STUDY ON THE RELATIONSHIP BETWEEN "COPPER MOSSES" AND THEIR SUBSTRATES

（いわゆる銅ごけと呼ばれる蘚苔類と基物土壌との関係についての研究）

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STUDY ON THE RELATIONSHIP BETWEEN "COPPER MOSSES" AND THEIR SUBSTRATES

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DEDICATE TO

MY FAMILY FOR THEIR CONTINUOUS ENCOURAGEMENT AND

COOPERATION

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INTRODUCTION

INTRODUCTION

The bryophytes, together with the lichens, are known as pioneer plants to play an important role in the process of soil formation (Yamane et al. 1978; Yagi 1997). They can be found out on the lava field, as a typical example, in the primary succession process (Tagawa 1965, 1973; Numata and Iwase 1975). Among the bryophytes, some peculiar mosses and hepatics have been studied with special interest because of their curious ecology. They seem to prefer the metal rich soils, lithosols, rocks and others as their substrates although such an environment is generally considered to be an adverse condition upon the plant growth. These mosses and liverworts have been called "copper mosses" because their substrates containing remarkable amounts of heavy metals such as copper, iron, lead, etc., have been found by many field investigations and researches.

"Copper mosses" have been described as bryophytes with strangely ecological characteristics and have attracted the interest of many scientists. Their distribution has been considered to depend on the occurrence of heavy metals in their substrates. These mosses and liverworts have been studied by Persson (1948), Mårtensson and Berggren (1954), Shacklette (1965a, 1965b) and many subsequent authors.

The study of "copper mosses" began in nineteenth century. Many studies of classical "copper mosses", such as genera *Scopelophila* and *Mielichhoferia* and similar bryophytes, have been performed based on the observations made in the field, in the herbarium, and in the laboratory (Persson 1948; Schatz 1955; Amakawa 1960; Shacklette 1961, 1965a; Lampton 1966; Zander 1966, 1967, 1985; Crundwell 1976, 1986; Shaw and Crum 1984; Corley and Perry 1985; Dirkse 1986; Reese 1989) and also based on culture tests in the laboratory (Ikenberry 1936; Meyer and Ford 1943; Url 1956; Noguchi and Furuta 1956; Nagano and Shimizu 1972; Coombes and Lepp 1974). Brown (1982) critically reviewed the literature on mineral nutrition in bryophytes including a discussion of "copper mosses". Later, Satake et al. (1988) reviewed the literature on some mosses and liverworts which were found in possible copper-rich places and which seemed to contain high amounts of copper in their shoots. They carried out studies on *Scopelophila cataractae* (Mitt.) Broth., one of the famous "copper mosses" which inhabit Japan. Using non-destructive methods (Satake et al. 1985, 1988), they found that the copper was localized in the cell walls of *S. cataractae*. Shaw (1987, 1993a, 1993b) also carried out studies on *Scopelophila cataractae* (Mitt.) Broth. using statistical techniques. Although some studies have looked at the relationship between the bryophytes and their substrates, a definitive explanation for the occurrence of "copper moss" species has not yet been obtained (Brown 1982; Warncke 1968; Iwatsuki and Mizutani 1972; Shaw 1987). The reasons why only a few studies have been done would be as follows. 1) The analytical techniques were immature in the past. 2) Because of the interdisciplinary study area, there have not been so many researchers. 3) Bryophytes have been less frequently sampled for chemical analysis than have vascular plants because of their lack of value as forage plants and 4) the difficulty of their identification (Shacklette 1965b). 5) "Copper mosses" are disjunctive species and their habitat are restricted (Shaw 1987). 6) Obtaining adequate clean "copper moss" samples was thwarted by the contamination of substrate soil particles (Mårtensson and Berggren 1954; Pigott 1958; Satake et al.

1988).

Bioremediation has been an active area of interest in the field of the soil remediation (Matsumoto 1996). "Phytoremediation" is a subclass of bioremediation, which uses plants to clean contaminated sites. Certain plants actively take up and concentrate heavy metals from the soil (Council for Promotion of Utilization of Organic Materials 1997; Japan Association of Industries and Environment 1998).

In this thesis, focusing on the studies which have been done on "copper mosses", the relationship between the bryophytes and the water-soluble elements in their substrates which have never been considered in the past, the washing methods for the bryophyte contamination and the differences among "copper mosses" were studied. Finally, bryophytes' ability of accumulating some heavy metals was also focused and its application for environmental restoration and environmental monitoring was discussed in this thesis.

CHAPTER I

Chemical Elements in the Substrates of *Scopelophila ligulata* (Spruce) Spruce and Other Mosses Growing on the Same Black Slate

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Chemical Elements in the Substrates of *Scopelophila ligulata* (Spruce) Spruce and Other Mosses Growing on the Same Black Slate

1.1. Introduction

Brown (1982) noted that only a few chemical analyses have been performed on the rock, soil or detritus beneath "copper mosses" by Persson (1956), Mårtensson (1956), Shacklette (1967) and Wilkins (1977). Wilkins (1977), Coker (1971) and some other authors (Warncke 1968; Shaw 1987; Brown and House 1978) used their several extraction methods to estimate exchangeable copper in the substrate, since total elemental content would overestimate the amount of copper available to the bryophyte. Pigott (1958) analyzed a water-extractable sulfate in the substrate of *Mielichhoferia elongata* (Hornsch.) Hornsch., known for long as one of the "copper mosses".

S. ligulata is known to prefer a strongly acidic substrate (Persson 1956; Noguchi 1956). The pH of the substrate of *S. ligulata* is significantly lower than that of *S. cataractae* (Persson 1956; Noguchi 1956). Nagano et al. (1969) pointed out that there was a close relationship between the pH of the substrate of *S. ligulata* and the limonite-like substance on rocks in the Chichibu district.

In this report, we discuss the relationship between the water-soluble chemical elements in the substrate of a "copper moss" *S. ligulata* which grow on a vertical face of black slate (Chichibu Paleozoic strata). For comparison, analyses were made using the substrates of four other non-copper mosses, namely, *Pogonatum nipponicum* Nog. et Osada, *Leucobryum juniperioideum* (Brid.) C. Muell., *Dicranella heteromalla* (Hedw.) Schimp. and *Campylopus* *umbellatus* (Arm.) Par., which grow on the same rock wall with *S. ligulata*.

1.2. Materials and Methods

The mosses and their substrates used in this study were collected at Urayama Valley, Chichibu, Saitama Prefecture, at about 320 m above sea level. A steep-rising rock wall of black slate is exposed on a road cut. The black slate contains well-developed clefts, with relatively closely spaced colonies of *S. ligulata*, about 1.5 meters wide. Adjacent to the *S. ligulata*, *P. nipponicum*, *L. juniperioideum*, *D. heteromalla*, and *C. umbellatus* were observed. Their boundaries with the *S. ligulata* colonies were not distinct. In addition to these species, the following bryophytes were also found on the rock wall: *Calypogeia tosana* (Steph.) Steph., *Jungermannia infusca* (Mitt.) Steph., *J. radicellosa*, *Lejeunea japonica* Mitt., *Porella stephaniana* (Mass.) Hatt., *Scapania stephanii* K. Muell., *Atrichum undulatum* (Hedw.) P. Beauv. var. *gracilisetum* Besch., *Brothera leana* (Sull.) C. Muell., *Dicranum viride* (Sull. et Lesq.) Lindb. var. *hakkodense* (Card.) Tak., *Grimmia pilifera* P. Beauv., *Herpetineuron toccoae* (Sull. et Lesq.) Card., *Hypnum plumaeforme* Wils., *Oncophorus crispifolius* (Mitt.) Lindb., *Pohlia proligera* (Kindb.) Lindb. ex Arn., and *Rhacomitrium heterostichum* (Hedw.) Brid.

Substrate samples were collected in areas where comparatively close colonies of each moss species were growing. A bamboo spatula was used to collect them with the moss. The total number of substrates sampled was 34: 13 of *S. ligulata*, 9 of *P. nipponicum*, 4 of *L. juniperioideum*, 3 of *D. heteromalla*, and 5 of *C. umbellatus*. These samples were placed in polyethylene bags and naturally dried indoors. Substrate samples were carefully separated from the mosses, sharply observing whether the moss bodies, such as leaves, stems and rhizoids, were present. If any were found, they were removed from the sample using the tweezers with non-metallic tips. Then the samples were sieved to obtain under 2 mm diameter grains which were further ground up by using a mortar and sifted out with a 32 mesh screen.

Leaching procedures to estimate the exchangeable or extractable elements in the substrates of some bryophytes including "copper mosses" have been developed by some authors. Warncke (1968) and Wilkins (1977) used an ammonium acetate leach (pH 7) for the substrate of the liverwort *Marchantia alpestris* (Nees) Burgeff and the "copper mosses" *Mielichhoferia elongata* etc., respectively. Wilkins (1977) suggested that leaching with 1N ammonium acetate solution was an acceptable technique for the estimation of plant-available iron, while he noted that this leaching method did not provide the best estimate of plant-available copper. Coker (1971) used a mixture of hot nitric / hydrochloric acid to leach the elements from the substrates of *M. elongata* and *Saelania glaucescens* (Hedw.) Broth. in Bomanss et Broth. The solutions were analyzed for calcium, chromium, cobalt, copper, iron, lead, lanthanum and yttrium using an atomic absorption spectrophotometer. He also tested the aqueous extracts of five grams of the substrate in fifty milliliters of deionized water and analyzed sulfate concentrations using UV spectrophotometer. His results of the eluted sulfate concentrations compared with that of Pigott (1958) (11,800 ppm). Coker (1971) noted that there were considerable differences in the analyses from the Scottish and English sites for *M. elongata* and that these reflected the possible range of edaphic conditions under which *M. elongata* could grow. Brown and House (1978) extracted copper in the substrates of the hepatic

Jungermannia gracillima Sm. (*Solenostoma crenulatum* (Sm.) Mitt.) by either deionized water, 2% EDTA or 1000 microgram / gram lead nitrate. The latter solution was used for comparison with copper and potassium displaced from the bryophyte by lead nitrate. They suggested that the bryophytes appeared to be capable of substantial accumulation of copper from the soil. While Brown and House (1978) did not comment the relationship between the copper concentration of the plant body and the concentrations of either water-soluble or 2% EDTA extractable copper in the substrate, Brown (1982) mentioned that plant available copper was possibly in a chelate form and EDTA or other complexing agents would be better extractants than an ammonium acetate (pH 7). Shaw (1987) used 1% hydrochloric acid to extract cations, namely copper, zinc, lead and iron, in the substrates of *S. cataractae* from six localities in the eastern United States. Four of the six localities were on mine tailings in the Piedmont of North Carolina and two were in undisturbed habitats. His chemical analyses of the substrates from these localities showed that five of the six populations grew on copper-enriched soil, but the one substrate sample low in copper was very high in iron.

The bryophytes would absorb the nutritive substances and also pollutants with the water-soluble form into their system through their whole surface of the body (Taoda 1974; Berg and Steinnes 1997; Nagano et al. 1998). The soil analysis using the water-soluble method (1:10 soil:water) has been adopted by the Environment Agency of Japan to estimate the environmental quality of soil. The Environmental Quality Standards for Soil were formulated in accordance with Article 9 of the Basic Low for Environmental Pollution Control on August 23, 1991, as the standards to protect human health and conserve the living environment.

Taking account of the above-mentioned issues, author used the distilled water as a solvent to extract the elements in the substrate of the bryophyte. The slurry ratio of soil to water was not 1:10 but 1:2.5 by weight, which was the same one to be used at the soil pH measurement throughout the world (Department of Applied Biochemistry and Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo 1995; Kennedy 1992; Malhi et al. 1995) because author intended to find the relationship between the water-soluble elements and the pH value.

Twenty-five milliliters of distilled water were added to ten grams of each sample. The mixtures were then stirred, eluted and allowed to stand for 24 hours. This standing time was determined to obtain the optimum pH value (Nagano and Wada 1974). The pH and the metal contents (iron, copper, zinc, etc.) of the supernatants were measured using a pH meter and an atomic absorption spectrophotometer, respectively. The barium sulfate turbidimetric method was used to measure the sulfate concentration (Japanese Standards Association 1993). The ambient temperature at the time of these measurements was 23-25°C.

1.3. Results and Discussion

The chemical analyses of the moss substrates are shown in Table 1-1. The pH values of the substrate of *S. ligulata* which formed colonies on the black slate were 3.37 - 4.46 (average 3.90). These values were slightly higher than those measured for the same species by Noguchi (1956) (range 2.4 - 4.3, average 3.50), those by Persson (1956) (range 2.50 - 4.53, average 3.44) and those by Nagano et al. (1969) (range 2.06 - 3.90, average 2.98).

Despite of this, the pH of the substrate of *S. ligulata* was much lower than those of *P. nipponicum*, *L. juniperioideum*, *D. heteromalla*, and *C. umbellatus* which were growing on the same rock wall. The pH values, as well as the concentrations of chemical composition such as iron and sulfate, differed between the substrates of *S. ligulata* and those of the four other mosses.

These two groups were compared by the amount of dispersion and bias in measured values using the *F*-test and the *t*-test, respectively. The *F*-test was carried out before the *t*-test according to the established method (Yonezawa et al.). *F*-tests and *t*-tests showed a significant difference between those two groups. The results of these calculations are shown in Table 1-2. No difference in the pH was observed by the *F*-test, but significant differences were found for the pH and the iron and the sulfate levels. Neither the *F*-test nor the *t*-test could effectively detect a difference between the concentrations of copper and zinc, since samples contained only minute amounts. The substrate of *S. ligulata* had a lower pH value (by a factor of about 1.5 on average) and higher concentrations of iron (by about five times on average) and sulfate (by about two times on average) than those of four other mosses.

Nagano (1976) pointed out that, with respect to the vegetation, the black slate could be divided into two different types due to their geological characteristics. The species that developed on the black slate in some places are similar to those growing on limestone, while in other places, the species strongly resembles those on the siliceous or mineralized rock. The latter rock type is generally distinguished from the black slate and referred to as siliceous black slate. This type of rock tends to have more silica and sulfate and less alumina and lime than the normal black slate. Furthermore, the pH values of the weathered surface layer of siliceous black slate varied from 2.8 to 6.4. In particular, the weathered rock soil contained a limonite-like substance that is strongly acidic (pH 2.8 - 5.4). Given the moss species appearing on it and the results of chemical analyses of their substrates, the black slate in this study may be considered to be a typical siliceous black slate.

To determine the correlation between the chemical elements in the substrates, the pH to the iron concentration, the pH to the sulfate concentration, and the iron concentration to the sulfate concentration were plotted on graphs with the regression curve which was fittest for the plotted points on each graph (Okuno 1984). These results are shown in Figs. 1-1, 1-2 and 1-3. The coefficient of determination (R^2) between each of two components was calculated. High correlations ($R^2 = 0.794$, $R^2 = 0.666$ and $R^2 = 0.810$, $p<0.001$) were observed between the pH and the iron concentration, the pH and the sulfate concentration, and the iron and the sulfate concentrations, respectively. The relationship between the pH and the iron concentration and the pH and the sulfate concentration were inversely proportional (Figs. 1-1 and 1-2), while the relationship between the iron and the sulfate concentrations was directly proportional (Fig.1-3). Although the relationships between the copper or zinc concentration and the other elements were also calculated, no relationship was found. Thus, the pH, the iron concentration and the sulfate concentration are not only important factors for the limonite-like substance in the substrate of *S. ligulata*, but are also closely related to each other.

This study area covered about 20 square meters on the same black slate.

In the field, a limonite-like substance was found adhered to the substrate of *S. ligulata*, but not to those of four other non-copper mosses. In spite this narrow area, the differences between the substrate of *S. ligulata* and those of the other mosses was discernible, based on the water-soluble chemical elements mentioned above. The relationship among these three elements was determined. Namely, the concentration of water-soluble sulfate was directly proportional to that of water-soluble iron, and the pH value was in inversely proportional to the concentrations of water-soluble sulfate and iron. These suggest that the water-soluble sulfate was leached from the permeable siliceous black slate, which was rich in sulfate, by the percolating rainwater. The interflow or the subsurface stormflow including sulfate would dissolve iron while slowly percolated downward through the siliceous black slate. When the subsurface water containing sulfate and iron reached some parts of rock face, evaporation occurred, leaving sulfate and iron on the rock face. *S. ligulata* appeared to prefer such a strongly acidic place, rich in the water-soluble sulfate and iron. It was not clear that the formation of the limonite-like substance was affected by the bioaccumulation of *S. ligulata*. Further studies on this subject will be required.

Noguchi and Furuta (1956) performed culture tests on the regeneration from leaves and the germination of spores on *S. ligulata* and *S. cataractae*, in order to clarify the reproductive mechanism of these two species. They also carried out several experiments using $CuSO_4$ and $FeCl_3$ to clarify the possible role of the copper-ion, iron-ion and sulfur in the lives of these two species. They noted that the spores of *S. ligulata* did not germinate in the medium containing more than 0.1% $CuSO_4$, but that they germinated well in media containing $FeCl_3$. Regrettably, the correlation between the presence of sulfur and the growth of plants or the germination of spores was not determined in their study. Further culture tests of *S. ligulata* using $FeSO_4$ and/or $Fe_2(SO_4)_3$ instead of $FeCl_3$ seem to be necessary.

1.4. Conclusions

Chemical analyses of elements eluted from the substrates of a "copper moss" *S. ligulata* and four other non-copper mosses which grew on the same black slate rock wall were studied. The following results obtained. The pH value and the water-soluble sulfate and iron levels of the substrate of *S. ligulata* are significantly different from those of four other mosses. A very high correlation of the above chemical data was observed. The relationships between the pH and the iron concentration and the pH and the sulfate concentration were inversely proportional, while the relationship between the sulfate and the iron concentrations was directly proportional. The water-soluble copper and zinc in the substrate of *S. ligulata* were present in most samples, but at much lower level than the water-soluble iron. Conversely, the water-soluble copper and zinc in the substrates of four other mosses were rarely detected.

Table 1-1. Chemical analyses of samples eluted from the moss substrates.

Moss species	Sample No.	pH value	Fe mg L^{-1}	Cu mg L^{-1}	Zn mg L^{-1}	SO_4^{2-} mg L^{-1}
Scopelophila ligulata (S.l.)	1	3.37	3.20	0.13	0.13	10.62
	2	3.76	7.68	0.64	0.13	17.54
	3	4.05	2.56	0.51	0.13	12.03
	4	3.94	6.78	0.38	0.26	16.00
	5	4.12	2.56	0.26	0.26	10.88
	6	3.96	4.48	0.26	0.13	8.32
	7	4.46	3.84	0.13	<0.01	11.14
	8	4.03	4.35	0.13	0.13	13.44
	9	3.49	8.06	0.10	<0.01	20.86
	10	3.44	6.14	0.08	<0.01	10.88
	11	3.79	5.89	0.26	0.13	8.32
	12	3.80	7.94	0.26	0.13	20.22
	13	4.08	3.84	0.38	0.05	10.37
	Average	3.87	5.18	0.27	0.12	13.12
	SD	0.31	2.01	0.17	0.08	4.22
Pogonatum nipponicum (P.n.)	1	5.07	2.18	0.05	<0.01	11.14
	2	5.33	0.38	<0.01	<0.01	5.38
	3	5.11	1.92	<0.01	<0.01	8.32
	4	5.18	0.38	0.05	<0.01	5.76
	5	5.67	0.51	<0.01	0.26	6.02
	6	5.53	1.92	0.03	<0.01	7.55
	7	5.57	0.64	<0.01	<0.01	5.12
	8	5.36	1.54	<0.01	0.13	5.25
	9	5.66	0.51	<0.01	<0.01	4.86
Leucobryum juniperioideum (L.j.)	1	5.01	1.54	<0.01	<0.01	4.86
	2	5.66	0.38	<0.01	<0.01	5.12
	3	5.55	0.26	<0.01	<0.01	4.48
	4	5.52	0.26	<0.01	<0.01	4.48
Dicranella heteromalla (D.h.)	1	5.80	1.54	<0.01	<0.01	6.91
	2	5.42	0.38	<0.01	0.13	6.02
	3	5.63	1.28	<0.01	<0.01	6.27
Campylopus umbellatus (C.u.)	1	5.64	1.92	<0.01	<0.01	7.55
	2	5.47	0.26	<0.01	<0.01	4.35
	3	5.25	1.79	<0.01	<0.01	7.94
	4	5.16	0.26	<0.01	<0.01	4.61
	5	4.94	2.30	<0.01	<0.01	5.38
non-copper mosses	Average	5.41	1.05	0.01	0.03	6.07
	SD	0.25	0.76	0.01	0.06	1.67

Other heavy metals, such as Cd, Pb, etc., were not detected. For convenience, 0.01 was used in calculation for <0.01. SD, standard deviation.

Table 1-2. *F*-test and *t*-test results of the chemical analyses for the substrates of *Scopelophila ligulata* and four other non-copper mosses growing on the same cliff face.

	pH (H_2O)	Fe	Cu	Zn	SO_4^{2-}
F-test	1.16	7.06**	-	-	6.35**
t-test	16.23**	7.09**	-	-	5.76**

Number of samples: *Scopelophila ligulata*, 13; *Pogonatum nipponicum*, 9; *Leucobryum juniperioideum*, 4; *Dicranella heteromalla*, 3; *Campylopus umbellatus*, 5. *, ** Significant levels at 0.05 and 0.01, respectively.

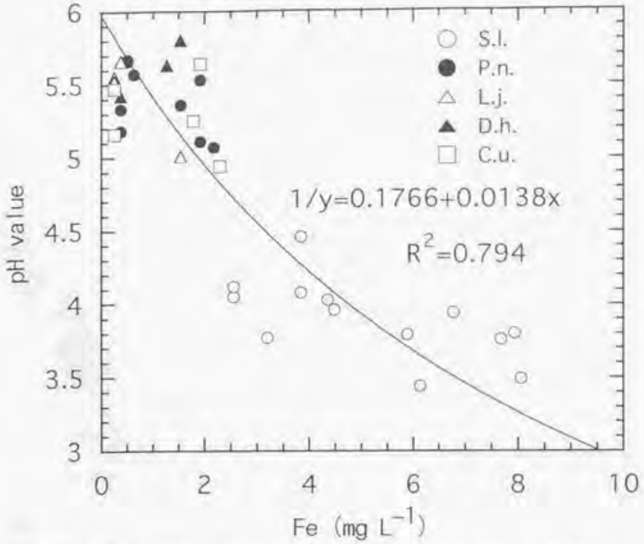

Fig.1-1. The relationship between water-soluble iron and pH for the moss substrates. For S.l.,etc. see Table 1-1.

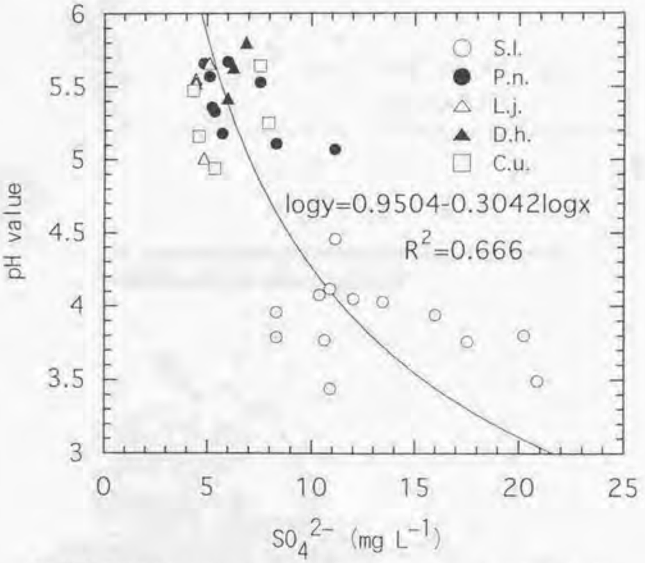

Fig.1-2. The relationship between water-soluble sulfate and pH for the moss substrates.

