (5ml) was implanted to the fresh medium (200ml), microorganisms reached their respective population peak. After the 16th day, constant population was maintained in all species (Fig.2). The number of microorganisms in the stationary phase were 1.1×10^{6} N · ml^{-1} for bacteria, 6. 0×101 N · ml^{-1} for C. *glaucoma*, 1.0×10^{1} N · ml^{-1} for *Lepadella* sp., 1.1×10^{1} N · ml^{-1} for *Philodina* sp., 6 N · ml^{-1} for *A. hemprichi*, 1.1×10^{5} N · ml^{-1} for *Chlorella* sp., and 8.0×10^{2} cm · ml^{-1} for *Tolypothrix* sp. Even after day 360, no changes were observed in the constituent species and its numbers.

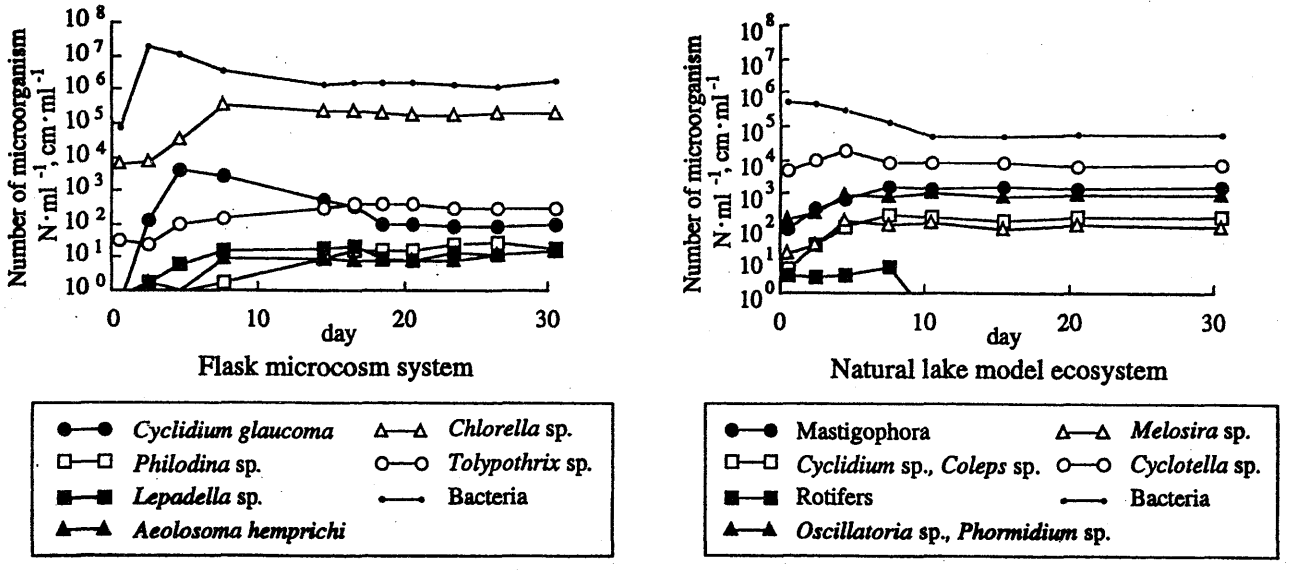

Fig. 2 Change in the number of microorganisms in flask microcosm system and natural lake model ecosystem (control system)

Natural lake model ecosystem

Many microorganisms appearance in the system, for example, *Trithigmostoma* sp., *Coleps* sp., *Cyclidium* sp. and *Vorticella* sp. as protozoa, *Keratella* sp., *Synchaeta* sp. and *Brachionus* sp. as metazoa, *Oscillatoria* sp., *Phormidium* sp., *Raphidiopsis* sp., *Microcystis* sp. and *Anabaena* sp. as blue-green algae, *Melosira* sp., *Cyclotella* sp., *Synedra* sp. and *Nitzschia* sp. as diatoms. The number of dominant microorganisms at the start of cultivation were 4.7×10^{1} N · ml^{-1} for mastigophora, 3.0 N · ml^{-1} for *Cyclidium* sp. and *Coleps* sp., 2.0 N · m l^{-1} for *Keratella* sp., *Synchaeta* sp. and *Brachionus* sp., 9.8×10^{1} cm · ml^{-1} for *Oscillatoria* sp. and *Phormidium* sp., 9.0 cm · ml^{-1} for *Melosira* sp., 2.8×10^{3} N · ml^{-1} for *Cyclotella* sp., 3.0×10^{5} N · ml^{-1} for bacteria. Rotifer disappeared from the control system after 7 days from the start of cultivation, but natural lake model ecosystem was maintained consisting of producer, decomposer and predator for 30 days (Fig. 2)

Biodegradation rates of LAS in the two systems

LAS decreased gradually in flask microcosm system. It took 6 days for decrease of LAS when initial LAS was 1.5 mg · l^{-1}. More than 2.5mg · l^{-1}, it took about 10 days. Time for decreasement became longer with increase in initial LAS concentration. In natural model ecosystem and flask microcosm system, biodegradation rates of LAS were almost the same (Fig. 3). In sterilized water, concentration of LAS did not decreased, which suggested biodegradation of LAS by microorganisms in systems. It was suggested that the flask microcosm system could be used to evaluate the biodegradation rate of LAS in a natural ecosystem.

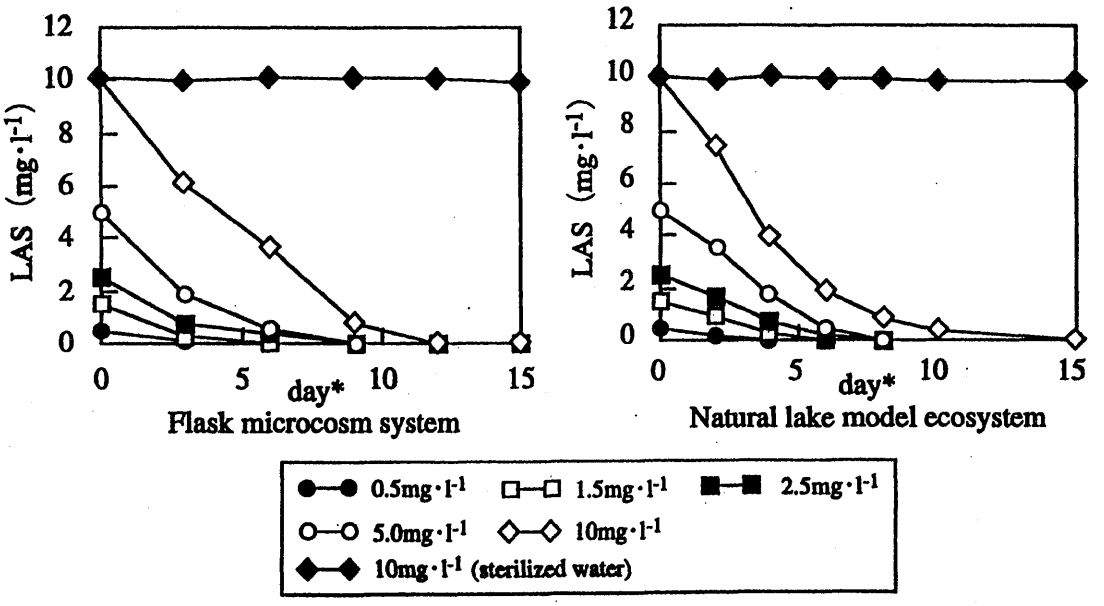

Fig. 3 *Time course of LAS in flask microcosm system and natural lake model ecosystem*
**days after addition of LAS*

Comparison of effect of LAS on microorganisms in the two systems

Flask microcosm system

When initial LAS was 0.5 and 1.5 mg · l^{-1}, changes in number of all microorganisms were

same as those of the control system (Fig. 4). At 2.5 mg · l^{-1}, the number of *C. glaucoma*

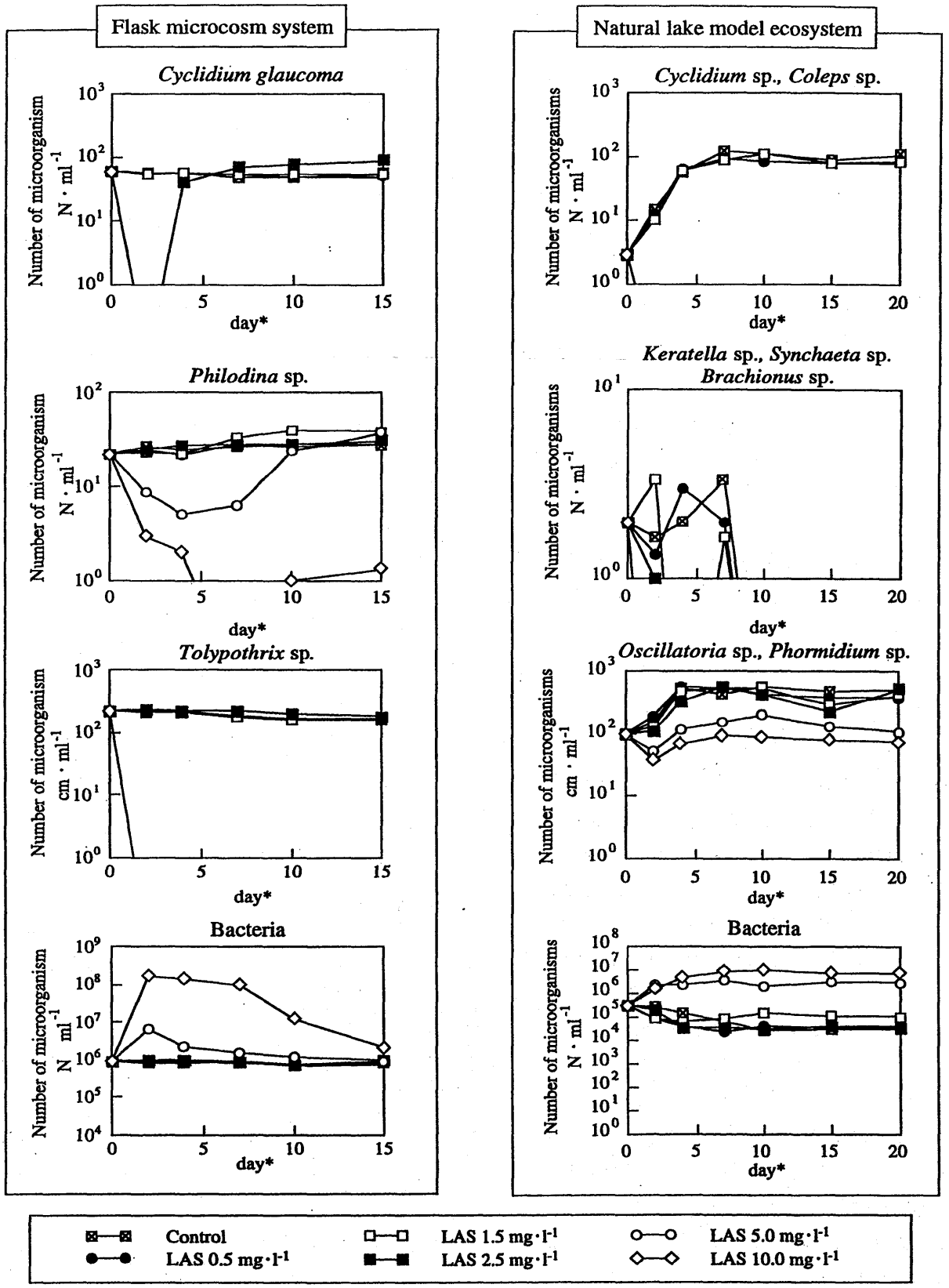

Fig. 4 *Effect of LAS on number of microorganisms in flask microcosm system and natural lake model ecosystem*
** days after addition of LAS*

decreased for 2 days after addition, and then the number slowly increased again. At 5.0 mg · l^{-1}, *C. glaucoma* and *Tolypothrix* sp. were eliminated from the system. The number of *Philodina* sp. and *A. hemprichi* decreased for 2 days after addition, after that the number slowly increased again until reaching the same number with the control. The number of bacteria was 10 times higher than that of control on day 2 after addition. At 10 mg · l^{-1}, the number of *Philodina* sp. and *Lepadella* sp. decreased for 2 days after addition but then increased slowly. *A. hemprichi* was eliminated from the system. The number of bacteria was 100 times higher than control from day 2 to 7 after addition. The effect of LAS on *Chlorella* sp. was not recognized in these LAS concentrations. No observed effect concentration (NOEC) of LAS on population density was less than 1.5 mg · l^{-1}.

Natural lake model ecosystem

When initial LAS was 0.5 and 1.5 mg · l^{-1}, changes in number of all microorganisms were same as those of the control system (Fig. 4). With the addition of LAS more than 2.5 mg · l^{-1}, *Cyclidium* sp. and *Coleps* sp. were eliminated from the system for 2 days after addition. At 0.5, 1.5 and 2.5 mg · l^{-1}, *Keratella* sp., *Synchaeta* sp. and *Brachionus* sp. disappeared from these system for 7 days after addition. When the LAS concentration was more than 5.0 mg · l^{-1}, these were eliminated from the system for 2 days after addition, meanwhile, *Oscillatoria* sp. and *Phormidium* sp. decreased for 2 days after addition, the number was not increased again. At 10.0 mg · l^{-1}, *Cyclotella* sp. decreased for 2 days after addition, then the number slowly increased again until reaching the same number with the control. At 10.0 mg · l^{-1} *Melosira* sp. decreased for 2 days after addition, the number was not increased again. At more than 5.0 mg l^{-1}, the number of bacteria was 100 times higher than control from day 2 after addition. No observed effect concentration (NOEC) of LAS on population density was less than 1.5 mg · l^{-1}.

Comparison of NOECs of LAS in the two systems

NOECs of LAS on bacteria as decomposer in flask microcosm system and natural lake model ecosystem were 10 mg · l^{-1} (Fig. 5). NOECs on LAS on blue green algae as producer in the two systems were 2.5 mg · l^{-1}. NOECs on LAS on protozoa as predator in the two systems were 1.5 mg · l^{-1}. NOEC on LAS on rotifer as predator in flask microcosm system was 2.5 mg · l^{-1}, but rotifer disappeared from the control system after 7 days from the start of cultivation so that the natural model ecosystem test could not clarify NOEC of LAS. It was suggested that flask microcosm test had higher reproducibility and stability than natural lake model ecosystem.

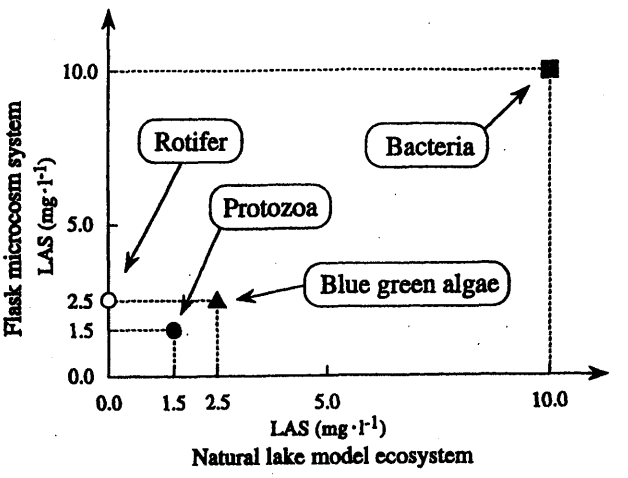

Fig. 5 Comparison of NOECs of LAS on micro-organisms in natural lake model ecosystem and flask microcosm system

Ecotoxicity assessment using microcosm test

Effectiveness of assessment was compared between flask microcosm and natural lake model ecosystem tests (Table 1). The assessment of destiny of LAS was effective in both tests. But natural lake model ecosystem did not show higher reproducibility and stability so that the assessment of toxicity on microorganisms and recoverability of the system were not effective. In both tests, NOECs of LAS on population density was less than 1.5 mg · l^{-1} (Table 2). It was suggested that flask microcosm test could provide precise ecological effects assessment of LAS on number of microorganisms because the system shows higher reproducibility and stability than natural lake model ecosystem. It was considered that safety concentration to aquatic ecosystem was that of assessment A. In assessment B, the concentrations were assumed to be the recoverable values for aquatic ecosystem to be able to accept surfactant without long term damage. In assessment C and D, the concentrations were assumed to be those which could lead to serious damage to aquatic ecosystem.

Table 1 *Comparison of effectiveness of assessment as flask microcosm system test and natural lake model ecosystem test*

Item of assessment	Flask microcosm system	Natural lake model ecosystem
Destiny of LAS	○	○
Toxicity on microorganisms	○	◉
Recoverability of the system	○	●

○ : effective ◉ : case by case ● : not effective

Table 2 *Evaluations of effects of LAS using flask microcosm system and natural lake model ecosystem*

Assessment	LAS (mg·l-1)	
	Flask microcosm system	Natural lake model ecosystem
A	1.5	1.5
B	2.5	-
C	10.0	10.0
D	> 10.0	> 10.0

Microcosm test is useful for assessment on ecosystem level containing material cycle, energy-flow and interaction of microorganisms, which could not be obtained with single-culture experiment. Above all, flask microcosm test was useful ecological effect assessment method, which can reflect natural aquatic ecosystem. Therefore, flask microcosm test is applicable for assessment of effect of chemical substance on aquatic ecosystem.

CONCLUSIONS

The purpose of our study is to evaluate the effect of LAS on aquatic ecosystem using flask microcosm system and natural lake model ecosystem, and the following results were obtained.

1) In the flask microcosm system and the natural lake model ecosystem, biodegradation rate of LAS were almost the same and NOECs of LAS were also below 1.5 mg · l-1.
2) It was suggested that flask microcosm test could provide precise ecological effects assessment of LAS on number of microorganisms because the system shows higher reproducibility and stability than natural lake model ecosystem.
3) It was concluded that flask microcosm test was useful ecological effect assessment method which can reflect natural aquatic ecosystem.

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17. Computer Simulation of a Microorganic Ecology (Microcosm) as a Self-sustaining System of Complexity

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INTRODUCTION

From the viewpoint of General System Thinking[1, 2], ecology is a nonlinear system of complexity structured by interactions among components and environment. A mathematical system analysis is a model "which is an imperfect and shorthand illustration of the real ecosystem"[3], which is very difficult or impossible to solve numerically. Since very powerful mathematics and information tools are now available in terms of heuristic mathematical theory (e.g. cellular automata, genetic algorithm, etc.) and recent computer systems are highly sophisticated, it may be possible to describe how ecological systems and their components are likely to interact using computer simulation techniques by providing a simplified model on the basis of principles in the ecological systems, i.e., self-organization, feedback in response to disturbance, emergent behavior, diversity and so on[4].

This study explores a microorganic closed-ecosystem[5-9] by computer simulation to illustrate symbiosis among populations in the microcosm. This artificial ecosystem consists of:

(1) *Protozoa Tetrahymena thermophila B*, heterotroph plankton as a consumer, abbreviated as "**Tetrahymena**",

(2) *Algae Euglena gracilis Z*, autotroph plankton as a primary producer which carries out photosynthesis and fixes CO_2 in the system, abbreviated as "**Euglena**", and

(3) *Bacteria Escherichia coli DH5alpha*, saprotroph bacteria as decomposer, which dissolve metabolic wastes or corpses into CO_2 and humus or biogenic salts (detritus food chain or organic detritus circuit), abbreviated as "**E-coli**".

MATERIALS AND METHODS

The simulation program is written as a procedure of **StarLogo**, which is a specialized version of *Logo language* (*pascal base*) distributed by the Epistemology and Learning Group, Media Laboratory, Massachusetts Institute of Technology (**http://el.www.media.mit.edu/**) and StarLogoT, which is a updated version of StarLogo by the Center for connected learning and Computer-based modeling, Tufts University (**http://www.ccl.tufts.edu/cm/**).

StarLogo has been used to look at many different phenomena in biology, cellular automata, Game strategic theory, mathematics, physics and social systems. The virtual microcosm is structured and operated by the following rules.

1) Living "*environment*" is defined as a lattice model, which consists of 10,201 square

patches, of which X- and Y-coordinates range from -50 to 50 (101 (horizontal) times 101 (vertical) equals 10,201 patches).

2) Each patch has its own attributes, **Nutrient**, **Detritus** and absolute coordinates (X,Y).

