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Accumulation Route and Chemical Form of Mercury in Mushroom Species

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Some papers were published on several species of fungi having more accumulating abilities of mercury than other land plants and a relatively small part of mercury being present as methylmercury in most species (STEGNAR et al. 1973, STIJVE and ROSCHNIK 1974). But, little information is available regarding the routes of mercury in fungi, and also no report on mercury speciation (chemical form and complexation) in them have been published, apart from methylmercury. In order to evaluate accurately their biological characteristics such as absorption, excretion, accumulation and toxicity (The Task Group on Metal Interaction 1978), the mercury speciation present in mushrooms, regardless of edible or nonedible, should be identified.

In this report, we present 1) contents of total and methylmercury in mushrooms near the acetaldehyde factory which had the mounds of sludge containing mercury, 2) data of exposure experiment of mercury vapor to raw mushrooms (Shiitake) on the market, and 3) data on mercury speciation of mercury other than methylmercury.

MATERIALS AND METHODS

Sampling and analysis. Mushrooms obtained from mountain forests near the acetaldehyde factory, Niigata Prefecture, Japan in September to November, 1979 were used. The area had some mounds of sludge containing mercury. Also, a kind of mushrooms, Shiitake on the market were used for experiment of mercury exposure.

A whole or part samples of each species, nearly always consisting of several fruit bodies, were cut finely using scissors. The materials were used for the analysis of total mercury, methylmercury and organic mercury, and for gel chromatography.

Measurements of total mercury and methylmercury have been previously described (MINAGAWA et al. 1979). Inorganic mercury was determined by the method that only inorganic mercury compound is reduced by tin(II) chloride (MINAGAWA et al. 1980). Organic mercury was measured as the difference between total and inorganic mercury.

Exposure of mercury vapor to mushroom. As shown in Fig.

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1A, environmental chamber for exposure of mercury vapor was prepared. At the 1st, 3rd, 5th and 7th day after the beginning of mercury exposure, mushrooms (Shiitake, 8.5 ± 1.2g) were sampled. The sites of samples, as shown in Fig. 1B, were analyzed. Total mercury in air samples of environmental chamber was analyzed as reported earlier (TAKIZAWA and MINAGAWA 1979).

Gel filtration chromatography. Mushrooms which were cut by scissor were homogenated in 1 : 9 wet weight (g) : volume (ml) of 0.05M Tris-HCl buffer, pH 8.0 using Polytron® (Willems High Frequency Generator). The homogenate was centrifuged at 105000 xg for 60min. The resulting supernatant fluid was first fractioned by gel filtration on a Sephadex G-75 or 200 superfine column (2.5 x 87cm) which was equilibrated with 0.05M Tris-HCl buffer, pH 8.0 containing 0.05M KCl and 0.02% NaN_3. Solvent of 0.05M Tris-HCl was used and flow rate was 12ml per hour and 4.2ml fractions were collected. Absorbancy measurements were also made at 250nm and 280nm. Protein concentration was determined by the Folin phenol reagent (Lowry et al. 1951) and total mercury and inorganic mercury were by the methods abovementioned. Sulfhydryl groups (SH) were measured by the method of SEDLAK and LINDSAY, 1968 with Ellman's reagent.

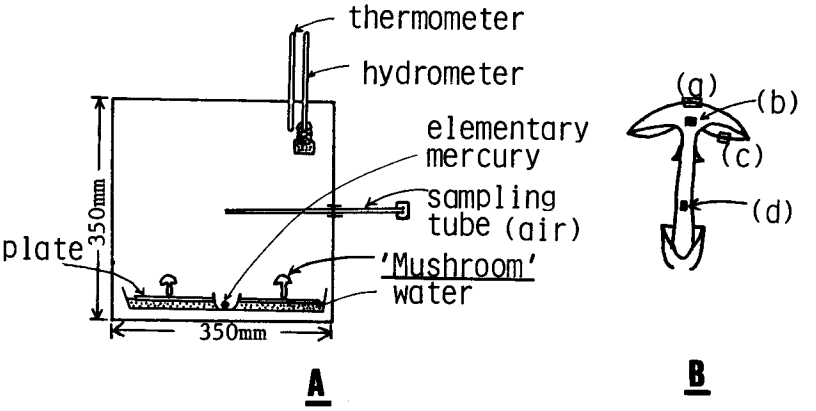

Fig. 1 Environmental chamber of mercury vapor to mushrooms (Shiitake) and its sites collected. A : a side of view of chamber and B analyzing sites of a mushroom (a) cuticle of cap, (b) flesh of cap, (c) plait of cap (d) flesh of stalk.

RESULTS AND DISCUSSION

The analytical results of total mercury and methylmercury in mushrooms obtained from near acetaldehyde factory are shown in Table 1. Mercury concentrations were expressed as wet and dry basis. Normally, it is desired that the latter value was used

TABLE 1

Total Mercury and Methylmercury Levels in Mushrooms from the surrounding Environment of acetaldehyde factory.