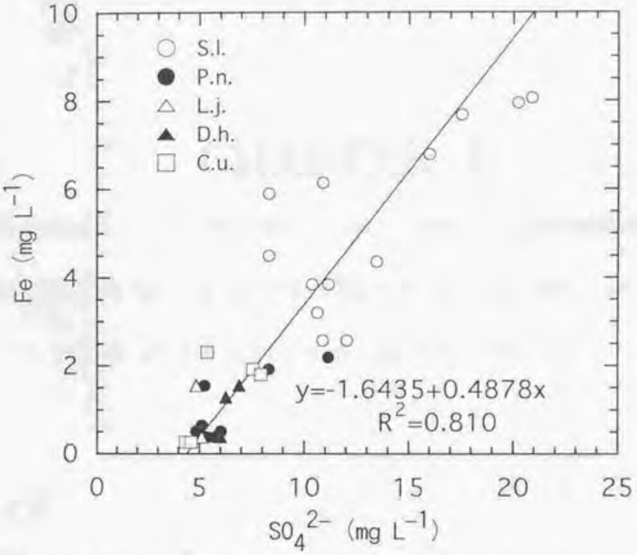

Fig.1-3. The relationship between water-soluble sulfate and water-soluble iron for the moss substrates.

CHAPTER II

Chemical Elements in the Substrate of *Scopelophila ligulata* (Spruce) Spruce and the associated mosses growing in the different habitat

CHAPTER Ⅱ

Chemical Elements in the Substrate of *Scopelophila ligulata* (Spruce) Spruce and the associated mosses growing in the different habitat

2.1. Introduction

"Copper mosses" have a peculiar ecology and have puzzled scientists including botanists, ecologists, applied biologists, geologists and others (Pant 1981). *Scopelophila ligulata* (Spruce) Spruce is a typical "copper moss" (Mårtensson and Berggren 1954; Kitagawa 1987; Noguchi and Ochi 1956) and its habitat has been reported to include substrates rich in heavy metals such as copper, lead, zinc and iron (Noguchi and Ochi 1956; Persson 1956), rocks covered with limonite (Noguchi and Ochi 1956; Noguchi and Furuta 1956; Nagano et al. 1969), and sulfurous springs (Persson 1948, 1956; Noguchi and Ochi 1956).

The studies including chemical analyses of bryophyte substrates were reviewed. These studies were summarized in Table 2-1. Chemical analyses of the substrates of "copper mosses" and similar bryophytes have been vigorously carried out by Persson (1956), Shacklette (1965, 1967) and others. However, the plant-substrate relationships have not yet been totally resolved (Warncke 1968; Shaw 1987; Brown 1982; Iwatsuki and Mizutani 1972; Takagi 1986). Especially the substrate analysis using the water-soluble method has not been adopted although the bryophytes would absorb the pollutants such as heavy metals with the water-soluble form into their tissues through their whole surface of the body (Taoda 1974; Gullvåg and Skaar 1974; Berg and Steinnes 1997).

Nagano et al. (1999) studied that the water-soluble chemical elements eluted from the substrates of *S. ligulata*, a "copper moss" and four other non-copper mosses, *Campylopus umbellatus* (Arm.) Par., *Dicranella heteromalla* (Hedw.) Schimp., *Leucobryum juniperioideum* (Brid.) C. Muell., and *Pogonatum nipponicum* Nog. et Osada, all of which grew on the same black slate rock wall. The results indicated that the pH value and the concentrations of water-soluble iron and sulfate in the substrate of *S. ligulata* were significantly different from those of the four non-copper mosses, and a high correlation was found among these three elements (Nagano et al. 1999).

This study was performed to determine whether or not the aforementioned observations by Nagano et al. (1999) could be applied to other habitat of *S. ligulata*.

2.2. Materials and Methods

2.2.1. Samples

Twenty samples were collected in Nakatsugawa, Chichibu in Saitama Prefecture, where *S. ligulata* is found in abundance. Hornfels outcropped at many locations in this region, about 670-720 m above sea level, facing a deep, V-shaped gorge. Eleven of them at two sites (A and B) were chosen for this study within this area.

Nagano et al. (1969) indicated that *S. ligulata* grew well on cliff faces, but not on cliff shelves of the same rock wall. In this study, particular attention was paid to such ecological characteristics of *S. ligulata* on cliff faces and those of other mosses on cliff shelves located very near the habitat of *S. ligulata*, but where *S. ligulata* did not grow. Twenty samples were collected; eleven of them were *S. ligulata* from the cliff faces; and nine were non-copper mosses from the cliff shelves.

Hornfels formed bedrock at both A and B sites. At site A, *S. ligulata*, along with small community of *Jungermannia vulcanicola* Steph., grew in a crowded group on the vertical cliff face. *Woodsia manchuriensis* Hook., *Nardia assamica* (Mitt.) Amak., *Dicranella heteromalla*, and *Pseudotaxiphyllum pohliaecarpum* (Sull. et Lesq.) Iwats. grew on a cliff shelf about 1.5m from the bottom of the rock and in an area about 0.65m in width, which contained some gravel. At site B, *S. ligulata* partially accompanied by *J. vulcanicola* and *Scapania stephanii* K. Muell. grew in crowded groups on the vertical cliff face, as well. *Dennstaedtia hirsuta* Mett., plantlets of *Gleichenia glauca* Hook., *N. assamica*, *D. heteromalla*, *Pogonatum nipponicum*, and *Pohlia proligera* (Kindb.) Lindb. ex Arn. grew on a cliff shelf about 1.8m from the bottom of the rock wall.

2.2.2. Sampling and Treatment

Substrate samples were collected in areas where comparatively dense colonies of each of the moss species were growing. A bamboo spatula was used to collect them along with the moss. These samples were placed in polyethylene bags and naturally dried indoors in the laboratory. Substrate samples were carefully separated from the mosses, closely observing whether plant bodies, such as leaves, stems, and rhizoids, were present. If any were found, they were removed from the sample using the tweezers with non-metallic tips. Then the samples were sieved to obtain under 2 mm diameter grains which were further ground up by using a mortar and sifted out with a 32 mesh screen.

The leaching method was the same as Nagano et al. (1999). Twenty-five milliliters of distilled water were added to ten grams of each sample. The mixtures were then stirred and eluted for one hour. They were allowed to stand for twenty-four hours to obtain the optimum pH value, following the procedure of Nagano and Wada (1974). The pH and the metal contents (iron, copper, zinc, etc.) of the supernatant liquids were measured using a pH meter and an atomic absorption spectrophotometer, respectively. The sulfate concentration was determined using the barium sulfate turbidimetric method (Japanese Standards Association 1993). The ambient temperature at the time of measurement was 23-25℃.

2.3. Results and Discussion

Analytical results of the moss substrates are presented in Table 2-2. The pH values of the substrate of *S. ligulata*, which formed colonies on vertical cliff faces of the hornfels, were 2.01 - 2.76 (average 2.50). These values tended to be much lower in comparison with the values measured of Persson (1948,1956), Nagano et al. (1969, 1999) and Noguchi (1956). For the heavy metals, similar results were obtained for copper and zinc concentrations to those determined by Nagano et al. (1999) in their investigation of the soluble chemical elements in the substrates of *S. ligulata* on black slate. The iron and sulfate concentrations tended, however, to be much higher in comparison with the measured values than in the study by Nagano et al. (1999).

F-tests and *t*-tests were undertaken to compare the substrates of *S. ligulata* and those of the other non-copper mosses. The *F*-test was carried out before the *t*-test according to the established method (Yonezawa et al. 1996). The results are shown in Table 2-3. The *t*-tests showed a significant difference between the two groups, except for the copper and zinc concentrations. The substrate of *S. ligulata* had a lower pH value (by a factor of about 1.7 on average) and higher concentrations of iron and sulfate (both by about two times on average) than those of the non-copper mosses on the cliff shelves. To explain the difference of the iron and sulfate levels between the cliff face and the cliff shelf on the same rock wall, Nagano et al. (1999) suggested that the interflow or subsurface stormflow dissolved iron, along with high concentrations of sulfate, slowly percolated downward through the rock, and deposited this iron and sulfate on the cliff face when evaporation occurred (Fig.2-1). Furthermore, it was remarkable that there was no significant difference in copper and zinc concentrations between the substrates of *S. ligulata* and those of the other non-copper mosses.

The maximum coefficient of determination (R^2) between each combination of two constituents was calculated. Highly negative correlations of R^2=0.837 (p<0.001) and R^2=0.901 (p<0.001) were found between the pH value and the iron concentration, and the pH value and the sulfate concentration, respectively. Also, a highly positive correlation of R^2=0.841 (p<0.001) was found between the iron and the sulfate concentrations. The pH versus iron concentration, the pH versus sulfate concentration, and the iron concentration versus sulfate concentration were plotted using a best-fit regression (Okuno 1984). These are shown in Figs. 2-2, 2-3 and 2-4. These results confirmed the relationships between three elements that were presented by Nagano et al. (1999).

No relationship was found between the copper and zinc concentrations and other elements, while a slightly positive correlation of R^2=0.260 (p<0.05) was found between the copper and the zinc concentrations themselves.

The pH value is worthy of additional discussion. The pH values from the cliff shelves, on which *S. ligulata* did not grow at all, were 3.70 - 4.90 (average 4.19), and those values with a few exceptions (pH values 4.82, 4.83 and 4.90 in Table 2) were in the range of the suitable pH values of the substrates of *S. ligulata* that were reported in several studies cited previously in this report. This would indicate that a factor other than pH controlled whether or not *S. ligulata* would grow in a particular location. In light of this, the pH value appears to be a secondary factor for the habitat of *S. ligulata*.

Several bryophytes have been described as "copper mosses". Although some studies have focused on the relationship between these "copper mosses" and their substrates, a definitive explanation has not yet been obtained. Although *S. ligulata*, along with *S. cataractae* (Mitt.) Broth. has been famous as a "copper moss", *S. ligulata* seems to prefer iron to copper that differs from *S. cataractae* which seems to be strongly related with copper. The water-soluble iron, in conjunction with the water-soluble sulfate in the substrate of *S. ligulata*, appear to be a major factor in fixing the pH value of its substrate. *S. ligulata* may grow well on such restrictive substrate where relatively higher water-soluble iron and sulfate ions exist, hence lower pH value.

2.4. Conclusion

In this report, we discussed the water-soluble chemical element concentrations in the substrates of *S. ligulata*. There was a significant difference in the pH value and the water-soluble iron and sulfate concentrations between the substrates of *S. ligulata* on cliff faces and those of other non-copper mosses on the cliff shelves located just above the *S.*

ligulata. Very high correlations ($p<0.001$) were found among the pH value, the concentration of water-soluble iron and that of water-soluble sulfate. Water-soluble copper and zinc were detected in all substrate samples, but no relationship was found except between the copper and the zinc concentrations themselves ($R^2=0.260$, $p<0.05$).

Table 2-1. Studies accompanied with bryophyte substrate analyses and their methods.

Author (s)	Pre-treatment		Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
	Method	Reagent etc.				
Mårtensson and Berggren (1954)	Digestion	A mixture of nitric, perchloric and sulfuric acid	Unknown	*Mielichhoferia elongata**	3	Cu
				*Dryptodon atratus**	2	
Persson (1956)	Ashed		SA	*Mielichhoferia elongata**	9	pH, Cu
				*Dryptodon atratus**	6	
				Scopelophila ligulata*	11	
				Mielichhoferia species	14	
Pigott (1958)	Water-extraction for sulfate Unknown for total copper		Unknown	*Mielichhoferia elongata**	1	pH, SO_4^{2-} Cu
Shacklette (1965b)	Ashed		CM & SSA	*Anomodon rostratus*, etc.; total 29 species (non "copper mosses")	38	33 elements including Cu, Pb, Zn, Fe, Al
Dykeman and De Sousa (1966)	Ashed	Concentrated nitric acid and potassium perchlorate	EM	*Pohlia nutans*	2	Cu
Shacklette (1967)	Ashed		CM & SSA	*Mielichhoferia macrocarpa**	2	30 elements including Cu, Pb, Zn, Fe, S
				Platydictya jungermannioides	2	
				*Mielichhoferia mielichhoferi**	1	
				*Mielichhoferia elongata**	2	
Warncke (1968)	Leaching	N-ammonium acetate solution at pH 7	Unknown	*Marchantia alpestris*	11	pH, Cu, Zn, Fe, Mn, Mg, Ca, K, Na
Hartman (1969)	Unknown		AAS	*Mielichhoferia mielichhoferi**	1	pH, Cu, SO_4^{2-}
Coker (1971)	Digestion	A mixture of hot nitric and hydrochloric acid	AAS	*Mielichhoferia elongata**	3	Cu, Pb, Fe, Ca, Cr, Co, La, Y
				Saelania glaucescens	3	
	Water-extraction for sulfate		UVS			SO_4^{2-}
Brooks and Yates (1973)	Digestion	A mixture concentrated nitric and hydrofluoric acid	AAS	*Fissidens rigidulus*	6	Cu
				Philonotis tenuis	1	
				Mniobryum wahlenbergii	1	
				Composite of various species	1	
Wilkins (1977)	Leaching	N-ammonium acetate solution at pH 7	AAS	*Mielichhoferia elongata**	1	Cu, Fe, Mg, Ca, Al, K, Na
				Coscinodon cribrosus	1	
				Grimmia atrata	1	
				Barbula sp.	1	
	Non-destructive		NAA	*Mielichhoferia elongata**	2	Cu
				Bryum argenteum	1	

Table 2-1. Continued.

Author (s)	Pre-treatment		Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
	Method	Reagent etc.				
Brown and House (1978)	Extraction	Either deionized water, 2% EDTA, or 1,000 microgram / gram lead nitrate	AAS	*Jungermannia gracillima* Sm. (= *Solenostoma crenulatum*)	2	pH, Cu
				Marsupella emarginata	1	
				Pohlia nutans	1	
Burton and Peterson (1979)	Digestion	2N hydrochloric acid	AAS	*Philonotis fontana*	5	Cu, Pb, Zn, Fe
				Scapania undulata	4	
				Fontinalis squamosum	2	
				Eurhynchium riparoides	2	
				Hygrohypnum ochraceum	1	
				Rhacomitrium aciculare	1	
				Jungermannia gracillima Sm. (=*Solenostoma crenulata*)	1 (site(s))	
Corley and Perry (1985)	Extraction	2M nitric acid and 10 vol. hydrogen peroxide solution	AAS	*Scopelophila cataractae**	1	pH, Cu, Zn
Empain (1985)	Digestion**	Nitric and perchloric acid**	Unknown	*Crustry cyanophyceae*	1	Cu, Pb, Zn, Cd, Co, Cr, Fe, Mn, Ni
				Brachymenium acuminatum	2	
				Brachymenium philonotula	3	
				Bryum sp.	2	
				Campylopus bequaertii	4	
Sotiaux et al. (1987)	Digestion	A mixture of nitric and hydrochloric acid	AAS	*Scopelophila cataractae**	7	pH, Cu, Pb, Zn, Cd, Fe, Mn, Ca, S
Shaw (1987)	Extraction	1% hydrochloric acid	AAS	*Scopelophila cataractae**	6	pH, Cu, Pb, Zn, Fe
Rumsey and Newton (1989)	Digestion	Concentrated nitric acid	AAS	*Scopelophila cataractae**	5	pH, Cu, Pb, Zn
Nagano et al. (1999)	Water-extraction	A slurry ratio of soil to water, 1 : 2.5 by weight	AAS	*Scopelophila ligulata**	13	pH, Cu, Zn, Fe, SO_4^{2-}
				Pogonatum nipponicum	9	
				Leucobryum juniperioideum	4	
				Dicranella heteromalla	3	
				Campylopus umbellatus	5	

SA, spectrum analysis using a microphotometer; CM & SSA, colorimetric method and semiquantitative spectrographic analysis; EM, electrolytic method; AAS, atomic absorption spectrophotometry; NAA, Neutron Activate Analysis; UVS, U.V.spectrophotometry; *, "copper mosses"; **, Empain (1985) described the method of digestion only for the bryophyte body.

Table 2-2. Analytical results of chemical elements eluted from substrates.

Site	Cliff Faces					Cliff Shelves				
	pH(H_2O) value	Fe mg L^{-1}	Cu mg L^{-1}	Zn mg L^{-1}	SO_4^{2-} mg L^{-1}	pH(H_2O) value	Fe mg L^{-1}	Cu mg L^{-1}	Zn mg L^{-1}	SO_4^{2-} mg L^{-1}
A	2.73	10.5	1.02	0.38	37.8	3.72	6.40	0.10	0.19	23.4
	2.76	9.22	0.20	0.13	37.4	4.82	5.12	0.67	0.38	16.4
	2.54	13.2	0.36	0.26	48.6	3.70	5.12	0.23	0.26	19.1
	2.69	8.22	0.69	0.06	38.4	4.07	5.12	0.08	0.19	16.0
	2.68	11.5	0.77	0.38	42.2	3.75	7.68	0.28	0.26	19.1
	2.60	11.9	0.90	0.26	44.8	-	-	-	-	-
B	2.56	9.60	0.13	0.26	45.8	4.13	2.54	0.13	0.19	14.5
	2.25	12.8	0.83	0.70	47.4	4.83	5.12	0.55	0.38	14.7
	2.01	13.6	0.29	0.26	37.3	4.90	6.40	0.73	0.13	15.7
	2.36	11.3	0.13	0.04	45.1	3.76	6.40	0.29	0.19	18.8
	2.27	11.8	0.23	0.19	46.5	-	-	-	-	-
Average	2.50	11.2	0.50	0.27	42.8	4.19	5.54	0.34	0.24	17.5
SD	0.24	1.71	0.34	0.18	4.37	0.52	1.44	0.25	0.09	2.85

Other heavy metals, such as Cd, Pb etc., were not detected. *Scopelophila ligulata* was found on the hornfels cliff faces. Mosses other than *S. ligulata* found on the cliff shelves nearby. SD, Standard deviation.

Table 2-3. *F*-test and *t*-test results of the chemical analyses of water-soluble element for the substrates of *Scopelophila ligulata* growing on the cliff faces and other non-copper mosses growing on the cliff shelves.

	pH (H_2O)	Fe	Cu	Zn	SO_4^{2-}
F-test	4.68	1.28	1.87	4.28	2.35
t-test	8.99**	8.38**	1.25	0.39	15.6**

Number of samples: *Scopelophila ligulata*, 11; other non-copper mosses, 9. *, ** Significant levels at 0.05 and 0.01, respectively.

Table 2-4. Correlation matrix of the water-soluble chemical elements in the substrates of *Scopelophila ligulata* and others.

Element	pH (H_2O)		Fe		Cu		Zn	
Fe	0.837***^{i}	Hyp						
Cu	0.020^{i}	Se-L	0.121^{d}	Hyp				
Zn	0.039^{d}	Log	0.064^{d}	Qua	0.260*^{d}	Str		
SO_4^{2-}	0.901***^{i}	Hyp	0.841***^{d}	Se-L	0.044^{d}	Hyp	0.040^{i}	Hyp

Values are maximum coefficient of determination (R^2) for the best-fit regression curve. *, **, *** Significant levels at 0.05, 0.01 and 0.001, respectively. ^{d}, directly proportional; ^{i}, inversely proportional. Exp, exponential curve; Hyp, hyperbola; In-L, inverse logarithmic curve; Log, logarithmic curve; Qua, quadratic curve; Root, square root curve; Se-L, semilogarithmic curve; Str, straight line.

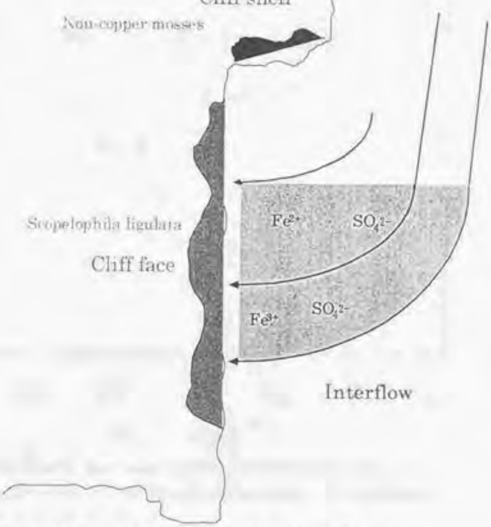

Fig. 2-1. Sampled substrates and interflow.

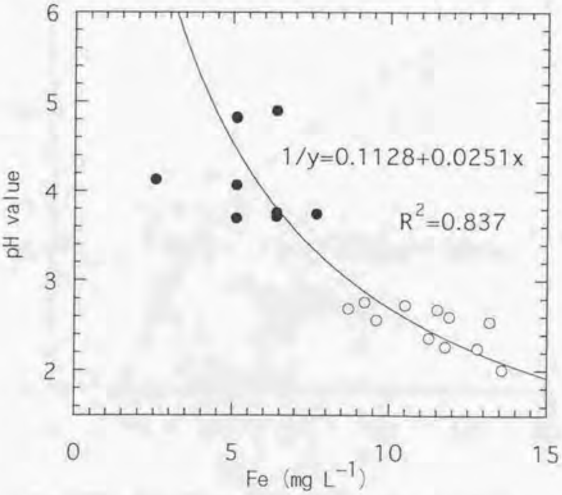

Fig.2-2. The relationship between water-soluble iron and pH for substrates from cliff faces and cliff shelves. Symbols: ○, Cliff faces; ●, Cliff shelves.

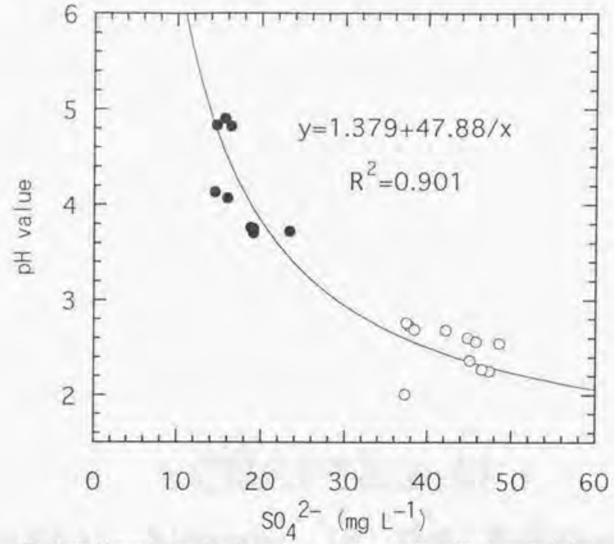

Fig.2-3. The relationship between water-soluble sulfate and pH for substrates from cliff faces and cliff shelves. The symbols are the same as Fig.2-2.

