

Characterization of Copper, Lead and Zinc in
Metal Tolerant Mosses, *Atrichum*
undulatum(Hedw.) P. Beauv., *Scopelophila*
cataractae(Mitt.) Broth and *Pohlia*
bulbifera(Warnst.) Warnst. In Heavy Metals
Pollution Areas

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Tatsuro Oda* and Takaharu Honjo* : **Characterization of Copper, Lead and Zinc in Metal Tolerant Mosses, *Atrichum undulatum* (Hedw.) P. Beauv., *Scopelophila cataractae* (Mitt.) Broth and *Pohlia bulbifera* (Warnst.) Warnst. in Heavy Metals Pollution Areas**

織田樹郎*・本浄高治* : 重金属汚染地域の金属耐性コケ植物, タチゴケ, ホンモンジゴケ及びキヘチマゴケにおける銅, 鉛及び亜鉛のキャラクタリゼーション

Abstract

The gregarious mosses like *Atrichum undulatum* (Hedw.) P. Beauv. of Polytrichaceae, *Scopelophila cataractae* (Mitt.) Broth. of Pottiaceae, and *Pohlia bulbifera* (Warnst.) Warnst. of Bryaceae, have been found in the areas of heavy metals pollution resulting from the copper roofs of temples and shrines in Japan, and from lead tiles of the ruins of Kanazawa Castle in Ishikawa Prefecture, the roofs of which are dissolved with acid rain. The distribution of *Scopelophila cataractae* was reported only along the side of the Pacific Ocean until now (Satake *et al.* 1989), but in this study, it turned out that the species also grows on the side of Japan Sea. Therefore, it became clear that the distribution of the moss was in a nationwide scope in Japan. Heavy metal tolerant moss, *Scopelophila cataractae*, is fairly specific for copper pollution, while *Pohlia bulbifera* is tolerant to lead in addition to copper and zinc.

The distribution state of heavy metals in vegetative organs of these moss growing on the polluted areas in which heavy metals are accumulated in high concentration has also been examined by means of histochemical techniques and several instrumental analytical methods such as x-ray fluorescence and atomic absorption analyses. These results are summarized as follows: 1) A qualitative analysis by x-ray fluorescence method revealed that the elements like Si, P, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Pb, Rb, and Sr were detected in plants and soils.

2) The stems of moss in which heavy metals were accumulated in high concentrations was cut with a microtome, and the small sections were stained with rubeanic acid for copper, dithizone for zinc, and sodium rhodizonate for lead in order to ascertain the distribution of the heavy metals in the tissues by means of microscopy. It was found that moss mentioned above accumulated a large amount of copper, lead, and zinc over the cell walls of cortex in the tissues.

3) In general, the accumulation of heavy metals in the tissues of gregarious moss (~20,000 ppm) on the dry base (110°C) increased when the apparent total concentration of heavy metals (~15,000 ppm) increased in the polluted areas.

Key words : acid rain, heavy metal, metal tolerant plant, moss, pollution.

Copper has been generally used in the buildings, because it is a shining material at first and becomes patina (basic copper carbonate) which looks beautiful. During the seventh century, copper was used as building materials of living houses, temples and shrines in Japan. In addition, copper was used as basic materials of gutters and rain pipes because of its corrosion-resistant nature. Zinc exists as galvanized sheet

steel around us, which is the cheapest building material and is hard to burn off, and it became one of the most popular building material after the Great Kanto Earthquake. Many metals are useful for us to spend comfortable and safety life, but they cause some hazardous effects in natural circumstances. Copper and zinc are essential metals of plants, but a large amount of them affects on the growth of plants in the environ-

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ments. The roofs of Kanazawa Castle in Kanazawa, Ishikawa Prefecture, and Zuiryūji Temple in Takaoka, Toyama Prefecture, have been tiled with lead metal. There is no established reason why lead was used as building materials by the feudal lord, Maeda, in the Edo period. Some of reliable opinions are based on the beautiful features of white lead composed of basic lead carbonate, which is described "as if the roof looks like covered with snow", a store of bullets, and the prevention of roofs from frozen destruction, etc. (Honjo and Nakanishi 1986). Though lead has some advantages as building materials, it gives bad effects to the environments around the buildings roofed over with lead, and the lead pollution becomes a serious problem (Honjo *et al.* 1984). An observation of moss growing on metal polluted areas evidently may reveal the distribution of metal tolerant species and the extent of pollutions (Markert 1993). Therefore, it is thought that these moss plants might be useful for evaluating the degree of heavy metal pollution.

In this paper, the gregarious moss growing on the polluted places was studied as an indicator plant of heavy metal pollution in the environments. The distribution of heavy metals in vege-

tative organs of the moss has also been investigated by means of the histochemical method.

Materials and Methods

1. Field study on moss plant

The research works were mainly carried out around Kanazawa Castle. Figures 1 and 2 show the sampling sites of moss plants. The names of sampling sites and moss growing in each area (April, 1991~March, 1992) are shown in Table 1. It was found that only a few species of moss are growing around the field investigated and described as follows: *Atrichum undulatum* (Hedw.) P. Beauv. of Polytrichaceae is a moss of light green color and normally growing in sunny greenbelts of Kanazawa Castle and Central Park, etc., where this type of moss alone grows around the buildings tiled with lead. *Pohlia bulbifera* (Warnst.) Warnst. of Bryaceae is moss of yellow green color and grows around almost all temples and shrines tiled with copper. This moss grows near the buildings of Kanazawa Castle tiled with lead, too. It is said that *Scopelophila cataractae* (Mitt.) Broth. of Pottiaceae is not distributed around the side of Hokuriku district in Japan (Satake *et al.* 1984), but the result of speciation of the moss picked up around Toyama

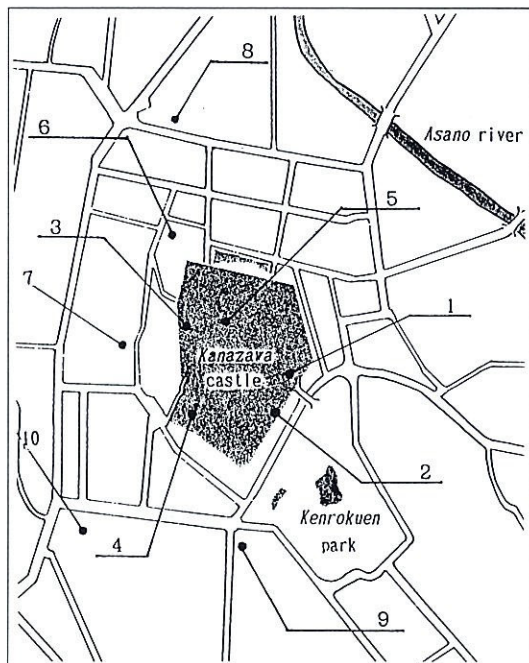


Fig. 1. Sampling sites around Kanazawa Castle.