Place collected	Species	Water Concent(%)	Total Hg		Methyl Hg		Methyl Hg/Total Hg (%)
			Wet(ppm)	Dry(ppm)	Wet(ppm)	Dry(ppm)	
1	*Collybia maculata*	94.1	0.33	5.64	0.03	0.51	9.1
2	*Lyophllum fumosum*	92.1	1.96	24.8	0.10_5	1.33	5.4
3	*Cortinarius*	91 9	0.26	3.12	nd	nd	-
4	*Rhodophyllus crassipes*	93.5	0.17	2.61	nd	nd	-
5	*Tricholoma ustale*	92.9	0.34	4.85	nd	nd	-
6	*Suillus bovinus*	93.8	0.03	0.44	nd	nd	-
7	*Collybia dryophila*	89.8	1.03	10.1	0.03	0.29	2.9
8	*Collybia maculata*	83.5	0.44	2.65	nd	nd	-
9	*Cortinarius variclor*	95.8	0.26	6.14	nd	nd	-
10	*Lepiota acutesquamosa*	92.2	0.11	1.44	nd	nd	-

Note: nd; Less than 0.005ppm for total mercury and less than 0.01ppm for methyl mercury.
All analyses were performed on condition of wet sample, and using reduction-volatilization and A.A. for total mercury and using gas chromatography for methylmercury.

because the water content is different in each mushroom. High concentration of total mercury was found in the majority of samples. Total mercury levels ranged from 0.44 to 24.8 μg/g, dry weight. The results for methylmercury conformed with the finding of STEGNAR et al. 1973, STIJVE and ROSCHNIK 1974 and STIJVE and BESSON 1976 who observed that this toxic compound is only present at a low percentage of total mercury content. There is no doubt that a significant degree of contamination has occured as a result of the relatively high mercury vapor concentrations and the contaminated soil around the mounds of slugde containing mercury. But it is important to know the route of mercury accumulation from the view of ecotoxicology.

Therefore, for the aim of investigating the route, exposure experiment of mercury vapor was carried out. The conditions of exposure chamber were as follows : temperature, 20°C ; humidity, 81% ; mercury concentration in air, 172 μg Hg/m^3. An inspection of the Fig. 2 reveals certain interesting features : first are the very high mercury levels, particular in the plait of the cup, followed by the cuticle of the cap, second is that increased mercury depend on the days of exposure, third is that the plait of the cap has great tendency to bind with mercury vapor originated from air. As indicator organisms in the study of mercury pollution , though different in species, the nearly everywhere growing species are available as suggested by Rauter 1975.

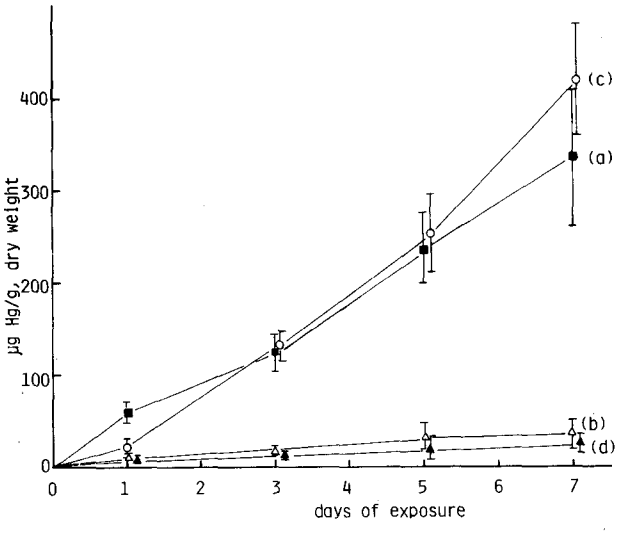

Fig. 2 Relationship between total Hg and days of exposure of mercury vapor in mushroom, Shiitake (*Lentinus edodes*)
○ : plait of cap (c), △ : cuticle of cap (a),
■ : flesh of cap (b), ▲ : flesh of stalk (d)