3) Components of protozoa, **Tetrahymena** and **Euglena** are defined a "turtles". Each turtle has its own attributes as follows:

① *struct ID (who, integer),*
② *body color (red for* **Tetrahymena**, *green for* **Euglena***)* ,
③ *X- and Y-coordinates,*
④ *heading direction,*
⑤ *clone generation,*
⑥ *life-span potential for cloning,*
⑦ *biomass in 10^{-9} gram,*
⑧ *breeding threshold in 10^{-9} gram carbon,*
⑨ *feeding rate in 10^{-9} gram carbon per hour,*
⑩ *digestive rate in % per hour,*
⑪ *energy consumption rate by metabolism in 10^{-9} gram carbon per hour,*
⑫ *energy consumption rate by moving in 10^{-9} gram carbon per hour,*
⑬ *energy production rate by photosynthesis in 10^{-9} gram carbon per event,*
⑭ *cell-cycle,*
⑮ *sexual reproduction switch ON/OFF,*
⑯ *terminal body mass in 10^{-9} gram carbon.*

E-coli is defined as "patch" which does not move by itself, but diluted by diffusion of water. E-coli has some parameters as follows;

① *doubling time in minutes,*
② *feeding rate in 10^{-9} gram carbon per hour,*
③ *peptone digestive rate in % per hour,*
④ *detritus dissolving rate in % per hour,*
⑤ *energy consumption rate by metabolism in % per hour,*
⑥ *death rate by background cell killing with environmental toxic in %.*

4) Each component of protozoa, **Tetrahymena** and **Euglena** lives its life by moving randomly, feeding (or consuming) a Nutrient or peptone from the "*environment*", and excreting its metabolic products to the "*environment*" as **Detritus**. **Tetrahymena** feeds **E-coli** as a source of living energy.

5) Each component of protozoa, **Tetrahymena** and **Euglena** breeds if it has more energy (or biomass) than the breeding threshold. Breeding energy threshold are inherited by its *clone* or *duplicate*.

6) Each component of protozoa, ***Tetrahymena*** and **Euglena** dies when its cloning generation reaches the life span potential. While, **E. coli** is an immortal bacterium, which has no life span potential. Their dead protoplasms are accumulated in the "*environment*" as **Detritus**.

7) Each component of protozoa, **Tetrahymena** and **Euglena** dies when its energy or biomass is less than terminal level. Their dead protoplasms are accumulated in the "*environment*" as **Detritus**.

8) Each component of immortal bacterium, **E. coli** dies when exposed to background environmental toxicants. Its dead protoplasms is accumulated in the "*environment*" as **Detritus**.

9) As a autotroph protozoa, each component of **Euglena** fixes part of the sunlight energy as a potential food energy (*photosynthesis process*), and produces glucose and oxygen from CO_2 + H_2O + light energy in presence of enzyme systems associated with chlorophyll (biogeochemical **Nutrient** cycle). The photosynthesis process is hindered by the overlapping of its *chlorophyll* with those of other components of **Euglena** as an intraspecific competition.

10) As a saprotroph bacterium, each component of **E. coli** breaks down the organic compounds of dead protoplasms or metabolic wastes (**Detritus**), and absorbs some of the decomposition products to reproduce itself. As a by products, it produces inorganic substances which may be recycled by the primary producer and soluble organic matters which may produce energy sources and/or may suppress or promote growth of other components of protozoa (**Detritus** food chain).

11) The **Detritus** food chain is inhibited by the overpopulation of **E. coli** (*self-crowding effect as an intraspecific competition*)[4].

12) Items 4) - 9) are local phenomena, which are the summation of interactions between each component of the species, ***Tetrahymena***, **Euglena** and **E. coli**, and its underlying "*environment*" or patch (Xcor,Ycor).

13) As a global parameter, "Sunbeam" is defined as a boolean (true or false) to switch the sunlight ON/OFF. Peptone is injected as an initial **Nutrient**.

14) As found by experimental study[5], predator - prey relationships between **Tetrahymena** and **E. coli** is included in the simulation. Predation pressure acts only if the population of prey (**E. coli**) is large enough relative to that of predator (**Tetrahymena**).

It is useful to investigate how some control mechanisms regulate the 1) storage and release of **Nutrients** and the 2) production and decomposition of organic substances (material cycles and energy flows) and generate a self-organizing homeostasis with no outside control

required.

Example output windows of the Microcosm Simulator, SIM-COSM Ver. 7.5 are illustrated in Figure 1a. These windows are prepared by the **StarLogo** graphic user interface as a standard unit. *Observatory window* is where each component of the species moves and the 10,201 patches are drawn as "*environment*". Spatial distributions of **Nutrient** and **Detritus** of the "*environment*" are illustrated by scaled-blue and scaled-gray, respectively.

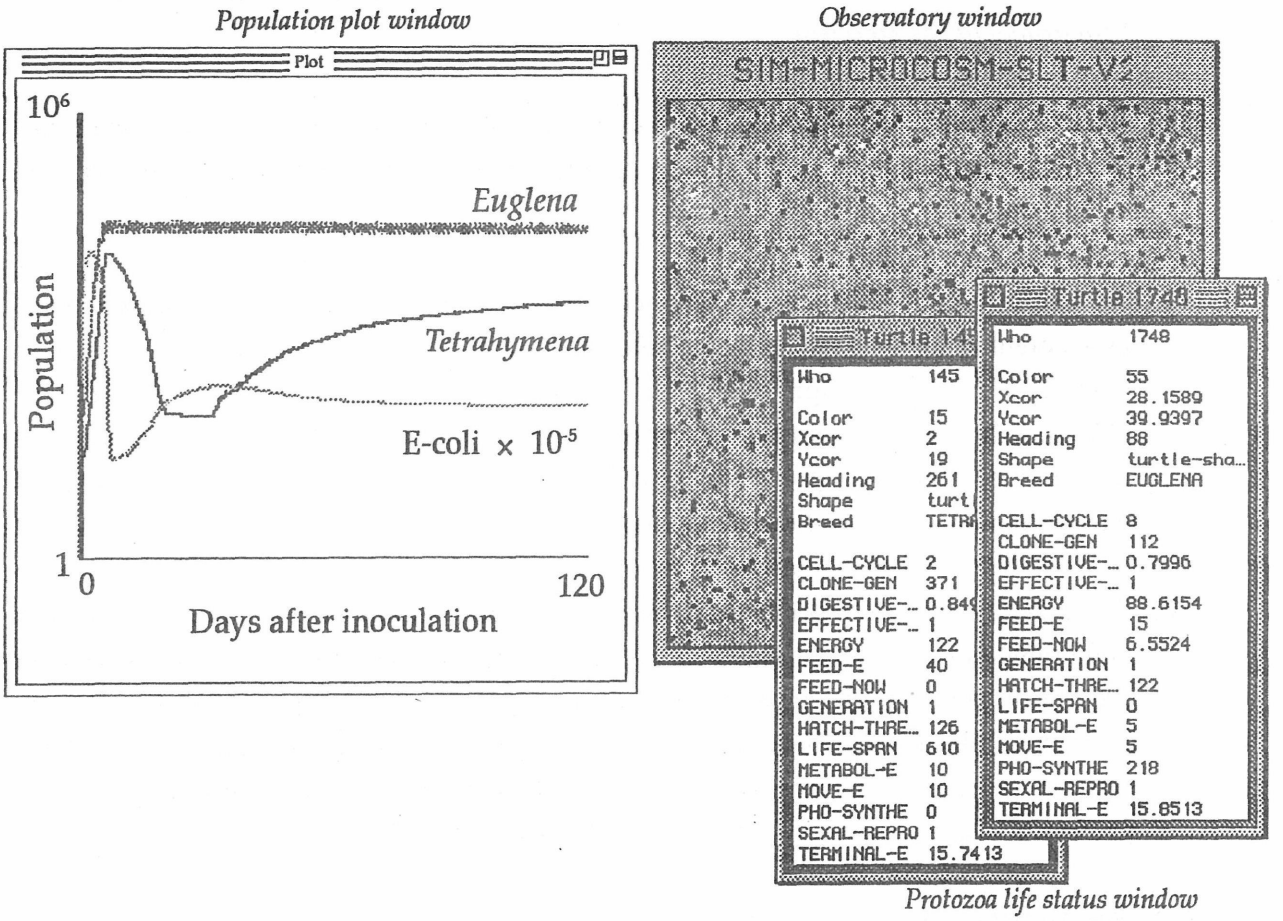

Figure 1-a Output windows of the Microcosm-simulator Ver. 7.5

Real time monitoring of the physical condition of a selected component is possible through *the individual's life status window*. Trends of population growth are shown in the *population plot window*, timed by the observer's clock (not by the biological clock of each species in the ecosystem). The operation interface of the simulator is shown in Figure 1b.

A life of **Euglena** in the simulator is illustrated in Figure 2 as an example. A struct of ID-256 is a component of **Euglena**, having age and energy of 3 and 126, respectively. It has life span potential and breeding threshold of 22 and 801, respectively, which are inherited deterministic parameters. Its coordinates (X,Y) are (24.7, 40.3), which are included in the patch (X=25,Y=40). Since its heading direction) equals -52° and step length r is 1, its future coordinates (Xcor ,Ycor) are (24.7 + sin -52° = 23.9, 40.3 + cos -52° = 40.9), which are included in the patch (24, 41). If) equals 180°, its next coordinates (X, Y) are (24.7, 39.9) in the patch (25, 40), which is occupied by another component. As a rule of intraspecific competition, the photosynthesis process is hindered during the next step by

overlapping of chlorophyll. It eats, moves, lives, wastes, breeds, and dies in due time. Global variables are as follows :

1) peptone as a primary culture medium of the "environment" (energy unit per patch) and total patch (10201 patches) is defined as 0.1 ml of water. 4.8 nano-gram of peptone per patch is almost equivalent to 500 mg peptone per 1 litter of water.
2) initial populations of species (**Tetrahymena**, **Euglena** and **E. coli**), (Ni) and
3) sunlight ON /OFF, which can be controlled as a forcing function by the operator of the simulator or a disturbance by the outside "*environment*". One cycle of calculation is calibrated to 1 hour, and sunlight is turned on and off every 12 hours.

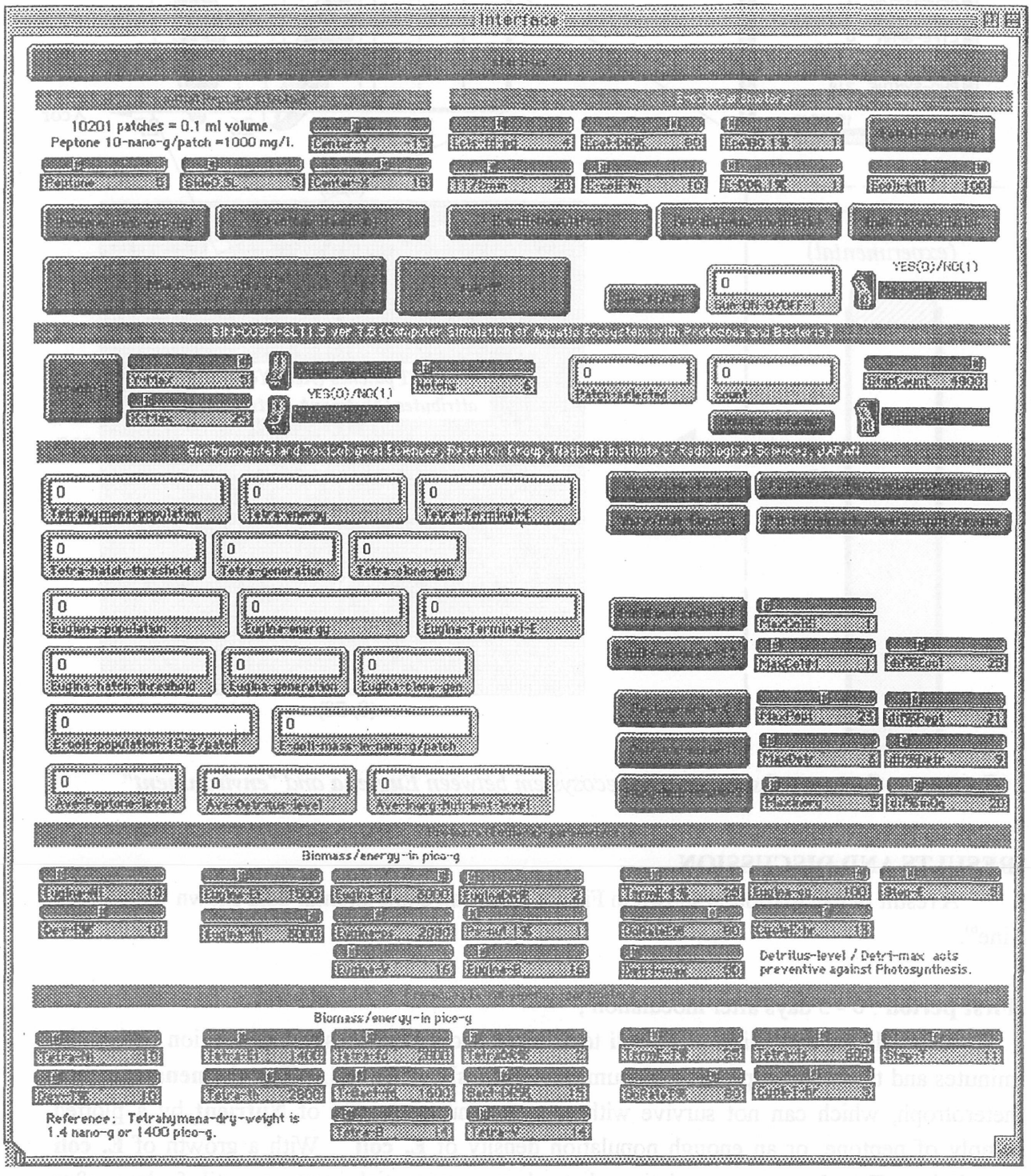

Figure 1-b Operation Interface of the Microcosm-simulator Ver. 7.5

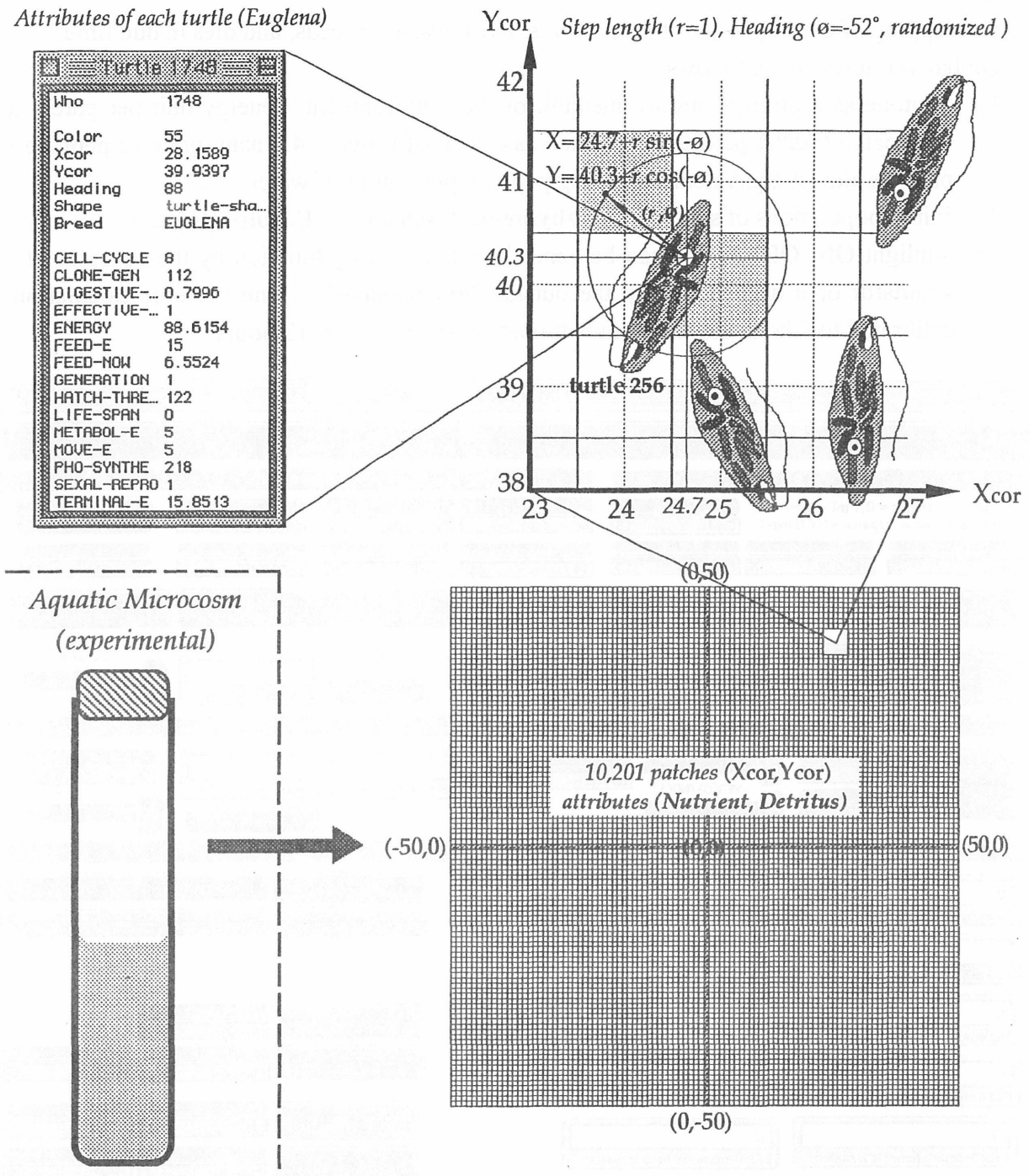

Figure 2 Conceptual scheme of the ecosystem between ***Euglena*** *and* ***"environment"***

RESULTS AND DISCUSSION

A result of calculation is shown in Figure 3 with an experimental data shown with dotted line[6].

First period : 0 - 5 days after inoculation ;

A population explosion of **E. coli** took place because of its short generation period, 20 minutes and the initial peptone is consumed voraciously by **E. coli**. **Tetrahymena** is truly a heterotroph, which can not survive without a continuous supply of **Nutrient** by a plotted supply of peptone, or an enough population density of **E. coli**. With a growth of **E. coli** population, **Tetrahymena** population showed an exponential increase until 5 days after

inoculation. **Euglena** is an autotroph protozoa, which is self-sufficient in food, as long as the sunlight and CO_2 is sufficient. **Euglena** population also showed an exponential increase until 5 days after inoculation. One of the selection pressures is overpopulation because overlapping of the chlorophyll may interrupt the photosynthesis process. It must be noted that **Euglena** acts as a heterotroph, when the sunlight is OFF.

Second period : 5 -25 days after inoculation ;

A sudden extermination of **E. coli** took place because of a shortage of peptone and predation by **Tetrahymena**. This sudden decrease of **E. coli** caused a delayed decrease of **Tetrahymena** population. The metabolic products and dead protoplasms of **Tetrahymena**, **Euglena** and **E. coli** were accumulated in the environment as organic **Detritus**. As a saprotroph bacteria, **E. coli** breaks down the **Detritus** to produce and some of the decomposition materials was absorbed by themselves to breed gradually, and release organic and inorganic substances into environment, which is a downstream of material cycle in the microcosm, or a "Detritus food chain". As Odum suggested that **Detritus** acts as a pollutant storage which might suppress or promote growth of other components of species[4], which should be investigated carefully in the future. From this point of view, **E. coli** may act to sustain the ecological system for a long period by activating the detritus food chain. Experimental observation found that **Tetrahymena** is a predator of **E. coli**, and its predation pressure is active only when the population of prey is more than the threshold level[5]. As shown in Figure 3 at 5 days after inoculation, 10^{-6} cells/ml of **E. coli** population should be

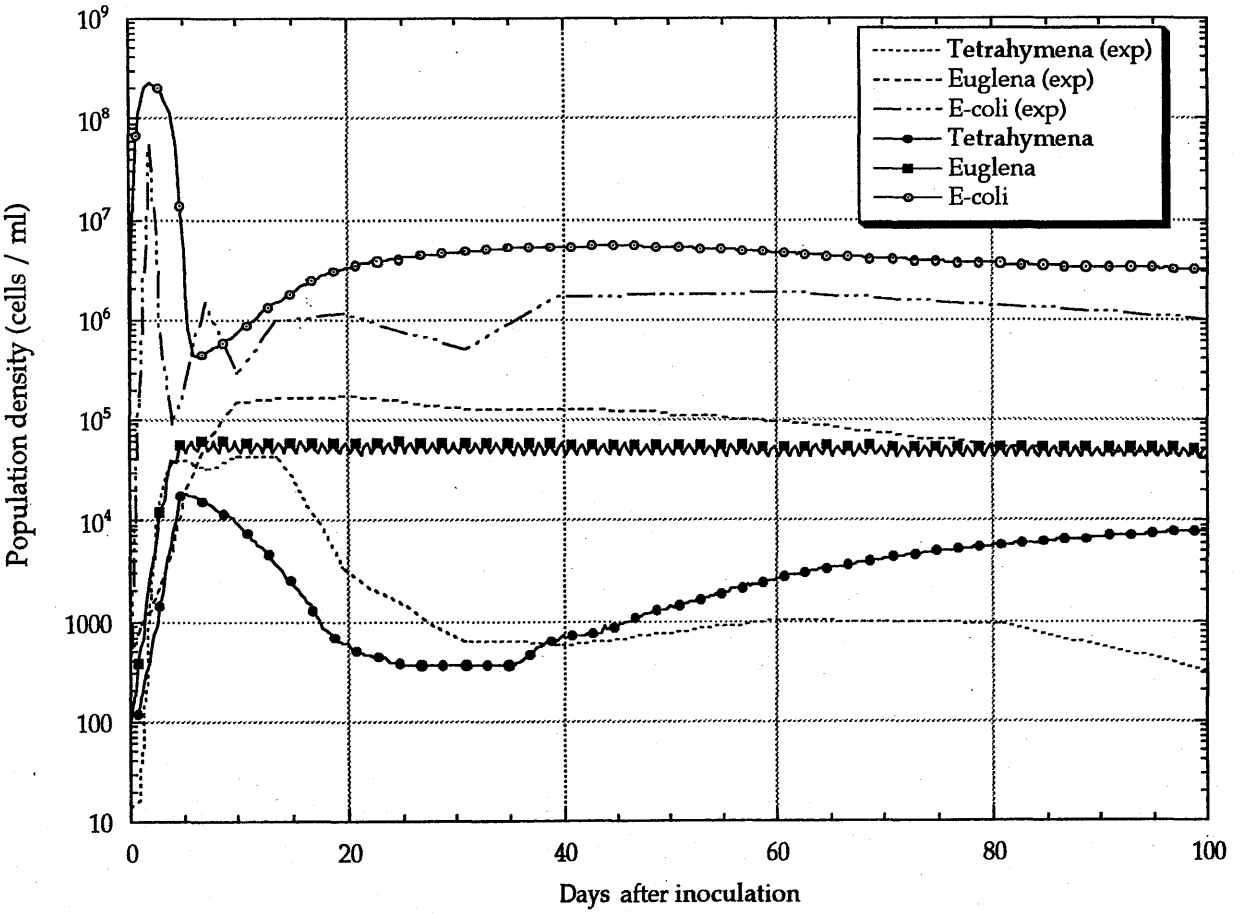

Figure 3 Population changes in the microcosm predicted by computer simulation

crucial for **Tetrahymena** to satisfy their energy requirement to support their lives. The population of **Tetrahymena** approached to the bottom line from 5 to 25 days after inoculation, which might be beneficial for **E. coli** to recover their population.