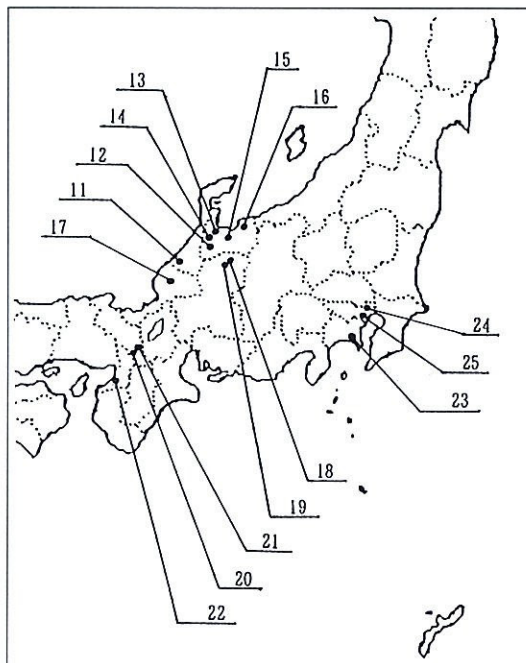


Fig. 2. Sampling sites outside Kanazawa Castle.

Table 1. Moss growing in the sampling sites and the polluted areas of heavy metals around the moss

No*Sampling site	heavy metal			Moss		
	Cu	Zn	Pb	<i>Atrichum undulatum</i>	<i>Pohlia bulbifera</i>	<i>Scopelophila cataractae</i>
1 Ishikawamon Gate			○	○	○	
2 Ishiotoshi			○	○		
3 Education Center			○	○	○	
4 Sanjukken Nagaya			○	○	○	
5 Energy Institution	○			○	○	
6 Ozaki Shrine	○				○	○
7 Oyama Shrine	○			○	○	○
8 Ichihime Shrine	○				○	○
9 Ishiura Shrine	○				○	○
10 Restaurant	○					○
11 Ogoya Mine	○				○	
12 Zuisenji Temple	○			○		
13 Gokoku Shrine	○			○	○	○
14 Zuiryuj Temple	○	○		○	○	
15 Toyama Museum	○			○		○
16 Ichihiko Shrine	○				○	
17 Eiheiji Temple	○				○	
18 Kamioka Mine	○	○	○	○	○	
19 Kamioka Castle	○			○	○	
20 Higashihonganji	○			○	○	
21 Yoshida Shrine	○	○	○	○	○	○
22 Osaka Castle	○				○	○
23 Great Buddha	○					○
24 Toshogu Shrine	○					○
25 Honmonji Temple	○					○

*The numbers correspond to Fig. 1 and Fig. 2

Museum in Toyama Castle turned out that it would be the moss of that type. Now it is sure that *Scopelophila cataractae* grows in about ten sites around Ishikawa and Toyama Prefecture (Yamaoka 1993). The color of *Scopelophila cataractae* is deep green. *Pohlia nutans* grows not only near the buildings tiled with copper but also near gutters, rain pipes and railings containing iron or zinc. *Brachythecium* B. S. G. sometimes grows along with *Atrichum undulatum*. *Racomitrium canescens* grows around thorny wires or the buildings made of galvanized sheet steel. Among these mosses, *Atrichum undulatum*, *Pohlia bulbifera* and *Scopelophila cataractae* were mainly studied. They are illustrated in Fig. 3.

2. Apparatus

A model W-103 T HONDA ultrasonic cleaner, a model DG-81 YAMATO drying oven and a HAYASHI DENKO electrothermal drier, were used for the preparation of moss and soil samples.

A model AE 163 METTLER electric balance

was used to weigh moss and soil samples.

A model QD-01 MITSUBISHI CHEMICAL INDUSTRIES quick digester, a model HF-120 TOMY SEIKO micro centrifuge, and a model Z-6100 and a model Z-8270 HITACHI polarized Zeeman atomic absorption spectrophotometers, were used to determine the concentration of heavy metals in the samples.

A model 711 EDAX X-ray fluorescence analyser and a model 902 D EDAX X-ray generator were used to measure the X-ray fluorescence spectrum. A model Mark III SOAD microcomputer was used for the background correction of X-ray fluorescence spectrum. A model SR-II TAIYO recipro shaker and a model CD-50 R TOMY SEIKO centrifuge, were used for the analysis of soil.

The pH was measured by a model B-112 HORIBA pH meter.

A model KCM-10 SHIMADZU cylinder microtome was used to prepare the sections of moss. A model BHC OLYMPUS microscope attached with a model PM-6 OLYMPUS camera was used to take photomicrographs of the sections of moss.

3. Reagents

Standard solutions (1000 µg/ml) of lead, copper and zinc in 0.1M nitric acid (KANTO CHEMICAL) were used by suitable dilution with deionized water.

Hydrochloric acid and sulfuric acid (KATAYAMA CHEMICAL), nitric acid (WAKO PURE CHEMICAL INDUSTRIES) and perchloric acid (60%, KATAYAMA CHEMICAL) were reagent grade materials.

Ammonium acetate of guaranteed reagent grade (KATAYAMA CHEMICAL) was used for the extraction of soil components.

Dyeing reagents such as rhodizonic acid disodium salt and rubeanic acid (NAKARAI CHEMICALS), pyridylazonaphthol (PAN) (WAKO PURE CHEMICAL INDUSTRIES) and ruthenium red (MERCK) were guaranteed reagent grade materials. Sodium thiosulfate (WAKO PURE CHEMICAL INDUSTRIES) of guaranteed reagent grade was used to mask heavy metals. An aqueous ammonia of extra pure grade (KATAYAMA CHEMICAL) was used

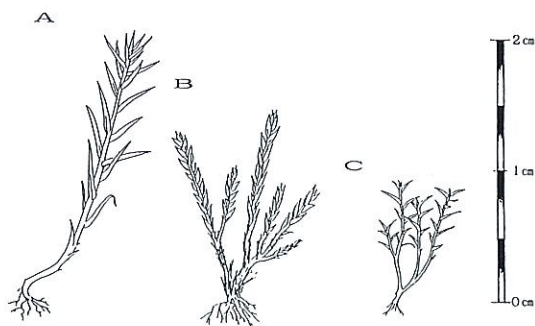


Fig. 3. Metal tolerant moss.

