In vitro Determination of Oxidation of Atmospheric Tritium Gas in Vegetation and Soil in Ibaraki and Gifu, JAPAN

MICHIKO ICHIMASA¹*, MASATOMO SUZUKI², HARUO OBAYASHI³, YOUICHI SAKUMA³ and YUSUKE ICHIMASA¹

¹Department of Environmental Sciences, Faculty of Science, Ibaraki University, Mito 310–8512, Japan
²Faculty of Education, Ibaraki University, Mito 310–8512, Japan
³National Institute for Fusion Science, Shimoishicho, Toki 509–5292, Japan

(Received, April 21, 1999)
(Revision received, August 9, 1999)
(Accepted, August 27, 1999)

Tritium/Tritium gas/Oxidation/Soil/Vegetation

To quantify the rate of oxidation of tritium gas (referred to as HT) to tritiated water in the environment, various woody and herbaceous plant leaves and roots, mosses and lichens taken from a forest and fields in Ibaraki prefecture, and a forest in Toki, Gifu prefecture, were investigated as to their ability to oxidize atmospheric HT in in vitro experiments.

The HT oxidation activity in vegetation was compared with that in the surrounding surface soil (0–5 cm in depth). The rate of oxidation of HT in woody plant leaves including pine needles was extremely low, only about 1/10000–1/1000 that in the surface soil, as well as in herbaceous plant leaves with some exceptions (Phalaris arundinacea and Vaccinium smallii), whereas the rate in mosses and lichens was 50–500 times that in pine needles. The HT oxidation activity in roots of several plants including Phalaris arundinacea, Pieris japonica and Lespedeza homoloba was quite high and comparable to that in the surrounding surface soil. These results suggest that mosses, lichens and the leaves or roots of particular plants with high HT oxidation activity can be used to monitor the accidental release of HT into the environment.

INTRODUCTION

Future nuclear fusion reactors are expected to use large amounts of tritium gas (HT) as fuel. When HT from a nuclear facility is accidentally released into the environment, tritium is rapidly deposited in soil, where it disperses predominantly as tritiated water (HTO), and can be detected in vegetation¹,². Generally, the rate of HT oxidation in plant leaves is very low³–⁶ and it has been shown in in vivo experiments using dwarf trees that almost all of tritium detected in plant leaves comes from that oxidized in the surface soil and roots in the surface
layer of the soil\(^7\). However, the oxidation activity of HT in some mosses and lichens was found to be remarkably high compared with that in plant leaves\(^5\), and about 1/100–1/20 that in surface soil, where the activity was highest in the top 0–5 cm\(^5\). The oxidation activity of HT in vegetation can be attributed to microorganisms adhered to the surface of the vegetation as that in soils is due to soil microbes with hydrogen oxidizing activity\(^9\). Recently, a chronic HT release field experiment was carried out in Canada\(^9\)–\(^13\). This follows acute HT release field studies done in France\(^14,15\) and Canada\(^16\)–\(^19\), to elucidate the environmental behavior of HT. The rate at which tritium is incorporated into the body depends on the chemical form of the molecule, and the limit for intake of HTO by workers is about 1/25,000 that of HT\(^20\). Thus, it is important in terms of health physics to quantify the rate of oxidation of HT to HTO in the environment for the assessment of committed doses. Vegetation with a high HT oxidation activity would be useful as an indicator in the environmental monitoring of accidental releases of HT. Thus, in this study, we investigate the HT oxidation activity of several kinds of vegetation and compare the values with that in neighboring soil in \textit{in vitro} experiments.