The population of **Euglena** reached to the carrying capacity level. Experimental observation suggested that the efficiency of photosynthesis may decline significantly with the lapse of time after inoculation[10]. Since the energy flow of the microcosm is sustained by the sunlight, perturbation of efficiency of the photosynthesis of **Euglena** should be crucial for the stability of microcosm.

Third period : 25 -100 days after inoculation ;

The population of **E. coli** recovered enough to feed **Tetrahymena** to the certain capacity level. Growth of **Tetrahymena** followed a sigmoid pattern (S-shaped), which overshoots the upper population size possible (0-5 days after inoculation) and undergoes oscillations after before converging to the capacity level. That is,

1) when population density of **E. coli** accumulated enough prior to population growth of **Tetrahymena** and there was enough time lags needed to start breeding, overshoots of the **Tetrahymena** population might occur, and
2) when time lags to adjust the overpopulation by altering birth and death rates, oscillations might occur before settling down at the capacity level of the ecosystem[11].

At 100 days after inoculation, computer simulation was not able to follow each perturbation of the population of microbial observed by experiment. This discrepancy might be caused by the interactive effects of metabolites of microbial in the batch culture microcosm, which are not taken into consideration in the model and left open as our future assignment.

FUTURE DIRECTIONS

This study showed that computer simulation is a valuable tool to illustrate symbiosis among populations in the microcosm, where a feedback mechanism acts in response to disturbances and interactions among species and environment as a self-organization.

In the simulation, the results of the population balance showed a probabilistic uncertainty and diversity. The selection pressure might be high in the first critical minutes of system operation, which consists of both intraspecific and interspecific competitions.

One of the future research directions is to investigate the *genome*, or blue print of each individual component of life. For example, some kinds of haploid protozoa undergo asexual reproduction to breed their clones when they have enough food to support their lives, but they shift their mode of reproduction to a self-conjugation or *autogamy* when they live close to the margin of starvation[12]. As a kind of sexual reproduction, *autogamy* introduces some genetic renovations, i.e., selection, crossover and mutation which may cause the diversity of the genotype of the population. In the field of information science, this kind of approach is well known as a genetic algorithm[13] and should be a valuable procedure to find out the strategy of survival and how *diversity and similarity* appeared in a chain of generations in a real ecosystem.

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18. Where Are the Radon Induced Lung Cancer Cases?

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ABSTRACT

High radon levels can be found in many Swedish dwellings. According to the Swedish authorities it is estimated that the mean radon concentration is 140 Bq/m^3 in detached houses and 75 Bq/m^3 in apartments. These values are high compared to the estimated world average of about 30 Bq/m^3. Thus, health effects from radon should be expected to be seen more clearly among the Swedish population.

Statistical data from 1988 show that in the Swedish population of about 8.4 million people there were 2710 lung cancer incidences. As lung cancer is considered to be fatal, most cases die within one year after diagnosis, the number of incidences is about the same as the number of deaths. Most of the lung cancer incidences occur among elderly smokers. Smoking is considered to be the main cause of lung cancer. All ages are exposed to radon in dwellings. As children are supposed to be more sensitive to radiation, childhood exposure should be specially taken into account. There are, however, very few lung cancer cases at younger ages and among non-smokers. Thus, the expected radon induced lung cancer cases mostly overlap with the smoking induced lung cancer cases, and occur at elderly ages. Due to the small number of lung cancer cases at lower ages, as well as in the non-smoking population, the risk from radon seems to be small compared with many other risks in the society. A cost effective method to decrease the risk for lung cancer seems to be to decrease the smoking habits.

INTRODUCTION

Risk estimations in Radiation Protection are based on extensive experimental data from various fields, among them from the atomic bomb survivors, from radiation accidents, form medical use of radiation as well as from animal experiments and biological data. These data have been used for making models for predicting new situations. The models are based on some assuptions and the validity of the model has to be proven in each situation. A inaccuracy in one of the parameters used can be transferred through the different steps in the model and cause serious errors in the results

Radon and its progeny is regarded as the largest single contributor to human irradiation. According to UNSCEAR[1] the mean radon values indoors in the world is estimated to be about 30 Bq/m^3. There are several countries, especially Sweden and Finland, where high radon levels in dwellings countrywide are especially high. According to the Swedish authorities[2], the mean values of radon is estimated to be as high as 140 Bq/m^3 in detached houses and 75 Bq/m^3 in apartments.

Radon is considered to cause mainly lung cancer. A dose response relationship is considered well established among miners[3]. The main cause of lung cancer is, however, considered to be tobacco smoking. Most of the miners were smokers.

To what extent indoor radon can cause lung cancer has been under debate for a long period. It has been questioned whether data from miners can be transferred to the general public. In order to shed light on this question, several epidemiological studies about indoor radon has been started during the last years. The results published until now are not quite conclusive yet, however.

The Swedish situation preserves certain interest due to the high radon levels countrywide. From nationwide radon surveys[4-7], it is estimated that the radon distribution in Swedish dwellings is distributed according to Table 1.

Table 1. Estimated distribution of indoor radon in Swedish dwellings[2, 4-7]

No of dwellings	Indoor radon concentrations (Bq/m^3)	Percentage of all dwellings (%)
ca 2.05 million	0 - 100	50
ca 1.55 million	100 - 200	38
ca 0.35 million	200 - 400	8
ca 0.11 million	400 - 800	3
ca 0.04 million	> 800	1
Total: 4.1 million		100

Thus, although most of the Swedish dwellings have rather low radon levels, there is an appreciable amount of dwellings with rather high radon levels. Thus, health effects from radon should be expected to be seen rather clearly from the Swedish situation.

From present risk evaluations, based on dosimetric calculations, epidemiological data from miners as well as from hitherto performed data on domestic radon, animal experiments and other biological data, the Swedish authorities estimate that the swedish indoor radon levels should give rise to 300-1200, most probably about 900 lung cancer cases per year[2].

The large span in the risk evaluations indicate a large uncertainty in the model. There are several factors that contributes to this uncertainty, as for example should the data be based on radon measurements or radon daughter measuements, or both. If radon daughters are measured, should also the size distribution of the aerosols, to which the radon daughters are attached, be determined. This size distibution is dependent on humidity and other pollutants present. An estimation of the cumulative exposure is dependent of a knowledge how the radon/radon daughter concentrations vary with time, the occupancy factor the people stay in their homes, other radon sources, inhalation rates, age dependence of sensitivity, how to separate the effect of smoking etc. Thus, it is obvious that there are several uncertainty factors in the model.

METHOD

The present approach is based on the real, not measured, radon situation in Sweden and is not based on any model used. Statistical data of lung cancer cases is taken from the Swedish cancer registry[8].

Age distribution of lung cancer incidences

The situation in 1988 is discussed. In the Swedish population of 8.4 million people, there were totally 2710 primary cancer incidences in the bronchial region (2617 bronchus and lung, 81 pleura and 12 trachea) distributed over age as shown in Figure 1.

Of these, 82 % were over an age of 60 years (68 % over an age of 65 years) and there were very few cases at younger ages. In fact, lung cancer is very rare at younger ages: 0,6 % below 35 years. Most of the lung cancer cases are smokers, and smoking is considered the main cause of lung cancer.

Age distribution at exposure

Whereas the age at exposure can be fairly well known among the miners; they were exposed as adults to radon and its daughters, during their working hours, which can be fairly well recorded, all ages are exposed in the homes. As children generally are supposed to be more sensitive to radiation, childhood exposure should be especially taken into account. According to Swedish statistics[8] about 12 % of the Swedish population of about 8.4 million people is of an age

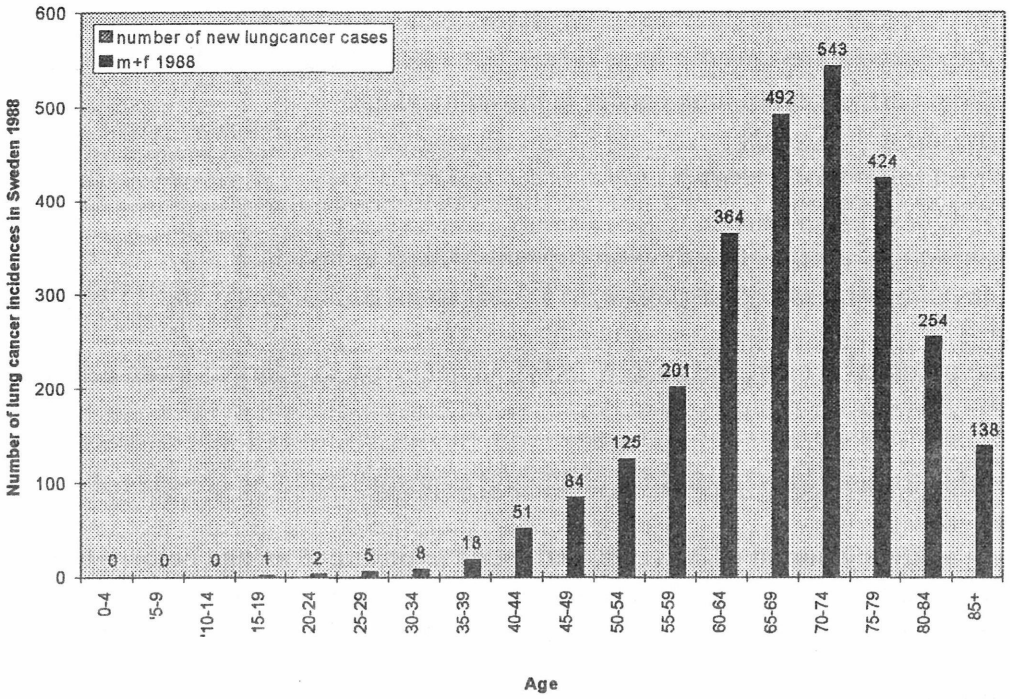

Fig. 1 Age distribution of lung cancer incidences

under 10 years. Assuming an even distribution of children in all dwellings, it could be estimated that 12 % och these children (i.e. about 120.000 children) are living in dwellings with an radon level more than 200 Bq/m^3 (and of these about 4 % , i.e. 40.000 children in dwellings with a radon level of more than 400 Bq/m^3, which is the Swedish action level for existing buildings).

Effects of childhood exposure

There is little data about the effect of childhood exposure due to radon. Although some of the radon epidemiological studies have long follow up times, the nationwide Swedish radon epidemiological study as long as 35 years in retrospect, these follow up times do not cover childhood exposure, however. From figure 1 follows that there are very few lung cancer cases below an age of 35 years. Thus, there is not enough data to give any statistical significance for following up childhood exposure, with follow up times of 35 years or less. In fact, the Swedish nationwide radon epidemiological study[9-10] did not include any study person below an age of 35 years. The age distribution among the 1360 cases in this study was 68 (5%) aged 35-44, 156 (11.5%) aged 45-54, 477 (35%) aged 55-64 and 659 (48.5%) aged 65-74. Thus, for about half of the cases, the follow up of radon exposure did not start before an age of 30 years.

As childhood exposure is of special interest of many reasons: children are supposed to be more sensitive to radiation, children are expected to have a long lifetime still to live and children at an age of less than ten are nearly all non-smokers (maybe passive smokers). A follow up of childhood exposure should thus give data about the effect of childhood exposure as well as provide a study group giving information about exposure to non-smokers.

Latency periods

From the study of miners, a latency period of 10-20 years has been found. It was also found that the risk of lung cancer decreases with age and time since exposure, expressed in the formula (1) [3]

$$(1) \quad \lambda_r(a)/\lambda_0(a) = s\,\gamma(a)\,(P_1 + 0.5P_2)$$

where $\lambda_r(a)$ is the excess age-specific lung cancer rate caused by a preceeding exposure
$\lambda_0(a)$ is the normal rate of lung cancer at the attained age
$\gamma(a) = 1.2$ for $a < 55$ years
$\gamma(a) = 1.0$ for $a = 55$-64 years
$\gamma(a) = 0.4$ for $a = 65$ years
s is a constant of proportionality with exposure, taken to be 0.025
P_1 is the potential α energy exposure, in WLM, incurred between 5 and 15 years before the age a
P_2 is the potential α energy exposure, in WLM, incurred 15 years or more before this age

Whether this formula also is valid for childhood exposure could be questioned.

Assuming a latency period of 20 years, childhood exposure at an age below 10 years of age, should result in lung cancer at an age below 30 years of age. Although there are some lung cancer cases at an age below 30 years (8 cases in 1988), little is known about the radon exposure in these cases.

It is well known, from the atomic bomb survivors for example, that cancer can occur after longer periods, 50 years or more, after exposure. Thus, it is possible that childhood exposure will give rise to lung cancer at older ages. The epidemiological studies performed until now, do not include the childhood exposure, however.

Smoking habits

Most of the lung cancer cases are smokers, and smoking is considered the main cause of lung cancer. In fact, there are so few non-smoking lung cancer cases (in the Swedish nationwide study only 178 of totally 1360 lung cancer cases) that no statistically significant dose response relationship could be established, although a weaker trend than for the smoking cases could be seen. In another study of non-smoking women in Missouri[11], no effect from radon could be seen.

In 1988 there were 2710 lung cancer incidences in Sweden. Most of them are smokers. Smoking is generally believed to be the main cause of lung cancer. According to the Swedish authorities, the present radon levels in Swedish dwellings should give rise to 300-1200 lung cancer cases per year with a most probable value of 900. Thus, it follows that there is a big overlap between the smoking induced lung cancer cases and the radon induced lung cancer cases. It seems that in most cases smoking is needed for lung cancer to occur.

Risk comparisons

It can be of interest to compare the effects of some risks in the society. According to Swedish statistics[12], see Table 2, in 1988 there were 772 traffic deaths and 5483 severely injured in the traffic. An appreciable number of these were young, for example 223 traffic deaths were of an age below 24 years. Of the 1590 suicides, 148 were of an age less than 24 years. It could be informative to compare these numbers with the number of lung cancer incidences (most of them deadly within one year), 2710, most of which are smoking related, and 3 occurring below an age of 24 years.

Among the cancer incidences, see Table 3, the lung cancer incidences is about 7 % of the total number. Whereas many cancer incidences are curable today, the prognosis of lung cancer is bad, mostly with death occurring within one year. Thus, the number of lung cancer incidences is about the same as the number of lung cancer deaths. It should in this context be pointed out that lung cancer is not the only smoking related disease. It is estimeted that there are about 10.000 smoking related deaths per year in Sweden, mainly heart and coronary diseases, which often occur at younger ages than lung cancer.

Table 2. Age distributions of deaths or severe injuries caused by traffic or suicides in Sweden 1988 (SCB 1988)[12]

Cause	Age (years)				
	0-24	24-45	46-65	>65	Total
Traffic deaths	223	192	165	192	772
Severely injured in traffic accidents	2232	1543	945	763	5483
Suicides	148	552	.....870.....		1590

Table 3. Age distributions of some cancer incidences in Sweden 1988 (SOS 1988)[8]

Type	Age (years)				
	0-24	25-44	45-64	>65	Total
All cancers	547	2423	9780	26519	39269
Lung, trachea and bronchus	3	82	774	1851	2710
Lymphatic leukemia	83	15	65	265	428

Radon mitigation

There is certainly a need to reduce the number of lung cancer incidences. Radon reduction methods has been considered a cost effective method, these methods being in most cases simple and cheap. There are, however, cases where radon reduction methods are not effective[13]. The reduction of smoking habits, especially preventing young people from start smoking seems to be another cost effective method to save more lives from lung cancer.

CONCLUSION

Most of the lung cancer cases occur among elderly smokers. Smoking is considered the main cause of lung cancer. There is a big overlap between smoking induced lung cancer cases and radon induced lung cancer cases.

High radon levels in Swedish dwellings is supposed to cause an appreciable amount of lung cancer cases, however:

Due to the small number of non-smoking lung cancer cases there is today little evidence for a correlation between lung cancer and radon in this group.

Due to the small number of lung cancer at younger ages there is today little evidence for a correlation between lung cancer and radon exposure at young ages.

The risks for young people and non-smokers cannot be ruled out but is most probably small related to many other risks in the society.

More data is needed - epidemiological as well as on a cellular or molecular biological level.

The risks from smoking is predominant. A cost effective method to reduce the number of lung cancer cases is thus to stop smoking and to prevent young people from start smoking. The risks from radon should not be neglected, especially as radon reduction methods mostly are cheap and easy to make.

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19. The Study on the Cytotoxicity of Gadolinium in Alveolar Macrophages

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INTRODUCTION

Recently rare earth elements have been utilized broadly with the rapid development of advanced technologies such as the semiconductor industry[1]. Gadolinium (Gd), one of lanthanide rare earth elements, has been used in the field of clinical medicine as a promising contrast agent for MRI and also used experimentally to study the physiology of the reticuloendothelial system. Gd combined with chelating agents can be applied to human as a contrast agent for MRI without apparent toxicity[2]. On the other hand, the experimental animals are treated with Gd due to its ability to block the reticuloendothelial system[3]. It inactivates macrophages, particularly as measured by reduced clearance of test particles from the blood and by decreased localization of circulating particles to resident macrophages. Macrophage-mediated immune and inflammatory responses are also suppressed in Gd-treated animals, such as the induction of tolerance to portal venous antigen and the development of lethal endotoxin shock. At present it is unclear whether the effects of Gd on macrophages in vivo are based on it's cytotoxic(lethal) effect or the functional block such as the decreased production of cytokines and NO. Recently it was reported that Gd induced remarkable apoptosis, one mode of interphase cell death, in alveolar macrophages cultured in vitro[4]. However, the extent of toxicity of Gd for macrophages and the mechanisms have not been fully elucidated. In the present study, we investigated the cytotoxic effect of Gd for mouse and rat alveolar macrophages (AM) and obtained very intriguing results.

MATERIALS AND METHODS

Animals:

Male Sprague-Dawley (SD) rats, Wistar rats, C3H mice and C57Black/6(B6) mice were purchased from SLC Co.Ltd (Shizuoka, Japan) and were housed in a controlled environment, with access to food and water *ad libitum*.

Alveolar macrophages (AM):

AM were harvested by repeated postmortem bronchoalveolar lavage with Dulbecco's PBS. The cells were washed and suspended in D-MEM supplemented with heat-inactivated fetal calf serum (10%) and antibiotics.

Cell culture:

The cells were plated in 96-well microculture plates at a cell density of $5x10^4$ per well and preincubated 2hr at 37 °C in a humidified atmosphere of 5% CO_2 in air to allow for the cells to adhere to the plates. Thereafter the culture plates were washed with fresh culture medium to remove non-adherent cells. The medium containing Gd and cadmium (Cd) at various

concentrations was added and then the incubation was started (this time point was defined as 0hr).

Gd and Cd preparation:

$GdCl_36H_2O$ and $CdCl_22H_2O$ were dissolved in sterile saline at a concentration of 100mM and then each preparation was diluted serially with culture medium.