A, *Atrichum undulatum* (Hedw.) P.Beuv. of Polytrichaceae. B, *Pohlia bulbifera* (Warnst.) Warnst. of Bryaceae. C, *Scopelophila cataractae* (Mitt.) Broth. of Pottiaceae

for the alkalization of dye solution.

Tartaric acid and potassium sodium tartrate of guaranteed reagent grade and sodium acetate of extra pure reagent grade (KANTO CHEMICAL), acetic acid of extra pure reagent grade (KATAYAMA CHEMICAL) were used to prepare buffer solutions. The pH standard solution "1.00-7.00" (pH = 6.8, HORIBA) was used as a buffer solution.

The ion test papers "Copper-Test", "Zinc-Test" and "Lead-Test" (MERCK) were used for the semi-quantitative determination of heavy metals. All stock solutions were prepared with deionized water.

4. Procedure

Semi-quantitative in situ analysis: A stem of moss was cut by a razor and the stem was stumped on the ion test paper moistened with buffer solution (pH = 6.8). The color of the test paper was compared with a printed series of the color scale varying the metal concentrations and the approximate concentration of heavy metals in the stem was determined.

Preparation of moss samples: Moss samples collected were identified with the reference sam-

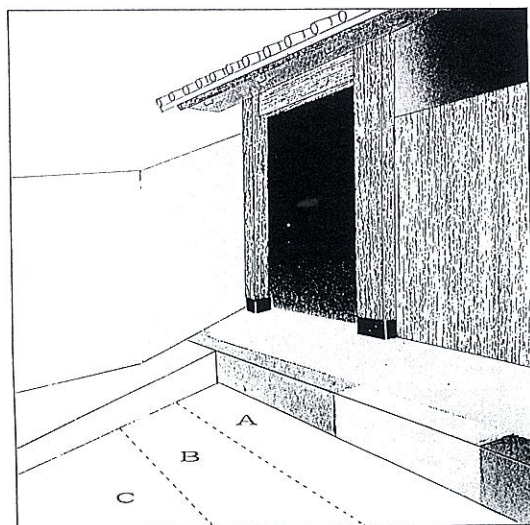


Fig. 4. View of the field around buildings caused by copper pollution.

A, *Pohlia bulbifera* alone or *Scopelophila cataractae* is seen. B, *Atrichum undulatum* is not seen. C, *Atrichum undulatum* is seen, but *Pohlia bulbifera* and *Scopelophila cataractae* is not seen.

ples, and were cleaned with deionized water by using ultrasonic cleaner. The moss sample was dried in a drying oven at 60°C for 2 days and in an electrothermal drier at 110°C for 6 hours. The concentration of metals in all moss samples were calculated on the basis of 110°C-dried weight.

Preparation of soil samples collected along with moss samples: Soil samples pulverrized with agate motor and pestle were sieved to 100-mesh (0.147 mm) after drying in a drying oven at 60°C for 2 days and in an electrothermal drier at 110°C for 6 hours. The metal concentrations of all soil samples were calculated on the basis of 110°C-dried weight.

Determination of weight loss of moss and soils on drying: The weight loss of moss samples was measured as the difference between the weight on air-drying and the weight on the 60°C-drying, and as the difference between the weight on the 60°C-drying and the weight on the 110°C-drying. In the case of soil samples, only the difference between the weight on the 60°C-drying and the weight on the 110°C-drying weight was measured.

Digestion of moss samples and the determination of concentration of heavy metals: About

0.01 g of moss sample dried at 110°C was accurately weighed, and taken into sample vial (10 ml). A 1 ml of concentrated nitric acid was added to the moss sample, and the mixture was digested by a quick digester with 50%-power for 20 minutes. Then, a 1 ml of the mixture of nitric acid (63%) and perchloric acid (60%) (3:1 v/v) was added to the sample vial, and the contents were decomposed by using digester with 40 %-power for 25 minutes and then 100%-power for 35 minutes.

The inside of the sample bottle was rinsed with a 1.0 ml of 1 M nitric acid solution, and the sample was centrifuged at 6400 r.p.m. for 1 minute to eliminate silicate. After centrifugation, the solution was diluted to 10 ml with deionized water. The concentration of metals was determined by AAS at 324.7 nm for copper, 213.8 nm for zinc and 283.3 nm for lead.

Determination of metals in each part of moss samples: A piece of 60°C-dried moss sample was cut in each 3 mm length from the top of the moss and was accurately weighed in a small vial. A 1 ml of the mixture of nitric acid and perchloric acid was added to the sample vial, and the concentration of metals was analysed as in the same way described above.

X-ray fluorescence analysis of soil samples: A 0.05 g of soil sample dried at 110°C was made to a tablet, inserted into the holder and shielded with mylar film, followed by measuring the X-ray fluorescence spectrum under the following conditions: voltage 20 V, current 32 μ A, energy 0~16 keV, count time 1000 seconds. The spectrum of metals contained in the sample were obtained.

Determination of the apparent total contents of metals in soil samples: About 0.005 g of soil sample dried at 110°C was accurately weighed into a sample vial. A 1 ml of the mixture of nitric acid and perchloric acid was added to the sample vial, and the mixture was analysed as in the same way described above.

Determination of the contents of available metals in soil samples: About 0.1 g of soil sample dried at 110°C was accurately weighed and was shaken with a 10 ml of 1 M ammonium acetate solution (pH = 7.3) for 20 minute. After the centrifugation at 2000 r.p.m. for 20 minute, the

solution was diluted with deionized water, if necessary, and the concentration of heavy metals in the solution was determined by AAS.

Determination of the contents of soluble metals in soil samples: About 0.1 g of soil sample dried at 110°C was accurately weighed and was shaken with a 10 ml of deionized water for 20 minutes. After the centrifugation at 2000 r. p. m. for 20 minutes, the solution was diluted with deionized water, if necessary, and the concentration of heavy metals in the solution was determined by AAS.

Analysis of acid rain dropped from metal roofs: Rain dropped from metal roofs was collected in P. E. T. (polyethylene terephthalate) bottle, and the pH and the concentrations of heavy metals in rain samples were measured.