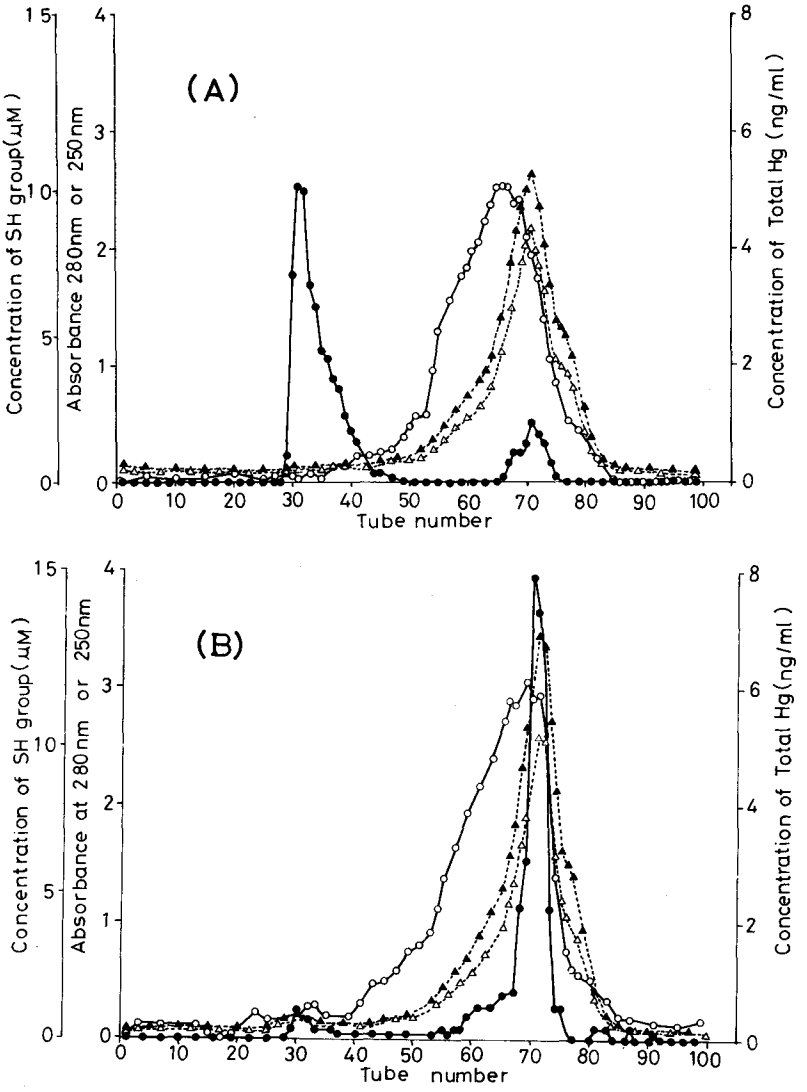

Fig. 3 Gel filtration chromatograms of water soluble fraction to which 1% cysteine was added or not in wildlife mushroom on Sephadex G-75. Chromatogram (A) : no addition of 1% cysteine, (B) : addition of 1% cysteine. Column : 2.5 x 87cm, 0.05M Tris-HCl, pH 8.0
●: Total mercury, ○: SH group, ▲: OD at 250nm, △: OD at 280nm.

More than 90 percent of the mercurials in the wild life mushrooms were readily soluble in 0.05M Tris-HCl buffer, pH 8.0, the remainder was fat-soluble or conjugated with tissue components. According to the separation of the mushroom supernatant by gel filtration on a column of Sephadex G-200 superfine, supernatant fraction from the mushroom in total mercury had two Hg peaks and two protein peaks in the elution profile. In the first small peak of tube No. 16 is the high molecular weight substance that elute in the void volume. The amount of mercury in the peak was very small. The second peak of the tube No. 32 is composed of a molecular or molecules 20,000 - 30,000 molecular weight. Our calculations indicate that this peak has roughly more than 90 percent of mercury of supernatant fraction mounted and that more than 95 percent of mercury may be present in organically associated forms that are not always reactive or measurable by the commonly employed techniques. This substance may not bind with proteins with sulfhydryl groups and has not the absorbancy both of 250 and 280nm. We confirmed this was almost entirely in the form of the inorganic mercury compound due to the following methods. 1) Generally, both organic and inorganic mercury were reduced by tin(II) chloride-cadmium(II) chloride mixture reagent in alkali solution, while only inorganic mercury compound was reduced by tin(II) chloride in alkali solution (MAGOS 1971, MINAGAWA et al. 1980). Therefore, this elution of tube No. 28 to 40 gave inorganic mercury compoumds. However, we found, in this case, the analytical method for organic mercury is not specific in the sense that the mercury is easily released from organomercury of higher molecule than methylmercury. 2) For this reason, gel filtration chromatography on Sephadex G-75 was carried out in the supernatant fraction to which 1% cysteine was added or not. As shown Fig. 3, it was found the binding force of this substance with mercury was weaker than that of cysteine, that is to say, it had not a carbon-mercury bond such as methylmercury. The mechanism by which mercury is accumulated remains still obscure. But, our experiments demonstrated that one of the main routes may be airborne. STIJVE and BENSSON 1976 suggested that mercury was chelated by reaction with the sulfhydryl groups of the protein. However, we do not think mercury in mushroom do bind with protein with sulfhydryl groups, based on the results of gel filtration chromatography.

Mushrooms may serve as strong scavenger of mercury in the ambient environment. Now we are carrying out ongoing investigation of the transfer rate from the part of the root as another main route.

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