Cytototoxicity assay:

MTT assay[5] was used to quantitate the cell survival. Briefly, 50μl of MTT solution at a concentration of 5mg/ml in PBS was added to each well at 72hr of culture and the incubation was continued further 1-2hr. Then the culture medium was removed carefully and 100μl of DMSO was added. Immediately the absorbance at a wavelength of 540nm(reference wavelength of 620nm) in each well was measured with a microplate reader.

RESULTS AND DISCUSSION

Figure 1 shows percent survival fractions of AM exposed to Gd or Cd at serially diluted concentrations. Cd decreased dose-dependently the survival of AM, although the dose-response curves were not identical between animals. On the other hand, the dose-response curves in Gd-treated AM were shown to be remarkably different between mouse and rat AM. Particularly Gd decreased exponentially the survival of SD rat AM at doses of up to 10μM, with less than 10% survival at 10μM Gd, whereas the survival of B6 mouse AM was not significantly affected at doses of up to 1000μM.

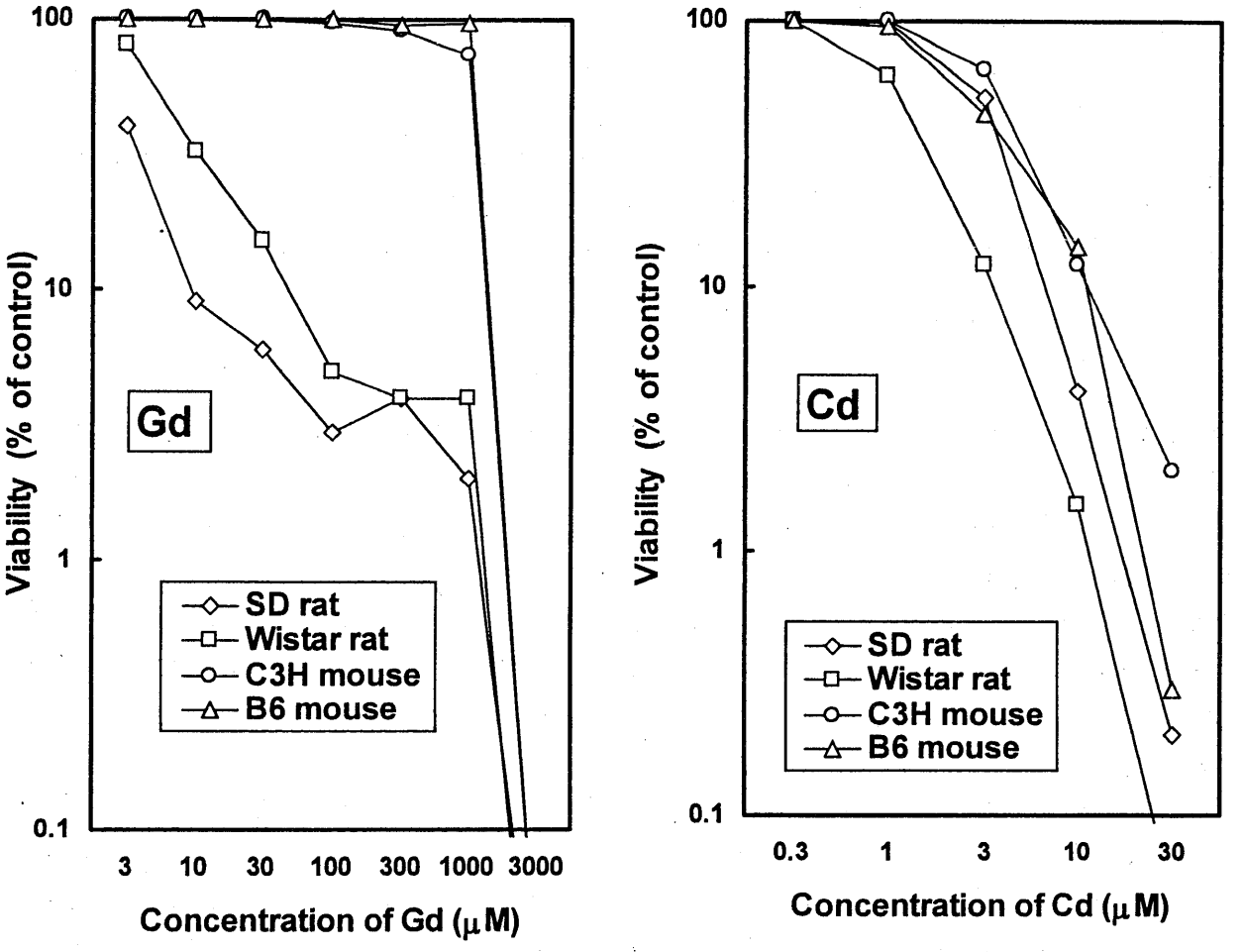

Fig. 1 Cytotoxicity of Gd and Cd for mouse and rat alveolar macrophages

Rare earth metals have been known to form insoluble colloid, dependent on the chemical form[1]. The primary biological mechanism by which Gd has been considered as a benign phagocytic blocker without apparent effects on non-phagocytic cells might be attributed to a characteristic of Gd to form colloid in vivo to be phagocytosed by macrophages. It was easy under an inverted phase-contrast microscope to identify particulate matters on the bottom of culture plates loaded with Gd. Furthermore, white cloudy substance (colloid) could be observed instantaneously when Gd solution in saline or distilled water was added at high concentration to culture medium. From these facts, it was suspected that a part of Gd might exist as colloid and the cytotoxicity of Gd might be dependent on the phagocytosis of the colloid. When Gd preparations at doses of up to 1000μM were centrifuged and filtered with 0.2μm filter in order to remove the particulate matters, the filtered solutions no longer had the cytotoxicity for rat AM, whereas the filtration did not alter at all the cytotoxicity of Cd samples for both mouse and rat AM (Fig.2).

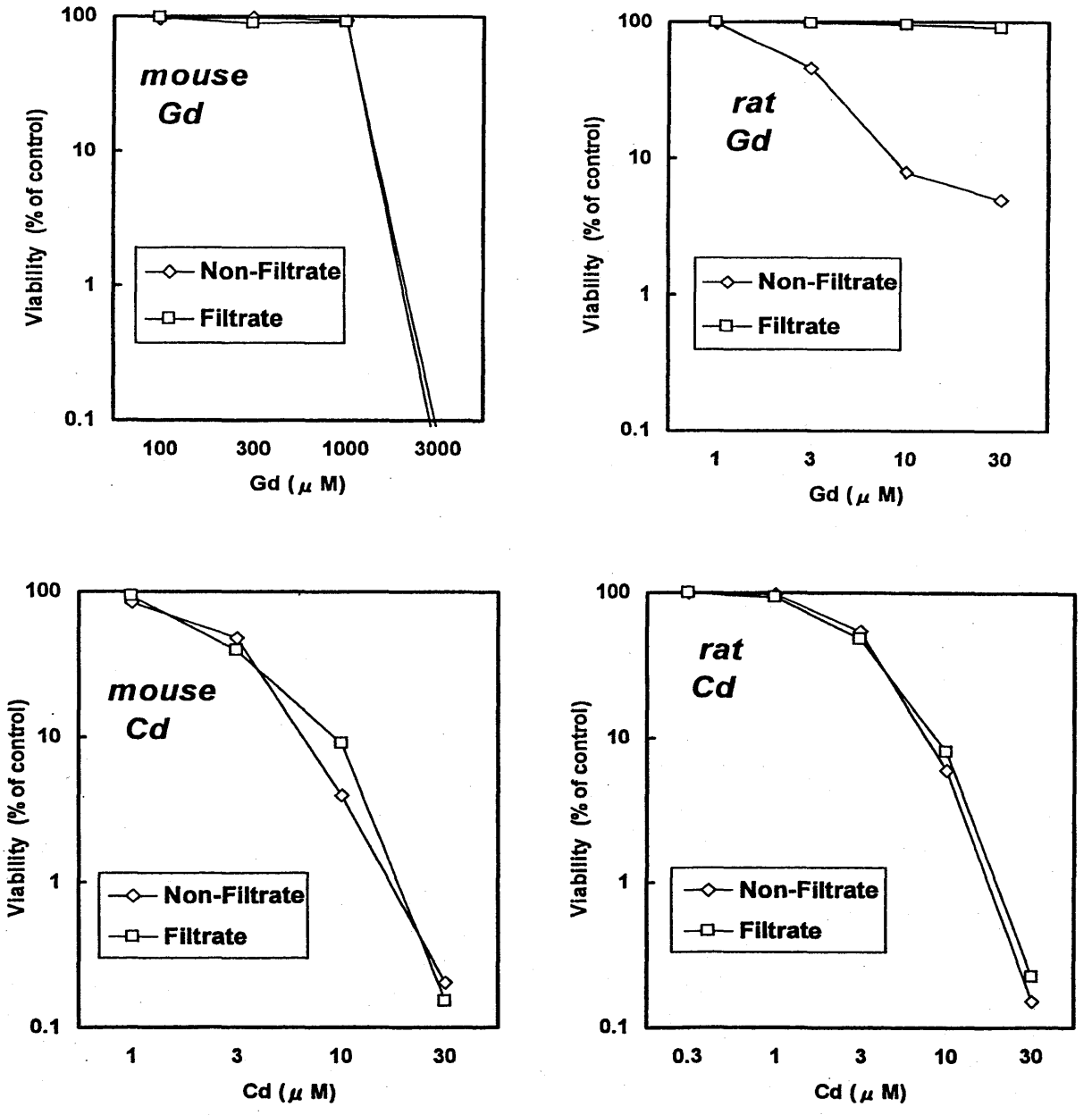

Fig. 2 The effect of filtration on the cytotoxicity of Gd and Cd for mouse and rat alveolar macrophages

Fig.3 shows the effect of NH_4Cl or chloroquine treatment on the cytotoxicity of Cd and Gd for mouse and rat AM. AM were treated with NH_4Cl or chloroquine at various concentrations throughout the culture period. NH_4Cl treatment had no effect on the cytotoxicity of Cd for both mouse and rat AM. On the other hand, the cytotoxic effect of Gd at doses of up to 1000μM for rat AM was disappeared by the treatments with NH_4Cl or chloroquine at proper concentrations. NH_4Cl or chloroquine are well known lysosomotropic agents with a pharmacologic action to elevate PH in lysosomes and consequently to suppress the acidic lysosome enzyme activities.

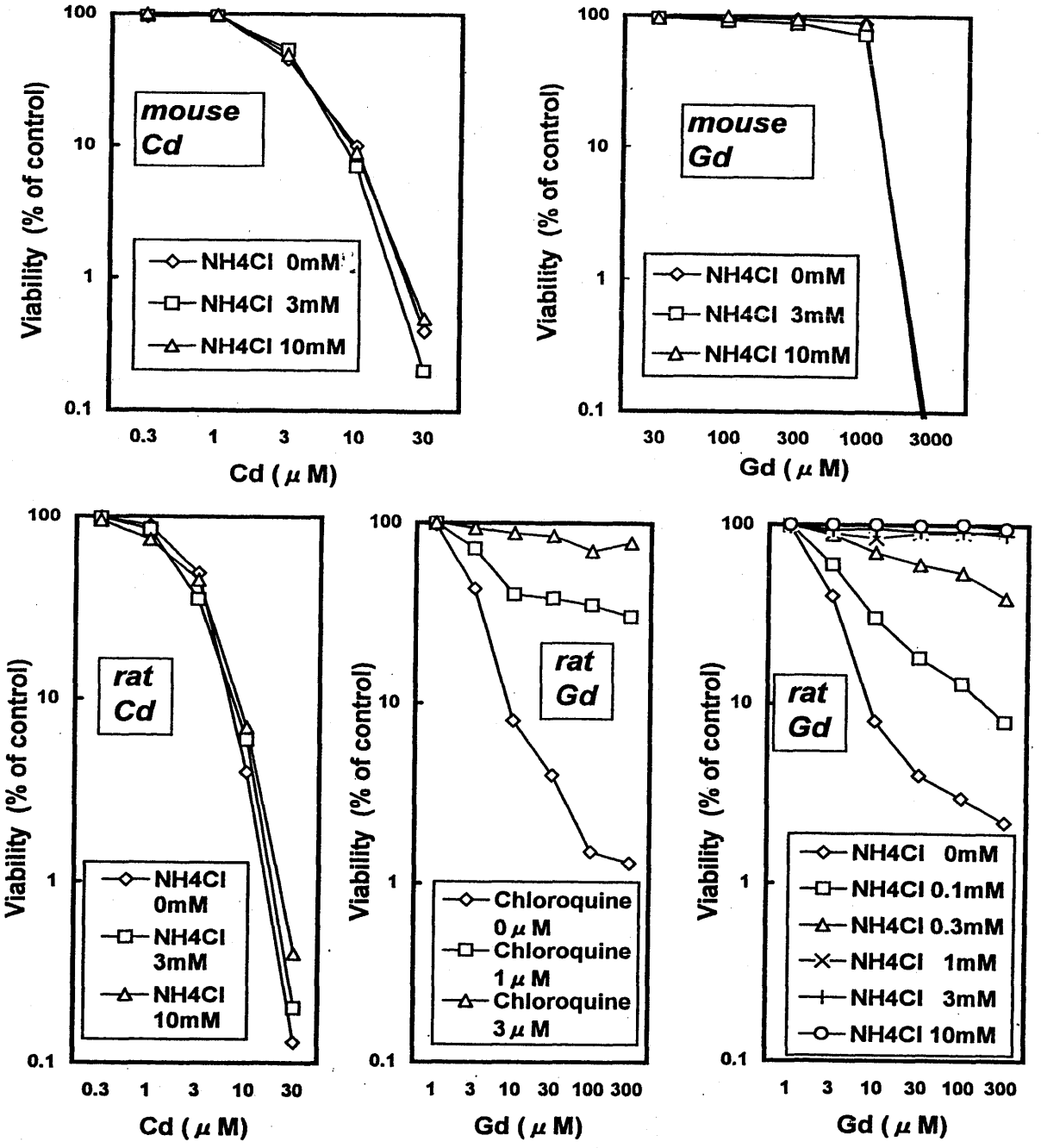

Fig.3 The effect of NH_4Cl or chloroquine treatment on the cytotoxicity of Cd and Gd for mouse and rat alveolar macrophages

Based on these facts, it was concluded that the decreased survival of rat AM exposed to Gd at doses of up to 1000μM was dependent on the phagocytosis by AM of particulate matters existing in Gd preparations and on the dissolution inside the phagolysosomes. The fact that Gd at doses of up to 1000μM did not remarkably affect the survival of mouse AM suggests the possibilities of lesser phagocytosis or dissolution of particulate matters by mouse AM or lower sensitivity of mouse AM to the soluble form of Gd after the dissolution of Gd colloid inside the cells. Preliminary experiments revealed no significant differences in the rate of phagocytosis of latex particles between mouse and rat AM. Also rat and mouse AM showed almost same sensitivities with respect to the cytotoxicity to the Gd preparation filtered at a dose of 3000μM which probably contained non-colloidal form of Gd over the level cytotoxic to AM. The cytotoxic action of Gd dissolved inside the phagolysosome may be different from that of Gd existing in a non-colloidal form in the culture medium. However, at present it is most possible explanation that the dissolution inside the phagolysosome of particulate matters existing in Gd preparations may be different between mouse and rat AM.

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20. Mutagenicity Testing with Embryo Cells of *Rhodeus ocellatus ocellatus* (Pices, Cyprinidae)

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INTRODUCTION

Mutagenicity assays play an important role in evaluating the genotoxicity of chemicals and predicting their carcinogenicity. Two major assays for cytogenetic damage are the in vitro chromosomal aberration test using mammalian cells (Ishidate and Odashima, 1977) and the in vivo micronucleus test (Schmid, 1975; Mavournin et al., 1990). Fish and other aquatic organisms are important for monitoring water pollution and for assessing the effect of pollutants on aquatic resources. For serious acid rain problems we need to understand the genotoxic effect of low pH on aquatic organisms.

We used metaphase and micronucleus analysis of embryonic cells of *Rhodeus ocellatus ocellatus* (Pices, Cyprinidae) (Ueda et al., 1991) to study the cytogenetic effects of mitomycin C, trichloroethylene (as model chemicals), and low pH.

MATERIAL AND METHODS

Fertilized eggs were treated in dechlorinated tap water containing 0.2-400 g/ml mitomycin C (MMC), 0.03-3000 g/ml trichloroethylene (TCE), and 15mM Bis-Tris or MES for low pH. They were kept at 17-19°C for one day (to gastrula stage). The embryos, from which the chorion and the york were removed, were transferred to a microtube (1.5 ml) that contained colchicine solution (Eagle's MEM medium + 5% fetal bovine serum + 15mM HEPES + 0.005% colchicine, at pH 7.0 with $NaHCO_3$). After 30 min at room temperature (to arrest and accumulate metaphase cells), the cells were spun at 1000 rpm for 5 min, the supernatant was removed, and 1 ml of hypotonic solution (0.6% sodium citrate aqueous solution) was added. The cells were stirred by gently pipetted with the micropipette tip and ejection from and treated with hypotonic solution for 3 min at room temperature. 0.5 ml of 1:3 acetic acid-ethanol was added. The cells were stirred as before with the micropipette tip and centrifuged at 1000 rpm for 5 min. The fixative was renewed several times and finally replaced with a small amount of 1:1 acetic acid ethanol. A drop of cell suspension was placed on a clean glass slide and air dried. The preparation was stained with 5% Giemsa in S rensen's phosphate buffer at pH 5.8 and rinsed with deionized water several times.

We also studied the effect of low pH on embryos that developed from eggs that were kept in freshwater for 10 min at 20°C prior to artificial fertilization.

All analyzable metaphases were scored for chromosome structural aberrations (CSAs), and 1000 interphase cells were scored for micronucleus induction. Chromatid and chromosome gaps, breaks, exchanges, and microchromosomes that did not appear in the standard karyotype were scored as CSAs, and numbers of metaphase with one or more CSA(s) were recorded.

RESULTS AND DISCUSSION

The frequency of cells with CSAs and micronuclei (MN) increased dose dependently after incubation of the embryos in MMC, with the first increase evident at 20 g/ml. At 200 g/ml all cells contained one or more CSA(s) (Table 1). 400 g/ml MMC inhibited development completely, and no metaphases were observed. For TCE exposure, CA and MN frequencies increased only at the highest concentration (3000 g/ml), which is much higher than is found in the environment (Table 2). We need to increase sensitivity of the assay and one possible approach is to increase number of cells to be analyzed by introduction of automatic analyzing systems. The sensitivity of the assay system might depend on chemical class (e.g., the rodent micronucleus assay is relatively insensitive to halogenated chemicals (Morita et al., 1997)).

Table 1 *Frequency of cells with chromosomal aberration(s) or micronuclei in R. ocellatus ocellatus embryos treated with mitomycin C.*

Experiment	Concentration (μg/ml)	Cells with CSA (%)	Cells with MN (%)
1	0	0/50 (0.0)	3/1265 (0.2)
	0.2	0/1 (0.0)	3/969 (0.3)
	2	5/50 (10.0)	3/1120 (0.3)
	20	7/50 (14.0)	9/1052 (0.9)
	50	34/52 (65.4)	31/1073 (2.9)
	100	51/57 (89.5)	59/1147 (5.1)
	200	47/47 (100.0)	101/1098 (9.0)
2	0	0/30 (0.0)	0/300 (0.0)
	0.2	1/49 (2.0)	6/1367 (0.4)
	2	2/38 (5.3)	20/900 (2.2)
	20	57/81 (70.4)	159/915 (17.4)
	200	2/2 (100.0)	34/186 (18.3)

We performed 6 series of experiments to evaluate the frequency of cells with CSA in embryos grown in water that ranged in pH from 4.5 to 7.5. In 5 of the 6 experiments, no cell with CSA was observed at any pH (Table 3). In one exceptional experiment, 7.0, 5.1, 5.7, and 2.3% cells had CSA(s) at pH 5.5, 6.0, 6.5, and 7.0, respectively (Table 3). Those results suggest that the eggs were not fresh. When aged eggs were used, the frequency of cells with CSAs increased at low pH (Table 4). The induction of CSAs by storage of unfertilized eggs in freshwater or preservative solution was

Table 2 *Frequency of cells with chromosomal aberration(s) or micronuclei in R. ocellatus ocellatus embryos treated with trichloroethylene.*

Experiment	Concentration (μg/ml)	Cells with CSA (%)	Cells with MN (%)
1	0	0/30 (0.0)	2/1000 (0.2)
	0.03	0/31 (0.0)	2/1004 (0.2)
	0.3	0/31 (0.0)	2/1001 (0.2)
	3	0/31 (0.0)	1/1010 (0.1)
	30	1/31 (3.2)	3/1012 (0.3)
2	0	0/30 (0.0)	6/1000 (0.6)
	0.03	0/25 (0.0)	6/1000 (0.6)
	0.3	0/30 (0.0)	1/1039 (0.1)
	3	0/29 (0.0)	5/1050 (0.5)
	30	0/30 (0.0)	4/1035 (0.4)
	300	0/27 (0.0)	11/1013 (1.1)
3	0	0/31 (0.0)	1/1007 (0.1)
	300	3/29 (10.3)	6/1000 (0.6)
	3000	12/31 (38.7)	15/1030 (1.5)

Table 3 *Frequency of cells with CSAs from the embryos of R. ocellatus ocellatus that developed at low pH.*

Experiment	pH						
	7.5	7	6.5	6	5.5	5	4.5
1	nt	0/50	0/53	0/51	0/61	nt	nt
2	nt	0/77	nt	nt	0/50	0/55	0/67
3	nt	0/50	nt	0/50	1/79	0/51	nt
4	nt	0/42	0/33	0/49	0/46	nt	nt
5	0/58	0/51	0/49	0/27	nt	nt	nt
6	nt	2/87	4/70	4/78	4/57	nt	nt

nt: not tested

reported (Ueda, 1996a) in *R. ocellatus ocellatus*. CSAs were also induced in *Oncorhynchus mykiss* and *Salmo trutta* embryo cells when the eggs were held in coelomic fluid (Ueda 1996b). In *O. mykiss* and *O. masou* embryos, chromosome aberration including, a high incidence of haploidy, hyperdiploidy, and mosaicism, were caused by prolonged retention of the eggs in the coelomic cavity (Yamazaki et al., 1989). Because the eggs in the exceptional experiment might have been overripe, we concluded that low pH did not induce the CSAs, but enhanced the clastogenicity of aging. In cultured mammalian cells, in contrast, low pH itself is reportedly clastogenic (Morita et al., 1992). It is possible that there, too, the low pH itself was not clastogenic, but merely enhanced the clastogenic effect of in vitro conditions. Genotoxicity assays that use cultured cells generally are highly sensitive, and unphysiological conditions might increase positive responses. The enhancement of genotoxicity at low pH might be due to inhibition of DNA repair enzymes (Morita et al., 1992). If that is the case, an environment that is acidified by acid rain or other factors might increase genetic damage to aquatic organisms. Therefore, when evaluating genotoxicity of environmental water samples, a study at artificial low pH might predict the effect of acidic contamination. For further study, we have to study genotoxic effects of chemicals at low pH.