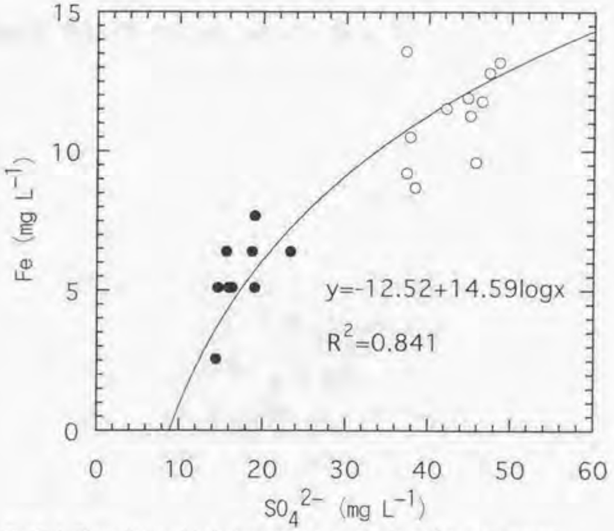

Fig.2-4. The relationship between water-soluble sulfate and water-soluble iron for substrates from cliff faces and cliff shelves. The symbols are the same as Fig.2-2.

CHAPTER III

Chemical Elements in the Substrates of *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch.

CHAPTER III

Chemical Elements in the Substrates of *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch.

3.1. Introduction

The habitat of *S. cataractae* (Mitt.) Broth. was reported in detail by Satake et al. (1988). It has been well established that *S. cataractae* occurs not only in natural surroundings but also grows in and around man-made edifices such as copper roofs, and bronze statues and garden lanterns (Satake et al. 1988). Meanwhile, *Mielichhoferia mielichhoferi* (Hook.) Wijk et Marg., taxonomically closed to *M. japonica* Besch, has been found on mineralized rock-faces in Europe, the Caucasus, and North America.

As yet, studies on the heavy metals in the substrates of "copper mosses" have been done limited and a definitive explanation for the occurrence of "copper mosses" has not yet been obtained (Brown 1982; Shaw 1987; Nagano et al. 1999; Aikawa et al. 1999). The water-soluble chemical elements eluted from the substrates of *S. ligulata* (Spruce) Spruce and other non-copper mosses were studied by Nagano et al. (1999) and Aikawa et al. (1999). The results indicated that the pH value and the concentrations of water-soluble iron and sulfate in the substrate of S. ligulata were significantly different from those of the non-copper mosses, and a high correlation was found among these three elements (Nagano et al. 1999; Aikawa et al. 1999).

This study was performed to determine whether or not the afore-mentioned observations could be applied to species of "copper mosses" other than *S. ligulata*, namely *S. cataractae* and *M. japonica*.

3.2. Materials and Methods

3.2.1. Samples

Seventeen samples of *S. cataractae* were collected from a mineralized rock-face, at about 700 meters above sea level, located in Tochigi Prefecture. In Japan, *S. cataractae* can be found west of the Kanto Region on the western part of Honshu Island, namely at the Nikko Toshogu Shrine in Tochigi Prefecture; the Chichibu District in Saitama Prefecture; the Yasukuni Shrine, the Zojyoji Temple and the Honmonji Temple in Tokyo; in Kamakura in Kanagawa Prefecture; in Saijyo in Ehime Prefecture on Shikoku Island; and in Hinokage in Miyazaki Prefecture on Kyushu Island.

Fourteen samples of *M. japonica* were collected from the mineralized rock-face located in Tochigi Prefecture where the *S. cataractae* samples were obtained. As mentioned above, *M. mielichhoferi* is found in Europe, the Caucasus, and North America. *M. japonica* is endemic to Japan and can be found in the north to central regions of Honshu and on Hokkaido. Thus, this species has a different geographical distribution from that of *S. cataractae*. Tochigi Prefecture is located on the northern boundary of the habitat of *S. cataractae*, while it is located on the almost southern boundary of that of *M. japonica*. Shacklette (1967) pointed out that *M. mielichhoferi* was one of the bryophytes which could indicate the type of metals contained in the substrate. This is based on the fact that *M. mielichhoferi* grows on clay slate, rich in iron pyrites, in the southern Appalachian Mountains and on mineralized rock-faces in several other places in North America.

3.2.2. Sampling and Treatment

Substrate samples were collected in areas where comparatively dense colonies of each of the moss species were found. A bamboo spatula was used to collect them along with the moss. These samples were placed in polyethylene bags and naturally dried indoors in the laboratory. Substrate samples were carefully separated from the mosses, closely observing whether moss bodies, such as leaves, stems, and rhizoids, were present. If any were found, they were removed from the sample using the tweezers with non-metallic tips. Then the samples were sieved to obtain under 2 mm diameter grains which were further ground up by using a mortar and sifted out with a 32 mesh screen.

The leaching method was the same as Nagano et al. (1999). Twenty-five milliliters of distilled water were added to ten grams of each sample. The mixtures were then stirred and eluted for one hour. They were allowed to stand for twenty-four hours to obtain the optimum pH value, following the procedure of Nagano and Wada (1974). The pH and the metal contents (iron, copper, zinc, etc.) of the supernatant liquids were measured using a pH meter and an atomic absorption spectrophotometer, respectively. The sulfate concentration was determined using the barium sulfate turbidimetric method (Japanese Standards Association 1993). The ambient temperature at the time of measurement was 23-25°C.

3.3. Results and Discussion

Analytical results of the substrates of *S. cataractae* and *M. japonica* are shown in Table 3-1. These two moss species were compared using the *F*-test and the *t*-test. These statistical results are shown in Table 3-2. The pH values of the substrate of *S. cataractae* were 3.59 - 4.88 (average 4.03), and those of *M. japonica* were 3.22 - 3.97 (average 3.62). The *t*-test showed a significant difference ($p<0.01$) between these two moss species. The pH value of *M. japonica* was slightly lower than that of *S. cataractae*.

Meanwhile, the water-soluble iron in the substrate of *S. cataractae* was <0.01 - 0.30 mg L^{-1} (average 0.07 mg L^{-1}) and that of *M. japonica* was <0.01 - 1.70 mg L^{-1} (average 0.40 mg L^{-1}). ("<0.01" was calculated as 0.01 mg L^{-1}, for convenience). The *t*-test showed a significant difference ($p<0.05$) between the two moss species. The water-soluble iron concentration in the substrate of *M. japonica* was slightly higher than that of *S. cataractae*, while the iron concentrations of both *S. cataractae* and *M. japonica* were much lower than that of the *S. ligulata* mentioned above. The water-soluble copper in the substrate of *S. cataractae* was 0.62 - 9.85 mg L^{-1} (average 3.76 mg L^{-1}) and that of *M. japonica* was 0.41 - 21.5 mg L^{-1} (average 8.09 mg L^{-1}). The *t*-test again showed a significant difference ($p<0.05$) between the two moss species. The water-soluble copper concentration in the substrate of *M. japonica* was higher than that of *S. cataractae*. The water-soluble zinc in the substrate of *S. cataractae* was 0.23 - 7.18 mg L^{-1} (average 1.68 mg L^{-1}), and that of *M. japonica* was 0.25 - 15.0 mg L^{-1} (average 4.89 mg L^{-1}). The *t*-test showed a significant difference ($p<0.05$) between the two moss species. The water-soluble zinc concentration in the substrate of *M. japonica* was higher than that of *S. cataractae*. The water-soluble sulfate in the substrate of *S. cataractae* was 7.60 - 60.5 mg L^{-1} (average 27.0 mg L^{-1}), and that of *M. japonica* was 9.20 - 478 mg L^{-1} (average 175 mg L^{-1}). The *t*-test showed a significant difference ($p<0.05$) between those two moss species. The water-soluble sulfate concentration in the substrate of *M. japonica* was higher than that of *S. cataractae*.

The maximum coefficient of determination (R^2) between two component combinations of the substrate constituents was calculated using computer-software and method developed by IBC CO., LTD. in Miyazaki, Japan. The results for *S. cataractae* are shown in Table 3-3 (Case 1). Correlation coefficients of R^2=0.262 (p<0.05) and R^2=0.517 (p<0.001) were found between the pH value and the sulfate concentration and the zinc concentration and the sulfate concentration, respectively. Those of *M. japonica* are also shown in Table 3-3 (Case 2). High correlations were observed between the elements. The iron and the sulfate (R^2=0.408, p<0.05), the copper and the zinc (R^2=0.440, p<0.01), and the zinc and the sulfate (R^2=0.733, p<0.001) were found to be directly proportional. The pH and the iron (R^2=0.507, p<0.01), the pH and the zinc (R^2=0.403, p<0.05), the pH and the sulfate (R^2=0.396, p<0.05), and the copper and the sulfate (R^2=0.471, p<0.01) were found to be inversely proportional. Accordingly, *S. cataractae* and *M. japonica* grew in the same vicinity, but not under the same chemical conditions of the water-soluble elements in the substrate. Thus, correlation analyses on thirty-one samples, *S. cataractae* plus *M. japonica* were undertaken. Those results are shown as Case 3 in Table 3-3. Significant relationships were found between all two pair combinations of the constituents, except between the iron and the copper concentrations. Indeed, very high correlations (p<0.001) were observed between each of two constituents combinations as follows. The iron and the zinc concentrations (R^2=0.332, Fig.3-1), the iron and the sulfate concentrations (R^2=0.465, Fig.3-2), the copper and the sulfate concentrations (R^2=0.343, Fig. 3-3), and the zinc and the sulfate concentrations (R^2=0.707, Fig.3-4) were directly proportional. The pH value and the iron concentration (R^2=0.321, Fig.3-5) was inversely proportional. Relationship between the pH value and the copper concentration, the pH value and the zinc concentration, and the pH value and the sulfate concentration was R^2=0.187 (p<0.05), R^2=0.140

($p<0.05$), and $R^2=0.268$ ($p<0.01$, Fig.3-6) with inversely proportional, respectively.

3.4. Conclusion

In this report, we discussed the water-soluble chemical element concentrations in the substrates of *S. cataractae*, and *M. japonica*. There was a significant difference in the pH value ($p<0.01$), the water-soluble iron concentration ($p<0.05$), the water-soluble copper concentration ($p<0.05$), the water-soluble zinc concentration ($p<0.05$), and the water-soluble sulfate concentration ($p<0.05$) between the substrate of *S. cataractae* and that of *M. japonica*. High correlations were obtained among these constituents in the substrates of *S. cataractae* and *M. japonica* as follows. The iron and the zinc concentrations ($p<0.001$), the iron and the sulfate concentrations ($p<0.001$), the copper and the zinc concentrations ($p<0.01$), the copper and the sulfate concentrations ($p<0.001$), and the zinc and the sulfate concentrations ($p<0.001$) were directly proportional. The pH value and iron concentration ($p<0.001$), the pH value and copper concentration ($p<0.05$), the pH value and zinc concentration ($p<0.05$), and the pH value and sulfate concentration ($p<0.01$) were inversely proportional.

Table 3-1. Analytical results on chemical elements eluted from the moss substrates.

Sample	pH value	Fe mg L^{-1}	Cu mg L^{-1}	Zn mg L^{-1}	SO_4^{2-} mg L^{-1}
Sc 1	3.59	0.10	1.03	0.58	7.60
2	3.78	0.01	3.08	0.43	8.20
3	3.80	0.12	8.05	0.65	15.4
4	3.82	0.10	5.68	5.36	50.4
5	3.84	0.30	6.10	0.23	23.0
6	3.92	<0.01	2.90	0.53	24.8
7	3.92	<0.01	9.85	2.45	20.4
8	3.92	<0.01	3.58	0.23	21.6
9	3.92	<0.01	1.27	0.47	12.0
10	3.94	0.20	5.76	7.18	48.6
11	3.99	0.10	1.93	1.25	41.5
12	4.01	<0.01	1.17	0.58	15.4
13	4.07	<0.01	4.00	0.50	17.7
14	4.08	<0.01	1.52	0.58	17.2
15	4.21	0.09	4.50	1.58	60.5
16	4.88	<0.01	2.82	3.78	51.0
17	4.82	0.10	0.62	2.10	23.2
Average	4.03±0.34	0.07±0.08	3.76±2.62	1.68±2.00	27.0±16.7
Mj 1	3.22	1.70	6.66	6.00	424
2	3.47	0.36	16.5	5.52	210
3	3.47	0.09	7.16	3.78	23.0
4	3.48	1.16	7.30	15.0	478
5	3.49	0.38	4.06	3.14	206
6	3.58	0.23	14.9	10.2	454
7	3.61	0.23	18.2	10.3	111
8	3.66	0.10	1.75	0.98	9.20
9	3.70	0.01	3.76	0.25	25.8
10	3.75	0.94	1.52	0.65	23.8
11	3.75	0.24	6.90	10.1	414
12	3.79	0.10	21.5	1.65	46.8
13	3.85	<0.01	0.41	0.58	13.4
14	3.97	<0.01	2.60	0.30	9.60
Average	3.63±0.19	0.40±0.51	8.09±6.86	4.89±4.79	175±188

Other heavy metals, such as Cd, Pb etc., were not detected in almost samples. For convenience, 0.01 was used in calculation for <0.01. Sc: *Scopelophila cataractae*; Mj: *Mielichhoferia japonica*; Average: average±standard deviation.

Table 3-2. *F*-test and *t*-test results of the chemical analyses of water-soluble element for the substrates of *Scopelophila cataractae* and *Mielichhoferia japonica*.

	pH (H_2O)	Fe	Cu	Zn	SO_4^{2-}
F-test	3.05	34.8**	6.84**	5.74**	128**
t-test	4.14**	2.39*	2.23*	2.35*	2.93*

Number of samples: *Scopelophila cataractae*, 18; *Mielichhoferia japonica*, 14. *, ** Significant levels at 0.05 and 0.01, respectively.

Table 3-3. Correlation matrix of the water-soluble chemical elements in the substrate of *Scopelophila cataractae* and *Mielichhoferia japonica*.

Case	Element	pH (H_2O)	Fe	Cu	Zn
1	Fe	0.032^{i} Hyp			
	Cu	0.148^{i} Hyp	0.100^{i} Qua		
	Zn	0.158^{d} Se-L	0.114^{i} Qua	0.099^{d} Hyp	
	SO_4^{2-}	0.262*^{d} Qua	0.122^{d} Qua	0.125^{d} In-L	0.517***^{i} Qua
2	Fe	0.507**^{i} Qua			
	Cu	0.181^{i} Hyp	0.041^{i} Hyp		
	Zn	0.403*^{i} Se-L	0.250^{i} Hyp	0.440**^{d} Log	
	SO_4^{2-}	0.396*^{i} Se-L	0.408*^{d} Qua	0.471**^{i} In-L	0.733***^{d} Exp
3	Fe	0.321***^{i} Hyp			
	Cu	0.187*^{i} Log	0.082^{d} Qua		
	Zn	0.140*^{i} Hyp	0.332***^{d} Qua	0.299**^{d} Qua	
	SO_4^{2-}	0.268**^{i} Hyp	0.465***^{d} Qua	0.343***^{i} Log	0.707***^{d} Qua

Case 1, *Scopelophila cataractae*, 17 samples; Case 2, *Mielichhoferia japonica*, 14 samples; Case 3, *S. cataractae* plus *M. japonica*, 31 samples. Values are maximum coefficient of determination (R^2) for the best-fit regression curve. *, **, *** Significant levels at 0.05, 0.01 and 0.001, respectively. ^{d}, directly proportional; ^{i}, inversely proportional. Exp, exponential curve; Hyp, hyperbola; In-L, inverse logarithmic curve; Log, logarithmic curve; Qua, quadratic curve; Root, square root curve; Se-L, semilogarithmic curve; Str, straight line.

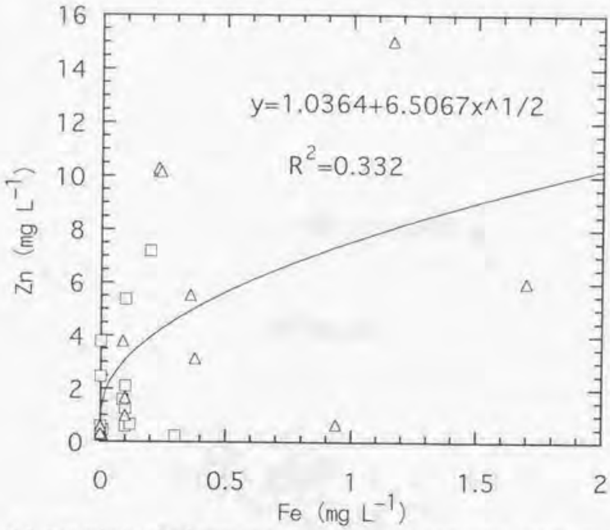

Fig.3-1. The relationship between water-soluble iron and water-soluble zinc for the moss substrates. Symbols: □,Scopelophila cataractae; △, Mielichhoferia japonica.

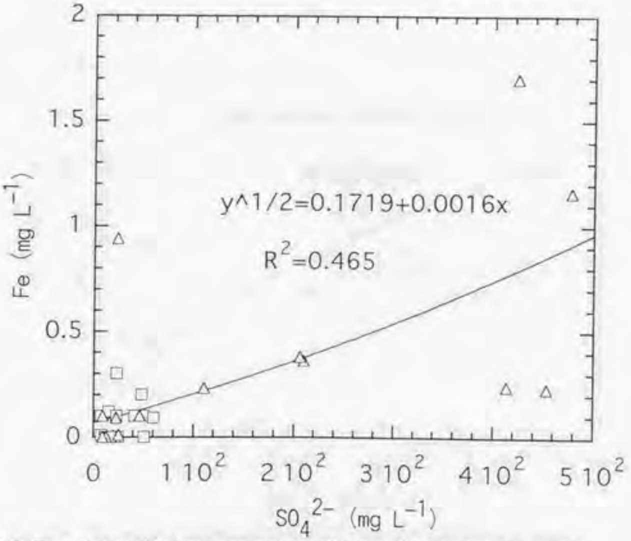

Fig.3-2. The relationship between water-soluble sulfate and water-soluble iron for the moss substrates. The symbols are the same as Fig.3-1.

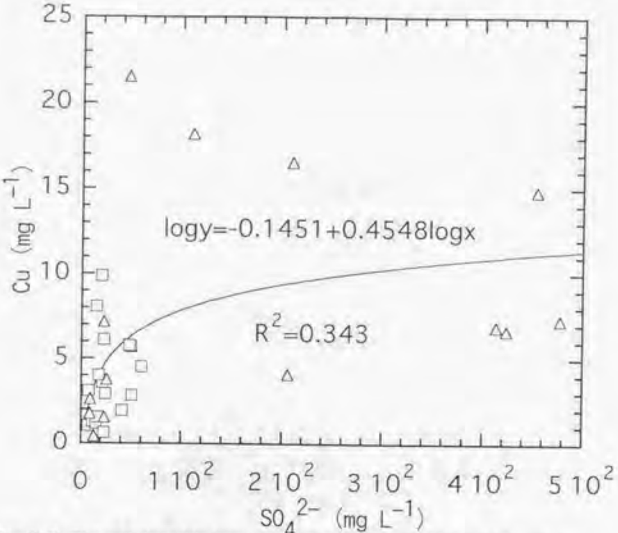

Fig.3-3. The relationship between water-soluble sulfate and water-soluble copper for the moss substrates. The symbols are the same as Fig.3-1.

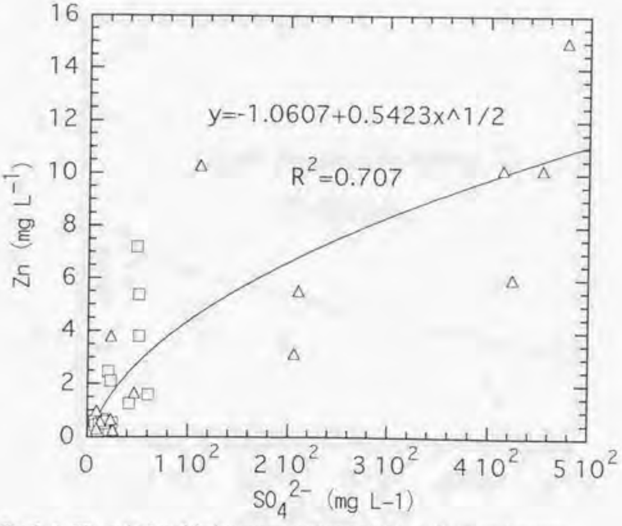

Fig.3-4. The relationship between water-soluble sulfate and water-soluble zinc for the moss substrates. The symbols are the same as Fig.3-1.

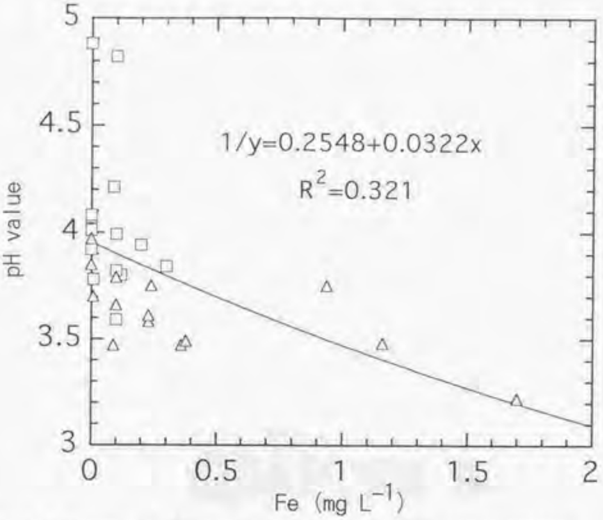

Fig.3-5. The relationship between water-soluble iron and pH for the moss substrates. The symbols are the same as Fig.3-1.

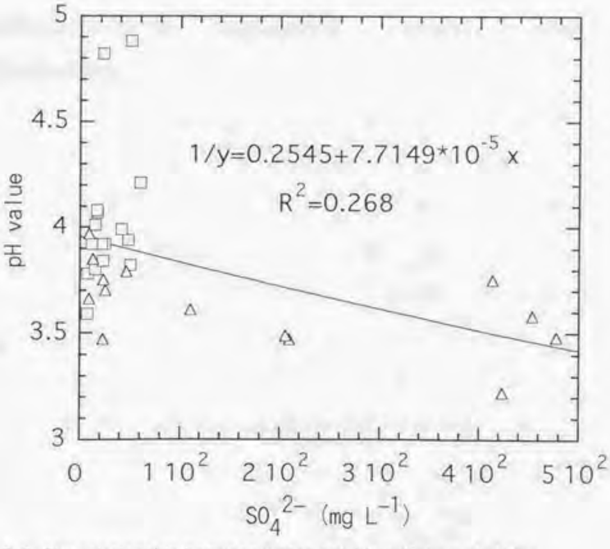

Fig.3-6. The relationship between water-soluble sulfate and pH for the moss substrates. The symbols are the same as Fig.1.

CHAPTER Ⅳ

Contents of Heavy Metal Elements in Copper Mosses: *Scopelophila ligulata* (Spruce) Spruce, *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. and Their Substrates.

CHAPTER Ⅳ

Contents of Heavy Metal Elements in Copper Mosses: *Scopelophila ligulata* (Spruce) Spruce, *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. and Their Substrates.