**MATERIALS AND METHODS**

Pine needles (\textit{Pinus thunbergii} Parl.), a lichen, \textit{Parmelia tinctorum} Nyl., and surface soil samples (0–5cm in depth) were collected from a coastal pine forest at Oarai in Ibaraki prefecture in May, and a lichen (\textit{Parmelia tinctorum} Nyl.), mosses \{\textit{Barbula unguiculata} Hedw., \textit{Glyphomitrium humillimum} (Mitt.) Card., \textit{Marchantia polymorpha} L., and \textit{Pogonatum inflexum} (Lind.) Lac.\}, herbaceous plant leaves (\textit{Trifolium repens}, \textit{L.}, \textit{Plantago asiatica} L. and \textit{Zoysia japonica} Steud.), and surface soil samples also taken from the campus of Ibaraki university in Mito in early June.

Woody plant leaves \{\textit{Vaccinium smallii} A Gray var. glabrum Koidz., \textit{Juniperus conferta} Parlat., \textit{Vaccinium oldhamii}, \textit{Smilax china} Linn., \textit{Rhododendron obtusum} (Lind.) Planchon var. kaempferi (Planchon) Wilson, \textit{Eurya japonica} Thunb., \textit{Quercus serrata} Murray, \textit{Lespedeza homoloba} Nakai, \textit{Pieris japonica} (Thunb.) D. Don, \textit{Ilex pedunculosa} Miq. \textit{Pinus thunbergii} Parl.,\}, herbaceous plant leaves [\textit{Phalaris arundinacea} L., \textit{Arundinaria pygmaea} (Miq.) Mitford var. glabra (Makino) Ohwi, \textit{Eragrostis ferruginea} (Thunb.) P. Beauv., \textit{Struthiopteris niponica} (Kunze) Nakai, \textit{Themedia triandra} Forssk. subsp. \textit{japonica} T. Koyama, \textit{Eragrostis curvula} (Schr. Nees), \textit{Lycophodium clavatum} L. var. \textit{nipponicum} Nakai, \textit{Pteridium aquilinum} (L.) Kuhn var. \textit{latiusculum} (Desv.) Und., \textit{Lespedeza homoloba} Nakai, \textit{Themeda triandra} Forssk. subsp. \textit{japonica} T. Koyama, \textit{Eragrostis curvula} (Schr. Nees), \textit{Lycophodium clavatum} L. var. \textit{nipponicum} Nakai, \textit{Pteridium aquilinum} (L.) Kuhn var. \textit{latiusculum} (Desv.) Und., \textit{Lespedeza homoloba} Nakai, \textit{Themeda triandra} Forssk. subsp. \textit{japonica} T. Koyama, \textit{Eragrostis curvula} (Schr. Nees)], and surface soil samples were collected from a forest at the Toki site of National Institute for Fusion Science (NIFS) in Gifu prefecture in May, September or January.

Soil samples were sieved (< 2 mm) three times before use. In a 12.5 ml glass vessel, 100 mg (fresh weight) of each sample was placed and sealed with a butyl rubber stopper and aluminum cap. Tritium gas, approximately 25 MBq, with a specific activity of 7.4 GBq/mmol (Amersham) was diluted with air, washed with 30 ml of sterilized distilled water to remove traces of contaminated HTO, and 7–15 kBq aliquots subsequently introduced into vessels with
OXIDATION OF TRITIUM GAS IN VEGETATION AND SOIL

a valved microsyringe. The samples were incubated at 30°C with constant shaking. The incubation time for plant samples was 15 minutes while that of soil samples was 2 minutes. In the case of soil samples, following incubation, 2 ml of cold sterilized distilled water was introduced into each vessel and the supernatant was obtained by centrifugation. The tritium activity in the supernatant was measured by liquid scintillation counting using a liquid scintillator cocktail, Opti-fluor (Packard). In the case of plant samples, the tritium activity was measured by liquid scintillation counting following the combustion of the sample in an automatic sample oxidizer (Packard) using Bio-fluor (Packard). Three incubation samples were prepared from each sample of soil and vegetation. The initial reaction rate of biological HT oxidation was calculated as described in our previous paper and expressed as percent oxidation per min per 100 mg fresh weight of sample. Unless otherwise indicated, plant samples were carefully washed with sterilized distilled water to devoid soil and excess moisture was removed before use with filter paper.