Table 4 *Frequency of cells with CSAs from the embryos of R. ocellatus ocellatus developed at low pH after eggs were aged (10 min at 20°C).*

Experiment	pH					
	7	6.5	6	5.5	5	4.5
1	0/50	0/50	0/50	1/38	3/25	nt
2	3/100	3/100	4/100	6/100	7/53	0/67
3	2/50	2/50	2/50	2/50	nt	3/29

nt: not tested

CONCLUSION

R. ocellatus ocellatus gamates can be obtained regularly throughout the year by controlling the temperature and light cycle, and artificial fertilization can be performed easily. Genotoxicity tests on the embryo require only a small amount of test water and high quality chromosome preparations, making this a useful system for assessing the cytogenetic effects of chemicals in a laboratory setting. One caveat is that attention must be paid to the age of the egg.

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21. Comparison of Cytotoxicity of Asbestos and Gamma-irradiation in MSTO-211H Cell Line

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INTRODUCTION

Airborne particulate matters deposited in the respiratory tract may induce various acute and chronic diseases in human. Particulate matters are phagocytosed by broncho-alveolar macrophages (BAM) in the respiratory tracts, and receive various biochemical alterations such as intracellular solubilization and degradation. Since these processes have been known to be very important for inhaled particles to exert their toxic effects, a primary culture of BAM collected from experimental animals may have potentially importance and advantage for the evaluation of cytotoxicity of airborne particles. However, the BAM cells need to be collected directly from animals just before the cytotoxicity test, since BAM are differentiated and non-proliferative cells and thus can not be maintained for longer period more than a few days under an ordinary culture condition[1, 2]. This suggests that the cytotoxicity test using BAM needs much time and labors cost. Furthermore, since cell death induced in BAM by exogenous toxicants are so called interphase cell death, the cytotoxicity assay using BAM is usually not sensitive compared with assay systems using proliferative cell death as an endpoint, especially for ionizing irradiation[3].

MSTO-211H cells are a cell line established from human mesothelioma[4], and demonstrated to have a phagocytic activity to the exogenous particulate matters[5]. We are investigating toxic effects of several particles on these MSTO-211H cells, and developing a new cytotoxicity test method for airborne particulate matters collected on air filters. Although the development itself has not been completed yet, data on the cytotoxicity of some environmental toxicants in this assay system have been accumulated. We describe the cytotoxiciy of γ-irradiation and asbestos (chrysotile) in this assay system, since one of the major purposes of this workshop is the comparison of toxic effects of environmental toxicants, especially those related to the inhalation toxicology.

MATERIALS AND METHODS

MSTO-211H cell line was obtained from American Tissue Culture Collections (ATCC CRL-2081) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum according to the directions of ATCC. Approximately 2.0 x 10^5 cells were inoculated into the 35mm culture dish, and incubated for 24 hours at 37 ℃ in 5% CO_2 and 95% air. Then the cells were loaded with chrysotile at concentrations of 3.2 – 25.0 μg/ml, or were irradiated with ^{137}Cs gamma source at doses of 2.5 – 20.0 Gy. At 24 and 48 hours after the start of exposure to asbestos or irradiation, non-adherent cells were recovered from the culture dish by aspirating the supernatant of culture medium. The cells adhering to the dish bottom were collected after the treatment with trypsin-EDTA solution. The cell numbers of adherent and non-adherent fraction

were counted with the haemocytometer, and cell survival (the living and dead cell number) in each fraction was determined by means of trypan-blue-dye exclusion test.

In some experiments, ^{3}H-thymidine (3.7 kBq/ml) was added to the culture medium at 24 hours after the exposure to asbestos or γ-irradiation, and the cells were continuously cultured for further 24 hours. At the end of culture, that is, 48 hours after the start of experiment, non-adherent cells were discarded by aspirating the supernatant. The adherent cells were washed with phosphate buffered saline, removed from the bottom of culture dish by pipetting after the treatment with trypsin-EDTA solution, and recovered onto the glass fiber filter according to a conventional cell harvesting method. The cells collected on the filter are introduced into the plastic scintillation vial, and the amount of ^{3}H incorporated into the cells was measured by a liquid scintillation counter.

RESULTS AND DISCUSSION

In order to find out a suitable endpoint for the evaluation of cytotoxicity of particulate matters, four types of endpoint were examined in the experimental group exposed to γ-irradiation. Those are: the number of living adherent cells, the number of dead adherent cells, the number of living non-adherent cells, and the number of dead non-adherent cells. In these endpoints examined, the number of living adherent cells seems to be the most sensitive indicator for the cytotoxicity of γ-irradiation. The number of living adherent cells decreased linearly with the doses of irradiation (Fig.1). In this endpoint, cytotoxicity is expressed as a suppressive effect of toxicant, γ-irradiation in this case, on the cell proliferation. Among the other endpoints examined the number of dead non-adherent cells also increased dose-dependently, and seemed to be able to use for a parameter of cytotoxicity. However, the absolute number of these cells was relatively small compared with the number of living adherent cells, and the accuracy and reliability of cell counting tend to be low. Therefore, the number of living adherent cells was used in the present study for the comparative evaluation of cytotoxicity of radiation and asbestos.

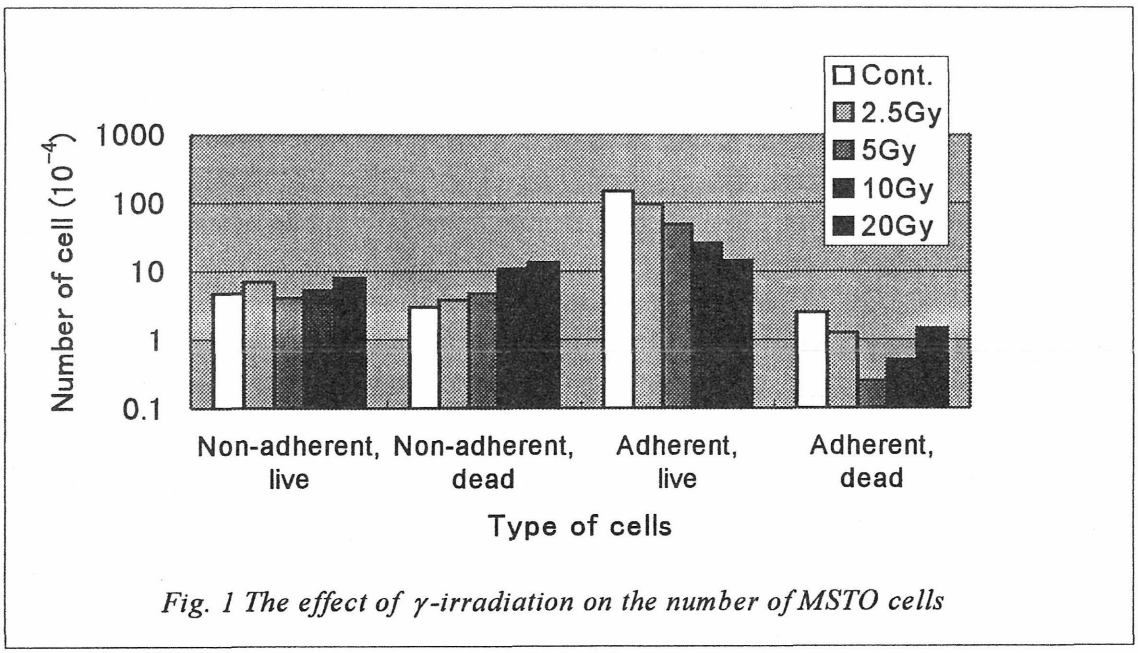

Fig. 1 The effect of γ-irradiation on the number of MSTO cells

Table 1 shows the number of living adherent cells in the γ-irradiated group 24 and 48 hours after the exposure, although the data for 48 hours are the same as those shown in Fig. 1. The number of cells was 1.5 x 10^5 at the start of incubation, and became 2.5-3.0 x 10^5 when the cells were exposed to radiation or asbestos. Therefore, the number of cells increased approximately 1.5 and 3 times for 24 hours after exposure in the 2.5 Gy irradiated group and control group, respectively. The γ-irradiation at doses of 5 Gy or more significantly inhibited the cell proliferation, and no significant increase in cell number was observed in these dose ranges. During the next 24 hours, as shown in Table 1, a slight degree of recovery was observed in the cells irradiated at 5.0 Gy. That is, in 5.0 Gy group, the cell number increased 1.7 times during this period, while it did not increased during the initial 24 hours after irradiation.

Table 1 The number of live adherent cells 24 and 48 hours after the irradiation

Radiation dose (Gy)	24 hours	48 hours	Ratio*
Control	87.0	150	1.7
2.5	43.8	96.0	2.2
5.0	27.5	47.8	1.7
10	27.3	26.0	1.0
20	21.3	14.5	0.7

* denotes the ratios of the cell number at 48 hours after exposure to that at 24 hours.

The cytotoxic effects of asbestos (chrysotile) were also evaluated with using the same endpoint as used for the γ-irradiation. The exposure to asbestos significantly inhibited the cell proliferation as shown in Fig. 2. As described above, the number of cells was estimated to be 2.5-3.0 x 10^5 at the time when they were exposed to radiation or asbestos. The cell proliferation was partially inhibited at a dose of 3.2 μg/ml, and was completely diminished at doses of 6.4 and 12.5 μg/ml. The exposure to 25 mg/ml asbestos decreased the cell number to 0.9 x 10^5. This value was significantly smaller than the initial cell number at the start of exposure, suggesting that the disappearance of cells through cell-lysis had happened during the exposure period of 24 hours.

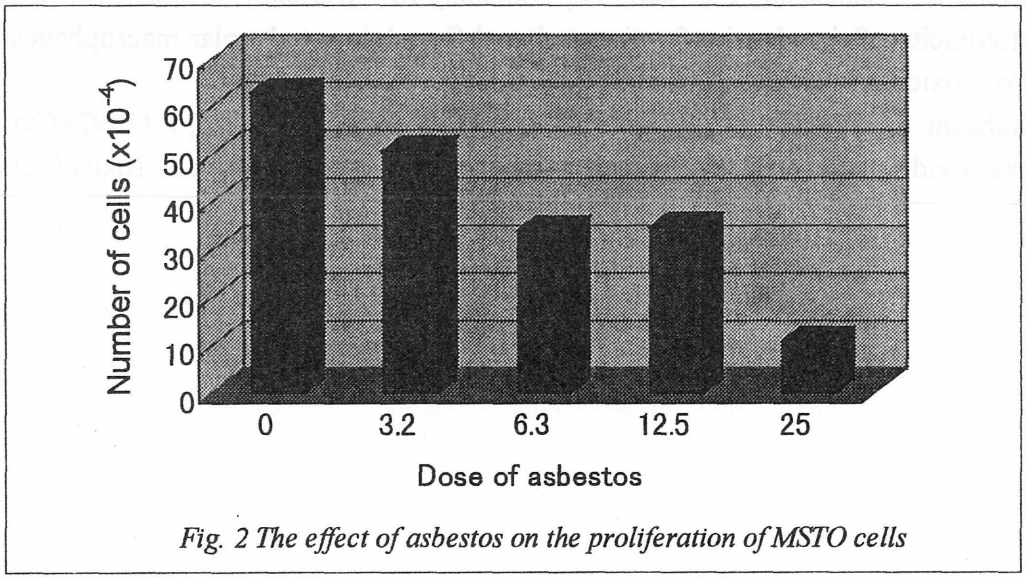

Fig. 2 The effect of asbestos on the proliferation of MSTO cells

Up to the present, the experiment was carried out only for one time point (24 hours after the exposure), and has not completed for other time points. Therefore, accurate comparison between asbestos and γ-irradiation is difficult. However, it may be possible to conclude that the proliferation of MSTO cells are partially interfered with the exposure to 2.5Gy g-irradiation or 3.2 μg/ml asbestos at a similar levels, and completely inhibited with the exposure to 5.0 Gy γ-irradiation or 6.4 μg/ml asbestos.

Usually the cytotoxicity tests using BAM are insensitive for the ionizing radiation except for those using a specific strain of mouse[3, 6]. However, the present assay system using MSTO cells is relatively sensitive for γ-irradiation. The toxicity of asbestos could be detected by this assay system. Preliminary experiment using fly ash particles and nickel oxide particles have showed that this assay system is also as sensitive as that using rat alveolar macrophages, although the data are not shown in this workshop[7, 8]. Considering these features, this assay system may be useful for the comparative evaluation of ionizing radiation and other toxic particles.

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22. Molecular Analysis of Radiation-induced Mutations in the HPRT of Normal Human Skin Fibroblasts

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INTRODUCTION

It has been reported that physical and chemical mutagens, such as ionizing radiation, ultraviolet light, ethyl methane sulfonate and *N*-ethyl-*N*-nitrosourea, induce different varieties of structural aberrations in hypoxanthine guanine phosphoribosyl-transferase gene (hprt) in various mammalian cells. While the conventional missense mutagens cause principally point mutations, ionizing radiation-induced aberrations show both point and deletion mutations. We have been investigating the molecular nature of the mutations induced by various types of ionizing radiation. In the present study, we compared mutational spectra in the hprt locus of normal human fibroblasts induced by α-particles to that induced by γ-rays.

EXPERIMENTAL METHODS

HPRT deficient mutants were isolated from normal human skin fibroblasts irradiated by γ-rays (60Co, 1～4Gy) or α-particles (238Pu, 0.2～0.8Gy, 3.5 MeV, LET 116keV/μm), and then analyzed by multiplex PCR of exons to determine the extent of deletions. In order to estimate the size of the deletion events, several sequenced tagged site (STS) primers for X chromosome (Xq26) specific markers were utilized to localize the breakpoints by PCR analysis(Yamada et al.). These markers are closely linked and spanned regions about 1.7 Mbp from the telomeric side and 1.7Mbp from the centromeric side of the *hprt* locus. cDNAs of mutants that displayed no deletion at multiplex PCR analysis were analyzed by reverse-transcribed (RT) PCR followed by sizing analysis on agarose gels. The cDNAs which size differed from that of the wild type were subjected to direct sequencing to determine the location of potential splice-site alterations. Point mutations in cDNAs that showed no significant change in size were analyzed by single strand conformational polymorphism (SSCP) analysis. The regions containing mutations were then sequenced.

RESULTS

One-hundred and one γ-rays- and 172 α-particles-induced HPRT deficient mutants were isolated and then analyzed by multiplex PCR of exons in *hprt* locus. Forty six γ-rays- and 79 α-particles-induced mutants had partial or total deletions of the *hprt* exon(s). The proportion of the deletion mutants increased in dose-dependent manner (Table 1). However, there was no obvious difference between the distribution of the deletion mutants induced by equitoxic dose of γ-rays (3Gy) and α-particles (0.2～0.4Gy)(Table 1, $p=0.265$). These deletion mutants were analyzed by PCR utilizing STS primers (Fig. 1a). The results indicated that the maximum size of the total

deletions could be greater than 2.7Mbp (Fig. 1b) and there were no apparent differences in size distribution of the total deletions induced by γ-rays and α-particles (Fig. 1b, p=0.259).

Table 1. Mutation spectra in spontaneous and radiation-induced hprt mutants

Treatment	Total number of mutants	Number of [a] normal pattern mutants (%)	Number of [b] partial deletion mutants (%)	Number of [c] total deletion mutants (%)	Statistical [d] results
Spontaneous [e]	23	21/23 (91)	1/23 (4)	1/23 (4)	
Gamma (Gy)					
Low dose (1-2)	41	24/41 (59)	12/41 (29)	5/41 (12)	p=0.001
Medium dose (3)	36	22/36 (61)	6/36 (17)	8/36 (22)	
High dose (4)	24	9/24 (38)	2/24 (8)	13/24 (54)	p=0.265
All dose	101	55/101 (54)	20/101 (20)	26/101 (26)	
Alpha (Gy)					
Low dose (0.2-0.4)	51	35/51 (69)	3/51 (6)	13/51 (25)	
Medium dose (0.5-0.6)	60	32/60 (53)	8/60 (13)	20/60 (33)	p=0.022
High dose (0.7-0.8)	61	26/61 (43)	6/61 (10)	29/61 (48)	
All dose	172	93/172 (54)	17/172 (10)	62/172 (36)	p=0.035

Note. Each mutant was confirmed by multiplex PCR exon analysis. The first number in each data set indicates number of mutants, and the number in parentheses indicates percentage of the mutants.
a) Normal pattern mutants consist of all mutants that show the same gel electrophoresis pattern as wild type.
b) Partial deletion mutants consist of either intragenic or end-deletions.
c) Total deletion mutants mean that mutants show total deletion pattern which lacks all *hprt* exons.
d) The *p* values from *chi* square tests for similarity between the spectra of mutants generated at each dose level are shown. A *p* value of less than 0.05 means that the spectra are statistically different.
e) Data of spontaneous mutants were derived from the previous study by Park *et al.*

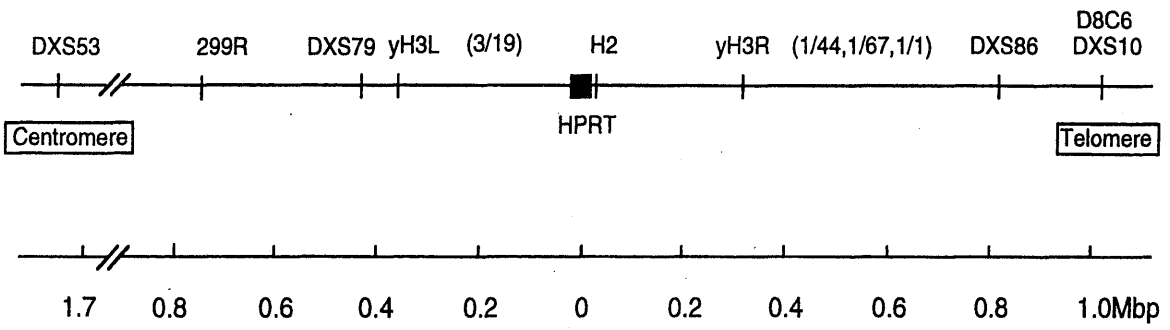

Fig. 1(a) Map of Xq26 region surrounding the human *hprt* locus. The upper bar shows the position of *hprt* locus (black square, 43kbp) and various sequence tagged site (STS) markers, and the lower scale indicates the approximate distances from the center of the hprt locus. These distances are derived from Cole et al. Exact distances from the hprt locus to those markers in parentheses are unknown.