4.1. Introduction

Some peculiar mosses and hepatics have been studied with special interest because of their peculiar ecological characteristics. They seem to prefer metal-rich soils, lithosols, rocks, etc. as their substrates although such an environment is generally considered to be unfavorable for plant growth. These mosses and liverworts have been referred to as "copper mosses" because their substrates containing remarkable amounts of heavy metals such as copper, iron, and lead, have been found by field investigations and researches. *Scopelophila ligulata* (Spruce) Spruce, *S. cataractae* (Mitt.) Broth. and *Mielichhoferia mielichhoferi* (Hook.) Wijk et Marg. were identified as one of the "copper mosses" long time ago (Persson 1948; Mårtensson and Berggren 1954; Noguchi and Ochi 1956). *S. ligulata* is widely distributed throughout Europe, the Azores, the Caucasus, China, the Himalayas, the Philippines, Indonesia (Java Island), Taiwan, Japan, North America, Guatemala and Ecuador. *S. cataractae* is distributed the Himalayas, the Philippines, Taiwan, in the Korean Peninsula, Bolivia, Indonesia (Java Island), India and North America. In Japan, it can be found in the western part of Honshu island, west of the Kanto region, and in Shikoku and Kyushu. *M. mielichhoferi* occurs in Europe, the Caucasus and North America. However, *M. japonica* Besch., which is a close relative of *M. mielichhoferi*, is unique to Japan and can be found in northern Honshu (north of the central regions of Japan) and in Hokkaido.

So far, studies on the heavy metal contents in the moss bodies and in their substrates of "copper mosses" have been done limited (Brown 1982; Shaw 1987; Nagano et al. 1999; Aikawa et al. 2000). Leaching procedures to estimate the amounts of exchangeable or extractable elements in the substrates of several bryophytes have been developed by some researchers, since total analyses would overestimate the amount of elements available to the bryophytes. However, among the leaching methods, the water-extraction method for heavy metals had never been applied, though it was considered that the bryophytes may absorb the nutritive substances and also pollutants in the water-soluble form into their system through the whole surface of their body (Taoda 1974; Berg and Steinnes 1997). In this study, the water-soluble chemical elements in the moss substrates were analyzed together with total heavy metal contents in the moss substrates and moss bodies of *S. ligulata*, *S. cataractae*, and *M. japonica*.

4.2. Materials and Methods

Six samples of *S. ligulata* were collected from the mineralized rock-face located along the Nakatsugawa Valley in Chichibu, Saitama prefecture, at about 680 meters above sea level, facing a deep, V-shaped gorge. Hornfels formed the bedrock at this site. Samples of both *S. cataractae* (3 samples) and *M. japonica* (3 samples) were collected from the mineralized rock-face located near Ginzandaira in Ashio-cho, Tochigi prefecture at about 700 meters above sea level. Rhyolite formed the bedrock at this site. All the samples of mosses and their substrates were collected from the sites which had not been disturbed by human activity. The substrates were collected using a bamboo spatula in areas where comparatively close colonies of each moss were growing.

The substrate samples were kept in polyethylene bags, taken to the laboratory, and dried at room temperature. The substrate samples were carefully separated from the mosses, by observing whether components of the moss bodies, such as leaves, stems, and rhizoids, were present. If any were found, they were removed from the samples using tweezers with non-metallic tips. Then the samples were sieved to obtain under 2 mm diameter grains which were further ground by using a mortar and sifted out with a 32 mesh screen. The moss bodies were first thoroughly washed with distilled water and then they were carefully separated using a pair of tweezers with non-metallic tips into a fresh green-colored upper part and a brown-colored lower part. Both parts were naturally dried indoors. Contents of the heavy metals such as iron, copper, zinc and lead in the moss bodies and substrates were determined with an atomic absorption spectrophotometer after wet digestion with a mixture of nitric / sulfuric acid (Department of Applied Biochemistry and Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo 1995).

Water-soluble elements in the substrates of the bryophytes were obtained as follows. Twenty-five milliliters of distilled water were added to 10 grams of each sample. The mixtures were then stirred and shaken for one hour and allowed to stand for 24 hours. After the pH measurement of the mixtures (Nagano and Wada 1974) using a pH meter, the supernatants were filtered. The barium sulfate turbidimetric method (Japanese Standards Association 1993) was used to measure the sulfate concentration in the filtrate. Then the filtrate was decomposed with a mixture of nitric / sulfuric acid (Department of Applied Biochemistry and Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo 1995). The heavy metal contents were determined using an atomic absorption spectrophotometer. The data obtained were statistically analyzed using *F*-test and *t*-test.

4.3. Results and Discussion

Tables 4-1 and 4-2 show the levels of chemical elements in both the moss substrates and the moss bodies.

4.3.1. Moss substrate

The pH values of the substrate of *S. ligulata* was lower than that of *S. cataractae* and *M. japonica* ($p<0.01$). These results corresponded to the findings of Nagano et al. (1969) who studied the habitat segregation between *S. ligulata* and *S. cataractae* in Chichibu, Saitama prefecture.

The content of water-soluble iron in the substrate of *S. ligulata* was much higher than that of the two other mosses ($p<0.01$), and also the water-soluble sulfate content in the former was higher than that of *S. cataractae* ($p<0.01$) and *M. japonica* ($p<0.05$). On the contrary the content of water-soluble copper in the former appeared to be lower than that in the latter although no significant differences were observed.

Nagano et al. (1999) examined the substrate of *S. ligulata* and that of four other non-copper mosses which grow on the same rock face of black slate. They reported that the pH value of the substrate of *S. ligulata* was lower while the concentrations of water-soluble iron and sulfate were higher than those in the four other mosses. Nagano et al. (1999) also found a high correlation among the pH value, the concentration of water-soluble iron and that of water-soluble sulfate. The pH value of the substrate of *S. ligulata*, in this study, was also significantly lower than that of *S. cataractae* and *M. japonica*, and the concentrations of water-soluble iron and sulfate in the substrate of *S. ligulata* were significantly higher than those of *S. cataractae* and *M. japonica*. Thus it can be concluded that *S. ligulata* prefers an iron and sulfate-rich environment as its habitat, which would provide more acidic conditions as compared with the habitats of *S. cataractae* and *M. japonica*.

The available data of total heavy metal contents in the substrates of classical "copper mosses" are as follows: *M. elongata* (Hornsch.) Hornsch. (Cu: 0.32 - 0.77 g kg^{-1}) and *Dryptodon atratus* (Mielichh.) Limpr. (Cu: 0.53 - 0.70 g kg^{-1}) in Sweden (Mårtensson and Berggren 1954); *M. elongata* (Cu: 0.03 - 0.45 g kg^{-1}) and *D. atratus* (Cu: 0.03 - 0.09 g kg^{-1}) in Europe, and *S. ligulata* (Cu: 0.01 - 0.42 g kg^{-1}) in Europe, West Java, India, Turkey and the Caucasus (Persson 1956); *M. macrocarpa* (Hook. ex Drumm.) Bruch et Schimp. ex Jaeg. et Sauerb. (Fe: 30.0 - 70.0 g kg^{-1}, Cu: 0.15 - 0.20 g kg^{-1}, Zn: 0.05 g kg^{-1}, Pb: 0.03 g kg^{-1}) in Alaska, *M. mielichhoferi* (Fe: 1.00 g kg^{-1}, Cu: 0.15 g kg^{-1}, Zn: <0.2 g kg^{-1}, Pb: <0.01 g kg^{-1}) in Michigan, and *M. elongata* (Fe: 10.0 - 20.0 g kg^{-1}, Cu: 0.01 g kg^{-1}, Zn: <0.2 g kg^{-1}, Pb: 0.01 - 0.07 g kg^{-1}) in Europe (Shacklette 1967); *M. mielichhoferi* (Cu: 0.10 g kg^{-1}) in Colorado (Hartman 1969); *M. elongata* (Fe: 10.0 - 18.0g kg^{-1}, Cu: 0.30 - 0.33 g kg^{-1}, Pb: 0.30 - 0.31 g kg^{-1}) in Scotland (Coker 1971); *M. elongata* (Cu: 0.03 - 0.04 g kg^{-1}) in Britain (Wilkins 1977); *S. cataractae* (Fe: 23.0 - 114 g kg^{-1}, Cu: 0.45 - 7.00 g kg^{-1}, Pb: 1.07 - 58.0 g kg^{-1}) in Europe (Sotiaux et al. 1987); *S. cataractae* (Cu: 1.80 - 7.00 g kg^{-1}, Zn: 0.60 - 9.80 g kg^{-1}, Pb: 4.60 - 8.30 g kg^{-1}) in Britain (Rumsey and Newton 1989). The total iron content (Fe: 123-212g kg^{-1}) in the substrate of *S. ligulata* in this study was higher than that of the above-mentioned "copper mosses" while the total copper, zinc, and lead contents in the former were within the range of those in the latter. The total iron, copper, zinc, and lead contents in the substrates of *S. cataractae* and *M. japonica* in this study were within the range of the contents in the substrates of "copper mosses" in the above-mentioned studies.

The total iron content in the substrate of *S. ligulata* was higher than that of *S. cataractae* and *M. japonica*, respectively ($p<0.01$). On the contrary the total copper content in the substrate of *S. ligulata* was lower than that of *M. japonica* ($p<0.01$) and also the total zinc content in the substrate of *S. ligulata* was lower than that of *S. cataractae* ($p<0.05$) and *M. japonica* ($p<0.01$), respectively.

4.3.2. Moss body

The total iron contents in both upper and lower parts of the moss body of *S. ligulata* were clearly higher than those of *S. cataractae* and *M. japonica*, respectively ($p<0.01$). On the contrary the total copper contents of the former were lower than those of the latter.

The limited data of total heavy metal contents in the bryophyte bodies of classical "copper mosses" are as follows: *M. macrocarpa* (Fe: 7.70 g kg^{-1}, Cu: 0.15 g kg^{-1}, Zn: 0.15 g kg^{-1}, Pb: 0.003 g kg^{-1} dry weight) in Alaska (Shacklette 1967); *M. elongata* (Fe: 6.80 - 8.20 g kg^{-1}, Cu: 0.14 - 0.15 g kg^{-1}, Pb: 0.67 - 0.68 g kg^{-1}) in Scotland (Coker, 1971); *S. cataractae* (Fe: 1.30 - 30.8 g kg^{-1}, Cu: 9.04 - 22.1 g kg^{-1}, Zn: 0.11 - 0.32 g kg^{-1}, Pb: 0.07 - 3.42 g kg^{-1}) in Japan (Satake et al. 1988); *S. cataractae* (Cu: 8.44 - 17.9 g kg^{-1}) in Japan (Satake et al. 1990). The total iron content in the moss body of *S. ligulata* in this study was much higher than that of the above-mentioned "copper mosses" while the total copper, zinc, and lead contents in the former were within the range of those in the latter. The total iron, copper, zinc, and lead contents in the moss bodies of *S. cataractae* and *M. japonica* in this study were within the range of the contents in the moss bodies of "copper mosses" in the above-mentioned studies.

Limited data of total heavy metal contents in the bryophyte bodies may have resulted from possible problem contamination. Some researchers were concerned about the contamination which might be caused by substrate particles when they analyzed the plant body of bryophytes. Mårtensson and Berggren (1954) reported that they tried to analyze the mosses, but it was not possible to free the tufts of alluvial sand, silt, and soil from the weathered schist. Pigott (1958) mentioned that attempts to determine whether copper was actively taken up by *Mielichhoferia* had failed due to the difficulty in obtaining adequate material which was completely free from soil particles. Shacklette (1965) considered the bryophyte sample contamination in detail, and stated that since many elements, with a few exceptions such as aluminum, silicon, titanium and zirconium, were generally concentrated in plant ash far in excess of the amounts found in the underlying soil or rock, the contamination of the plant sample with soil would lead to a decrease of the percentages of these elements in the ash. Coker (1971) suggested that the use of ultrasonic cleaning methods may enable to obtain plant samples which would be almost completely free from contamination with soil. Satake et al. (1988) used non-destructive analyses such as energy-dispersive X-ray fluorescence spectrometry and X-ray microanalysis in combination with scanning and transmission electron microscopy to address the problem of bryophyte sample contamination.

In this study, to estimate the contamination with substrate particles, the moss samples were separated into upper and lower parts after washing with distilled water thoroughly for many hours, then the heavy metal contents in those two parts were compared. Paired-sample *t*-test was carried out to confirm the difference between the total heavy metal contents in the upper part and those in the lower part of the moss body of *S. ligulata*, *S. cataractae* and *M. japonica*. The *t*-test could effectively detect differences only in the copper contents (upper part > lower part, $p<0.05$) of *S. ligulata*, and the iron contents (upper part < lower part, $p<0.01$) and lead contents (upper part < lower part, $p<0.01$) of *M. japonica*, respectively. However it was not possible to determine whether contamination had occurred. In the case of *S. ligulata*, the copper content of the moss body was almost equal to that of its substrate. On the contrary, in the cases of *S. cataractae* and *M. japonica*, the copper content of the moss body was higher than that of the substrate. Therefore, even if there was any contamination of the moss body with the substrate, there was no overestimation of the content of copper in the moss body.

4.3.3. Differences between "copper mosses" and other bryophytes

Regarding the zinc and copper contents, the common bryophytes generally contained relatively high levels of zinc in both bodies and substrates (Shacklette 1965; Bowen 1979). High zinc contents in the bodies and substrates were also observed in most of the vascular plants (Bowen 1979). Shacklette (1965) performed an analytical study on 29 species of bryophytes and their substrates. These bryophytes were non "copper mosses" but showed a different adaptability to the substrate on which they grew. In 17 species of bryophytes in that study a wide range of choice for the substrates was available. The total copper and zinc contents (g kg^{-1} dry weight) were as follows: bryophyte bodies, Cu 0.01 - 2.00 and Zn 0.05 - 20.0; bryophyte substrates, Cu 0.005 - 0.05 and Zn 0.025 - 4.00. The ratios of zinc contents to copper contents (Zn / Cu) in the bryophyte bodies were 1.33 - 133 (average 20.1) and those in their substrates were 1.25 - 571 (average 34.8). However, in the cases of *S. ligulata*, *S. cataractae* and *M. japonica* in this study, their moss bodies and substrates contained more copper than zinc while the zinc contents of the three mosses (moss bodies: 0.04 - 0.50 g kg^{-1}, moss substrates: 0.04 - 0.35 g kg^{-1}) were within the range of the values in the bryophyte bodies of the above-mentioned 17 species and their substrates, respectively. The ratios of Zn / Cu were as follows: *S. ligulata*, moss bodies 0.11 - 1.59, moss substrates 0.09 - 0.72; *S. cataractae*, moss bodies 0.01 - 0.05, moss substrates 0.08 - 0.56; *M. japonica*, moss bodies 0.03 - 0.04, moss substrates 0.43 - 0.56. These values were very low compared with those reported by Shacklette (1965) as mentioned above. This phenomenon was considered to be one of the peculiar characteristics of these three kinds of bryophytes known as "copper mosses."

The lead content in the moss bodies of all three species was not different from that of the bryophytes in general (Bowen 1979).

4.3.4. Differences between three "copper mosses"

The ratios of water-soluble iron to water-soluble copper in the substrate of *S. ligulata* were 10.3 - 46.1, values which were higher than those of *S. cataractae* (0.003 - 0.10) and *M. japonica* (0.01 - 0.62). The contents of iron and copper in both upper and lower parts of the moss bodies were also completely different between *S. ligulata* and the two other "copper mosses."

The ratios of total iron content to total copper content of *S. ligulata* were 207 - 770, values which were much higher than those of *S. cataractae* (0.13 - 4.39) and *M. japonica* (0.48 - 4.93). *S. ligulata* appeared to show a closer relationship with iron than copper, although it had been referred to as one of the "copper mosses" (see Figs.4-1 and 4-2).

S. ligulata contained an average of 0.24 g kg^{-1} copper in both its substrate and moss body. *M. japonica* contained an average of 3.40 g kg^{-1} copper in its substrate and 9.00 g kg^{-1} copper in its moss body. While *S. cataractae* contained an average of 7.10 g kg^{-1} copper in its substrate and 15.2 g kg^{-1} copper in its moss body. These results indicate that the high values of copper in both substrate and moss body are characteristic of *S. cataractae* among the bryophytes known as "copper mosses." Satake et al. (1990) also reported that *S. cataractae* accumulated a large amount of copper in the protonemata and shoots, and contained 8.44 - 17.9 g copper kg^{-1} moss body (dry weight).

Although in the substrate of *S. cataractae* the iron content was several times higher than the copper content, in the moss body, the copper content was higher than the iron content (see Figs.4-1 and 4-2). Copper is usually toxic to the vascular plants except when the concentration is low. Thus, the intake of large amounts of copper by *S. cataractae* is worth noticing.

4.4. Conclusions

The total and water-soluble element contents in the substrates and heavy metal contents in the moss body were analyzed in this study. The results of these chemical analyses were as follows. The iron contents in the moss body and substrate of *S. ligulata* were higher than those of *S. cataractae* and

M. japonica. On the contrary the copper contents of the former were lower than those of the latter. The pH values of substrate of *S. ligulata* were lower than those of *S. cataractae* and *M. japonica.* The concentrations of water-soluble iron and sulfate in the substrate of *S. ligulata* were higher than those of *S. cataractae* and *M. japonica.* On the contrary, the water-soluble copper concentrations in the substrate of *S. ligulata* were lower than those of the others. Although in the substrate of *S. cataractae* the iron content was several times higher than the copper content, in the moss body the copper content was higher than iron content.

Table 4-1. Analytical results of chemical elements in the substrates of *Scopelophila ligulata*, *Scopelophila cataractae* and *Mielichhoferia japonica*.

Sample	Soluble						Total			
	pH(H_2O)	Fe	Cu	Zn	Pb	SO_4^{2-}	Fe	Cu	Zn	Pb
Sl-1	2.73	10.5	1.02	0.38	<0.5	37.8	212	0.22	0.08	0.09
Sl-2	2.76	9.22	0.20	0.13	<0.5	37.4	187	0.24	0.10	0.08
Sl-3	2.54	13.2	0.36	0.26	<0.5	48.6	209	0.28	0.16	0.09
Sl-4	2.69	8.70	0.69	0.06	<0.5	38.4	142	0.35	0.08	0.05
Sl-5	2.68	11.5	0.77	0.38	<0.5	42.2	181	0.28	0.09	0.20
Sl-6	2.60	11.9	0.90	0.26	<0.5	44.8	123	0.05	0.04	0.03
	**a, **b	**a, **b		*a		**a, *b	**a, **b	**b	*a, **b	*b
Average	2.67±0.08	10.8±1.70	0.66±0.32	0.25±0.13	<0.5	41.5±4.51	176±36.0	0.24±0.10	0.09±0.04	0.09±0.06
Sc-1	3.59	0.10	1.03	0.58	<0.5	7.60	54.0	2.20	0.27	0.25
Sc-2	3.78	0.01	3.08	0.43	<0.5	8.20	20.0	14.0	0.34	0.06
Sc-3	3.80	0.12	8.05	0.65	<0.5	15.4	24.0	5.20	0.19	0.04
	**a	**a		*a		**a	**a		*a	*c
Average	3.72±0.12	0.08±0.06	4.05±3.61	0.55±0.11	<0.5	10.4±4.34	32.7±18.6	7.10±6.10	0.27±0.08	0.12±0.12
Mj-1	3.47	0.09	7.16	3.78	<0.5	23.0	64.0	3.30	0.34	0.41
Mj-2	3.66	0.10	1.75	0.98	<0.5	9.20	92.0	3.00	0.27	0.65
Mj-3	3.75	0.94	1.52	0.65	<0.5	23.8	54.0	3.90	0.35	0.35
	**b	**b				*b	**b	**b	**b	*b, *c
Average	3.63±0.14	0.38±0.49	3.48±3.19	1.80±3.19	<0.5	18.7±8.21	70.0±19.7	3.40±0.50	0.32±0.04	0.47±0.16

Soluble: concentration of water-soluble element (mg L^{-1}) in the substrate; Total: total heavy metal content (g kg^{-1} dry weight) in the substrate; Sl: *Scopelophila ligulata*; Sc: *Scopelophila cataractae*; Mj: *Mielichhoferia japonica*; Average: average±standard deviation. *, ** Significant levels at 0.05 and 0.01, respectively, by *t*-test. a, b, and c: Significant difference between *S. ligulata* and *S. cataractae*, *S. ligulata* and *M. japonica*, and *S. cataractae* and *M. japonica*, respectively.

Table 4-2. Analytical results of total element contents in the moss bodies of *Scopelophila ligulata*, *Scopelophila cataractae* and *Mielichhoferia japonica*.

Sample	Upper				Lower			
	Fe	Cu	Zn	Pb	Fe	Cu	Zn	Pb
Sl-1	69.0	0.22	0.35	0.09	105	0.18	0.17	0.12
Sl-2	91.0	0.28	0.37	0.10	151	0.24	0.25	0.13
Sl-3	111	0.35	0.53	0.10	176	0.25	0.28	0.10
Sl-4	60.0	0.29	0.17	0.11	80.0	0.24	0.11	0.09
Sl-5	125	0.37	0.04	0.10	99.0	0.22	0.18	0.12
Sl-6	34.0	0.13	0.07	0.03	77.0	0.10	0.05	0.08
Average	**a, **b 81.7±33.8	*b 0.27±0.09 *d	0.26±0.19	**a 0.09±0.03	**a, **b 115±40.1	*b 0.21±0.06 *d	*a 0.17±0.09	**b 0.11±0.02
Sc-1	8.80	6.20	0.28	0.05	29.0	6.60	0.30	0.13
Sc-2	3.40	17.0	0.35	0.02	3.30	26.0	0.27	0.05
Sc-3	4.00	10.0	0.50	0.03	5.30	25.0	0.39	0.07
Average	**a 5.40±3.00	11.1±5.50	0.38±0.11	**a 0.03±0.02	**a 12.5±14.3	19.2±10.9	*a 0.32±0.06	0.08±0.04
Mj-1	12.0	9.80	0.28	0.08	29.0	9.90	0.27	0.17
Mj-2	20.0	6.30	0.26	0.12	35.0	7.10	0.22	0.19
Mj-3	5.30	11.0	0.32	0.05	24.0	9.60	0.25	0.15
Average	**b 12.4±7.40 **d	*b 9.00±2.40	0.29±0.03	0.08±0.04 **d	**b 29.3±5.50 **d	*b 8.90±1.50	0.25±0.03	**b 0.17±0.02 **d

Upper: total heavy metal content (g kg^{-1} dry weight) in the upper part of the moss body (fresh green color); Lower: total heavy metal content (g kg^{-1} dry weight) in the lower part of the moss body (brown color); Sl: *Scopelophila ligulata*; Sc: *Scopelophila cataractae*; Mj: *Mielichhoferia japonica*; Average: average ± standard deviation. *, ** Significant levels at 0.05 and 0.01, respectively, by *t*-test. a, b, and c: Significant difference between *S. ligulata* and *S. cataractae*, *S. ligulata* and *M. japonica*, and *S. cataractae* and *M. japonica*, respectively. d: Significant difference between the upper part and the lower part of the moss body by paired-sample *t*-test.

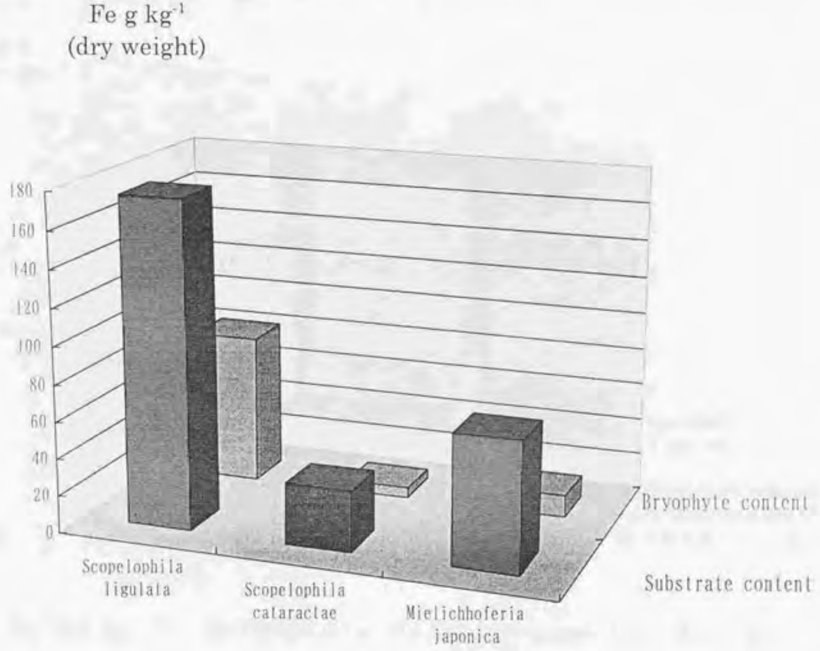

Fig.4-1. Fe content in the bryophytes and their substrates.