RESULTS

Comparative study of the HT oxidation in vegetation and soil of two regions in Japan

Table 1. HT oxidation in soil, plant leaves, mosses and lichens at Mito and Oarai in Ibaraki Prefecture

| Sample Type               | HT Oxidation Activity (Relative ratio<sup>a</sup>)          |
|---------------------------|-------------------------------------------------------------|
| Surface soil              | 1.0000                                                      |
| Woody plant leaves:       |                                                             |
| Pinus thunbergii          | 0.00003 ± 0.000001                                          |
| Herbaceous plant leaves:  |                                                             |
| Zozysia japonica          | 0.0003 ± 0.0002                                              |
| Trifolium repens          | 0.0011 ± 0.0002                                              |
| Plantago asiatica         | 0.0006 ± 0.0002                                              |
| Moss:                     |                                                             |
| Marchantia polymorpha     | 0.0113 ± 0.0022                                              |
| Pogonatum inflexum        | 0.0071 ± 0.0003                                              |
| Glyphomitrium humillium   | 0.0322 ± 0.0047                                              |
| Barbula unguiculata       | 0.0581 ± 0.0080                                              |
| Lichen:                   |                                                             |
| Parmelia tinctorum<sup>b</sup> | 0.1198 ± 0.0033                           |
| Parmelia tinctorum<sup>c</sup> | 0.0700 ± 0.0246                           |

<sup>a</sup>HT oxidation is represented as the relative oxidation rate to the oxidation rate of HT in neighboring surface soil (0–5 cm in depth). Most values given in this table were calculated from the rates in our previous experiments<sup>6</sup>. Mean ± S.D.

<sup>b</sup>obtained from poplar bark.

<sup>c</sup>obtained from pine bark.
was intended.

Table 1 shows the summary of our previous experiments on HT oxidation in soil and vegetation at Mito and Oarai in Ibaraki Prefecture in which HT oxidation was recalculated as the relative ratio of the oxidation rate in vegetation to that in surrounding surface soil. The HT oxidation in woody and herbaceous plant leaves was quite low, less than 1/1000 that in soil, while that in mosses was remarkably high, about 1/100–1/20 that in soil. High HT oxidation activity was also observed in the lichen, *Parmelia tinctorum*.

Table 2 shows the HT oxidation in surface soil (point B, the meteorological observation post of NIFS, the same sampling point in every sampling month) and vegetation such as plant leaves, mosses and lichens at the Toki site of NIFS. The first three, the next five and the last

| Table 2. HT oxidation in surface soil, plant leaves, mosses and lichens at the Toki site of NIFS in Gifu prefecture |
|---------------------------------------------------------------|
| **HT oxidation activity**                                     |
| **(Relative ratioa)** |
| Surface soil        | 1.0000                        |
| Woody plant leaves: |                                |
| *Vaccinium smallii*  | 0.0113 ± 0.0019               |
| *Juniperus conferta* | 0.0014 ± 0.0002               |
| *Smilax china*      | 0.0009 ± 0.0001               |
| *Vaccinium oldhami* | 0.0015 ± 0.0007               |
| *Rhododendron obtusum* | 0.0008 ± 0.0004              |
| *Eurya japonica*   | 0.0004 ± 0.0001               |
| *Quercus serrata*  | 0.0003 ± 0.00001              |
| *Lespedeza homoloba* | 0.0003 ± 0.0001              |
| *Pieris japonica*  | 0.0003 ± 0.0001               |
| *Ilex pedunculosa* | 0.0003 ± 0.0001               |
| *Pinus thunbergii* | 0.0001 ± 0.0001               |
| Herbaceous plant leaves:                                     |
| *Phalaris arundinacea* | 0.0405 ± 0.0129              |
| *Arundinaria pygmaea* | 0.0012 ± 0.0005              |
| *Pteridium aquilinum* | 0.0002 ± 0.0003              |
| *Themeda triandra* | 0.0004 ± 0.0001               |
| *Eragrostis farraginea* | 0.0007 ± 0.0003              |
| *Eragrostis curvula* | 0.0003 ± 0.00004             |
| *Struthiopteris niponica* | 0.0006 ± 0.0003              |
| *Lycopodium clavatum* | 0.0003 ± 0.0001              |
| Moss:                                                         |
| *Ditrichum pallidum* | 0.1816 ± 0.0081               |
| *Hypnum plumaeforme* | 0.0522 ± 0.0160               |
| Lichen:                                                       |
| *Cladia aggregata*   | 0.0561 ± 0.0320               |
| *Cladonia rangiferina* | 0.0049 ± 0.0027              |