Observation of heavy metals accumulated in the tissue of moss: A few pieces of moss samples were wrapped with "kimwipes" (KIMBERLY-CLARK), and were held between the artificial materials as elder divided (SHIMADZU). After ratching it on the cylinder microtome, each 10 μ m of elder with moss was sliced off by a razor. The cross sections of sliced moss were collected in deionized water to prevent them from drying, and were transferred on a slide glass with a micro pipette. The tissues of moss were observed by using optical microscope after dyeing them with organic or inorganic reagents.

Results and Discussion

1. There is only one kind of moss mentioned earlier sometimes around buildings made of heavy metals, but usually two or more species of gregarious moss were found in the areas of heavy metals pollution of the buildings. A typical example of this state is shown in Fig. 4. In the seriously contaminated areas below the copper roof (A in Fig. 4), *Pohlia bulbifera* was mainly found and *Scopelophila cataractae* or *Pohlia nutants* were found here and there but *Atrichum undulatum* was never found there. *Atrichum undulatum* was not found in a belt within a distance of several decimeters outer from just under the copper roof (B in Fig. 4). *Atrichum undulatum* grows outer this belt (C in Fig. 4) but *Pohlia bulbifera* or *Pohlia nutants* was found rarely. Sometimes *Racomitrium canescens* was

found near *Atrichum undulatum*. It is assumed that the order of the tolerance of these moss for copper is as follows: *Pohlia bulbifera*, *Scopelophila cataractae*, *Pohlia nutans*, *Atrichum undulatum*, *Racomitrium canescens*.

2. Species of other plants growing along with metal tolerant moss: In the fields of heavy metals pollution, some plants were found beside the moss mentioned above, as follows: *Athyrium yokoscense* (Honjo et al. 1984), *Equisetum arvense*, *Pueraria lobata* and some creepers were found near the buildings tiled with lead in Kanazawa Castle. These plants are used as an indicator plant of heavy metals pollution. Around the Kamioka mine areas, Gifu Prefecture, contaminated by zinc and lead, *Athyrium yokoscense* and *Pueraria lobata* grow in colony state. In the areas of Ichihiko Shrine in Kurobe City, Toyama Prefecture, *Athyrium yokoscense* grows along with *Pohlia bulbifera* in line. This type of moss was usually found near the place of heavy metals pollution. It is assumed that moss is stronger tolerant to heavy metals than other metal tolerant plants. This might be related to the differences of the vegetative organs between moss and other higher plants.

3. Relationship between the concentrations of

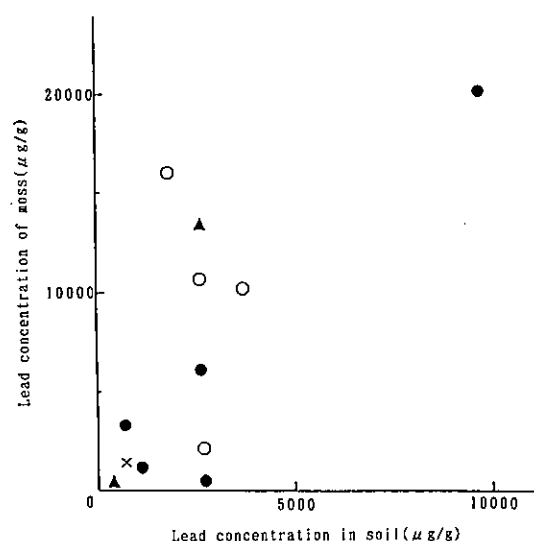


Fig. 5. Relationship between the concentration of lead contained in moss samples and that of lead in soil samples where moss grows.

●: *Atrichum undulatum*, ▲: *Pohlia bulbifera*, x: *Scopelophila cataractae*, ○: other moss.

heavy metals in moss samples and in soil samples: Moss samples collected were digested by quick digester, and then the concentration of heavy metals in the samples was determined by AAS. The concentration of metals obtained was calculated on the basis of 110°C-dried weight. Metals in soil samples were examined by X-ray fluorescence analysis. The apparent total concentration of each heavy metal in a soil sample was correlated to the results obtained by AAS after digestion and the intensity of X-ray fluorescence spectrum peaks. The concentration of metals obtained is calculated on the basis of 110°C-dried weight. Figs. 5, 6 and 7 are the plots, whose x-axis shows the concentration of lead, copper and zinc in soil samples, and y-axis shows those in moss samples, respectively. Three kinds of moss indicate different types of characters. In the areas polluted by lead, copper and zinc, it tends that the larger the concentration of heavy metals in soils, the larger that in moss. The concentration of heavy metals in soil and moss samples was measured, and the results (Figs. 5~7) show that when the apparent total concentration of metal in soils increases, the concentration of heavy metals in moss samples also increases.

The state of colony of moss depends on the difference of the concentration of heavy metals in soils around moss, and it is considered that the concentration of heavy metals in environment is

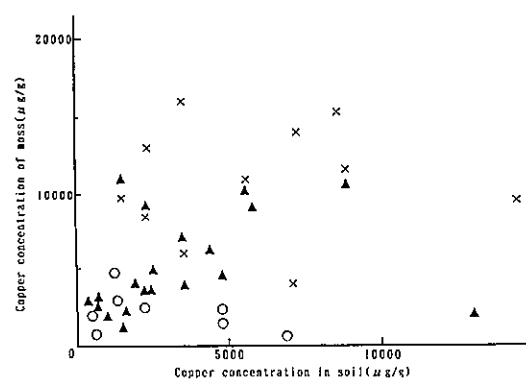


Fig. 6. Relationship between the concentration of copper contained in moss samples and that of copper in soil samples where moss grows.

●: *Atrichum undulatum*, ▲: *Pohlia bulbifera*, x: *Scopelophila cataractae*, ○: other moss.

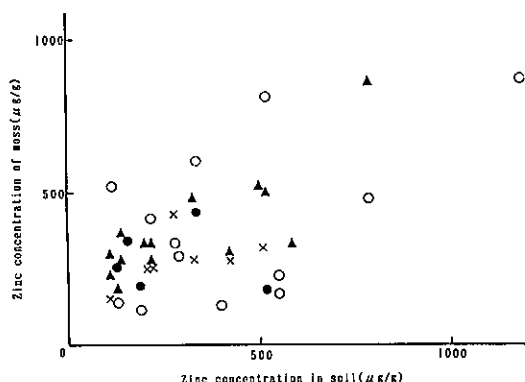


Fig. 7. Relationship between the concentration of zinc contained in moss samples and that of zinc in soil samples where moss grows.