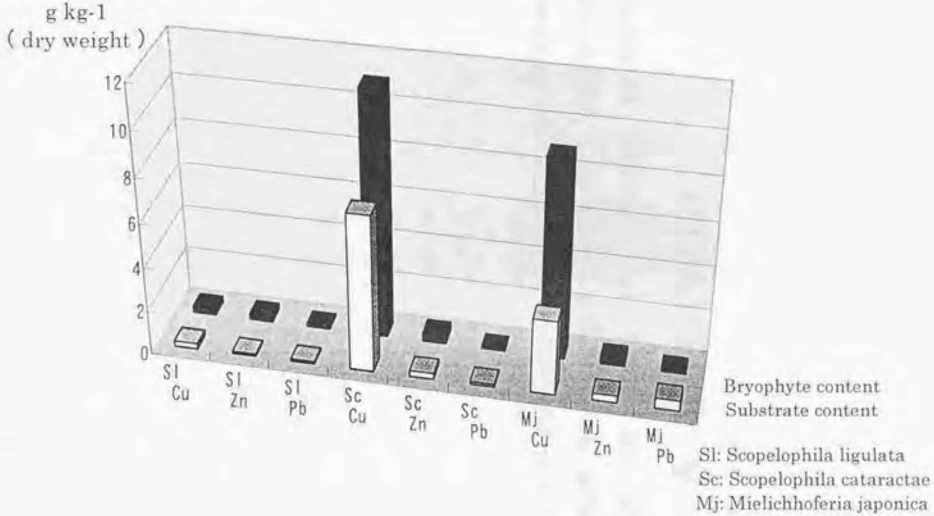

Fig. 4-2. Cu, Zn and Pb content in the bryophytes and their substrates.

CHAPTER V

Chemical species of iron in the bryophyte substrates and their pH values, and efficiency of cleaning methods for contaminant of bryophytes

CHAPTER V

Chemical species of iron in the bryophyte substrates and their pH values, and efficiency of cleaning methods for contaminant of bryophytes

5.1. Introduction

"Copper mosses" have been said, for a long time, to be related to the substrate which contain large amounts of heavy metals such as copper, zinc, iron and lead, while we have not a definite explanation for the reason why "copper mosses" grow well in such harmful conditions which are generally injurious to the plants.

In the past, analytical techniques were limited and obtaining adequate clean "copper moss" samples was thwarted by the contamination of substrate soil particles (Mårtensson and Berggren 1954; Pigott 1958; Satake et al. 1988). Thus, few studies with analytical results of the bryophyte body have been published. These studies are summarized in Table 5-1, which includes some methods of cleaning for bryophyte body adopted in each study. Recently some bryophytes, but not classical "copper mosses", are studied with their aquatic environment. These aquatic bryophytes are used as biological monitor of water quality or biogeochemical indicator of botanical prospecting on mineralized area. The typical studies of those are also summarized with their cleaning methods in Table 5-2.

In this study, the analyses of the water-soluble elements and total elemental contents in the substrates, as well as the total elemental contents of the bryophyte bodies of three species belonging to classical "copper mosses" or similar to that which were growing in mineralized areas in Saitama Prefecture, were performed. The study was focused on the relationships between the pH values and the water-soluble elements, especially sulfate and iron (Fe^{2+} and Fe^{3+}), in the substrates, and also focused on the cleaning methods for the bryophyte bodies to minimize the effects of contamination from the substrate particles.

5.2. Materials and Methods

All samples of *Scopelophila ligulata* (Spruce) Spruce, *Campylopus* sp. which is a close relative of *C. schwarzii* Schimp., and *Jungermannia vulcanicola* Steph. were collected from mineralized areas in Chichibu, Saitama Prefecture. All samples of bryophytes and their substrates were collected from mineralized rock faces, which had not been disturbed by human activity.

Substrate samples were collected in areas where relatively close colonies of each moss were growing. A bamboo spatula was used to collect them with the bryophyte. These substrate samples, stored in polyethylene bags, were taken to the laboratory and naturally dried at room temperature. The substrate samples were carefully separated from the bryophytes, sharply observing whether the bryophyte bodies, such as leaves, stems and rhizoids, were present. If any were found, they were removed from the sample using tweezers with non-metallic tips. Then, the samples were sieved to obtain under 2 mm diameter grains which were further ground using a mortar and pestle and screened at 32 mesh. Five grams of bryophyte samples were first thoroughly washed and unfastened with distilled water stirring constantly for fifteen minutes. They were then washed with 500 ml of 2% disodium dihydrogen ethylenediamine tetraacetate dihydrate (EDTA) for one hour using a vertical shaker (250 rpm). They were finally cleaned ultrasonically at 38 kHz for 30 minutes using deionized water with 0.8% neutral detergent, followed by washing and rinsing again in deionized water.

After wet digestion with a mixture of nitric/sulfuric acid (Department of Applied Biochemistry and Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo 1995), the heavy metal concentrations, such as iron, copper etc., in the bryophyte bodies and their substrates were determined using an inductively coupled plasma atomic emission spectrophotometry.

The water-soluble elements in the bryophyte substrates were examined using the method of Nagano et al. (1999) and Aikawa et al. (1999). A slurry ratio of 1:2.5 by weight soil to water, which was the same one to be used for the soil pH measurement, was used, so that the relationship between the water-soluble elements and the pH value could be determined. Fifty milliliters of distilled water were added to twenty grams of each sample. The mixtures were then stirred and shaken for one hour and allowed to stand for twenty-four hours. Nagano and Wada (1974) determined this to be the necessary standing time to obtain the optimum pH value. After the pH and EC were determined, the supernatants were filtered. The barium sulfate turbidimetric method was used to measure the sulfate concentration of the filtrate (Japanese Standards Association 1993). Then, the filtrate was decomposed with a mixture of nitric/sulfuric acid. The heavy metal and aluminum contents were measured using an inductively coupled plasma atomic emission spectrophotometry.

5.3. Results and Discussion

The analytical results of the water-soluble elements and the elemental contents in the substrates are shown in Tables 5-3 and 5-4, respectively. The ferrous and ferric iron concentrations with their ratios in the substrates are shown in Table 5-5. The analytical results of the elemental contents in the bryophyte bodies are shown in Table 5-7.

5.3.1. The water-soluble chemical elements in the bryophyte substrate

The pH of the substrate of *S. ligulata* was 2.89 on average, which was very similar to the values of Noguchi (1956), Persson (1956), and Nagano et al. (1969, 1999). That of *Campylopus* sp. was 3.08 which falls within the pH range of 2.20 to 3.20 measured by Nagano et al. (1969). That of *J. vulcanicola* was 2.86, which was the same as that determined by Nagano et al. (1969). No significant difference was observed for water-soluble elements among three bryophytes except EC (electric conductivity). EC showed a significant difference between *S. ligulata* and *Campylopus* sp., and *Campylopus* sp. and *J. vulcanicola*, respectively ($p<0.05$). The water-soluble iron concentration in the substrate of *S. ligulata* was higher than those of *Campylopus* sp. and *J. vulcanicola*, although there was no significant difference among them. No correlation was obtained between any combination of elements by regression analysis.

5.3.2. The total elemental contents in the bryophyte substrate

Regarding the total elemental contents in the substrates of *S. ligulata*, *Campylopus* sp. and *J. vulcanicola*, no significant difference was found among these three bryophytes (Table 5-4). In order to determine the relationship among the total elemental contents in the substrates, correlation analysis was undertaken. No high correlation was observed among three bryophytes, because of nearly equal values measured for three bryophytes.

The total elemental contents in the substrates of *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. accompanied with *S. ligulata* were studied in Chapter 4 as mentioned before. The total iron contents in the substrates of *S. ligulata*, *Campylopus* sp. and *J. vulcanicola*, as shown in Table 5-4, were higher than those of *S. cataractae* and *M. japonica* by one order of magnitude, as shown in Table 4-1, while the total copper contents in the substrates of former three bryophytes were lower than those of latter two mosses by one order of magnitude.

5.3.3. The chemical species of iron in the bryophyte substrates

Since it seemed to be important to recognize the chemical species of iron in the substrates of *S. ligulata*, *Campylopus* sp. and *J. vulcanicola*, the chemical form of water-soluble iron and total content iron in the substrates were examined. The analytical results are shown in Table 5-5. The ratios of ferrous iron form (Fe^{2+}) in the water-soluble iron were almost 80 to 90 %, although those in the total content iron in the substrates of *S. ligulata*, *Campylopus* sp. and *J. vulcanicola* were about 15 to 20 %. The ferrous iron form was dominant in the water-soluble iron, though the ferric iron form (Fe^{3+}) was dominant in the total content iron in the substrates.

Dissolution of 1 mg, 10 mg, 100 mg, 1 g, 10 g, and 50 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$), ferric sulfate ($Fe_2(SO_4)_3 \cdot 9H_2O$), and copper sulfate ($CuSO_4 \cdot 5H_2O$) into one liter of distilled water each, were examined. These yield pH and EC values shown in Table 5-6. Relationships among the concentration of iron/copper ions, the concentration of sulfate ions, the pH value, and the EC value are shown in Figs. 5-1 to 5-6. Especially, the relationships between the concentration of iron (or copper) ions and the pH value, and concentration of sulfate ions and the pH value are shown in Figs. 5-1 and 5-2, respectively. If we put Fe^{3+} values of three mosses in Table 5-5, on the ferric sulfate line in Fig. 5-1 and also put SO_4^{2-} values of those in Table 5-3 on ferric sulfate line in Fig. 5-2, then we get suitable pH values for each moss shown in Table 5-3. These pH values are comparable with those of three bryophyte substrates. Fe^{3+} and SO_4^{2-} ions seem to be most important factors to give a definite pH value of the bryophyte substrates in this study.

5.3.4. The efficiency of cleaning methods for contaminant and total elemental contents in the bryophyte body

The analytical results of the elemental contents in the bryophyte bodies are shown in Table 5-7. The cleaning method for bryophyte contaminant is very important matter to analyze the bryophyte body. Some cleaning methods for terrestrial bryophytes including "copper mosses" are shown in Table 5-1, and also those for aquatic bryophytes are shown in Table 5-2. Many researchers addressed this problem but no effective resolution seemed to be obtained on the wet digestion method of analysis. Satake et al. (1988) mentioned that most of the previous data of bryophyte analyses have been insufficient because of difficulty to eliminate the contaminant in spite of careful washing, and they studied *Scopelophila cataractae* (Mitt.) Broth. using non-destructive analyses to settle the issue of sample contamination and determined that a remarkable amount of copper was contained in the cell wall of moss bodies.

Primarily, the efficiency of washing with huge water and/or 0.8% neutral detergent and/or ultrasonic cleaning at 38 kHz for 30 minutes as cleaning method was examined in this study, however no good result was obtained. Generally all ash percentages above 10% were assumed to be due to contamination from the substrate (Shacklette 1965b, 1967; Brooks and Yates 1973; Shacklette and Erdman 1982). All of the samples treated by above-mentioned procedure were less than 90% as ignition loss, that is more than 10% as ash percentages. Secondly, a solution of 2% EDTA was examined as cleaning agent in this study. The bryophyte samples were washed with 500 ml of 2% EDTA for one hour using a vertical shaker (250 rpm), followed ultrasonic cleaning at 38 kHz for 30 minutes using deionized water with 0.8% neutral detergent, and followed washing and rinsing again in deionized water. This cleaning method yielded an acceptable effect, scilicet less than 10% as ash percentages of bryophyte (Table 5-7). Regarding ignition loss, and total contents of iron, aluminum and titanium, the *t*-test showed significant differences between two data groups of all three bryophyte species, no washing (A in Table 5-7) and washing (B in Table 5-7) with 2% EDTA solution.

Titanium would be a good indicator of contamination in bryophyte sample, since titanium compounds are insoluble in water (Shacklette 1965b; Bowen 1979; Satake et al. 1988). Thus, it seems that the bryophytes hardly absorb titanium into their body. Shacklette (1965b) stated that his 38 bryophyte samples (non "copper mosses") averaged 297 mg kg^{-1} Ti in the dry sample (15.15% in ash), and the substrates averaged 3,000 mg kg^{-1} Ti, while he also stated that several bryophyte had a higher titanium content in their bodies than their substrates. Satake et al. (1988) studied the relationships between the concentration of titanium and that of other elements such as Cu, Zn, Fe, Al, etc. in the shoots of *S. cataractae*. Satake et al. (1988) reported that the concentrations of Cu, Zn and P showed inverse correlations with the concentration of Ti, while the concentrations of Fe, Al, etc. increased with that of Ti.

To determine the relationships among the ignition losses of bryophyte samples and the total elemental contents in the bryophytes, correlation analyses were carried out. These results were shown in Figs. 5-7 to 5-17. High correlations (p<0.001) were observed as follows. Titanium and iron concentrations (R^2=0.696), titanium and lead concentrations (R^2=0.557), titanium and aluminum concentrations (R^2=0.867) were directly proportional. Titanium concentration and ignition loss (R^2=0.724), ignition loss and iron concentration (R^2=0.983), ignition loss and aluminum concentration (R^2=0.840) were inversely proportional. The relationships between titanium and iron concentrations, and titanium and aluminum concentrations were consistent with findings of Satake et al. mentioned above.

Rühling and Tyler (1970) studied the capacity of *Hylocomium splendens* (Hedw.) Br. et Sch. to sorb heavy metal ions from dilute solutions and also the percent retention of heavy metals, originally held by the moss tissues by leaching extractions with five different solutions including 0.05 M EDTA (about 2% EDTA solution). Ten g of moss samples were extracted with 2×1 litters of these solutions for 2×24 hours. Their results of per cent retention of original amounts of copper, iron, lead and zinc in the moss tissues after leaching extractions with 0.05 M EDTA were 42%, 78%, 6% and 15% respectively. Thus, iron in the moss tissues was not extracted so much, while lead and zinc in those were almost quantitatively complexed and released by EDTA. The data of zinc and lead of two groups in Table 5-7, no washing (A) and washing (B) with 2% EDTA solution, indicate no significant influence of extraction with EDTA for moss tissues of all three bryophyte species.

With due regard for the studies of Shacklette (1965b), Satake et al. (1988), and Rühling and Tyler (1970), EDTA cleaning method would be useful one to eliminate the contaminant attached to the bryophyte.

5.4. Conclusions

The results of the chemical analyses in this chapter revealed the following. It seemed that Fe^{3+} and SO_4^{2-} ions were most important factors to give a definite pH value of the bryophyte substrates in this study. *S. ligulata*, *Campylopus* sp. and *J. vulcanicola* accumulated a huge iron in their body in due consideration of their contaminant from substrate particles. A very high correlation ($p<0.001$) was found between ignition loss and the concentration of titanium, the concentrations of titanium and iron, the concentrations of titanium and lead, the concentrations of titanium and aluminum, ignition loss and the concentration of iron, and ignition loss and aluminum. Ignition loss and the concentration of titanium in the bryophyte may be a good indicator of contaminant in certain cases. The concentration of copper in all three bryophytes washed with 2% EDTA solution was higher than those without washing. These indicated the accumulation of copper into the cell of bryophyte. The solution of 2% EDTA was a favorable agent to eliminate the bryophyte contaminant from substrate particles.

Table 5-1. Studies accompanied with bryophyte analyses and their methods.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Shacklette (1965b)	Dry cleaning with compressed air followed by rinsing in tap water and then distilled water, followed by further dry cleaning with compressed air.	CM & SSA	*Anomodon rostratus*, etc.; total 29 species (non "copper mosses")	38	33 elements including Cu, Pb, Zn, Fe, Al and Ti
Dykeman and De Sousa (1966)	Unknown.	EM	*Pohlia nutans*	2	Cu
Shacklette (1967)	Dry cleaning with compressed air followed by rinsing in tap water and then distilled water, followed by further dry cleaning with compressed air.	CM & SSA	*Mielichhoferia macrocarpa**	2	30 elements including Cu, Pb, Zn, Fe, S and Ti
			Platydictya jungermannioides	2	
			*Mielichhoferia mielichhoferi**	1	
			*Mielichhoferia elongata**	2	
Coker (1971)	Ultrasonic cleaning at 40 kHz for 2 minutes.	AAS	*Mielichhoferia elongata**	3	Cu, Pb, Fe, Ca, Cr, Co, La, Y
			Saelania glaucescens	3	
Gullvåg et al. (1974)	(Culture test) Non-destructive analyses.	EMA	*Rhytidiadelphus squarrosus* *Hylocomium splendens*	1 (set)	Pb
Usui et al. (1975)	Unknown.	AAS	*Nardia assamica* (=*Nardia sieboldii*)	2	Cu, Cd
Wilkins (1977)	Manual cleaning with a needle, followed by washing with tap water and then deionized water.	AAS	*Mielichhoferia elongata**	1	Cu, Fe, Mg, Ca, Al, K, Na
			Coscinodon cribrosus	1	
			Grimmia atrata	1	
			Barbula sp.	1	
	Non-destructive analysis.	NAA	*Mielichhoferia elongata**	2	Cu
			Bryum argentcum	1	
Lee et al. (1977)	Washing in running tap water followed rinsing in distilled water.	AAS	*Aerobryopsis longissima*	8 (sites)	Cr

Table 5-1. Continued.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Boyle (1977)	Unknown	Unknown	*Pohlia nutans*	5	31 elements including Cu, Pb, Zn, Cd and Co
Brown and House (1978)	Washing with deionized water.	AAS	*Jungermannia gracillima* Sm. (= *Solenostoma crenulatum*)	2	Cu
			Marsupella emarginata	1	
			Pohlia nutans	1	
Empain (1985)	Washing in a continuous flow of deionized water and agitating with compressed air vigorously.	Unknown	*Crustry cyanophyceae*	1	Cu, Pb, Zn, Cd, Co, Cr, Fe, Mn, Ni
			Brachymenium acuminatum	2	
			Brachymenium philonotula	3	
			Bryum sp.	2	
			Campylopus bequaertii	4	
Satake et al. (1988)	Non-destructive analyses.	EDXRF XMA	*Scopelophila cataractae**	38 (sources)	Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb
	Washing with purified water.	ICP			
Satake et al. (1990)	Washing with purified water.	ICP	*Scopelophila cataractae**	5 (sites)	Cu
Aikawa et al. (1999)	Washing in running tap water and then unfastening with distilled water stirring constantly.	AAS	*Scopelophila ligulata**	6	Fe, Cu, Zn, Pb
			*Scopelophila cataractae**	3	
			*Mielichhoferia japonica**	3	

AAS: atomic absorption spectrophotometry; CM & SSA: colorimetric method and semiquantitative spectrographic analysis; CVAAS: cold vapour atomic absorption spectrophotometry; EDXRF: energy-dispersive X-ray fluorescence spectrometry; EM: electrolytic method; EMA: electron microscope analysis; ES: emission spectrography; Fl: fluorimetry; GS: gamma spectrometry; ICP: inductively coupled plasma atomic emission spectrometry; NAA: neutron activate analysis; SA: spectrum analysis using a microphotometer; XMA: X-ray microanalysis in combination with scanning and transmission electron microscopy; XPS: X-ray photoelectron spectroscopy; ZEMA: Zeeman effect mercury analyzer. *, classical "copper mosses".

Table 5-2. Studies accompanied with aquatic bryophyte analyses and their methods.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Pickering and Puia (1969)	Washing in three successive 200ml aliquots of distilled water for at least one hour.	GM	*Fontinalis antipyretica*	1 (set)	Zn (culture test)
Whitehead and Brooks (1969)	Washing.	ES & GS (for U)	Composite samples of mixed bryophytes	14	Be, Cu, Pb, U
Brooks and Yates (1973)	Washing in running water.	AAS	*Fissidens rigidulus* *Philonotis tenuis* *Mniobryum wahlenbergii* Composite of various species	6 1 1 1	Cu
McLean and Jones (1975)	According to the method by Premi & Cornfield (1968)*	AAS	*Marsupella emarginata* *Brachythecium rivulare* *Fontinalis squamosa* *Scapania undulata* *Eurynchium riparioides*	9 (sites)	Cu, Pb, Zn
Empain (1976)	Washing in river water, followed washing in deionized jet water in laboratory. If necessary, centrifuge was used to separate attached particles.	AAS	*Platyhypnidium riparioides* *Cinclidotus danubicus* *Cinclidotus nigricans* *Fontinalis antipyretica*	3 (river sites)	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn
Burton and Peterson (1979)	Washing thoroughly in the tap water to remove large particles of sand and gravel, separated into individual stems and washed in several changes of deionized water.	AAS	*Philonotis fontana* *Scapania undulata* *Fontinalis squamosum* *Eurhynchium riparoides* *Hygrohypnum ochraceum* *Rhacomitrium aciculare* *Jungermannia gracillima* Sm. (=*Solenostoma crenulata*)	5 4 2 2 1 1 1 (site(s))	Cu, Pb, Zn, Fe
Mouvet (1980)	Washing in river water, followed washing thoroughly in deionized water in laboratory.	AAS	*Fontinalis antipyretica*	21 (sites)	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn.

* I could not obtain this reference.