*aMean ± S.D.*
three kinds of woody plant leaves in Table 2 were collected in May, September and January, respectively. In the case of herbaceous plant leaves, the first three, the next three and the last two kinds of plant leaves were collected in May, September and January, respectively. Mosses and the lichen, _Cladonia aggregata_, were collected in January while a lichen, _Cladonia rangiferina_, was collected in September. The HT oxidation activity in surface soil at point B collected in May, September and January was 0.5824 ± 0.0562, 0.6756 ± 0.2715 and 1.0086 ± 0.1408% oxidized HT/min/100 mg soil, respectively. The oxidation activity of HT in various kinds of woody plant leaves was very low and around 1/1000–1/10000 that in soil except that in _Vaccinium smallii_ leaves. In herbaceous plant leaves, the HT oxidation activity was almost as low as that in woody plant leaves except for _Phalaris arundinacea_, a grass, in which the activity was comparable to that of some mosses and lichens. The HT oxidation activity in mosses and lichens was considerably higher than that in plant leaves. _Ditrichum pallidum_ showed the highest activity, about 1/5 that in the surface soil.

To confirm the results of our previous _in vivo_ experiments\(^7\), the HT oxidation activity in plant leaves and their roots was compared with that (0.5824 ± 0.0562% oxidized HT/min/100 mg soil) in the surface soil of point B (Table 3) using three species of the Gramineae family (_Phalaris arundinacea_, _Eragrostis curvula_ and _Arundinaria pygmaea_) collected in May and a

| Table 3. HT oxidation activity in plant leaves and their roots compared with that in the surrounding surface soil at the Toki site of NIRS |
|-----------------------------------------------|
| HT oxidation activity (Relative ratio\(^a\)) |
| Surface Soil | 1.0000 |
| *Phalaris arundinacea* | |
| Leaves | 0.0405 ± 0.0129 |
| Roots | 1.3341 ± 0.0311 |
| *Eragrostis curvula* | |
| Leaves | 0.0022 ± 0.0038 |
| Roots | 0.0304 ± 0.0115 |
| *Arundinaria pygmaea* | |
| Leaves | 0.0012 ± 0.0005 |
| Roots | 0.1403 ± 0.0053 |
| *Struthiopteris niponica* | |
| Leaves | 0.0006 ± 0.0003 |
| Roots | 0.2632 ± 0.0332 |
| *Lycopodium clavatum* | |
| Leaves | 0.0003 ± 0.0001 |
| Roots | 0.0177 ± 0.0093 |
| *Pieris japonica* | |
| Leaves | 0.0003 ± 0.0001 |
| Roots | 0.5471 ± 0.0331 |

\(^a\)Mean ± S.D.
fern (*Struthiopteris niponica*), a lycopodium (*Lycopodium clavatum*) and an evergreen tree of *Ericaceae* (*Pieris japonica*) collected in January. Among them, *Phalaris arundinacea* showed extremely high HT oxidation activity in roots and the activity in its leaves was quite high compared with that in the plant leaves tested. The activity in the roots of the other two Gramineae was rather low and differed. In *Pieris japonica*, the oxidation activity in roots was about one half that in surface soil in spite of the very low activity in leaves.