● : *Atrichum undulatum*, ▲ : *Pohlia bulbifera*, x : *Scopelophila cataractae*, ○ : other moss.

inferred from the state of gregarious moss. Therefore, the distribution of moss in the environment seems to correlate with the concentration of heavy metals in soils.

4. Concentration of heavy metals in rain water dropped from metal roofs: The tissues of moss can not be clearly classified into the parts of root, stem and leaves. Therefore, it is difficult to conclude whether moss sucks up water and inorganic nourishments in soils through its organs as higher plants do. That is, the moss may directly absorb heavy metals from the surface of the organ. In the present study, the origin of heavy metals coming over the leaves of moss is considered to be acid rain which dissolves heavy metals used in buildings. This phenomenon is seen on the lead roofs of Zuiryuj Temple. The lead roof became gradually thin and had to be recast at intervals of about 60 years. The pH and the concentration of heavy metals in rain water flew down from the edge of the metal roofs were 11.2 ppm Pb at pH 4.4 at Ishikawamon Gate, 10.9 ppm Pb at pH 4.4 at Sanjukken Nagaya, 13.9 ppm Pb at pH 4.9 at Education Center of University, 55.3 ppm Cu and 0.5 ppm Zn at pH 4.9 at Ozaki Shrine, 0.3 ppm Cu at pH 4.7 at Oyama Shrine and 0.75 ppm Zn at pH 4.9 at Energy Institution of University, respectively.

5. Distribution of heavy metals in a section of moss samples: *Atrichum undulatum* growing on

lead pollution areas was cut with a design knife in each 3 mm length from the top of the organ. The amount of lead in each section was ascertained by AAS after digestion. Fig. 8 shows the cutting sites of moss and the concentration of lead found in each section. Three sections from the top of the stems (section A, B and C) were light green in color, and the result of determination of lead shows that the concentration of lead in the tissues was nearly equal. The section D of stem was brown in color, and the concentration of lead was found to be about three times higher than those of section A, B and C. Section E was dark brown in color and without leaf. The concentration of lead in section E was nearly equal to section D. The sections look older in the order of section E, section D, sections C, B, and A. Therefore, it is assumed that lead accumulation in vegetative organs occurs gradually, and it takes a long time.

6. Structures of the tissues of *Atrichum undulatum*

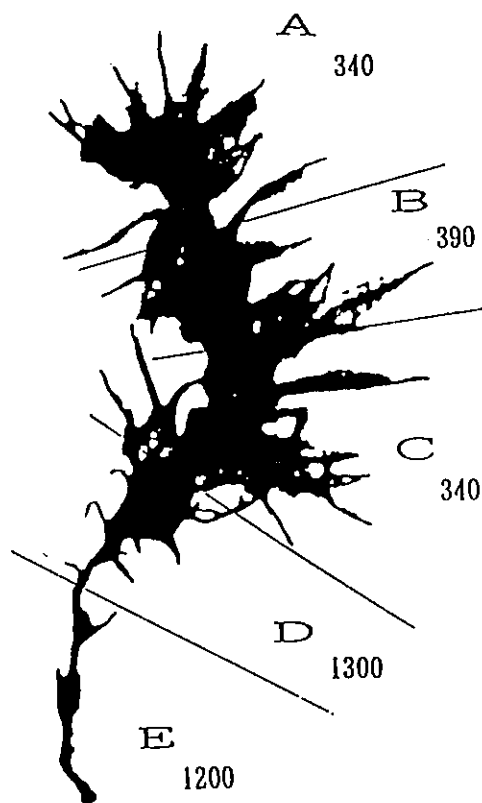


Fig. 8. Distribution of lead in the tissues of *Atrichum undulatum*. The figure shows the concentration of lead ($\mu\text{g/g}$) in each part of moss.

tum, *Pohlia bulbifera* and *Scopelophila cataractae*: The structures of the tissues of three species of moss are shown in Fig. 9. *Atrichum undulatum* belongs to V genus in taxonomy and has five layers structure composed of epidermis, cortex, leptome, hydrome sheath and hydrome, as shown in Fig. 9-A. *Pohlia bulbifera* belongs to IV genus and has four layers structure, as shown in Fig. 9-B. The structure is similar to that of V genus without hydrome sheath. *Scopelophila cataractae* has more primitive structure than that of another moss. It belongs to III genus and has three layers structure, as shown in Fig. 9-C.

7. Accumulation of lead in the tissue of moss: In order to show lead distribution in the tissues of moss, an aqueous dyeing solution of rhodizonic acid disodium salt (about 1% w/v) was dropped on the cross sections, and the tissues were dipped into 0.1 M tartaric acid solution (pH = 2.8) after 1 minute. The red colored state of the cross sections of *Atrichum undulatum* and

Pohlia bulbifera is shown in Fig. 10. It was found that lead was distributed over the cell walls and inner cells belonging to cortex.

8. Accumulation of copper in the tissues of moss: In order to dye copper distributed in the tissues of moss, ethanol solution of rubeanic acid (about 1% w/v) was dropped on the cross sections of moss, and the tissues were dipped into 1 M aqueous ammonia after 1 minute. The black colored state of the cross sections of *Pohlia bulbifera* and *Scopelophila cataractae* is shown in Fig. 11. It was found that copper was distributed over the cell walls alone.

9. Accumulation of zinc in the tissues of moss: In order to dye zinc distributed in the tissues of moss, first of all, 1 M sodium thiosulfate was dropped on the cross sections of moss for masking of lead, and then the tissues were dipped into ethanol solution of PAN (about 3% w/v) for 1 minute. After then, the tissues were rinsed with standard pH solution (HORIBA "1.00-7.00", pH = 6.8). The rose colored state of the cross

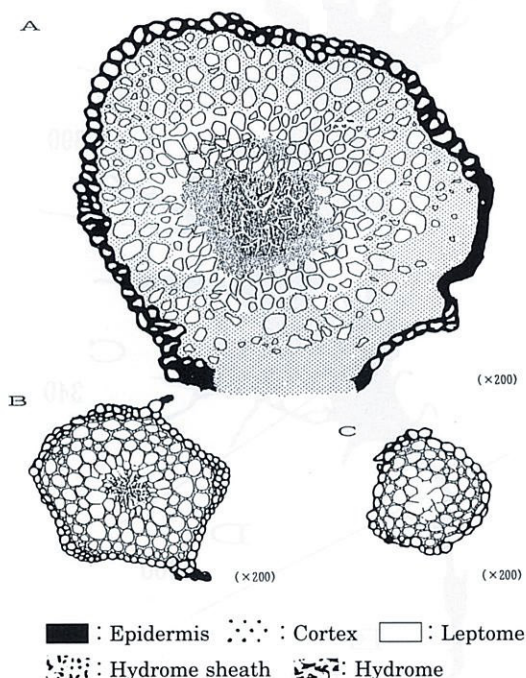


Fig. 9. Structure of the tissues of three kinds of moss.