Table 5-2. Continued.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Say et al. (1981)	Washing in river water, followed rinsing in distilled water in laboratory. 2 cm tips of the shoots were removed in deionized water and washed with several changes of deionized water.	AAS	*Fontinalis squamosa* *Rhynchostegium riparioides* *Fontinalis antipyretica* *Amblystegium riparium*	6 (sites)	Ca, Mn, Cu, Zn, Cd, Pb, Cr^{6+}
Shacklette and Erdman (1982)	Washing in clear spring water, followed washing in flowing tap water in laboratory.	Fl	*Pohlia* sp.	4	U
			Brachythecium rivulare	4	32 additional elements including Cu, Pb, Zn and Cd by spectrographic methods
			Mnium punctatum	2	
			Marchantia polymorpha	1	
			Cratoneuron filicinum	1	
			Philonotis fontana	4	
			Bryum sp.	2	
			Brachythecium lamprochryseum	6	
			Aulacomnium palustre	1	
			Drepanocladus fluitans	2	
			Cratoneuron falcatum	1	
			Dichodontium pellucidum	1	
Whitton et al. (1982)	Washing in stream water, followed rinsing in distilled water in laboratory. 1 cm tips of the shoots were removed in deionized water. Samples were restricted to 1 cm tips, because older parts of the plant are often encrusted with Mn- and Fe-rich deposits.	AAS	*Scapania undulata*	50	Ca, Mn, Fe, Cu, Zn, Cd, Pb
Say and Whitton (1983)	Washing in river water, followed rinsing a stream of distilled water in laboratory. 2 cm tips of the shoots were removed in deionized water and washed with several changes of deionized water.	AAS	*Fontinalis antipyretica*	52 (sites)	Ca, Mn, Fe, Cu, Zn, Cd, Pb

Table 5-2. Continued.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Wehr and Whitton (1983a)	Washing in river water, followed rinsing a stream of distilled water in laboratory. 2 cm tips of the shoots were removed in deionized water and washed with several changes of deionized water.	AAS	*Rhynchostegium riparioides*	105 (sites)	Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Ba, Pb
Wehr and Whitton (1983b)	Washing in river water, followed rinsing a stream of distilled water in laboratory. 2 cm tips of the shoots were removed in deionized water and washed with several changes of deionized water.	AAS	*Rhynchostegium riparioides* *Amblystegium riparium* *Fontinalis antipyretica*	7 1 1 (sites)	Na, Mg, Ca, Mn, Fe, Zn, Cd, Ba, Pb
Satake et al. (1983)	Washing in running water, followed washing again by deionized water in laboratory. Ultrasonic cleaning of same samples for ten minutes in 0.1% of polyethylene glycol monolauryl ether solution (EDXRF analysis).	ZEMA CVAAS EDXRF XPS XMA	*Jungermannia vulcanicola* *Scapania undulata*	3 2	Hg
		ICP	*Jungermannia vulcanicola*		Na, Mg, Al, Si, P, K, Ca, Ti, Mn, Fe, Cu, Zn, Sr
Satake et al. (1984a)	Washing in running water, followed washing again by distilled water in laboratory. 1 cm tips from each shoot of bryophyte were cut and used for further treatment because the attached particles (contaminant) were less frequent on the apical part (1 cm) than the basal part.	ICP	*Jungermannia vulcanicola*	5 (sites)	Na, Mg, Al, Si, P, K, Ca, Ti, V, Mn, Fe, Cu, Zn, Sr
Satake et al. (1984b)	Washing in running water.	EDXRF ICP	*Ectropothecium subobscurum* *Vesicularia inflectans* *Lopholejeunea* sp.	1 1 1	B, Na, Mg, Al, Si, P, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Mo, Cd, Sb

Table 5-2. Continued.

Author (s)	Removal method of contamination from the substrate	Analysis Method	Bryophyte species	Number of Samples	Analyzed Elements
Satake et al. (1984c)	Washing in running water. Non-destructive analysis.	XMA EDXRF ZEMA	*Jungermannia vulcanicola*	2 (sites)	Hg
Erdman and Modreski (1984)	Washing in the stream, followed washing in flowing tap water then finally rinsing in distilled water in laboratory.	AAS	*Brachythecium rivulare* *Drepanocladus fluitans* *Brachythecium lamprochryseum* *Bryum* sp.	9 (sites)	Cu, Co
Satake et al. (1987)	Washing in running water, followed washing again by purified water in laboratory.	EDXRF ICP	*Pellia endiviiforia*	7 (sites)	B, Na, Mg, Al, P, K, Ca, Ti, Mn, Fe, Cu, Zn, Sr, Cd, Pb
Soma et al. (1988)	Non-destructive analysis.	XPS	*Pohlia ludwigii*	1	Pb
			Scapania undulata	1	
			Pellia endiviifolia	1	
	Unknown.	ICP	*Pohlia ludwigii*	1	Na, Mg, Al, P, K, Ca, Mn, Fe, Cu, Zn, Pb

AAS: atomic absorption spectrophotometry; CM & SSA: colorimetric method and semiquantitative spectrographic analysis; CVAAS: cold vapor atomic absorption spectrophotometry; EDXRF: energy-dispersive X-ray fluorescence spectrometry; EM: electrolytic method; EMA: electron microscope analysis; ES: emission spectrography; Fl: fluorimetry; GM: gravimetrical measurement; GS: gamma spectrometry; ICP: inductively coupled plasma atomic emission spectrometry; NAA: neutron activate analysis; SA: spectrum analysis using a microphotometer; XMA: X-ray microanalysis in combination with scanning and transmission electron microscopy; XPS: X-ray photoelectron spectroscopy; ZEMA: Zeeman effect mercury analyzer.

Table 5-3. Analytical results of chemical elements eluted from the substrates of *Scopelophila ligulata*, *Campylopus* sp. and *Jungermannia vulcanicola*.

Sample	pH (H_2O) value	EC dS m^{-1}	Fe mg L^{-1}	Cu mg L^{-1}	Zn mg L^{-1}	Pb mg L^{-1}	Al mg L^{-1}	SO_4^{2-} mg L^{-1}
Sl-1	3.08	978	17.2	0.72	0.11	0.01	1.01	43.7
Sl-2	3.00	1,420	31.4	2.20	0.38	0.06	1.98	74.7
Sl-3	2.58	1,070	30.5	0.85	0.13	0.05	2.44	64.3
Average	2.89±0.27	*a 1,156±233	26.4±7.95	1.26±0.82	0.21±0.15	0.04±0.03	1.81±0.73	60.9±15.8
Csp-1	3.15	433	3.12	0.57	0.25	0.01	1.33	55.9
Csp-2	3.01	583	6.96	0.66	0.30	0.01	1.74	41.1
Csp-3	3.09	756	18.1	0.85	0.49	0.13	2.77	36.5
Average	3.08±0.07	*a, *b 591±162	9.39±7.78	0.69±0.14	0.35±0.13	0.05±0.07	1.95±0.74	44.5±10.1
Jv-1	3.05	1,255	24.5	1.90	0.56	0.02	2.74	75.7
Jv-2	2.79	1,385	13.2	2.11	0.60	0.02	1.38	61.6
Jv-3	2.74	823	14.5	0.72	0.11	0.04	1.43	46.0
Average	2.86±0.17	*b 1,154±294	17.4±6.18	1.58±0.75	0.42±0.27	0.03±0.01	1.85±0.77	61.1±14.9

Sl: *Scopelophila ligulata*; Csp: *Campylopus* sp.; Jv: *Jungermannia vulcanicola*; Average: average±standard deviation. * Significant level at 0.05 by *t*-test. a and b: significant difference between Sl and Csp, and Csp and Jv, respectively.

Table 5-4. Analytical results of total elemental contents (g kg^{-1}) in the substrates of *Scopelophila ligulata*, *Campylopus* sp. and *Jungermannia vulcanicola*.

Sample	Fe	Cu	Zn	Pb	Cd	Al	Ti
Sl-1	205	0.14	0.12	0.13	0.002	8.80	0.50
Sl-2	187	0.06	0.09	0.17	0.001	7.60	0.87
Sl-3	156	0.05	0.27	0.38	0.002	11.1	0.45
Average	183±24.8	0.08±0.05	0.16±0.10	0.23±0.13	0.0017 ± 0.0006	9.17±1.78	0.61±0.23
Csp-1	223	0.18	0.14	0.04	0.001	3.60	0.65
Csp-2	240	0.27	0.26	0.05	0.003	3.20	0.44
Csp-3	130	0.10	0.22	0.45	0.001	14.1	0.51
Average	198±59.2	0.18±0.09	0.21±0.06	0.18±0.23	0.0017 ± 0.0012	6.97±6.18	0.53±0.11
Jv-1	189	0.14	0.14	0.08	0.001	7.00	0.29
Jv-2	237	0.48	0.24	0.43	0.001	5.10	0.42
Jv-3	147	0.07	0.08	0.43	0.001	9.70	0.35
Average	191±45.0	0.23±0.22	0.15±0.08	0.31±0.20	0.001	7.27±2.31	0.35±0.07

Sl: *Scopelophila ligulata*; Csp: *Campylopus* sp.; Jv: *Jungermannia vulcanicola*; Average: average ± standard deviation. No significant difference was found among three bryophytes.

Table 5-5. The chemical species of iron and their concentrations in the substrates.

Samples	Water-soluble iron (mg L^{-1})			Total content iron (g kg^{-1})		
	Fe^{2+}	Fe^{3+}	$Fe^{2+}/(Fe^{2+}+Fe^{3+})$	Fe^{2+}	Fe^{3+}	$Fe^{2+}/(Fe^{2+}+Fe^{3+})$
Sl	27.5	3.90	0.876	37.8	153	0.198
Csp	15.9	1.92	0.892	27.9	151	0.156
Jv	12.0	2.50	0.828	30.3	117	0.206
Average	18.5±8.06	2.77±1.02	0.865±0.033	32.0±5.20 *	140±20.2	0.187±0.027

Sl: *Scopelophila ligulata*; Csp: *Campylopus* sp.; Jv: *Jungermannia vulcanicola*. Average: average±standard deviation.

* Significant difference between Fe^{2+} and Fe^{3+} at level of 0.05 by paired-sample *t*-test.

Table 5-6. The pH and EC values of dissolution of ferrous sulfate, ferric sulfate, and copper sulfate.

	Solution (g L^{-1})	Fe or Cu (mg L^{-1})	SO_4^{2-} (mg L^{-1})	pH	EC (dS m^{-1})
$FeSO_4 \cdot 7H_2O$	0.001	0.20	0.35	5.70	7.3
	0.01	2.01	3.45	5.60	15.0
	0.1	20.1	34.5	5.40	88
	1	201	345	4.70	633
	10	2,009	3,455	3.91	4,000
	50	10,043	17,274	3.48	12,400
$Fe_2(SO_4)_3 \cdot 9H_2O$	0.001	0.20	0.51	5.00	7.8
	0.01	1.99	5.13	4.00	33.2
	0.1	19.9	51.3	3.30	359
	1	199	513	2.60	1,450
	10	1,987	5,127	1.83	8,700
	50	9,937	25,636	1.25	30,900
$CuSO_4 \cdot 5H_2O$	0.001	0.25	0.38	5.70	5.9
	0.01	2.54	3.85	5.50	15.8
	0.1	25.5	38.5	5.40	106
	1	255	385	5.00	671
	10	2,545	3,847	4.43	4,000
	50	12,725	19,234	3.95	12,800

Solution: dissolution of indicated grams of $FeSO_4 \cdot 7H_2O$, $Fe_2(SO_4)_3 \cdot 9H_2O$, and $CuSO_4 \cdot 5H_2O$ into one liter of distilled water each.

Table 5-7. Analytical results of total elemental contents in the bryophyte bodies of *Scopelophila ligulata*, *Campylopus* sp. and *Jungermannia vulcanicola*.

	Scopelophila ligulata (S.l.)			*Campylopus* sp. (C.sp.)			*Jungermannia vulcanicola* (J.v.)		
	A		B	A		B	A		B
IL	80.7		90.0	88.5		95.1	79.2		89.5
	80.6		90.3	88.2		95.5	79.7		90.3
	80.1		90.5	88.3		95.4	79.5		90.5
	80.5±0.32	***	90.3±0.25	88.3±0.15	***	95.3±0.21	79.5±0.25	***	90.1±0.53
Fe	91.8		47.4	49.4		20.8	105		43.9
	91.9		42.5	49.8		17.6	97.1		48.8
	94.9		52.5	48.8		18.0	97.5		45.4
	92.9±1.76	***	47.5±5.00	49.3±0.50	***	18.8±1.74	99.9±4.45	***	46.0±2.51
Cu	0.051		0.151	0.070		0.113	0.145		0.507
	0.080		0.098	0.063		0.039	0.050		0.110
	0.051		0.093	0.029		0.033	0.115		0.254
	0.061±0.017		0.114±0.032	0.054±0.022		0.062±0.045	0.103±0.049		0.290±0.201
Zn	0.018		0.043	0.079		0.066	0.022		0.083
	0.018		0.047	0.049		0.012	0.029		0.033
	0.031		0.035	0.018		0.028	0.026		0.057
	0.022±0.008	*	0.042±0.006	0.049±0.031		0.035±0.028	0.026±0.004		0.058±0.025
Pb	0.016		0.006	0.015		0.003	0.017		0.029
	0.015		0.007	0.020		0.002	0.018		0.012
	0.019		0.007	0.026		0.002	0.017		0.020
	0.017±0.002	**	0.007±0.001	0.020±0.006	**	0.002±0.001	0.017±0.001		0.020±0.009
Al	0.558		0.473	0.455		0.167	0.771		0.484
	0.677		0.402	0.449		0.202	0.755		0.440
	0.669		0.504	0.658		0.165	0.783		0.458
	0.635±0.067	*	0.460±0.052	0.521±0.119	**	0.178±0.021	0.770±0.014	***	0.461±0.022
Ti	0.125		0.062	0.134		0.022	0.211		0.120
	0.134		0.061	0.134		0.024	0.205		0.116
	0.143		0.082	0.160		0.024	0.215		0.125
	0.134±0.009	***	0.068±0.012	0.143±0.015	***	0.023±0.001	0.210±0.005	***	0.120±0.005

A: without washing in the solution of 2% EDTA; B: washing with 2% EDTA; IL: Ignition loss. Unit: IL, %; other elements, g kg^{-1} dry weight. *, **, *** Significant of 0.05, 0.01 and 0.001 levels of probability between A and B, respectively, by *t*-test.

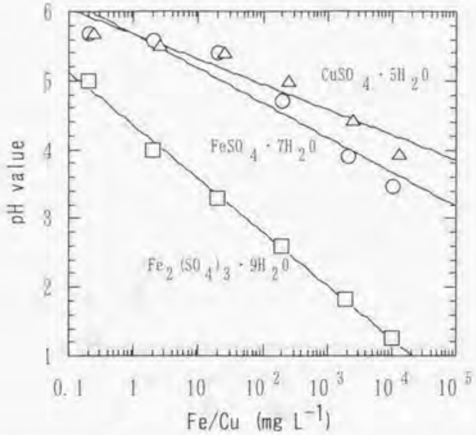

Fig.5-1. The relationship between iron (or copper) content and pH of ferrous sulfate, ferric sulfate and copper sulfate solution.

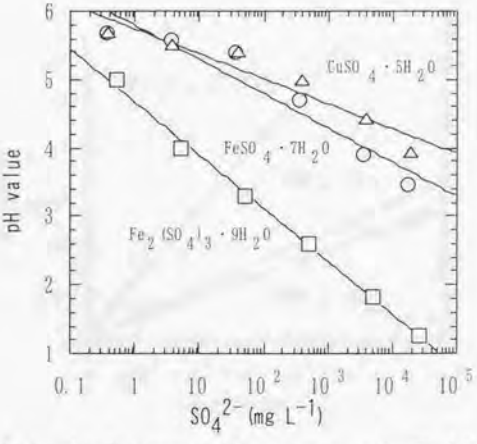

Fig.5-2. The relationship between sulfate content and pH of ferrous sulfate, ferric sulfate and copper sulfate solution.

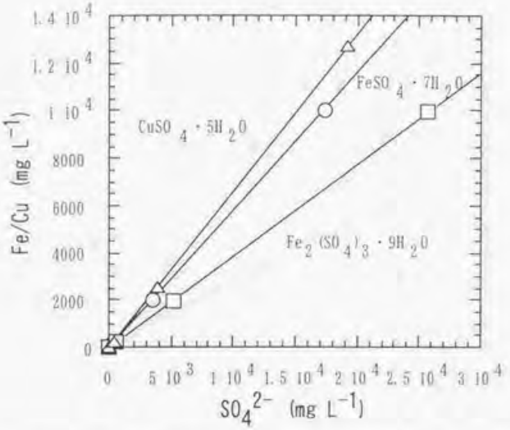

Fig.5-3. The relationship between sulfate content and iron (or copper) content of ferrous sulfate, ferric sulfate and copper sulfate solution.

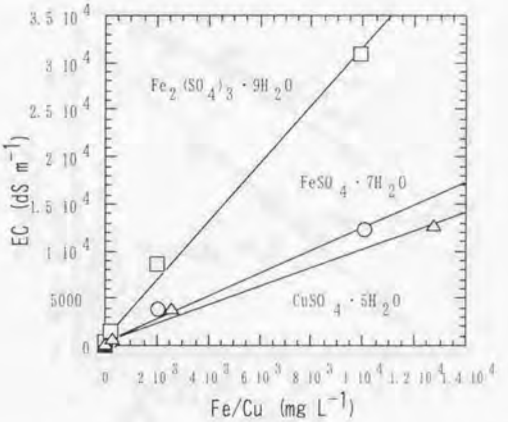

Fig.5-4. The relationship between iron (or copper) content and EC of ferrous sulfate, ferric sulfate and copper sulfate solution.

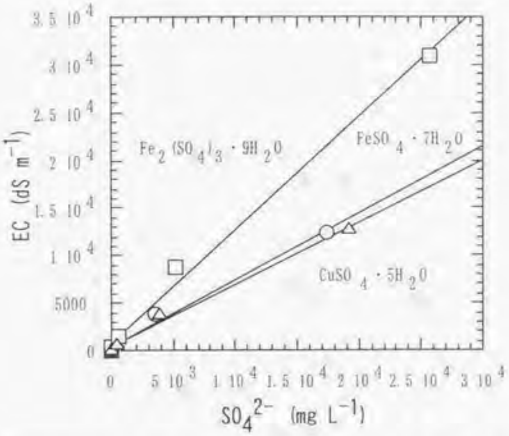

Fig.5-5. The relationship between sulfate content and EC of ferrous sulfate, ferric sulfate and copper sulfate solution.

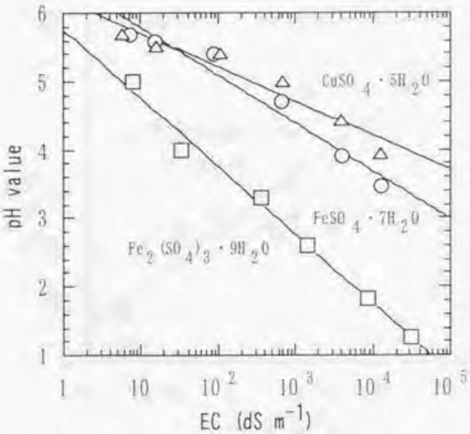

Fig.5-6. The relationship between EC and pH of ferrous sulfate, ferric sulfate and copper sulfate solution.

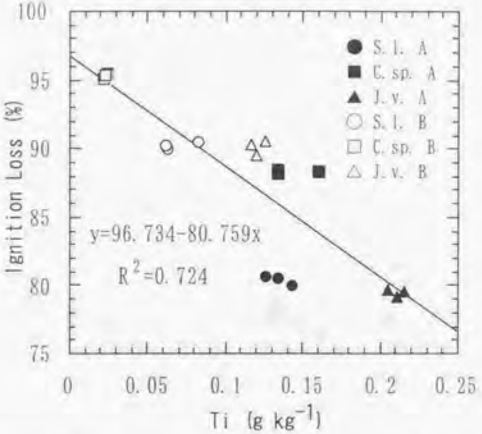

Fig. 5-7. The relationship between titanium content and ignition loss of the bryophytes. For S.l., etc. see Table 5-7.

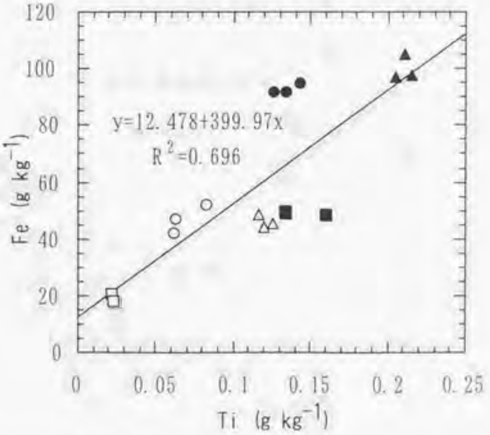

Fig. 5-8. The relationship between titanium content and iron content in the bryophytes. Symbols are the same as Fig. 5-7.

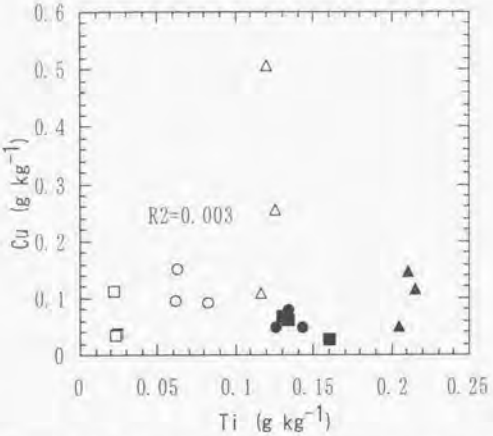

Fig. 5-9. The relationship between titanium content and copper content in the bryophytes. Symbols are the same as Fig. 5-7.

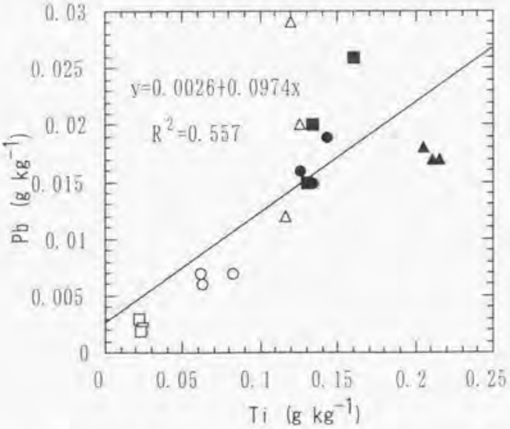

Fig. 5-10. The relationship between titanium content and lead content in the bryophytes. Symbols are the same as Fig. 5-7.

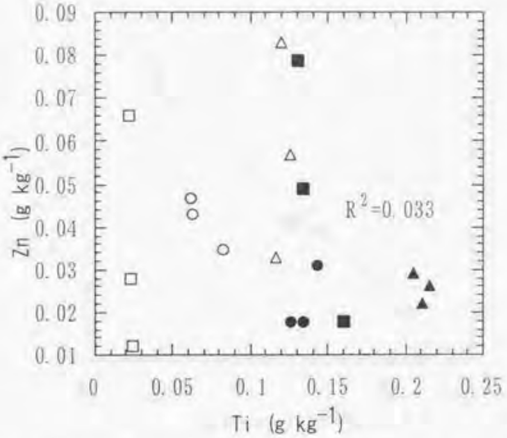

Fig. 5-11 The relationship between titanium content and zinc content in the bryophytes. Symbols are the same as Fig. 5-7.

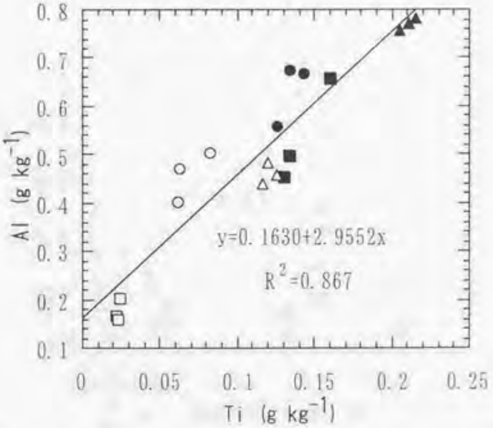

Fig. 5-12. The relationship between titanium content and aluminum content in the bryophytes. Symbols are the same as Fig. 5-7.

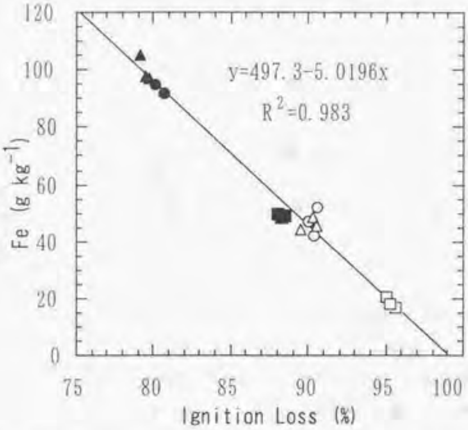

Fig. 5-13. The relationship between ignition loss and iron content in the bryophytes. Symbols are the same as Table 5-7.