The distribution of HT oxidation activity between various parts of plants and root-associated soils obtained from the same sampling point in September at the Toki site of NIFS was determined using three gramineous plants and a leguminous plant, *Lespedeza homoloba* (Table 4). The HT oxidation activities of roots and associated soil also differed dependent on the plants. Both the HT oxidation activity in roots and associated soil of *Lespedeza homoloba* was about twice that in the surrounding surface soil (point B), but that in roots and associated soil of the three gramineous plants was rather low compared with that in surrounding soil except in the root-associated soil of *Eragrostis ferruginea*.

**Table 4.** HT oxidation activity in leaves, roots and the associated soil of plants at the Toki site of NIFS

| Plant                  | HT oxidation activity (Oxidized HT%/min /100 mg sample*) | Relative ratio |
|------------------------|----------------------------------------------------------|----------------|
| **Surface soil**       |                                                          | 1.0000         |
| **Themeda triandra**   |                                                          |                |
| Leaves                 | 0.0003 ± 0.0001                                          | 0.0004         |
| Withered leaves        | 0.0009 ± 0.0001                                          | 0.0013         |
| Roots                  | 0.1086 ± 0.0777                                          | 0.1607         |
| Associated soil        | 0.3454 ± 0.0305                                          | 0.5112         |
| **Eragrostis ferruginea** |                                                      |                |
| Leaves                 | 0.0005 ± 0.0002                                          | 0.0007         |
| Withered leaves        | 0.0001 ± 0.0003                                          | 0.0001         |
| Roots                  | 0.0861 ± 0.0161                                          | 0.1274         |
| Associated soil        | 0.6640 ± 0.0056                                          | 0.9828         |
| **Eragrostis curvula** |                                                          |                |
| Leaves                 | 0.0002 ± 0.0003                                          | 0.0003         |
| Withered leaves        | 0.0002 ± 0.0003                                          | 0.0003         |
| Roots                  | 0.1736 ± 0.1084                                          | 0.2570         |
| Associated soil        | 0.2542 ± 0.0088                                          | 0.3763         |
| **Lespedeza homoloba** |                                                          |                |
| Leaves                 | 0.0002 ± 0.0001                                          | 0.0003         |
| Roots                  | 1.1340 ± 0.1464                                          | 1.6785         |
| Associated soil        | 1.1510 ± 0.0881                                          | 1.7037         |

*a*Mean ± S.D.
DISCUSSION

The HT oxidation activity detected in soil and vegetation samples may be primarily due to the action of soil- and vegetation-associated bacteria with hydrogenases, because hydrogenases which catalyze the production or consumption of molecular hydrogen with suitable electron donors or acceptors have been found in a wide variety of bacteria and in certain eucaryotes such as a protozoa and a fungus. However, hydrogenases in most known hydrogen-oxidizing bacteria isolated from soil or root nodules are unable to oxidize an ambient atmospheric mixing ratio of hydrogen as low as 0.5 ppmv significantly. Tritium gas oxidation activities in various samples of plant and soil collected at the Toki site of NIFS in Gifu were determined by in vitro experiments to compare with values obtained in Ibaraki prefecture. There were no significant regional differences. In fact, almost the same results were obtained; the highest HT oxidation activity was in soil, followed by mosses and lichens, and finally pine needles. These results also suggest a ubiquitous distribution of tritium gas-oxidizing bacteria in soils, mosses and lichens.

Sweet and Murphy reported that the deposition velocity for HT in loblolly pine needles was about 1/100000 that in the soil from atmospheric release of HT. Pine needles have been used as an environmental monitor for tritium because of their common distribution and ease of collection. However, the present results suggest the possible use of mosses, lichens and leaves or roots of special plants with high HT oxidation potential for monitoring the accidental release of HT into the environment, just as mushrooms are used for monitoring radionuclide-contamination in soil. With relatively small amounts of these plants, the passages of HT plume might be traced comparatively easily.