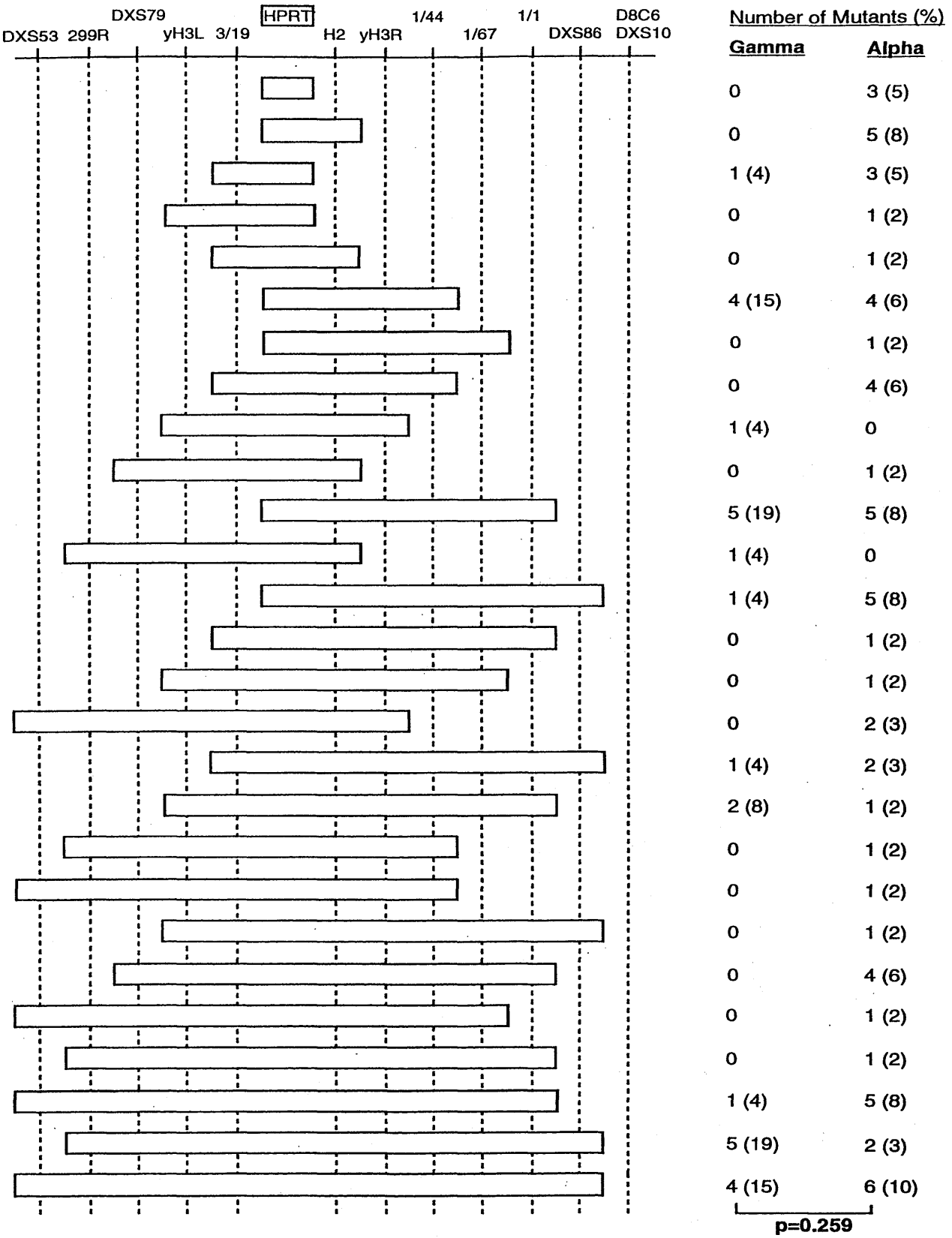

Fig. 1(b) Extent of deletions, in the region around the hprt locus, for total deletion mutants induced by γ-ray or α-particle. The patterns of deletions are shown by unfilled rectangles. The number and percentage of mutants, from γ-ray or α-particle exposure experiments, are indicated on the right side. The **p** value for the comparisons of γ-ray and α-particle-induced mutation were calculated by computer programs for analysis of mutation spectra (Cariello et al.). A **p** value less than 0.05 indicates that we can reject the hypothesis that the spectra are the same

The analysis of partial deletions indicated five α-particle-induced intragenic deletion mutants lacked only exon 2 and 3 and there were no 3'-end deletions in γ-ray-induced mutants except 3 mutants which lacked exon 9 (Fig. 2a). There were statistically significant differences between γ-ray and α-particle-induced partial deletion pattern (Fig. 2b, p=0.000). These findings indicate that certain regions of the *hprt* locus appear to be especially sensitive to radiation-induced deletion mutations and the deletion pattern may be dependent on the LET. The mechanisms for such a phenomenon, however, remain unclear.

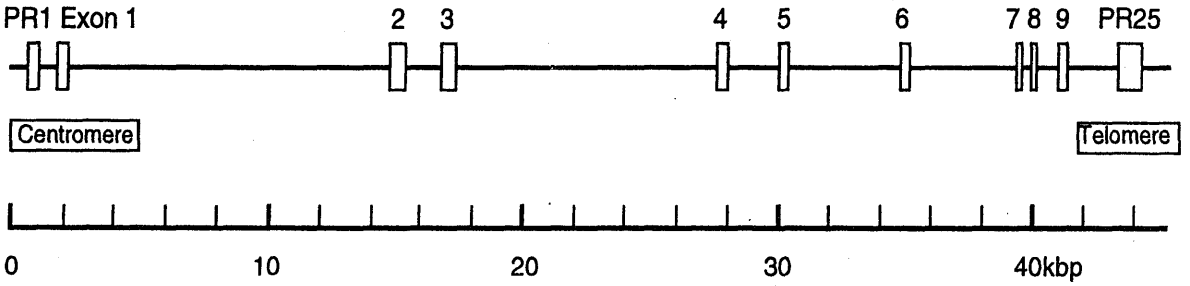

Fig. 2(a) Map of the human hprt locus. The upper bar shows the position of hprt exons, Pra and PR25 markers, and the lower scale indicates the distances from the centromere site of the hprtlocus. This distance is derived from Edward et al.

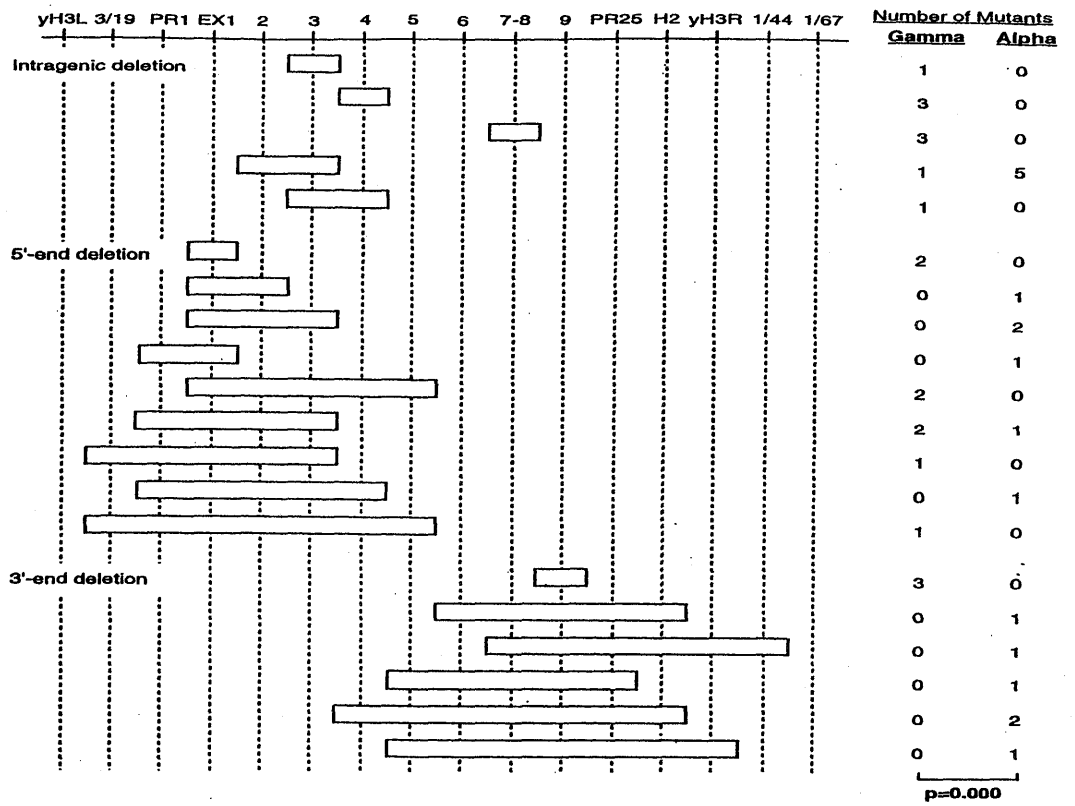

Fig. 2(b) Extent of deletions, in the exon(s) of *hprt* locus and in the region around the *hprt* locus, for partial (intragenic, 5'-end and 3'-end)deletion mutants induced by γ-ray or α-particle. The patterns of deletions are shown by unfilled rectangles. The number of mutants, from γ-ray or α-particle exposure experiments, are indicated on the right side. The **p** value for the comparison of g-ray and a-particle-induced mutation were calculated by computer programs for analysis of mutation spectra (Cariello et al.). A **p** value less than 0.05 indicates that we can reject the hypothesis that the spectra are the same.

To study the structure of the partial deletion mutation, we identified and sequenced the breakpoints of the intragenic deletions. The breakpoints of 4 γ-rays-and one α-particles-induced partial deletion mutants were mapped by PCR analysis utilizing intron specific primers and the structure of the breakpoints was determined by direct sequencing. Fig. 2c shows junction sequences of partial deletion mutations, with wild type upstream and down stream sequences. Only a few base pair homologies were found at the gamma-ray induced deletion breakpoints and they occurred in AT-rich regions. On the other hand, no base pair holomogies were found at the alpha-particle induced breakpoint. These results suggest that most of the intragenic deletions seem to result from non-homologous recombination, possibly by slippage-misalignment mechanism between short repeat sequences.

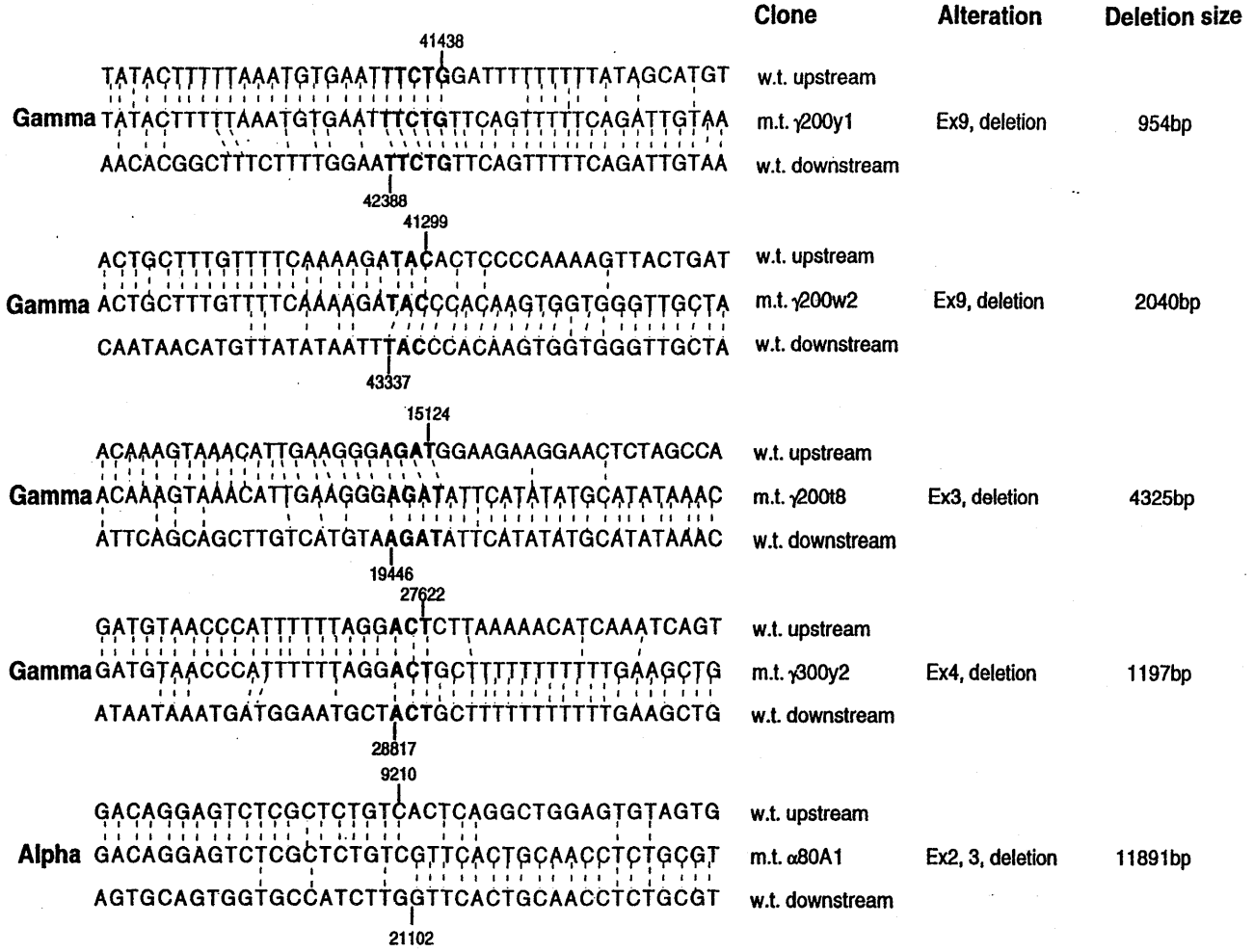

Fig. 2(c) Junction sequences of partial deletion mutants (m.t.), with wild type (w.t.) upstream and downstream sequences. Position numbers of the w.t. Sequences were referred from Edward et al. Vertical bar between bases indicate homology. Bold bases show overlapped sequences.

The cDNAs of mutants showing normal multiplex PCR patterns were analyzed by RT-PCR. Twenty-six γ-rays- and 40 α-particles-induced mutants produced cDNAs that were either shorter or normal in size when compared to the wild type (Table 2). Seven of the shorter cDNA mutations were sequenced and these data indicated various forms of exon skipping or aberrant splicing

involving exons 2 through 8 (Fig. 3a, 3b). Five cDNAs of normal size were also characterized and indicated 2 mutants with frame shifts involving either 1～2 base pair deletions, 2 mutants with transversions and a single transition mutation (Fig. 4).

Table 2. cDNA production in radiation-induced mutants

	Total number of mutants	Number of RT-PCR analysed mutants	Normal size[a] cDNA (%)	Small size cDNA (%)	No product (%)
Gamma	55	35	19/35 (54)	7/35 (20)	9/35 (26)
Alpha	93	48	33/48 (69)	7/48 (15)	8/48 (17)

Note. *hprt* cDNA products were obtained by reverse transcriptase (RT)-PCR from radiation-induced "normal pattern" mutants. The first number in each data set indicates number of mutants, and the number in parentheses indicates percentage of the mutants.
a) Normal size cDNA means that the size is the same as the wild type cDNA.

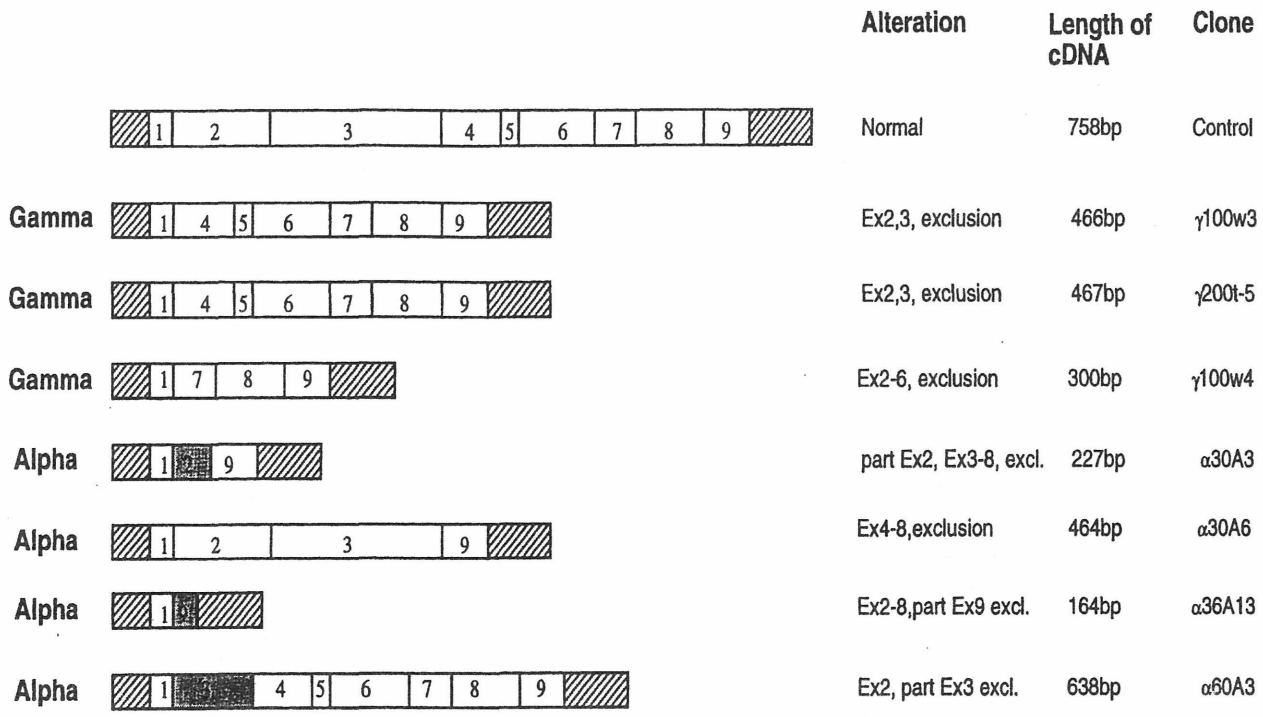

Fig. 3(a) Schematic description of the small size cDNA from γ-ray or α-particle-induced mutants.

Numbered columns indicate exons and hatched columns indicate untranslated flanking regions. Shaded columns show partial exclusion of the exon.

	Junction sequence of cDNA (5'→3')	Sequence alteration of genomic DNA (m.t.) (5'→3')		Clone
Gamma	Ex1 ¦ Ex2 ¦ Ex4 CGTG:ATTA:CCAGTCAA 130 423	Not comfirmed		γ100w3
Gamma	Ex1 ¦ Ex4 GCGTCGTG:ATGACCAG 126 419	Ex1 ¦ Intron 1 GTCGTG:**a**gtgagcag	(a insertion)	γ200t5
Gamma	Ex1 ¦ Ex7 GAGTCGTG:CTTGCTGG 126 584	Ex1 ¦ Intron 1 GTCGTG:gtga**a**cag	(g:c→a:t transition)	γ100w4
Alpha	Ex2 ¦ Ex9 TACCTAAT:CATGTTTG 177 708	Intron 8 ¦ Ex9 ttataa:CATGTTT	(g:c→a:t transition)	α30A3
Alpha	Ex3 ¦ Ex9 GCTATTGT:GTTTGTGT 417 711	Not comfirmed		α30A6
Alpha	Ex1 ¦ Ex9 GAGTCGTG:ATTAGTGA 126 721	Ex1 ¦ Intron 1 GTCGTG:**a**gtgagcag	(a insertion)	α36A13
Alpha	Ex2 ¦ Ex3 ATGATGAA:AGGAGATG 141 263	Ex3 GTGATG**C**AGGAGA	(A:T→C:G transversion)	α60A3

Fig. 3(b) Sequences of junction site of cDNA and mutated splice sites of g-ray or a-particle-induced mutation affecting mRNA splicing. The hprt cDNA whichh size was different from wild type cDNA were sequenced. Mutated splice sites of genomic DNA were also amplified by PCR and then sequenced. The positions in the cDNA are numbered according to Jolly *et al.* Exon sequences are indicated in capital letters and intron sequences are indicated in small letters. Mutated bases are shown in bold and underlined letters

	Sequence alteration in cDNA	Position	Local sequence (w.t.) (5'→3')	Clone
Gamma	AA deletion	484,485	TGGAAAG**AA**TGTCTTG	γ100t2
Gamma	C deletion	585	GTCGCAAG**C**TTGCTGG	γ400C14
Gamma	G:C→T:A transversion	563	GTATAATC**C**AAAGATG	γ200y5
Gamma	A:T→C:G transversion	473	CTCAACTT**T**AACTGGA	γ300B2
Gamma	A:T→G:C transition	714	AATCATGT**T**TGTGTCA	γ400C5

Fig.4 Sequence analysis of radiation-induced point mutation. The cDNA which size was same as wild type cDNA were sequenced. Positions in the cDNA are numbered according to Jolly *et al.* Affected base(s) are indicated in bold and underlined letters

CONCLUSION

HPRT deficient mutation assay and PCR method are available for molecular analysis of mutations in cultured cells. Ionizing radiation induce various types of mutations including point mutations and deletions, and the radiation-induced deletions extend to varying degrees into to the upstream and downstream regions beyond the *hprt* locus. Further investigations are required to elucidate the mechanisms of radiation-induced mutations and the differences in the α-particles and γ-rays irradiation.