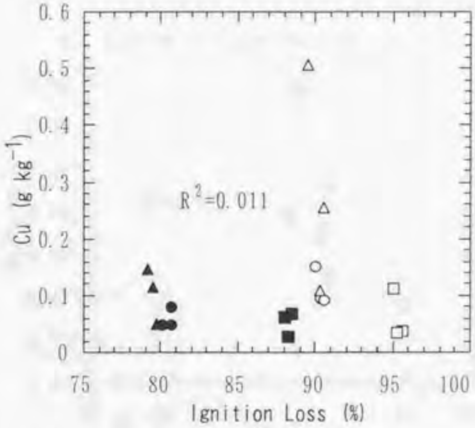

Fig. 5-14. The relationship between ignition loss and copper content in the bryophytes. Symbols are the same as Table 5-7.

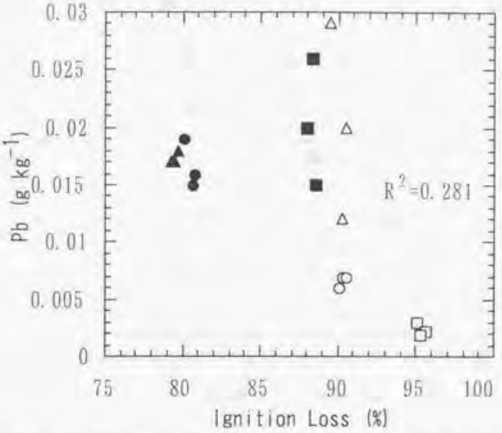

Fig. 5-15. The relationship between ignition loss and lead content in the bryophytes. Symbols are the same as Table 5-7.

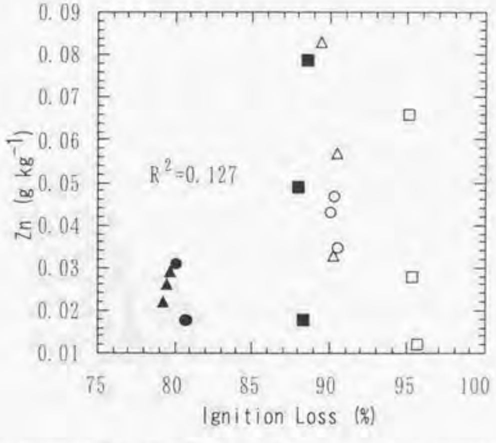

Fig. 5-16. The relationship between ignition loss and zinc content in the bryophytes. Symbols are the same as Table 5-7.

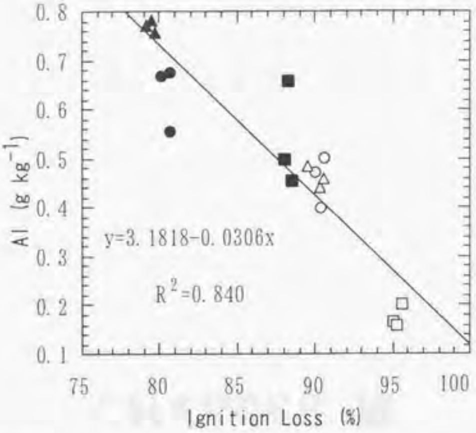

Fig. 5-17. The relationship between ignition loss and aluminum content in the bryophytes. Symbols are the same as Table 5-7.

CHAPTER Ⅵ

Copper Mosses and Their Substrates in Mineralized Areas

CHAPTER Ⅵ
Copper Mosses and Their Substrates in Mineralized Areas

6.1. Introduction

Regarding the chemical analyses of the substrate of "copper mosses", as shown in Table 2-1, only a few studies have reported (Aikawa et al. 1999). These limited studies have used the following techniques for substrate analysis. Mårtensson and Berggren (1954) digested samples with a mixture of nitric, perchloric, and sulfuric acid. Persson (1956) performed spectrum analysis on ashed samples using a microphotometer. Pigott (1958) determined the water-extractable sulfate and copper content by an unknown method. Shacklette (1965b, 1967) utilized the colorimetric method and semiquantitative spectrographic analysis for air-dried samples. Warncke (1968) used a 1N-ammonium acetate solution at pH 7 to leach samples, followed by an unknown analytical method. Hartman (1969) applied an unknown pre-treatment followed by analysis using atomic absorption spectrophotometry. Coker (1971) digested samples with a mixture of hot nitric and hydrochloric acid followed by atomic absorption spectrophotometry analysis. Brooks and Yates (1973) digested their samples with a mixture concentrated nitric and hydrofluoric acid followed by analysis using atomic absorption spectrophotometry. Wilkins (1977) leached samples with 1N ammonium acetate solution at pH 7 followed by atomic absorption spectrophotometry analysis. Brown and House (1978) extracted their samples with either deionized water, 2% EDTA, or 1,000 microgram/gram lead nitrate followed by analysis using atomic absorption spectrophotometry. Corley and Perry (1985) digested samples with 2M nitric acid and 10 vol. hydrogen peroxide solution followed by atomic absorption spectrophotometry analysis. Sotiaux and De Zuttere (1987) digested samples with a mixture of nitric and hydrochloric acid followed by analysis using atomic absorption spectrophotometry. Shaw (1987) extracted samples with 1% hydrochloric acid followed by analysis using atomic absorption spectrophotometry. Finally, Rumsey and Newton (1989) digested samples with concentrated nitric acid followed by atomic absorption spectrophotometry analysis.

In this chapter, the analyses of the water-soluble elements and total elemental contents in the substrates, as well as the total elemental contents of the bryophyte bodies of five species belonging to classical "copper mosses" or similar to that which were growing in mineralized areas, were performed, focusing on the relationships between the afore-mentioned mosses and their substrates.

6.2. Materials and Methods

All samples of *Scopelophila ligulata* (Spruce) Spruce, *S. cataractae* (Mitt.) Broth., *Mielichhoferia japonica* Besch., *Campylopus* sp. and *Jungermannia vulcanicola* Steph. were collected from mineralized areas in Japan. Two samples of *S. ligulata* were collected; the one from a location near Mt. Sobo, Miyazaki Prefecture, and the other from Chichibu, Saitama Prefecture. *Campylopus* sp. grew in the same vicinity of the habitat of *S. ligulata* in Saitama Prefecture mentioned above. Two samples of *S. cataractae* and *M. japonica* were obtained from Saitama Prefecture and Tochigi Prefecture, respectively. Two samples of *J. vulcanicola* were sampled; one from near Takachiho Gorge, Miyazaki Prefecture and the other from Chichibu, Saitama Prefecture. This liverwort is widely distributed from Hokkaido to

Kyushu in Japan (Iwatsuki and Mizutani 1972). All samples of bryophytes and their substrates were collected from mineralized rock faces, which had not been disturbed by human activity.

The substrates used for analysis were collected in areas where relatively close colonies of each moss grew. A bamboo spatula was used to collect them with the bryophyte. The substrate samples, stored in polyethylene bags, were taken to the laboratory and naturally dried at room temperature. The substrate samples were carefully separated from the bryophytes, sharply observing whether the bryophyte bodies, such as leaves, stems and rhizoids, were present. If any were found, they were removed from the sample using tweezers with non-metallic tips. Then, the samples were sieved to obtain under 2 mm diameter grains which were further ground using a mortar and pestle and screened at 32 mesh.

The bryophyte samples of five grams were first thoroughly washed and unfastened with distilled water stirring constantly for fifteen minutes. They were then washed with 500 ml of 2% EDTA for one hour using a vertical shaker (250 rpm). They were finally cleaned ultrasonically at 38 kHz for thirty minutes using deionized water with 0.8 % neutral detergent, followed by washing and rinsing again in deionized water. After wet digestion with a mixture of nitric/sulfuric acid (Department of Applied Biochemistry and Department of Biotechnology, Graduate School of Agriculture and Life Sciences, The University of Tokyo 1995), the heavy metal concentrations, such as iron, copper, etc., in the bryophyte bodies and their substrates were determined using an inductively coupled plasma atomic emission spectrophotometry.

The water-soluble elements in the bryophyte substrates were examined using the method of Nagano et al. (1999). A slurry ratio of 1:2.5 by weight soil to water, which was the same one to be used for the soil pH measurement, was used, so that the relationship between the water-soluble elements and the pH value could be determined. Fifty milliliters of distilled water were added to twenty grams of each sample. The mixtures were then stirred and shaken for one hour and allowed to stand for twenty-four hours. Nagano and Wada (1974) determined this to be the necessary standing time to obtain the optimum pH value. After the pH and EC measurements were determined, the supernatants were filtered. The barium sulfate turbidimetric method was used to measure the sulfate concentration of the filtrate (Japanese Standards Association 1993). Then, the filtrate was decomposed with a mixture of nitric/sulfuric acid. The heavy metal and aluminum contents were measured using an inductively coupled plasma atomic emission spectrophotometry.

6.3. Results and Discussion

The analytical results of the water-soluble elements, the elemental contents in the substrates, and the elemental contents in the bryophyte bodies are shown in Tables 6-1, 6-2, and 6-3, respectively. To determine the relationship between the chemical elements in the substrates and bryophyte bodies, correlation analysis was undertaken. The results of these calculations are shown in Tables 6-4 to 6-9.

6.3.1. The water-soluble chemical elements in the bryophyte substrate

The pH values of the two samples of *S. ligulata* were 2.68 and 2.59, which were very similar to the values of Noguchi (1956), Persson (1956), and Nagano et al. (1969, 1999). Those of *Campylopus sp.* were 2.51 and 3.25, which almost fall within the pH range of 2.20 to 3.20, measured by Nagano et al. (1969). The values of *S. cataractae* and *M. japonica* were 4.54 and 4.70, and 3.53 and 4.47, respectively. Those were consistent with the measured values for each of the two species by Nagano et al. (1969). The pH values of the substrates of *J. vulcanicola* were 2.72 and 4.02, which were the same as that determined by Nagano et al. (1969). The pH values of the substrates of *S. ligulata*, *Campylopus* sp. and *J. vulcanicola* were virtually identical, and they were significantly lower than those of *S. cataractae* and *M. japonica*.

High correlations were observed between the pH and the EC (R^2=0.839, p<0.001), the pH and the iron concentration (R^2=0.926, p<0.001), and the pH and the sulfate concentration (R^2=0.579, p<0.05), respectively (Table 6-4). These were plotted using a best fit curve (Figs. 6-1, 6-2, and 6-3). The relationships were all inversely proportional.

The EC values of *S. cataractae* and *M. japonica* were lower than those of the other bryophytes. A very high correlation (R^2=0.904, p<0.001) was found between the EC and the sulfate concentration (Fig. 6-4). Thus, EC measurements are useful in estimating the water-soluble sulfate concentration. The water-soluble iron concentrations in the substrates of *S. cataractae* and *M. japonica* were lower than those of the other bryophytes (Table 6-1). The water-soluble copper concentrations in the substrates of *S. cataractae* and *M. japonica* were higher than those of the other bryophytes (Table 6-1). The water-soluble sulfate concentrations in the substrates of

S. cataractae and *M. japonica* were somewhat lower than those of the other bryophytes. In addition to the previously mentioned relationships, the water-soluble sulfate correlated strongly with the water-soluble aluminum concentration (R^2=0.701, p<0.01). It is remarkable that the pH values and the water-soluble copper concentrations in the substrates of *S. cataractae* and *M. japonica* were higher than those of the other bryophytes. On the contrary, the EC values, and the water-soluble iron and sulfate concentrations in the substrates of *S. cataractae* and *M. japonica* were lower than those of the others.

6.3.2. The total elemental contents in the bryophyte substrate

The iron contents in the substrates of *J. vulcanicola*, and *Campylopus sp.*, as shown in Table 6-2, were higher, followed by *S. ligulata* sampled both in Miyazaki and Saitama. Those of *S. cataractae* and *M. japonica* were much lower than the other bryophytes. Meanwhile, the copper contents in the substrates of *S. cataractae* were higher than those of the other bryophytes by one order of magnitude.

In order to determine the relationship among the total elemental contents in the substrates, correlation analysis was undertaken. The results of these calculations are shown in Table 6-5. Typically, high inverse correlations were observed between the iron content and the copper content (p<0.001), the iron content and the zinc content (p<0.01).

6.3.3. The total elemental contents in the bryophyte body

The iron content in the moss bodies of *S. ligulata* sampled both in Miyazaki and Saitama were much higher than that of the other bryophytes.

Additionally, the iron content in the moss bodies of *S. cataractae* and *M. japonica* was much lower than that of the other bryophytes. Conversely, the copper contents in the moss bodies of *S. cataractae* and *M. japonica* were higher than those of the other bryophytes. Typically, these values were higher than those in their substrates, respectively. Satake (1983) reported a high concentration of aluminum in the bryophyte body of *J. vulcanicola*.

The correlation matrix of the elemental contents in the bryophyte bodies is shown in Table 6-6. The iron content had negative relationship ($R^2=0.544$, $p<0.05$) with the copper content. The same trend was found between the water-soluble iron and copper in the substrates ($R^2=0.679$, $p<0.01$) and the total iron and copper content in the substrates ($R^2=0.897$, $p<0.001$). These relationships are illustrated in Figs. 6-5, 6-6, and 6-7.

6.3.4. The relationship between the water-soluble chemical elements, total elemental contents in the bryophyte substrates, and those in the bryophyte bodies

To determine the relationship between the water-soluble chemical elements and the total elemental contents in the substrates, the water-soluble chemical elements in the substrates and total elemental contents in the bryophyte bodies, and total elemental contents in the substrates and those in the bryophyte bodies, correlation analyses were carried out. The results of these calculations are shown in Tables 6-7, 6-8, and 6-9.

Correlations were observed as follows. (1) In the substrates of the bryophytes, the following relationships between the water-soluble chemical elements and total element contents in the substrates were observed. The pH and total zinc, the pH and total aluminum, the EC and total iron, the water-soluble iron and total iron, the water-soluble copper and total copper, the water-soluble copper and total zinc, the water-soluble copper and total aluminum, and the water-soluble sulfate and total iron were found to be directly proportional. The pH and total iron, the EC and total zinc, the EC and total aluminum, the water-soluble iron and total copper, the water-soluble iron and total zinc, the water-soluble copper and total iron, the water-soluble aluminum and total aluminum, and the water-soluble sulfate and total aluminum were inversely proportional. (2) In the bryophyte bodies, the following relationships between the water-soluble chemical elements in the substrates and total elemental contents were determined. The pH and total copper, the pH and total aluminum, the EC and total iron (R^2=0.836, p<0.001; Fig.6-8), the water-soluble iron and total iron (R^2=0.880, p<0.001; Fig.6-9), the water-soluble copper and total copper (R^2=0.889, p<0.001; Fig.6-10), the water-soluble copper and total aluminum, the water-soluble zinc and total lead, the water-soluble zinc and total cadmium, and water-soluble sulfate and total iron were directly proportional. The pH and total iron, the EC and the total copper, the EC and total aluminum, the water-soluble copper and total iron, the water-soluble sulfate and total copper, and the water-soluble sulfate and total aluminum were inversely proportional. (3) In looking at the total elemental contents in the substrates and those in the bryophyte bodies, the following can be said. The total iron in the substrates and that in the bryophyte bodies, the copper in the substrates and that in the bryophyte bodies, total zinc in the substrates and total lead in the bryophyte bodies, and total aluminum in the substrates and total copper in the bryophyte bodies were directly proportional. The total iron in the substrates and total copper in the bryophyte bodies, total iron in the substrates and total lead in the bryophyte bodies, total iron in the substrates and total aluminum in the bryophyte bodies, and total aluminum in the substrates and total iron in the bryophyte bodies were inversely proportional.

6.4. Conclusions

The results of the chemical analyses in this chapter revealed the following. A very high correlation was found among the pH, the EC and the water-soluble iron in the substrate. Also a very high correlation was found between the EC and the water-soluble sulfate. Typically, a high correlation was observed between the water-soluble iron concentration in the substrates and the total iron content in the bryophyte bodies, and the water-soluble copper concentration in the substrates and total copper content in the bryophyte bodies. *S. ligulata* contained higher iron contents in its moss body, while *S. cataractae* contained higher copper in its moss body than the other bryophytes. *S. ligulata* seems to prefer an iron-sulfate rich environment, while *S. cataractae* does a copper-sulfate rich environment, which include a case of suppliance as metal rich solution from certain headwaters to the mosses.

Table 6-1. Analytical results of chemical elements eluted from the substrates of *Scopelophila ligulata*, *Scopelophila cataractae*, *Mielichhoferia japonica*, *Campylopus* sp. and *Jungermannia vulcanicola*

Sample		pH	EC	Fe	Cu	Zn	Pb	Cd	Al	SO_4^{2-}
1	Sl	2.68	1,871	6.42	1.17	0.93	<0.5	<0.01	5.78	78.8
2	Sl	2.59	1,190	6.71	1.47	0.07	<0.5	<0.01	0.20	32.4
3	Sc	4.54	440	0.20	7.48	0.40	<0.5	<0.01	1.57	11.0
4	Sc	4.70	153	0.15	4.01	0.06	<0.5	<0.01	0.14	4.81
5	Mj	3.53	720	2.11	1.42	1.08	<0.5	<0.01	1.81	33.2
6	Mj	4.47	132	0.69	3.29	0.09	<0.5	<0.01	0.11	5.84
7	Csp	2.51	1,201	6.70	0.13	0.05	<0.5	<0.01	0.24	29.3
8	Csp	3.25	645	1.78	0.77	0.45	<0.5	<0.01	3.75	27.9
9	Jv	2.72	1,293	4.42	0.09	0.14	<0.5	<0.01	0.74	34.4
10	Jv	4.02	609	0.18	3.40	0.46	<0.5	<0.01	7.35	66.0

Samples: 1 and 9, Miyazaki Prefecture; 2, 3, 5, 7, 8 and 10, Saitama Prefecture; 4 and 6, Tochigi Prefecture. Jv, *Jungermannia vulcanicola*; Sl, *Scopelophila ligulata*; Csp., *Campylopus* sp.; Mj, *Mielichhoferia japonica*; Sc, *Scopelophila cataractae*. Unit: EC, dS m^{-1}; Fe to SO_4^{2-}, mg L^{-1}.

Table 6-2. Analytical results of total elemental contents in the substrates of *Scopelophila ligulata*, *Scopelophila cataractae*, *Mielichhoferia japonica*, *Campylopus* sp. and *Jungermannia vulcanicola*.

Sample		Fe	Cu	Zn	Pb	Cd	Al
1	Sl	248	0.987	0.225	0.812	0.001	18.8
2	Sl	231	0.353	0.071	0.101	0.001	13.8
3	Sc	25.5	5.410	0.106	0.060	0.001	20.6
4	Sc	61.7	2.650	0.422	0.294	0.001	89.5
5	Mj	153	0.100	0.228	0.134	0.002	11.6
6	Mj	93.4	0.228	0.565	0.637	0.001	77.6
7	Csp	396	0.202	0.045	0.451	0.001	9.70
8	Csp	223	0.176	0.142	0.042	0.001	3.60
9	Jv	406	0.251	0.051	0.130	0.001	7.90
10	Jv	170	1.170	0.574	0.230	0.002	31.3

Unit: g kg^{-1} dry weight. Abbreviations are the same as Table 6-1.

Table 6-3. Analytical results of total elemental contents in the bryophyte bodies of *Scopelophila ligulata*, *Scopelophila cataractae*, *Mielichhoferia japonica*, *Campylopus* sp. and *Jungermannia vulcanicola*.

Sample		Fe	Cu	Zn	Pb	Cd	Al
1	Sl	56.4	0.127	0.298	0.084	0.003	0.473
2	Sl	57.7	0.308	0.089	0.007	0.002	0.402
3	Sc	8.90	8.877	0.444	0.046	0.001	1.194
4	Sc	3.60	3.305	0.097	0.040	0.002	1.370
5	Mj	15.5	0.230	0.407	0.067	0.004	1.203
6	Mj	12.8	3.947	0.199	0.072	0.001	1.038
7	Csp	46.7	0.315	0.298	0.002	0.001	0.165
8	Csp	20.8	0.113	0.066	0.027	0.002	0.202
9	Jv	41.6	0.056	0.035	0.012	0.003	0.384
10	Jv	25.7	0.123	0.068	0.016	0.002	0.239

Unit: g kg^{-1} dry weight. Abbreviations are the same as Table 6-1.

Table 6-4. Correlation matrix of the water-soluble elements in the substrates.

	pH	EC	Fe	Cu	Zn	Al
EC	0.839***i Root					
Fe	0.926***i Hyp	0.780***d Str				
Cu	0.748**d Root	0.431*i Qua	0.679**i Se-L			
Zn	0.042 Hyp	0.163 In-L	0.093 Hyp	0.118 In-L		
Al	0.100 Hyp	0.623**d Hyp	0.025 Hyp	0.090 Hyp	0.788***d Log	
SO_4^{2-}	0.579*i Hyp	0.904***d Hyp	0.359 In-L	0.290 Se-L	0.323 Qua	0.701**d Qua

Values are maximum coefficient of determination (R^2) for the fittest regression curve. *, **, *** Significant levels at 0.05, 0.01 and 0.001, respectively. d, directly proportional; i, inversely proportional. Exp, exponential curve; Hyp, hyperbola; In-L, inverse logarithmic curve; Log, logarithmic curve; Qua, quadratic curve; Root, square root curve; Se-L, semilogarithmic curve; Str, straight line.

Table 6-5. Correlation matrix of the elemental contents in the substrates.

	Fe	Cu	Zn	Pb	Cd
Cu	0.897***i				
	Hyp				
Zn	0.599**i	0.060			
	Hyp	Log			
Pb	0.092	0.083	0.206		
	Hyp	Qua	Log		
Cd	0.066	0.119	0.182	0.069	
	Qua	Hyp	Se-L	Qua	
Al	0.402*i	0.258	0.613**d	0.541*d	0.025
	Log	Log	Qua	Hyp	Hyp

Values and abbreviations are the same as Table 6-4.

Table 6-6. Correlation matrix of the elemental contents in the bryophyte bodies.

	Fe	Cu	Zn	Pb	Cd
Cu	0.544*i				
	Log				
Zn	0.027	0.645**d			
	Root	Hyp			
Pb	0.265	0.130	0.327		
	Exp	Exp	Se-L		
Cd	0.056	0.432*i	0.183	0.172	
	Hyp	Log	Hyp	Hyp	
Al	0.676**i	0.580*d	0.222	0.503*d	0.057
	Se-L	Se-L	Qua	Log	Hyp

Values and abbreviations are the same as Table 6-4.

Table 6-7. Correlation matrix of the elemental contents and the water-soluble elements in the substrates.

	Fe [a]	Cu [a]	Zn [a]	Pb [a]	Cd [a]	Al [a]
pH [b]	0.821***i	0.367	0.531*d	0.019	0.066	0.565*d
	Root	Root	Hyp	Qua	Hyp	Root
EC [b]	0.585**d	0.125	0.490*i	0.148	0.034	0.894***i
	Qua	Qua	Hyp	Qua	Hyp	Hyp
Fe [b]	0.641**d	0.514*i	0.456*i	0.102	0.110	0.393
	Qua	Hyp	Se-L	Qua	Hyp	Log
Cu [b]	0.923***i	0.805***d	0.686**d	0.027	0.064	0.411*d
	Exp	Str	Hyp	Se-L	Hyp	Log
Zn [b]	0.067	0.110	0.204	0.151	0.315	0.170
	Qua	Hyp	In-L	Hyp	Str	Exp
Al [b]	0.051	0.058	0.197	0.186	0.239	0.583*i
	Hyp	Hyp	Hyp	Hyp	Hyp	Hyp
SO_4^{2-} [b]	0.459*d	0.166	0.242	0.150	0.157	0.842***i
	Log	Exp	Hyp	Qua	Qua	Hyp

Values and abbreviations are the same as Table 6-4. [a], the elemental contents in the substrates; [b], the water-soluble elements in the substrates.