In the 1994 HT chronic release experiment in Canada, HT was released into an experimental area, consisting of three cultivated plots and one natural plot, continuously using a delivery system over a 12-day period. The average HT air concentration was $2.0 \times 10^5$ Bq/m$^3$ and the average ratios of HTO concentration in soil water and air moisture to HT concentration in air (20 cm above the ground) were 0.0014 and 0.0011 (Bq/ml) / (Bq/m$^3$) for a cultivated plot. Under these conditions, ratios of dose due to inhalation and skin absorption of HTO to that due to inhalation of HT was calculated to be about 300. HTO concentrations in natural soil water during the release were several times higher than those in cultivated soil water and in air moisture. The oxidation activity of HT in the surface soil (0–5 cm in depth) in the natural plot determined in in vitro laboratory experiments was 1.56 ± 0.08%/min/100 mg soil, and several times higher than that in the surface soil in the cultivated plots. The occurrence rates of HT-oxidizing bacteria in the natural plot surface soil was three times that in the cultivated plot surface soil. As described in this paper, HT oxidation activity in surface soils at the Toki site of NIFS was in the range 0.58–1.00%/min/100 mg soil and about one half that in the surface soil in the natural plot in the HT chronic release experiment in Canada. Thus, assuming the same meteorological conditions and HT air concentration as the HT release experiment in Canada, HTO concentration in air moisture may be estimated at about one half that in Canada by a calculation using HT oxidation activity in surface soil. These
results suggest the usefulness of previous *in vitro* determination of HT oxidizing activity in soil and vegetation for the assessment of committed doses in the case of HT release by accident or during the normal operation of nuclear facilities.

ACKNOWLEDGEMENTS

This work was supported in part by the research program at National Institute for Fusion Science.