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23. Photogenotoxicity of Polycyclic Aromatic Hydrocarbons

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), mostly produced during the incomplete combustion of organic materials, are among the major environmental pollutants being found in the waters, air, soils, and sediments [1,2]. Some PAHs are known to possible carcinogen after the metabolic activation conditions. However, recent toxicological studies, particularly in the discipline of aquatic toxicology, have presented evidence that PAHs may become toxic or substantially more toxic upon co-exposure to UV light [3]. We employed a series of *in vitro* genotoxicity assays to assess the photogenotoxicity of PAHs existing in environment - neutral red uptake assay using BALB 3T3 cells (Photo-NRU) assay, single cell gel (SCG) assay, mammalian cell mutation assays, chromosomal aberration assay, and cell transformation assay using BLAB 3T3 cells. We evaluated the photogenotoxicity of commercially available PAHs. We also investigated the photogenotoxicity of ash and river sediment, which were known to contaminate with PAHs.

MATERIAL AND METHODS

Chemical and light source

Eight PAHs, naphthalene, anthrancene, phenanthrene, pyrene, benzo[a]pyrene, benzo[a]anthracene, naphthacene, diphenylanthracene were purchased from Wako Pure Chemical industry. The ash used in experiments were collected from the 10 points of incinerators. The river sediments were collected from several points of SAKAWA River (Kanagawa). The extraction of PAHs from ash/river sediments was performed with ethyl acetate. In all experiments, sunlight simulator (SOL 500, Dr Hönle, Martinsried, Germany) was used for light source of irradiation. The intensity of irradiation was measured with a UVA meter (type no. 37, Dr Hönle)

Photo-NRU assay

The method of Photo-NRU assay was followed EEC/COLIPA joint validation project for the detection of phototoxicity [4]. The BALB 3T3 cells (mouse fibroblast cell line) were treated with chemicals under the irradiation of sun simulated UV at the intensity of 1.6mW/cm^2 for 50 minutes (about 5J/cm^2).

In vitro genotoxicity assays

The induction of primary DNA damages were evaluated by SCG assay to detect strand breads using Chinese Hamster fibroblast CHL/IU cells. Clastogenicity were evaluated by chromosome aberration assay and *in vitro* micronucleus assay using CHL/IU cells and BALB 3T3 cells. Gene mutations were evaluated by mouse lymphoma assay using L5178Y cells and HPRT gene mutation assay using Chinese hamster V79 cells. Cells were treated with chemicals under the irradiation of sun simulated UV at the intensity of 1.0 to 1.6mW/cm^2 for 50 minutes (about 3 to 5J/cm^2).

RESULTS AND DISCUSSION

PAHs

In the Photo-NRU assay, 6 of 7 PAHs indicated about 30 (phenanthrene) to 70,000 (benzo[a]pyrene) fold of enhancement of cytotoxicity. Among these PAHs, diphenylanthracene was tested for DNA damage and clastogenicity. About 10,000-fold enhancement of genotoxicities were observed in SCG assay and *in vitro* micronucleus assay. Benzo[a]pyrene was tested for DNA damage, clastogenicity, mutagenicity and cell transformation induction. Similar to diphenylanthracene, benzo[a]pyrene exhibited about 10,000 fold enhancement of DNA damage and clastogenicity, however, enhancement of mutagenicity and cell transformation induction were not detected. Therefore, Photo-NRU assay, SCG assay, and *in vitro* micronucleus assay are suitable for the photogenotoxicity testing on PAHs and other phototoxicants.

Ash extracts

In the Photo-NRU assay, 9 of 10 ash extracts indicated 5 to 108 fold of enhancement of cytotoxicity under the irradiation. Among them, two ash extracts were tested clastogenicity. By *in vitro* micronucleus assay, these two ash extracts significantly induced micronuclei by UV irradiation, but neither without irradiation nor exogenous metabolic activation.

River sediment extracts

In the Photo-NRU assay, river sediment extracts clearly increased cytotoxicity by UV irradiation. The intensity of phototoxicity was well correlated to the results of chemical analysis of river water.

CONCLUSION

Some PAHs strongly enhanced cytotoxicity (70,000 fold) and genotoxicity (10,000 fold) in cultured cells by the sun simulated UV irradiation. Therefore, photogenotoxicity of PAHs existing in environment can be detected by *in vitro* bioassay systems, *i. e.*, Photo-NRU assay, SCG assay, and *in vitro* micronucleus assay. Using these assay systems, photogenotoxicity was detected in ash/river sediment.

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24. Genotoxic Monitoring System for Water Pollution Using Aquatic Organisms, Sea Urchins

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INTRODUCTION

Bioassay systems using sea urchins have been widely used to assess pollution of marine environment. For this purpose, developmental toxicity such as fertilization or hatching rate has been mainly used, however, the geno-toxicological analyses have not been well established. In the geno-toxicological analyses, the micronucleus assays using several aquatic organisms, such as fishes, amphibians, and mollusks have been developed in recent years and provide a feasible approach to field monitoring in aquatic environment.

In the present study, we aim to develop micronucleus assay using sea urchins, apply this assay to the field water from river or sea and clarify the problems of this assay in evaluating the water pollution.

MATERIALS AND METHODS

Sea urchins, *Hemicentrotus pulcherrimus* and *Clypeaster japonicus* were used as materials. Eggs and sperm were obtained by injecting acetylcholine chloride into the body cavity of sexually mature adults. The fertilized eggs were cultured in the artificial sea water to the early blastula stage.

The embryos were treated with model chemicals (mitomycin C (MMC), vinblastine, 1-β-D-arabinofuranosylcytosine (Ara-C)), or the field sample from river or sea. The osmolality of the water from river was adjusted by artificial seawater (jamarin U). After treatment for overnight, the embryos were dissociated into their component cells in 1M urea by pipetting. The dissociated cells were fixed with 1:9 acetic acid-methanol and preparation was made by air-drying. The cells were analyzed microscopically after staining with acridine orange. 4,000 cells were analyzed in each group.

RESULTS

1. Induction of micronuclei in the blastomeres by model chemicals

When the embryos were treated with MMC, vinblastine and Ara-C, micronuclei were induced clearly in the blastomeres and a linear dose-response relationship was observed between the frequency of micronucleated cells and concentration of MMC (Fig. 1), vinblastine, and Ara-C.

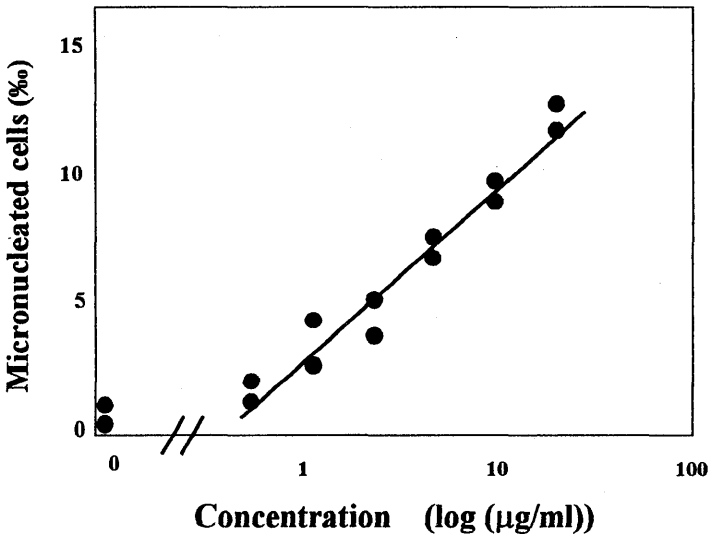

Fig. 1 Frequency of micronucleated cells induced by MMC

2. Application to the water collected from the river and sea

The samples from several places in the Sakawa River in Kanagawa prefecture were examined by this system. The chemical analysis showed that some variables, such ad COD, increased in the samples collected at downstream compared with those at upstream, whereas induction of micronucleus in sea urchin embryonic cells was not observed.

The sea water from Tokyo Bay was assayed during two years of 1996 and 1997. The sea water induced developmental toxicity and micronuclei constantly at the frequency of 4-25 ‰, which were very high because MMC, used as a positive control, induced about 6 ‰ at 10 μg/ml. Since the induced frequency of micronucleated cells treated with the seawater collected from Tokyo Bay was too high to explain as chemical pollutant, we objected this phenomenon to be clarified. Our findings are as follows.

3. Problems in evaluating pollution of environmental water

Osmolality out of many factors was considered as the most important factor in this case, because there was the river near the collecting points. In fact, salt concentration of the sample water was about 1/2 of that of normal seawater. While the induced micronuclei decreased by adjusting salt concentration, also the micronuclei were induced by diluted artificial seawater which was prepared with unpolluted distilled water. From these results, micronuclei observed after treatment with the sample seawater collected from Tokyo Bay were found to be induced by hypotonicity of the sample.

DISCUSSION

1. The sea urchin micronucleus assay was developed using embryos at the blastula and gastrula stages. This system could apply to the water from river or sea. Since we could not detect micronuclei in the water collected from the Sakawa River, higher sensitivity of the system is required to detect low levels of contamination. One of the ways to resolve is to increase the sample size (number of cells analyzed) by automation of analysis using an image analyzer or a flowcytometer.
2. The hypotonic condition (also may be other unphysiological conditions) to the organisms increased the frequencies of micronucleated embryonic cells. Therefore, even if the micronuclei are induced in the field water at high frequency, the water is not always polluted by chemicals. Accordingly, we have to be careful to interpret the sea urchin micronucleus assay results and we have to take account of other information of the sample, such as osmolality, pH, temperature as well as the results of chemical analysis.

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General Discussion

Chairperson (Dr. Kobayashi) : Good afternoon, ladies and gentlemen. We now start the final session of the workshop for General Discussion. Thank you Dr. Nakamura. As for the convenience of discussion, we would like to proceed with the general discussion in the following order. Firstly, Ecological Aspects, secondly, Biological Aspects, and then finally, Integrated Overall Issues. We would like to invite comments or discussions or additional information firstly on Ecological Aspects. We understand Dr. Schell has prepared some presentation on this point. May I invite Dr. Schell?

Dr. Schell : Thank you, Mr. Chairperson. We came here as a diverse group of scientists with many interests but focused on the global problems of comparative health effects of technology. There must be some way we can continue the good work initiated and the fruitful discussions we have had with our friends and colleagues around the world.

I have tried to look into the ecological aspects of environmental toxicants and to evaluate what might be of importance for the future. All the participants provided very interesting posters, papers and open discussions and my comments will only reinforce what you are already considering. My basic message is really to collaborate and to encourage interactions among the scientists who are here and those who have shown such excellent approaches to a wide range of investigations. The key to the future lies with the young people who can provide solutions to the problems we have posed at these meetings.

I would like to take a few minutes to review one or two aspects of health effects of environmental toxicants in ecosystems. The source, flux, and deposition of many chemicals and radionuclides influence the global ecosystem including man. It is my desire to focus more clearly on the ecosystem and its several aspects. As such, I encourage the consideration of an integrated modeling program to look at the transfer and transport of radionuclides and trace elements into the terrestrial, interface and aquatic ecosystems. The results of which may be a new field of inquiry.

Here is one approach to develop a set of models to provide information on the transport and deposition of organic and inorganic contaminants, their toxicity to man and to other biota. A linked integrated model could provide a framework for understanding contaminant input in the past and to project such input into the future. We must model a terrestrial system and some kind of interface system leading to an aquatic system where dose-to-man from food sources dominates. In practice, we usually have only a single team working on any given subject but without overall coordination. The complex model building and formulation requires collaboration among many people and one goal for the future could be more interaction amongst scientists with diverse technical backgrounds. To simplify such model building, commercially designed software is available for problem solution but what is lacking is problem definition and insight. Without such software, programming the computer and solving the many equations takes months to years. Thus, most people do not even contemplate such models. Realistic ecosystem models are much too complex for any single group. In considering modeling goals, metaphorically, we are all worried about water that is too deep when we fear we cannot swim! My feeling is: we should try to swim a little because with practice, we could become expert swimmers. We can accomplish many goals by collecting together a mix of people who are interested in the same general subject, as we have experienced here this week.

Modeling provides a systematic method for organizing and using information. In defining data for use in models, we often use single values giving a deterministic solution to the model. However, to establish error limits we need many values and a probabilistic approach in modeling which can define the range to be placed on end point parameters such as chemical dose. How can one model such a complex ecosystem? After defining the logic structure of the system under investigation, we can lump variables and coefficients into aggregates to limit the complexity. However, the practicality of which coefficients to lump together must come by determining which values are most in fluential to the total model. This is done through several runs using data over the range of values where we can determine a parameter sensitivity index. Experimental measurements must accompany the validation process to test the reality of the models before wide application.

An important policy question is: How do we manage a contaminated zone such as that found after the Chernobyl Accident? In delving into the policy questions, we found that political and social issues became more important to the affected population than the technical issues. Measurements of human dose while most important initially, were questioned by the affected population. Displaced people wanted to return to their ancestral homes. Children, for example, were affected by the radioiodine and cesium, which was recognized only significantly later by a series of health effects. The Byelorussian government set up schools at resort areas in the winter where children from highly contaminated areas could spend part of the year in an uncontaminated area. We must focus on policy and technical issues, which are important and utilize the experience gained at Chernobyl.

We must develop a global vision of toxic input and then divide this picture into parts, which can be comprehended. Once armed with this vision, we can handle most any problem using contemporary scientists and the latest computer equipment. Let's take a look into the future and the goals we would like to reach. We must first establish the goals, then we must break down the problem into useful parts. Through the stimulation of young people using conceptual models, we can gain insight into the global ecosystem and to health effects of environmental toxicants to mankind. Thank you.

Chairperson (Dr. Polikarpov) : Thank you Dr. Schell for your very good presentation which illustrated a new and excellent proposal. You are the king of models.

Chairperson (Dr. Kobayashi) : As to the ecological approach, we have had very useful suggestions. And I take all the materials presented by Dr. Schell will be included in the proceedings. Is there any discussion on this particular point?

Dr. Maubert : I would like to say something not exactly discussion on this, but this is general comments. This meeting, in my opinion, was the first of its kind because we put together many disciplines One about the study of comparative risks of pollutants. Comparison between asbestos and tobacco, or tobacco and radiation for example. It is very difficult to see what dose of asbestos is equivalent to what dose of radiation, and we need standards for that. Now we have another goal for improvement of a global vision of ecosystems as Dr. Schell said before me. Everything is interdependent. It is nonsense to conceive an animal by itself, for macrophage by itself and a human being also by itself. It is therefore very important to promote a cross-fertilization of techniques, putting together people in charge with chemical pollutants, radioecologists and so on. But, lets not to go too far in that way, because some disciplines may still be too far apart, for example, if you compare global circulation models in the ocean, and genetic mutation induced by

radiation, that cannot be right away in the same group! Anyway, I enjoyed this meeting, like many other people here. Again, it is the first of its kind, and one-day we will say : That meeting in Chiba in 1998, I was there!

Chairperson (Dr. Kobayashi) : Thank you Dr. Maubert. That was a concrete proposal to set up a task force or a working group. If there is no response to this, in view of the time constraint, we would like to move into the second topic of "Biological Aspect". I have here a question from Dr. S. Takahashi (NIRS) to Dr. Seitz as follows: "In the Toxicology Session, there was a little confusion between deterministic and stochastic effects of toxicants including radiation. I appreciate if Dr. Seitz would explain again how deterministic and stochastic effects are incorporated in the "Assessment of Health and Environmental Effects from Radioactive and Chemically Toxic Waste." Could you answer this question, Dr. Seitz?

Dr. Seitz : In the context of a health effects assessment and comparison within the IAEA project, I am looking for ways to simplify some of the complexities involved in data regarding the many types of potential health effects. In general, I am trying to classify radioactive and chemical toxicants into two basic categories of effects (namely, "no-threshold" and "threshold"). For radiation, "no-threshold" and "threshold" would be assumed to be equivalent to the ICRP and IAEA terms "stochastic" and "deterministic" effects, respectively. For chemical toxicants, as generally endorsed by many national and international organizations, "no-threshold" effects would be assumed for genotoxic carcinogens and germ cell mutagens and "threshold" effects would be assumed for toxicants posing other toxic effects. In the case of epigenetic carcinogens, there are differences in assumptions from national and international organizations regarding threshold or no-threshold effects. Thus, appropriate categorization of effects from epigenetic carcinogens will likely need to be considered on an individual basis. Consistent with current trends, if there is sufficient scientific information indicating that a specific toxicant may not behave in accordance with the above assumptions, then exceptions can be made in respect of the above general approach. In terms of comparing threshold and no-threshold effects, practical applications to test a number of potential comparison approaches is one part of the new Coordinated Research Project which is part of my overall project. Some potential approaches are briefly introduced in my paper.

Chairperson (Dr. Kobayashi) : Thank you Dr. Seitz. I would like to encourage anyone to speak up. Yes, Dr. Patrick, please.

Dr. Patrick : I would like to make a simple point arising out of this morning's session on Toxicological Studies. Dr. Nakamura has just highlighted the main problem as I see it, and this is : how we can select toxicological tests to compare the effects of very different modalities, e.g. ionizing radiation and cytotoxic substances? Can we use one test to compare these different things? Each paper this morning pointed out the difficulty of this problem. For example, the viability of alveolar macrophages is a very sensitive test for certain kinds of particle, but it is insensitive to radiation, as demonstrated many times before and illustrated very well this morning. If we look at a complete different model system - double strand breaks of DNA - we find that this is of course sensitive to radiation, for radiobiologists have used this system for many years, but Dr. Takahashi has found that it is insensitive to asbestos fibers. Then we had a very comprehensive paper by Dr. Ogiu on carcinogenesis from nitroso compounds in vivo, showing that one compound is effective in one organ system, and another different compound is effective not in that organ but

in another. So in effect we have the same problem at the level of DNA, at the level of the cell, and of the whole animal. The same problems arise in each case, and we do not have a single test to compare these very different modalities and toxicants. Now what I would like to suggest is something very simple, that is, we can make some progress with the problem by concentrating on the mechanisms of toxicity, and these of course vary widely according to a system we are looking at. I would like to illustrate what I am saying by referring to the system that I talked about in my lecture, the response of alveolar macrophages. We can classify the responses from these cells as a hierarchy of effects:

(1) There are materials which will make the macrophage elicit a functional response by producing reactive oxygen species like superoxide anion, nitric oxide and other reactive nitrogen species, also cytokines and so forth. Such a material may be cytotoxic at the same time, or if it may not, but we can see this kind of functional response as the first level in the hierarchy.

(2) Next, There are materials which produce effects on the cells which kill them with no functional response, where the cell is killed by necrosis. If the cell dies by necrosis, then it can affect the organ by inflammation or other reactions due to cell death.

(3) On the other hand, it has been found in recent years that cells can die by a process of programmed cell death or apotosis, which means that a cell can die quietly and not affect the rest of the organ.

(4) Finally, We can have agents, in this case ionizing radiation, which at normal doses - doses we are mainly interested in - produce no response whatsoever.

Now to a toxicologist, it is always a problem if you do not get a response over the dose range of interest. Toxicologists usually have to use higher and higher doses in order to show some response. However, we could just say that there is no response for this particular cell type following reasonable doses of radiation. Bearing all this in mind, and having selected the alveolar macrophage as the cell to use, we cannot say very much about radiation effects, but we can determine the effects of asbestos fibers, or lanthanides and so on. Thus we come to the question of how we should actually select which test system would are going to use. The above hierarchy refers to only one test system. I should like to say something very simple about how we might proceed to choose a test or tests, with which to compare very different agents. When we use such tests we can obtain two kinds of information:

(1) We would hope to provide predictive information, i.e. about the safety of some new compound, which has been developed, such as a mineral fiber, and whether it is likely to be toxic to man.

(2) We may also be able to test some hypothesis, and one of the advantages of test systems using cell biology techniques, for example, is that they can contribute to understanding the mechanisms involved in toxic action.

Depending on the selection of the test system, we may improve our understanding of the mechanism of action in the particular cell: how is DNA broken, how is the cell triggered into apotosis, and so on. On the basis of this improved understanding, we might then go back and modify the test system, or select a new test system. Novel test systems are being introduced all the time, because new methodologies are being continually introduced in modern biology. What I have tried to suggest here is that there is some kind of cycle by which we can continue to improve our choice of test systems in toxicology. We cannot expect that if we choose the appropriate cell system today, it will be the best system in some years' time. The cycle should lead to improvements in the type of test system we should use in environmental toxicology, by

concentrating as much on the mechanistic side as on simply predicting what the effects would be from any one relevant system.

Chairperson (Dr. Kobayashi) : Is there any argument on this presentation or any question? Everybody agrees to his suggestion? Apparently, so. May I invite Professor Hsie to give us your opinions?