A, *Atrichum undulatum* ($\times 200$). B, *Pohlia bulbifera* ($\times 200$). C, *Scopelophila cataractae* ($\times 200$).

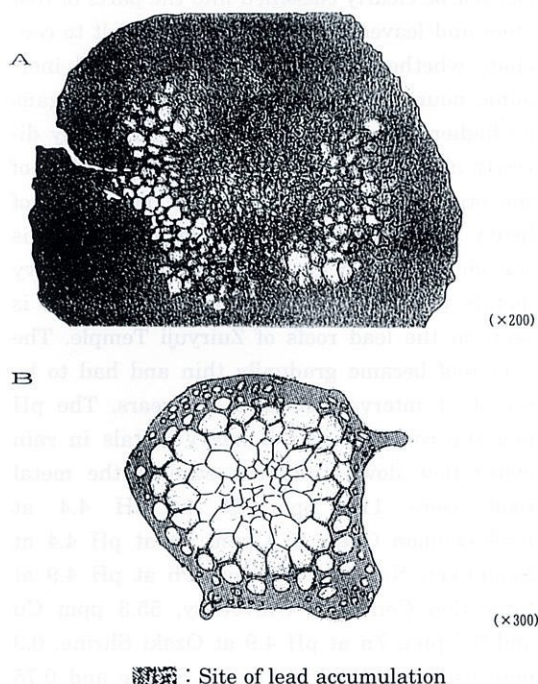


Fig. 10. Cross sections of moss dyeing with rhodizonic acid disodium salt.

A, *Atrichum undulatum* ($\times 200$). B, *Pohlia bulbifera* ($\times 300$).

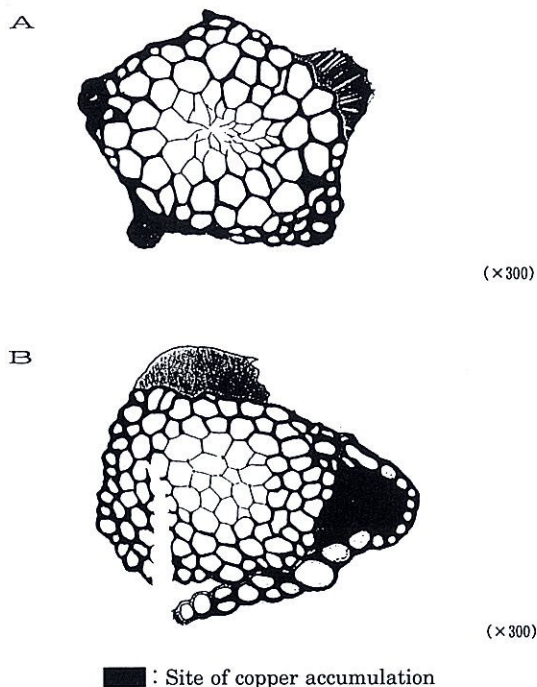


Fig. 11. Cross sections of moss dyeing with rubeanic acid.

A, *Pohlia bulbifera* (×300). B, *Scopelophila cataractae* (×300).

sections of *Pohlia bulbifera* and *Scopelophila cataractae* is shown in Fig. 12. It was found that zinc was distributed over the inner cells alone.

10. Distribution of functional groups forming metal complex in the tissues of moss: In the case of ferns, lead is mainly distributed over the cell walls of cortex as its complexes with pectic acid of carboxyl group of hemicellulose in the tissues (Honjo *et al.* 1988; Sakai *et al.* 1991). The results obtained by histochemical method described above, indicate that lead and copper are distributed over the cell walls and cortex, while zinc is distributed over the inner cell. Ruthenium red combines with carboxyl groups to develop highly sensitive purple color. Therefore, the cross sections of *Atricum unduratum*, *Pohlia bulbifera* and *Scopelophila cataractae* were stained with dilute aqueous solution of ruthenium red in order to ascertain the distribution state of heavy metals in the tissues of moss. The colored sites of the sections with ruthenium red are shown in Fig. 13.

All of the cell walls of three species of moss

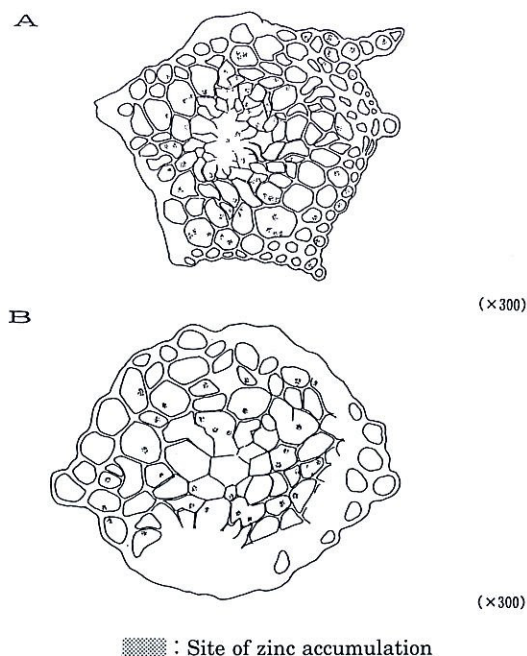


Fig. 12. Cross sections of moss dyeing with pyridylazonaphthol.

A, *Pohlia bulbifera* (×300). B, *Scopelophila cataractae* (×300).

turned purple color. This fact suggests that lead and copper in the tissues were adsorbed by complex formation with carboxyl groups of hemicellulose composed of cell walls. Zinc and a part of lead were found in the tissues irrelevant to hemicellulose.