REFERENCES

1. Sweet, C. W. and Murphy, C. E. Jr. (1984) Tritium deposition in pine trees and soil from atmospheric release of molecular tritium. Environ. Sci. Technol. 18: 358–361.
2. Okada, S. and Momoshima, N. (1993) Overview of tritium: characteristics, sources, and problems. Health Phys. 65: 595–609.
3. Belot, Y. (1986) Tritium in plants: A review. Radiat. Prot. Dosim. 16: 101–105.
4. Spencer, F. S. and Dunstall, T. G. (1986) Molecular tritium conversion in vegetation, litter and soil. Radiat. Prot. Dosim. 16: 89–93.
5. Murphy, C. E. Jr. (1989) Controlled environmental estimates of HT uptake by vegetation. Proc. 3rd Japan-US workshop P-133 on Tritium Radiobiology and Health Physics, Ed. S. Okada, pp. 64–73, (Nagoya; IPPJ-REV-3).
6. Ichimasa, M., Ichimasa, Y., Yagi, Y., Ko, R., Suzuki, M. and Akita, Y. (1989) Oxidation of atmospheric molecular tritium in plant leaves, lichens and mosses. J. Radiat. Res. 30: 323–329.
7. Ichimasa, M., Ichimasa, Y. and Akita, Y. (1989) *In vivo* fixation of atmospheric tritium gas in pine and zelkova trees and their surroundings. J. Radiat. Res. 30: 330–337.
8. Ichimasa, M., Ichimasa, Y., Azuma, Y., Komuro, M., Fujita, K. and Akita, Y. (1988) Oxidation of molecular tritium by surface soils. J. Radiat. Res. 29: 144–151.
9. Ichimasa, Y., Ichimasa, M., Jiang, H., Katsuno, K., Noguchi, H., Yokoyama, S., Amano, H. and Atarashi, M. (1995) *In vitro* determination of HT oxidation activity and tritium concentration in soil and vegetation during the chronic HT release experiment at Chalk River. Fusion Technol. 28: 877–882.
10. Davis, P. A., Workman, W. J. G., Amiro, B. D., Spencer, F. S., Noguchi, H., Amano, H., Ichimasa, Y. and Ichimasa, M. (1995) Overview of the 1994 chronic HT release experiment at Chalk River. Fusion Technol. 28: 840–845.
11. Amano, H., Atarashi, M., Noguchi, H., Yokoyama, S., Ichimasa, Y. and Ichimasa, M. (1995) Formation of Organically bound tritium in plants during the 1994 chronic HT release experiment at Chalk River. Fusion Technol. 28: 803–808.
12. Noguchi, H., Yokoyama, S., Kinouchi, N., Murata, M., Amano, H., Atarashi, M., Ichimasa, Y. and Ichimasa, M. (1995) Tritium behavior on a cultivated plot in the 1994 chronic HT release experiment at Chalk River. Fusion Technol. 28: 924–929.
13. Davis, P. A., Galeriu, D. C., Spencer, F. S. and Amiro, B. D. (1995) Evolution of HTO concentrations in soil, vegetation and air during an experimental chronic HT release. Fusion Technol. 28: 833–839.
14. Djerassi, H. and Gulden, W. (1988) Overview of the tritium release experiment in France. Fusion Technol. 14: 1216–1221.
15. Paillard, P., Calando, J. P., Clerc, H., Gros, R. and Belot, Y. (1988) Tritium release experiment in France results concerning HT/HTO conversion in the air and soil. Fusion Technol. 14: 1226–1230.
16. Brunham, C. D., Brown, R. M., Ogram, G. L. and Spencer, F. S. (1988) An overview of experiments at Chalk
River on HT dispersion in the environment. Fusion Technol. 14: 1159–1164.
17. Ogram, G. L., Spencer, F. S. and Brown, R. M. (1988) Field studies of the HT behavior in the environment: 2. The interaction with soil. Fusion Technol. 14: 1170–1175.
18. Spencer, F. S., Ogram, G. L. and Brown, R. M. (1988) Field studies of the HT behavior in the environment: 3. Tritium deposition and dynamics in vegetation. Fusion Technol. 14: 1176–1181.
19. Noguchi, H., Matsui, T. and Murata, M. (1988) Tritium behavior observed in the Canadian release study. Fusion Technol. 14: 1287–1292.
20. International Commission on Radiological Protection (1979) Limits for intakes of radionuclides by workers. ICRP Publication 30, Part 1, Pergamon Press, Oxford.
21. Friedrich, B. and Schwartz, E. (1993) Molecular biology of hydrogen utilization in aerobic chemolithotrophs. Annu. Rev. Microbiol. 47: 351–383.
22. Payne, M. J., Chapman, A. and Cammack, R. (1993) Evidence for an [Fe]–type hydrogenase in the parasitic protozoan Trichomonas vaginalis. FEBS Lett. 317: 101–104.
23. Marvin-Sikkema, F. D., Kraak, M. N., Veenhuis, M., Gottschal, J. C. and Prins, R. A. (1993) The hydrogenosomal enzyme hydrogenase from the anaerobic fungus Neocallimastix sp. L2 is recognized by antibodies, directed against the C-terminal microbody protein targeting signal SKL. Eur. J. Cell Biol. 61: 85–91.
24. Schuler, S. and Conrad, R. (1990) Soils contain two different activities for oxidation of hydrogen. FEMS Microbiol. Ecol. 73: 77–83.
25. Takashima, Y., Momoshima, N., Inoue, M. and Nakamura, Y. (1987) Tritium in pine needles and its significant sources in the environment. Appl. Radiat. Isot. 38: 255–261.
26. Hisamatsu, S., Katsumata, T. and Takizawa, Y. (1998) Tritium concentration in pine needle, litter and soil samples. J. Radiat. Res. 39: 129–136.
27. Ban-nai, T., Muramatsu, Y., Yoshida, S., Uchida, S., Shibata, S., Ambe, S. and Suzuki, A. (1997) Multitracer studies on the accumulation of radionuclides in mushroom. J. Radiat. Res. 38: 213–218.