Dr. Hsie : I would like to say something biological and something non-biological and in fact, maybe somewhat social and political. After listening to the wonderful presentation for the last three days and taking the one day private tour and briefing at NIRS, I feel really at home being with NIRS because I have been long associated with the Biology Division at Oak Ridge National Laboratory (ORNL) (1972-1988). In many respects, NIRS is similar to ORNL in terms of scientific missions. While NIRS has done a marvelous job in the ecological risk of ionizing radiation and other studies, ORNL has excelled itself in studying the genetic and somatic effects, basic and applied induced by radiation and environmental chemicals. I am pleased to say, that with my limited interaction in NIRS, I am indeed most impressed with the elegant work of Drs. Takahashi and Yamada and their colleagues on molecular and cellular studies of radiation effects. I strongly recommend to Drs. Takahashi and Yamada to establish a collaborative program with Drs. Sofuni and Hayashi at National Institute of Health Sciences who study the genetic effects of environmental chemicals. These two teams take complementary approaches studying the biological effects and they can complement each other. The second thing I would like to talk about is to suggest NIRS establish a formal graduate program. One of the secrets of success of URNL's Biology Division is the establishments of the University of Tennessee - Oak Ridge Graduate School of Biomedical Sciences at Oak Ridge by Dr. Alex Holleander, the then Director of the Biology Division thirty-four years ago. While the senior scientists at GRNL benefit from the privilege of having graduate students, the University of Tennessee is most pleased to have a no-cost high caliber graduate school in biomedical sciences at Oak Ridge only thirty five miles away from the main campus at Knoxville. I feel NIRS can model itself after ORNL to team up with Chiba University to establish a formal graduate program (or graduate school) for radiological sciences or for biomedical sciences with an emphasis on radiological sciences. I will be most honored if I can be part of the proposed endeavor and hope that my sincere constructive recommendation will receive positive criticism and actions.

Chairperson (Dr. Kobayashi) : Thank you Professor Hsie. I think we would be able to show the result of a new system to have graduate students at NIRS in the next meeting. May I invite any discussion on this point? May we move into the third issue: Overall Aspect. I have two prepared proposals, one is from Dr. Seitz and another from Professor Polikarpov. May I firstly, invite Dr. Seitz? Would you please present your views?

Dr. Seitz : I also would like to thank NIRS and all the organizers, because it is very informative for me as I tended to look at the these activities from the assessment point of view. It is very valuable for me to understand more about some of the problems with generation of toxicology data and a variety of issues associated with basic human and ecological toxicology research. In the next few minutes, I would like to highlight some things that I think are important for any kind of assessment that would be considered a comparative assessment in the context of this workshop. There are three basic points that I would like to highlight; multi-disciplinary nature of assessments,

the need to simplify the problem and the importance of identifying potential bias in the assumptions.

The first point to emphasize, as mentioned by other lecturers, and myself is the multi-disciplinary nature of assessments. As we can see in this workshop, there are people involved in different disciplines, including ecology, toxicology, physics, water resources and many others. In terms of new assessment projects, it is first necessary to understand the diverse types of wastes that are associated with energy systems. And I guess, fortunately or unfortunately, when we look at the waste from electricity generation, you find many of the types of contaminants and types of health effects that could be observed. Thus, this type of comparative assessment provides good fertile ground for research, because there are so many different contaminants and health effects that need to be considered. Once the waste is understood, it is necessary to consider how contaminants move in the environment and how humans or flora and fauna can be exposed to the contaminant. For example, there was a paper on obtaining data on how much exposure would occur, which emphasizes that you need to begin to understand how a person may become exposed to those materials. Then, finally, we need to look at health effects and ecological effects. This all emphasizes the idea that we have this very broad scope of different disciplines involved.

So, as this workshop reflects, step one is to seek a contribution from people from other disciplines, and I only support the benefits of getting people together from the chemical side and radiological side as well as people with backgrounds in human health and environmental effects. This has worked well in small meetings related to my project, where we brought diverse groups of people together. These are senior people with vast experience and they have all benefited from the sharing of information.

The next issue from my list focuses on the fact that you have all of this complexity and all of these technical issues to resolve. Somehow, you need to simplify everything into a manageable problem. As you try to simplify it, it is clear that still there are many areas that need to be investigated. So, at some point, research needs to start focusing on what the critical information is needed to support the assessment. Let's try to identify the most important parts, and let's try to create some reference sets of conditions that simplify some of the complexity in order that we can do an assessment. This is my second issue, somehow we need to limit data needs and the complexity of assessments. That is, we need to start drawing lines and saying that these are areas where simplifying assumptions are needed in order to conduct an assessment.

Now I will again show Occam's Razor. I don't understand Latin, but do understand its English translation, namely "what can be done with less is done in vain with more". Let's try to keep this thought in mind for whatever you are doing, basic data collection, creating models or conducting a comparative assessment. One way to help with this, and we have seen this in some of the papers at this meeting, is the idea of probabilistic assessments. I would like to refer to them as sensitivity and uncertainty analysis. Sensitivity analyses have been very valuable in assessments that I have been involved in, as a way to justify why some research needs to be done and also to justify why another research activity, based on information that you have available, may not be necessary. By using these kinds of analyses, you can begin to focus your attention on the data that are most important for the assessment. This can be very useful, because you can justify why an activity is needed to support comparative assessment. For example, when you come to decisions regarding funding, sensitivity analysis can help justify why one item of work needs to be done by showing the influence that the new information can have on the conclusions.

I just would like to make a comment, my undergraduate degree was in mathematics and statistics, and I very much support the idea of statistical methods and probabilistic approaches.

But, when you get involved in very complex systems like this, you need to have a warning in your mind that if you get to the point, where you have to start making up information due to a lack of data, such as creating distributions for inputs, then you may be going a little bit too far with the technique. That is, you do not want to apply a technique, for the sake of it, if you do not have the information to support it. This is something applicable for all kinds of assessments. We modelers often say, "garbage in, garbage out". For example, if we do not have some basic research to collect data, we may not have a basis to conduct an assessment. But it is the same when we use more sophisticated techniques. If you do not have the detailed information to support that technique, the technique may be of no more value than a simple calculation, and in the worst case may provide misleading results. I want to emphasize that sophisticated techniques may still have some value, but remember that the results may be more a reflection of your assumptions than behavior of the system.

Another example of simplification can be provided for ecological assessment. There are so many different flora and fauna that you may need to consider. This is one area where we need some simplifying assumptions. The Rio Conference highlighted the term "bio-diversity" as a measure of sustainable development for the ecological context. I think one valuable thing could be done is to define bio-diversity in terms that can be interpreted for the modeling process. For example, we need to do an ecological assessment for "critical species" in respect of maintaining "bio-diversity" in an area. Thus, bio-diversity may be quantified in terms of identifying the critical species that need to be protected.

The final point involves the identification of potential bias in the assumptions used for an assessment. I have been involved in a number of assessments for years and presenting results for university professors, the public and peers at other laboratories. In all cases, the key things that you need to understand are potential uncertainty and bias in conclusions. Basic research should help to identify assumptions that may bias results. For example, the question whether there is a threshold or not for health effects can bias the results (e.g., you can come to different conclusions if you assume there is or is not a threshold for the effect from a given contaminant). For solid waste, this is a very relevant issue due to the relatively small amounts of contaminants that would potentially be released to the environment. Another example of a potential source of bias is the use of safety (uncertainty) factors when identifying levels for threshold effects based on animal data. From this meeting, we learned those uncertainty factors could change, and the estimated threshold could change, for example, safety factors can vary depending on which organization interprets the data and thresholds could change depending on which type of rat or mouse was used in an experiment. All of these factors need to be at least recognized when one is doing an assessment, especially in respect of how they can result in a change in the conclusions of the assessment.

On a different subject, before I conclude, I was not sure about Dr. Takahashi's question earlier, but to address the issue of how to combine risks from different agents. There are two concepts being seen more and more often, the idea of margin of exposure and margin of protection. An example, margin of exposure, involves comparing the actual projected exposure with the level of exposure where you observe an effect or with levels observed in the natural environment. An example of margin of protection is a comparison of an acceptable regulatory concentration with the concentration of a contaminant that you predict. That is something you possibly could use as the common basis, although we know its present uncertainty in multinational assessments where you will not have common standards for acceptable concentrations. And, finally, I would like to support the idea of the development of mechanistic models for human toxicology as a supplement

to support animal studies. For example, models are being developed that estimate the toxicity or mode of action in the human body of a given substance based on its chemical structure (so called, physiologically based, pharmacokinetic (PB-PK) models and quantitative structure activity relationships (QSARs)). These models reflect the growing trend to understand the mechanisms of action for a substance in addition to simply observing tumors or other effects in laboratory animals.

Chairperson (Dr. Kobayashi) : Thank you very much Dr. Seitz. Any comment or question? Dr. Takahashi? Everybody seems to be impressed.

Dr. Takahashi : I agree with him.

Chairperson (Dr. Kobayashi) : So, thank you very much for pointing out clearly some essential principles in the assessment. May I then invite another prepared presentation. Professor Polikarpov, please.

Dr. Polikarpov : My comments and propositions. From my point of view, the Workshop is very successful. It brings together experienced scientists and full of energy growing young scientists. The big proportion of young representatives at the Workshop is very promising to continue and develop the success of this the 1st Workshop on this subject - Comparative effects studies of ionizing radiation and chemical pollutants. I think that the next step should be compilation and, of course, production of data on comparative effects of ionizing radiation and chemical / physical agents. Probably, the best form to fulfill such task internationally might be a Working Group on this subject for (a) collection and generalization of existing data (dispersed in different institutions) as well as (b) critical analysis of data on comparative effects of radiation /other factors. The same Working Group, or may be better, a specialized Working Group (or sub-group) should manage work on assessment of separate contribution of action by ionizing radiation and action of other factors, which influence simultaneously and in a mixture. As regarded to Eco-ethical principles in scientific work, I would underlined excellent impression from the key-note address by Dr. K. Morimoto (Osaka Univ.) on "Life Style" and P-18 by Dr. Anita Enflo "Where are the Radon Induced Lung Cancer Cases?" and the lecture in this afternoon session by Dr. Takizawa.

The Japan science is very experienced in management of dangerous wastes, and according to my personal impression during this visit, the Science in Japan follows Eco-ethics in the best way, possible for the moment. May I illustrate on only one short example the applicability of concrete data to the conceptual model on combined effects (which I proposed at the Workshop)? According to B-4, Dr. S. Fuma et al. "Ecological effects of Radiation and Other Environmental Stress on Aquatic Microcosm", we can plot their data on my Fig. 1 and obtain:
(a) confirmation of coincidence of received and predicted effect as well as
(b) make a table of equivalent doses, produced the same effect (extinction of 1-2 species):
γ ray 500 Gy = UV 50 k*erg*/mm^2 = Cu 100 mM = Ni 100 mM = Cd 300 mM = Mn 10,000 mM.
May I draught your attention to a promising method on assessment of separate contribution of ionizing radiation and other factors in a combination, which was proposed by IBSS, Sevastopol (Dr. V. Tsytsugina)? Also, an interesting work on modeling of state of "health of an ecosystem" Prof. Y. Kutlakhmedov is fulfilling (at Institute of Cellular Biology and Genetical Engineering, Kiev) with use of radiotracers under action of ionizing irradiation and other factors. I do wish

every success in developing of the subject of this successful 1st Workshop! Thank you, the Organizing Committee and participants of the Workshop! And finally, my e-mail address is: <GGP@iur.sebastopol.ua>.

Chairperson (Dr. Kobayashi) : Thank you very much, Professor Polikarpov. Are there any question and discussion? So far, we have had major contribution during this session from the invited guests. Dr. Ohmomo, may I have your comments or any impression?

Dr. Ohmomo : At first, I would like to express my thanks to the speakers and also the organizer of the workshop. I studied a lot during these three days, my background is radioecology, I have been mainly engaged in the work on the migration of radionuclides in the environment. I know, the biological effect is very important, but actually it had been difficult and impossible for me to cover all fields. In that meaning, this is very much impressive for me to join this workshop, and study biological effects and a lot. And, during these three days, I had one question, there are so many stress or agent including mental shock and temperature shock and so on. What is the most sensitive and best index of the biological response? This is my question. Thank you.

Chairperson (Dr. Kobayashi) : Thank you for your crucial question. However, I am afraid this question may better be handled at some other occasions. Are there any other comments? May I invite Dr. Muramatsu?

Dr. Muramatsu : During these three days, studies on several toxicants including radionuclides and heavy metals were presented from different aspects. I think there was a variety of interesting papers. But, many of them were not directly connected to each other. Therefore, it was difficult to understand the whole story starting from the source term of toxic substances, through environmental behavior and on to biological effects. Dr.Takizawa has categorized the toxicants as "traditional" and "modern" hazards. Now, we should consider what the "future" hazards are. We can not be sure there will be no Chernobyl-like or Minamata-like events with other toxicants in the near future. There are still several substances, which are not well studied, and we must accumulate knowledge on these substances as related to the environment. For instance, use of rare earth elements (lanthanoides) in advanced technological devices is increasing and these elements are expected to be released into the environment. Both behaviors in the environment and the biological effects are not well known for lanthanoides. Therefore, we should collect more data on these elements in relation to the environmental and toxicological studies. I would just like to introduce an example from our group in NIRS. My subgroup has started to cooperate with Dr.Takahashi's subgroup (toxicology) and we have developed a method to analyze trace elements including lanthanoides in understanding their environmental behavior and their distribution in animal bodies. We would like to expand this study to learn about the biological effects of the elements. This is just one example. I believe that there are several other approaches for cooperation between scientists of different fields to allow "comparative evaluation of health effects of environmental toxicants". Thank you.

Chairperson (Dr. Kobayashi) : Thank you. Is there anyone who would like to comment? If not, I myself would like to speak a little. Firstly, as Dr. Takizawa illustrated in his presentation, there is a field of science, which is called as "Human Ecology". This subject field deals with not

only the traditional environmental ecology, but also with the human activity within the area of concern. For example, as Dr. Nakamura showed on his schematic OHP figure, all the integrated approach will be utilized for risk assessment of environmental stress on human, which is the one typical study of human ecology. Secondly, I feel that we are missing in this meeting one important element, that is a contribution from developing countries. Although the theme of the present meeting is the health risks from high-tech, any influence of high-tech will inevitably involve developing countries as well. Possibly in future meetings of this sort, we may be able to have active participation from developing countries. Yesterday in the NHK TV program, it was shown that in a southern island of Philippines, children are suffering form so-called Minamata disease due to mercury release from the traditional gold mining. As I mentioned before, Minamata disease is the one very typical case where total comprehensive and integrated approach was successful, starting with the ecological and epidemiological studies up to the risk management at administration level. In this connection I may also speak, as the third topic, about an international program. IAEA under Regional Cooperative Agreement in Asia and Pacific region (RCA) in cooperation with the United Nations Development Program (UNDP) is planning to initiate a cooperative research project on the elucidation of mechanisms and counter-measures for air and water pollution. So, there are many future area of subject where we get together to work to solve problems.

I thank you for the very constructive suggestions given during this session, Dr., Schell, Dr. Maubert, Dr. Seitz, Dr. Patrick, Dr. Hsie and Professor Polikarpov. Professor Polikarpov has made a beautiful conclusion that the Workshop is successful. I join with him in a sense that during this meeting it has been made clear what we know, what we do not know, and what we should do. I wish to thank all of you who are present here for your kind assistance and cooperation.

Program

Wednesday, January 28, 1998

Ⅰ. Opening Session

1. Opening remarks 10:00-10:10

Yasuto Sasaki (or Jiro Inaba), Director General, NIRS, Japan

Ⅱ. Key-note Address

Chairpersons: A.Hsie (USA), Y. Ohmomo (IES)

1. G. Polikarpov (Ukraine) 10:10-10:50

Biological Aspect of Radioecology: Objective and Perspective

2. K. Morimoto (Osaka Univ.) 10:50-11:30

Life Style and Health : Chromosome Alterations and Immunological Potentials as Health Indices of Overall Lifestyles

Lunch Break 11:30-13:00

Ⅲ. Special Lecture 13:00-14:00

Chairpersons: G. Polikarpov (Ukraine), S. Takahashi (NIRS), R. Seitz,(IAEA)

Assessment of Health and Environmental Effects from Radioactive and Chemically ToxicWaste

Discussions 14:00-14:15

Ⅳ. Session A: Environmental Behavior of Toxicants

Chairpersons: W. R. Schell (USA), Y. Muramatsu (NIRS)

1. S. Yoshida and Y. Muramatsu (NIRS) 14:15-14:45

Behavior of Trace Elements and Radionuclides in Soil-Plant Systems

2. W. R. Schell (USA) 14:45-15:15

Behavior and Modeling of Radionuclide and Non-Radionuclide Toxicants in the Environment

(Break 15:15-15:30

3. S. Hisamatsu, (IES) 15:30-16:00

Behavior of Tritium in the Environment:Tritium in the Food and Human Body

4. A. Kudo, (Kyoto Univ.) 16:00-16:30

Fate of Nagasaki $^{239+240}$Pu and Interaction of ^{239}Pu and ^{237}Np with Bentonite and Sulfate Reducing Anaerobic Bacteria

5. Y. Shibata, (NIES) 16:30-17:00

Chemodynamics of Arsenic in Marine Environment

6. Round Discussions 17:00-17:15

Thursday, January 29, 1998

V. Session B: Effects on Eco-system

Chairpersons: H. Jones (UK), K. Komura (Kanazawa Univ.)

1. Z. Kawabata, (Ehime Univ.) 9:30- 10:00

Evaluation of the Effects of Biological Perturbations on an Ecosystem Using Aquatic Microcosms

2. H. Jones (UK) 10:00-10:30

The Ecotron Controlled Environment Facility: Microcosm Studieson the Effects of Elevated CO_2 on Terrestrial Communities

(Break 10:30-10:45

3. I. Aoyama, (Okayama Univ.) 10:45-11:15

Ecotoxicity Assessment and Bioassay of Chemicals

4. S. Fuma, (NIRS) 11:15-11:45

Ecological Effects of Radiation and Other Environmental Stress on Aquatic Microcosm

I. Lunch Break 11:45-13:15

VI. Session C: Modeling and Methodology in Environmental Risk Studies

Chairpersons: H. Maubert (IPSN), Y. Nakamura(NIRS)

1. T. Homma,(JAERI) 13:15-13:45

Accident Consequence Assessments and Their Uncertainty

2. H. Maubert and Philippe Renaud (IPSN) 13:45-14:15

Study of a Widespread Radioactive Contamination: Example of the Radioecological Consequences of the Tchernobyl Accident in France

3. S. Kumazawa,(JAERI) 14:15-14:45

A Hybrid-Scale Theory to be Applied in Health Risk Assessment

4. Round Discussions 14:45-15:00

(Break 15:00-15:15

Poster Session 15:15- 18:00

Short presentation within 3 minutes for Poster (10-12 posters)

Reception(Banquet) 18:30-20:30

Friday, January 30, 1998

Ⅶ. Session D: Toxicological Studies (Environment Stress and Inhalation Hazard)

Chairpersons: G. Patrick (UK), Y. Takizawa (NIMD)

1. K. Furuya, (Science University of Tokyo) 9:30- 9:55
   Chemical Characterization of Particle Matters and Their Cytotoxicity
2. A. Hsie (USA) 9:55-10:20
   Molecular Markers of Gene Mutations Induced by Ionizing Radiation and Radiomimetic Chemicals in Mammalian Cells

Ⅱ. 3. S. Takahashi, (NIRS) 10:20-10:45
   Comparison of Toxic Effects of Radiation and Environmental Pollutants in the Assay Systems Using a Transgenic Mouse, Cultured Alveolar Macrophages, and DNA Double Strand Break Analyses

(Break 10:45-11:00

Chairpersons:R. Seitz(IAEA), K.T.Suzuki (Chiba Univ.)

4. G. Patrik,(UK) 11:00-11:25
   The Use of Primary Culture of Alveolar Macrophages to Predict the Different Toxic Effects of Inhaled Materials
5. S. Hirano (NIES) 11:25-11:50
   Effects of Yttrium Chloride on the Lung
6. T. Ogiu,(NIRS) 11:50-12:15
   Chemical and Radiation Carcinogenesis in Experimental Animals
7. Round Discussions 12:15-12:30

Ⅲ.

Ⅳ. Lunch Break 12:30-14:00

Ⅷ. General Discussions 14:00-15:30

Chairpersons: G. Polikarpov (Ukraine), S. Kobayashi

Commentator

1. Y. Takizawa, Director General, 14:00-14:20
   National Institute for Minamata Disease
2. Y. Nakamura, NIRS 14:20-14:30
3. Comments and Discussions 14:30-15:30
   R. Seitz, G. Patrik, W.R. Schell
   K. Morimoto, S. Hirano, Z. Kawabata, etc,

Ⅸ. Closing Session 15:30-15:35

Closing Remarks J. Inaba, Deputy Director General, NIRS

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