The distribution of heavy metals in the vegetative organs of moss was also ascertained from the intensity of peaks of X-ray fluorescence spectra. Fig. 14 shows the correlation between the concentration of heavy metals and ratios of the X-ray intensity of calcium used as inner-standard vs. those of lead, copper and zinc. The shape of calcium-lead curve and calcium-copper curve showed similar pattern, but that of calcium-zinc curve gave different pattern. This fact shows that the accumulating mechanism and accumulating sites of zinc in the tissues of moss are different from the other metals. In the case of ferns, *Athyrium yokoscense* accumulated copper on the exterior cell walls, whereas zinc was accumulated throughout the cells including exterior cell walls (Honjo 1987). Judging from

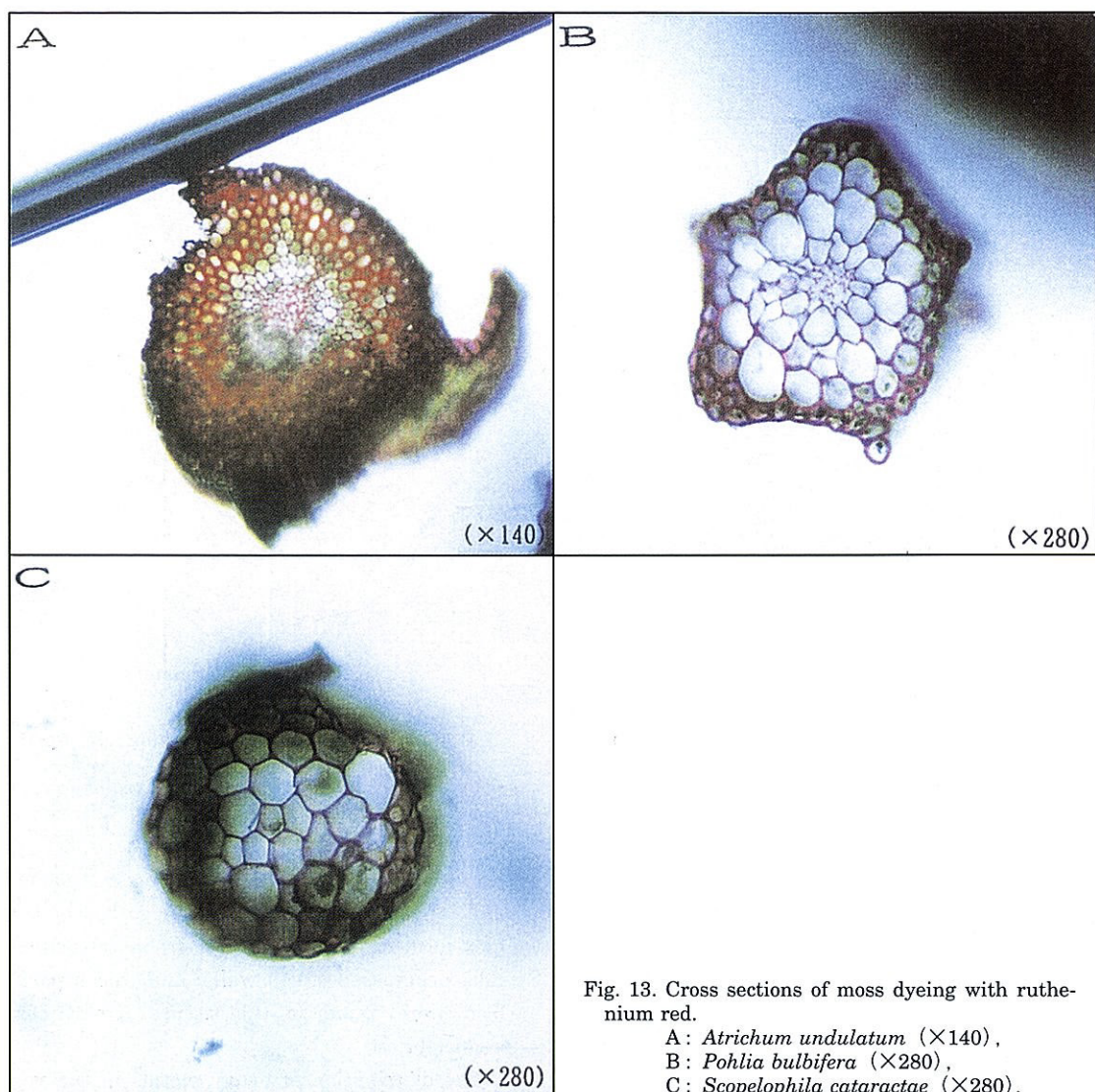


Fig. 13. Cross sections of moss dyeing with ruthenium red.

A: *Atrichum undulatum* ($\times 140$),
 B: *Pohlia bulbifera* ($\times 280$),
 C: *Scopelophila cataractae* ($\times 280$).

the results by histochemical methods, zinc may exist as intracellular species.

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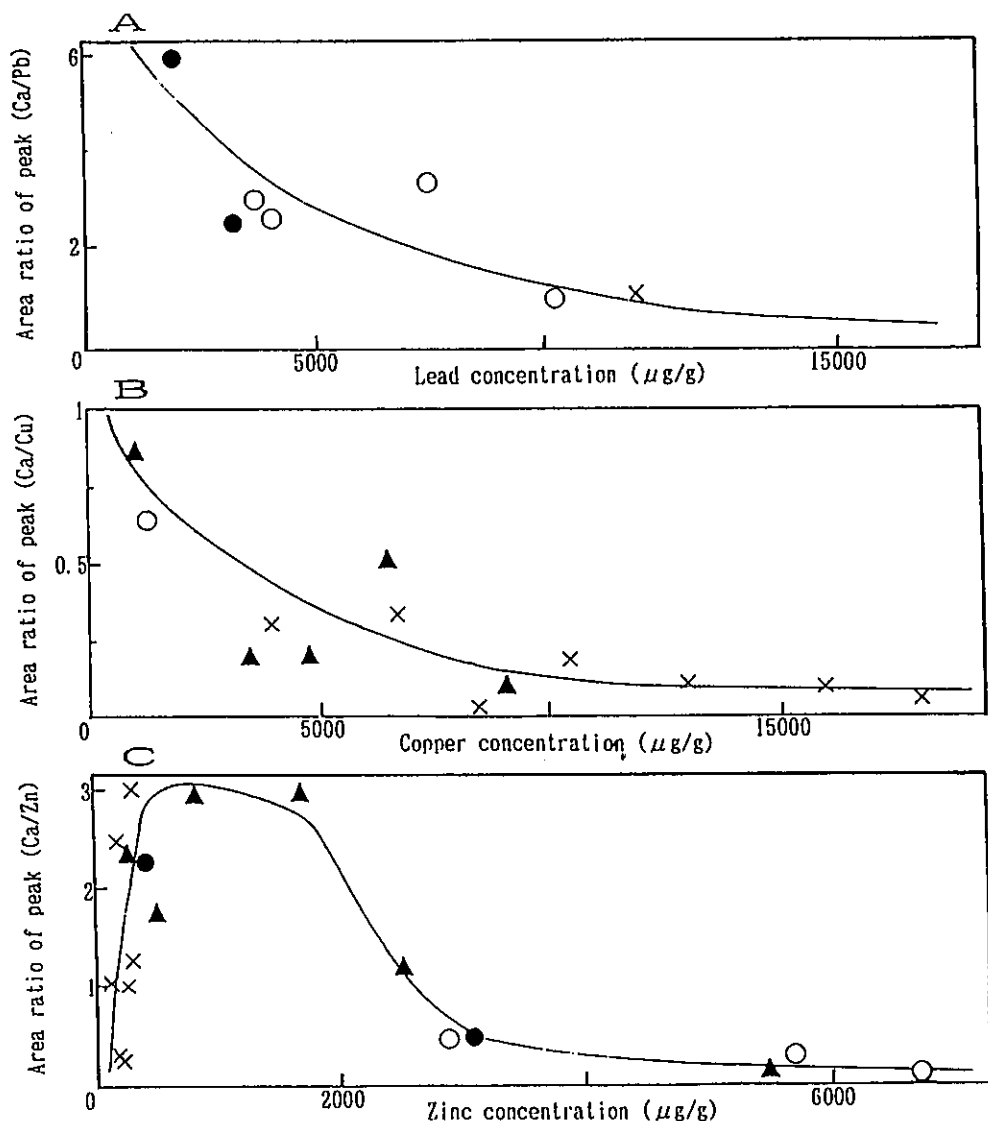


Fig. 14. Relationship between the concentration of heavy metals and the intensity of x-ray fluorescence spectrum (calcium/heavy metals) in moss samples.

A : Lead, B : Copper, C : Zinc, ● : *Atrichum undulatum*, ▲ : *Pohlia bulbifera*, x : *Scopelophila cataractae*, ○ : other moss.

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摘 要

近年、酸性雨が屋外の建造物の金属を溶かし、土壌環境汚染を引き起こして問題となっている。そこで、酸性雨により金沢城内（鉛瓦）、神社仏閣や一般家庭（銅屋根、トタン屋根および雨樋）の建造物から溶け出した重金属を含む土壌で生育しているコケの異常な植生を指標として、土壌重金属汚染を評価できないか検討した。重金属を使った建造物より滴り落ちる雨水を分析し、銅はpH 4.7~4.9では0.3~55.3 ppm、亜鉛はpH 4.9で0.5~0.75 ppm、鉛はpH 4.4~4.9で10.9~13.9 ppm 雨水に含まれていること、またコケが生育している周辺の土壌も分析し、土壌重金属汚染が著しいこと（乾燥重量で~15,000 ppm）を確認した。金沢市周辺での調査の際、土壌重金属汚染地域には、これまで日本海側では確認されていなかったホンモンジゴケを含む僅か三種類のコケだけが生育していることを見出した。そして、主として、スギゴケ科(Polytrichaceae)

のタチゴケ (*Atrichum undulatum* (Hedw.) P. Beauv.) は鉛、亜鉛汚染地域、カサゴケ科 (Bryaceae) のキヘチマゴケ (*Pohlia bulbifera* (Warnst.) Warnst.) は鉛、亜鉛、銅汚染地域、センボンゴケ科 (Pottiaceae) のホンモンジゴケ (*Scopelophila cataractae* (Mitt.) Broth.) は銅汚染地域で生育分布していることを明らかにした。つぎに、これら三種の組織中の重金属含量を定量し、その多くが周辺土壌に比べて体内に高濃度の重金属を集積しており（乾燥重量で~20,000 ppm）、重金属に耐性があることを明らかにした。さらに、これらのコケ組織中の重金属分布を横断切片の有機試薬染色一顕微鏡観察により、鉛は表皮から茎の中心部に向かって細胞質および細胞壁、銅は細胞壁周辺に表皮側ほど、亜鉛は細胞内部に多く分布していること、またコケ植物の細胞壁にカルボキシル基が多量に存在することを確認した。そして、銅と鉛がこのカルボキシル基と金属錯体を形成して細胞壁周辺に分布しているが、亜鉛の場合は水溶性化学種として細胞質内部に分布していること、これら重金属のコケの組織内分布状態と重金属汚染土壌で生育しているコケ種とが密接な関係にあることが分かった。

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○ 竹田孝雄 広島県ササ類植物誌 B5判, 230頁, 1995年1月15日; シンセイアート出版部 (〒727 広島県庄原市西本町 1-1-14), 6,000円。

ササ類の分類といえは、いづれの県においても調査・研究の狭間に置かれる植物群である。広島県では、その難題に広島県三原市に生まれ育ち、長らく広島県で高校教諭を務められた著者が何と古稀近くになって挑戦され、10余年間にわたり資料を集積、観察記録をまとめられたが、残念なことに、1990年81才で他界された。この本は、中村慎吾氏ほか3名の教え子たちが著者の没後5年間をかけて、遺稿を整理、加筆・修正してまとめあげたものである。本文はササ類植物概説(図解を含め4頁)、広島県のササ類植物—その種類と分布(21頁)、広島県産ササ類検索表(挿図を含め4頁)、ササ・スズササ・アズマザサ・スズタケ・ヤダケ・メダケ各属の解説(181頁)からなり、各属の解説では85種類についてそれぞれ2頁ずつが当てられ、白黒写真・メッシュ法による分布図・標本データを示すという構成で、広島県のササ類の全貌を浮かび上がらせている。分類はおもに鈴木貞雄博士の「日本タケ科総目録」(1978)により、用いられた標本はすべて比和町立自然科学博物館に収められている。特筆すべきは、この本は、教え子たちが著者の採集地に赴いて標本写真を撮影し直して取替え、属の解説を補筆し、生態と分布のメモをまとめあげ、巻頭に17枚のカラー写真、本文中に頁大の10枚の線画を描き入れるなど、献身的な努力の結果出来上がったということである。われわれは、地方における植物愛好者たちのこのようなスクラムによって植物自然史が支えられていることを忘れてはいけぬ。なお、プラント第4号(1989年7月)には著者の植物研究回顧録が掲載されている。(清水建美)