Table 6-8. Correlation matrix of the water-soluble elements in the substrates and the elemental contents in the bryophyte bodies.

	pH [a]	EC [a]	Fe [a]	Cu [a]	Zn [a]	Al [a]	SO_4^{2-} [a]
Fe [b]	0.862***i	0.836***d	0.880***d	0.469*i	0.078	0.194	0.715**d
	Hyp	Str	Str	Exp	Hyp	Hyp	Hyp
Cu [b]	0.600**d	0.580*i	0.388	0.889***d	0.129	0.318	0.734**i
	Qua	Log	Se-L	Qua	Hyp	In-L	Log
Zn [b]	0.054	0.031	0.003	0.307	0.297	0.039	0.034
	Hyp	Hyp	Hyp	Hyp	Qua	Hyp	Hyp
Pb [b]	0.362	0.179	0.331	0.384	0.436*d	0.088	0.128
	In-L	In-L	Hyp	Log	Hyp	Hyp	In-L
Cd [b]	0.131	0.184	0.108	0.217	0.572*d	0.230	0.278
	Qua	Log	Se-L	Qua	Qua	Hyp	Log
Al [b]	0.566*d	0.465*i	0.328	0.441*d	0.041	0.176	0.525*i
	Qua	Se-L	Root	Root	Qua	Qua	Hyp

Values and abbreviations are the same as Table 6. [a], the water-soluble elements in the substrates; [b], the elemental contents in the bryophyte bodies.

Table 6-9. Correlation matrix of the elemental contents in the substrates and in the bryophyte bodies.

	Fe[a]	Cu[a]	Zn[a]	Pb	Cd[a]	Al[a]
Fe[b]	0.668**d	0.328	0.321	0.105	0.048	0.583*i
	Log	Exp	Hyp	Qua	Hyp	Hyp
Cu[b]	0.932***i	0.817***d	0.118	0.040	0.094	0.448*d
	Hyp	Qua	Hyp	Hyp	Qua	Log
Zn[b]	0.247	0.254	0.087	0.168	0.017	0.140
	Hyp	Qua	Hyp	Hyp	Hyp	Hyp
Pb[b]	0.464*i	0.078	0.653**d	0.396	0.034	0.149
	Exp	Exp	In-L	Qua	Hyp	Exp
Cd[b]	0.243	0.193	0.054	0.029	0.228	0.104
	Hyp	Hyp	Qua	Hyp	Str	Root
Al[b]	0.654**i	0.278	0.244	0.023	0.004	0.384
	Root	Str	In-L	Hyp	Qua	Root

Values and abbreviations are the same as Table 6-4. [a], the elemental contents in the substrates; [b], the elemental contents in the bryophyte bodies.

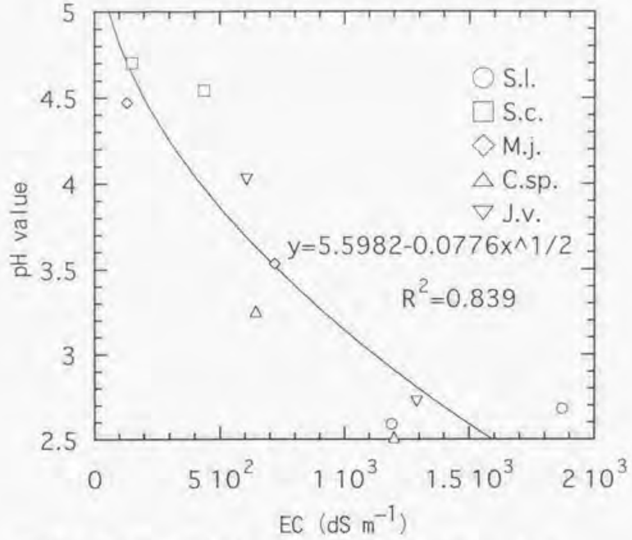

Fig.6-1. The relationship between EC and pH for the bryophyte substrates. For S.l., etc. see Table 6-1.

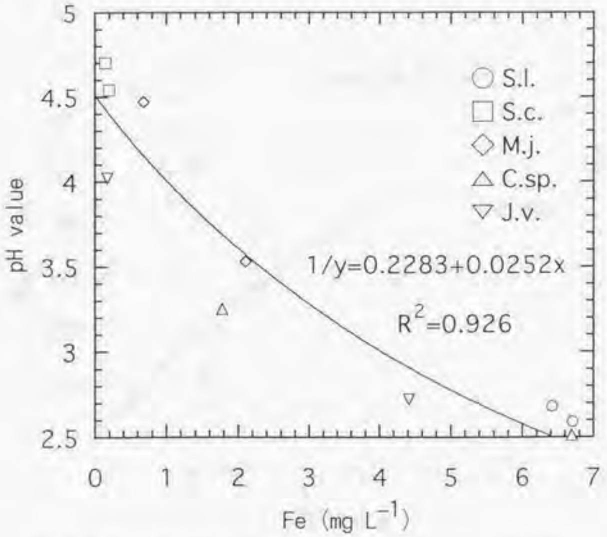

Fig.6-2. The relationship between water-soluble iron and pH for the bryophyte substrates.

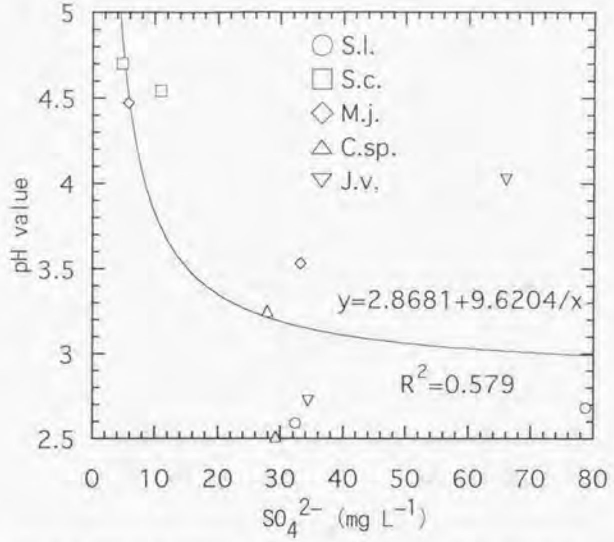

Fig.6-3. The relationship between water-soluble sulfate and pH for the bryophyte substrates.

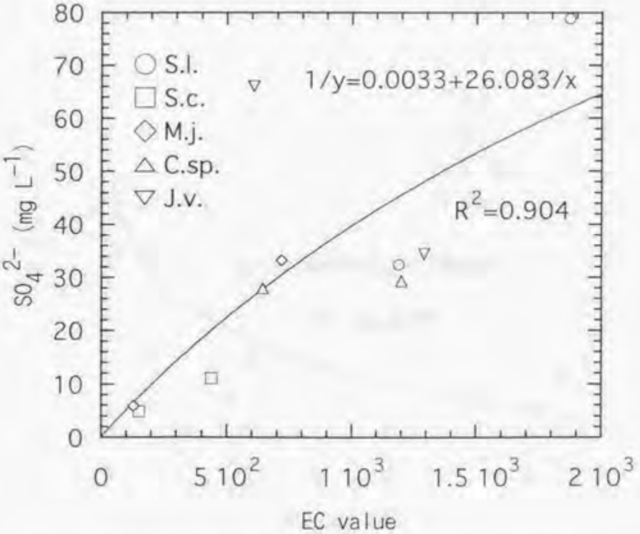

Fig.6-4. The relationship between water-soluble sulfate and EC for the bryophyte substrates.

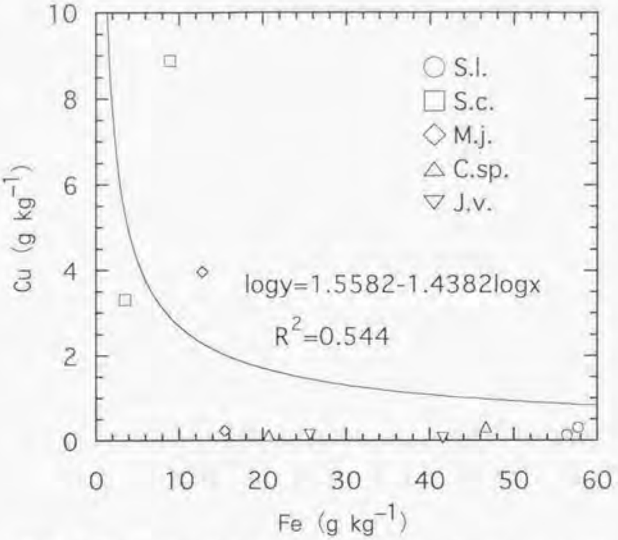

Fig.6-5. The relationship between iron content and copper content in the bryophytes.

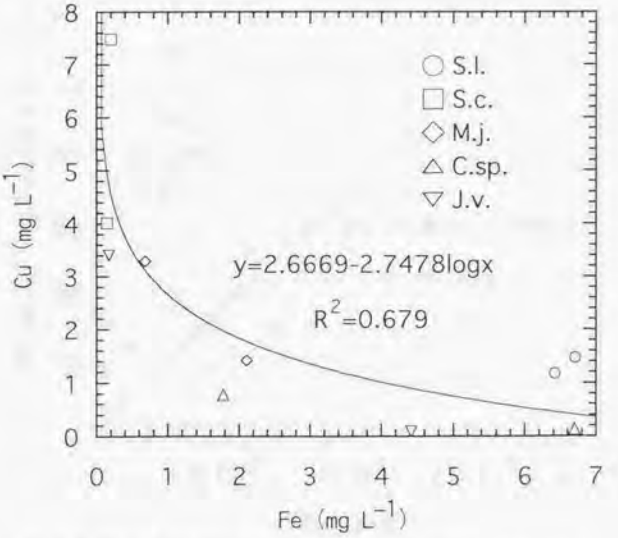

Fig.6-6. The relationship between water-soluble iron and water-soluble copper for the bryophyte substrates.

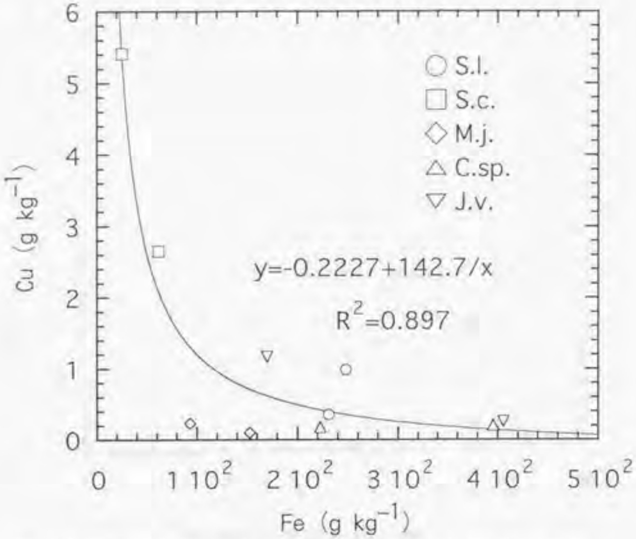

Fig.6-7. The relationship between iron and copper in the bryophyte substrates.

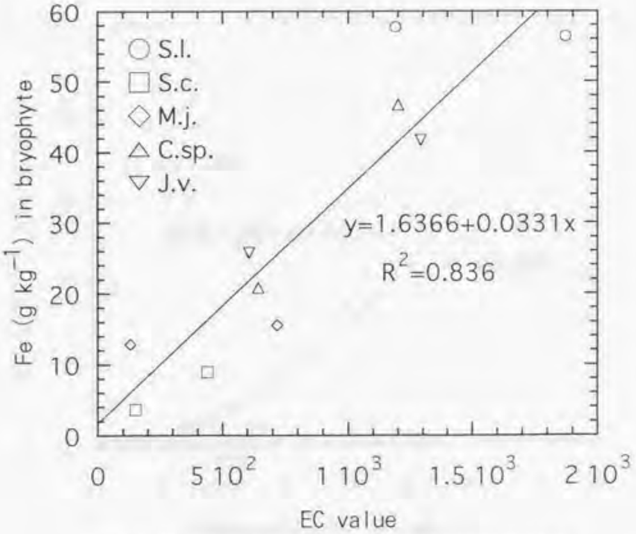

Fig.6-8. The relationship between EC and iron content in the bryophytes.

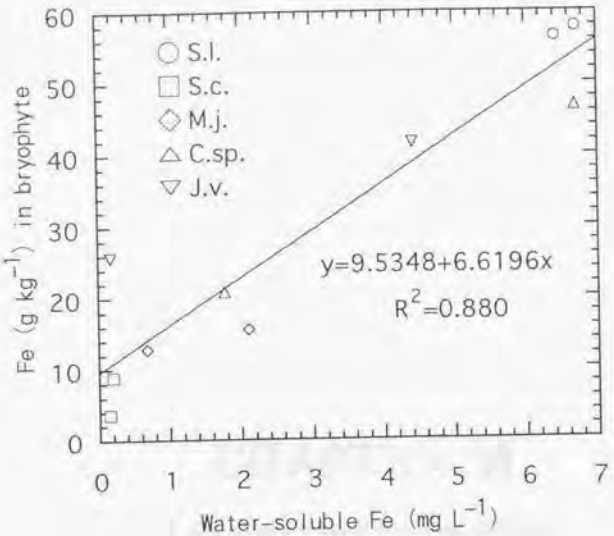

Fig.6-9. The relationship between water-soluble iron and iron content in the bryophytes.

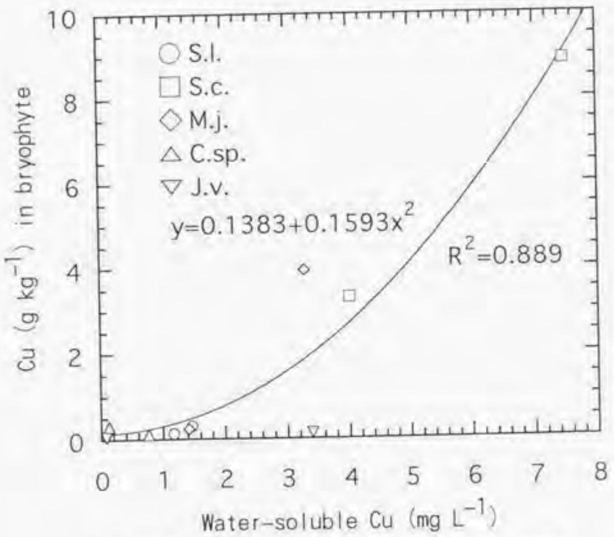

Fig.6-10. The relationship between water-soluble copper and copper content in the bryophytes.

CHAPTER Ⅶ

Comprehensive Discussion

Chapter Ⅶ

Comprehensive Discussion

7.1. Statistical review using multiple regression analysis

Whitton, Say and Jupp (1982), Say and Whitton (1983), and Wehr and Whitton (1983a, 1983b) studied the accumulation of heavy metals by aquatic bryophytes, *Scapania undulata* (L.) Dum., *Fontinalis antipyretica* Hedw., *Rhynchostegium riparioides* (Hedw.) C. Jens. and *Amblystegium riparium* (Hedw.) Br. Eur., to estimate aqueous metal in the river. They used multiple stepwise regression analysis to quantify the apparent influence of environmental variables in the effluent water. Their results by multiple regression analysis were much better than those by bivariate regression analysis

Multiple regression analysis according to established methods (Akimoto 1984; Fujimori 1986) was applied to calculate the relationships between the water-soluble elements in all 116 substrate samples in these studies. When the iron, copper and zinc concentrations were taken as the independent variables and the sulfate concentration as the dependent variable, a high multiple correlation coefficient (R=0.845, p<0.001) was obtained. Also, when the same three metal concentrations above were taken as the independent variables and the pH value as the dependent variable, high multiple correlation coefficient (R=0.645, p<0.001) resulted. Furthermore, when the sulfate concentration was added to the independent variables, a slightly higher multiple correlation coefficient (R=0.653, p<0.001) was determined. These heavy metal ions, in conjunction with the sulfate ions, appear to be a major factor in fixing the pH value of each bryophyte substrate.

The multiple linear regression analysis was also used to calculate the relationships between the water-soluble elements and the iron and copper contents in the bryophyte bodies. The samples were 31 of *S. ligulata*, *S. cataractae*, *M. japonica*, *Campylopus* sp. and *J. vulcanicola*, which were analyzed both water-soluble elements and elemental contents in these studies. When the iron, copper, zinc and sulfate concentrations, and the pH value were taken as the independent variables and the iron content in the bryophytes as the dependent variable, a high multiple correlation coefficient (R=0.722, p<0.001) was obtained. Also, when the same five elements above were taken as the independent variables and the copper content in the bryophytes as the dependent variable, high multiple correlation coefficient (R=0.777, p<0.001) was obtained. In case of the zinc content and the lead content in the bryophytes as the dependent variable, multiple correlation coefficient was R=0.550 (p<0.01) and R=0.409 (p<0.05), respectively. These results were better than those by simple bivariate regression analysis. Especially, the iron and copper content in the bryophytes was well predicted by each multiple regression analysis.

7.2. General review

Persson (1956) and Noguchi (1956) pointed out that the pH values of the substrates of "copper mosses" were lower than those of non-copper mosses. Noguchi (1956) mentioned that authors such as Schatz (1955), Persson (1948) and others seemed to put more weight on the pH of the soils where *Scopelophila ligulata* (Spruce) Spruce grew than on the physiological role of metallic ions such as copper and iron. Though Noguchi (1956) tried to confirm this by carrying out the culture test in the laboratory, the results were not satisfactory.

Schatz (1955) suggested that "sulfur mosses" might be a more appropriate term than "copper mosses" given the generally sulfur-rich substrates. Schatz's opinion was based on comparative biochemical considerations regarding the photosynthesis. He speculated that sulfide and sulfur might be utilized as hydrogen donors for photosynthesis by these "copper" or "sulfur mosses", thus these would be a distinct physiological group constituting a "missing link" between anaerobic photosynthetic sulfur bacteria and aerobic green plants. Though Schatz's speculation seems to be a very attractive one, it has never been confirmed experimentally (Noguchi and Ochi 1956; Nagano et al. 1969; Brown 1982).

Shaw (1987) studied the substrates of *S. cataractae* at 6 sites in the eastern United States of America. He pointed out 3 types of heavy metal contents in those substrates as follows. First, one of six substrates of *S. cataractae* contained little copper (12.0 mg kg^{-1}) but was instead enriched with iron (328.3 mg kg^{-1}). Second, four substrates were indeed enriched with copper (170.0-4,400 mg kg^{-1}) but they also generally contained high concentrations of zinc (60.0-1,800 mg kg^{-1}) and lead (4.7-9,000 mg kg^{-1}). Third, the other substrate was enriched with copper (163.8 mg kg^{-1}) but it also contained a very high concentration of iron (250.0 mg kg^{-1}). Thus, Shaw (1987) suggested that *S. cataractae* would be better considered a "metal moss" than a "copper moss."

My findings, through a series of studies, would coincide with Shaw's opinion, although the difference between *S. ligulata* and *S. cataractae* was distinguished clearly. The habitat segregation between *S. ligulata* and *S.*

cataractae was discerned. That is, *S. ligulata* would prefer an iron-sulfate environment, while *S. cataractae* would prefer a copper-sulfate environment, which include a case of suppliance as each metal rich solution from certain headwaters. Thus, "metal sulfate mosses" would be better for the name of those mosses.

7.3. Possible availability of "copper mosses" for environmental restoration and environmental monitoring

The National Risk Management Research Laboratory of the U.S. Environmental Protection Agency is attempting to demonstrate and evaluate phytoremediation efficacy for lead contamination in the field using *Brassica juncea* (Indian mustard) at a site in Trenton, New Jersey (Rock 1997). Growth chamber studies were conducted by Blaylock et al. (1997). *Zea mays* (corn) and *Ambrosia artemisiifilia* (ragweed) are also considered to be good lead accumulators (Raskin et al 1997). Chaney et al. (1997) mentioned that a hypertolerance to metals was the key plant characteristic required for hyperaccumulation.

"Copper mosses" have long been considered to be related to their substrates, which contain large amounts of heavy metals such as copper, zinc, iron and lead. The reason why "copper mosses" grow well in such conditions, which are generally harmful to plants, is not well understood. Thorough investigation of the ecology and environment of "copper mosses" is useful in the application to environmental problems caused by heavy metals.

The photos in Appendixes of this thesis are some views around the coal mine area in Santa Catalina, Brazil. Within that area, there are many abandoned mines, in which the open pit diggings have been one of the critical point sources of water pollution around there. Japan International Cooperation Agency (JICA) has assisted Brazilian Government for mine pollution control project in which author was engaged for about ten years. That project included the effluent water quality monitoring at typical point in the mine area. The monitoring program consisted of many automatic measuring eqipments which were state-of-the-art, while those were hardly maintained in the developing country. Thus, if we could have an effectual medium to estimate the environmental condition instead of the mechanical measuring which requires much labor, that method will be most favorable for all of us engaged in environmental issues. As shown in photographs, many mats of bryophytes were spontaneously growing around the drainage where drain water was strongly acidic and contained much iron, sulfate, BOD substances, etc. Those bryophytes probably accumulated certain metals in their bodies. Thus, those would be possibly used in the field of biological monitoring as taking account of cost-benefit analysis. Those also would be likely candidates for further study in the field of bioremediation for water pollution, and furthermore in that of phytoremediation for soil or lithosol contamination, which can be hardly remedied by ordinary method, in those abandoned mine areas.

SUMMARY

SUMMARY

Chemical Elements in the Substrates of *Scopelophila ligulata* (Spruce) Spruce and Other Mosses Growing on the Same Black Slate

Scopelophila ligulata (Spruce) Spruce, a species of "copper moss", is known to prefer a strongly acidic substrate. The soluble chemical elements in the substrate of *S. ligulata* and those of four other non-copper mosses which grow on the vertical rock face of the same black slate (Chichibu Paleozoic strata), were examined. The following results were obtained. The pH and the concentrations of water-soluble iron and sulfate in the substrates of *S. ligulata* showed a significant difference from those of the other mosses. A high correlation was found among the pH, the concentration of water-soluble iron and that of water-soluble sulfate. The concentrations of water-soluble copper and zinc in the substrate of *S. ligulata* were much lower than those of water-soluble iron.

Chemical Elements in the Substrate of *Scopelophila ligulata* (Spruce) Spruce and the associated mosses growing in the different habitat

Scopelophila ligulata (Spruce) Spruce has been called one of the species of "copper mosses". Authors focused on the substrate pH and the water-soluble elements such as iron, copper, zinc and sulfate found in the substrates of *S. ligulata* and other non-copper mosses which grew on same rocks but segregate each other on vertical cliff faces and on cliff shelves, respectively. The following results were obtained. The pH value and the water-soluble iron and sulfate concentrations of the substrates showed a significant difference ($p<0.01$) between the *S. ligulata*, which grew on cliff faces, and other mosses found on cliff shelves immediately above the habitat of *S. ligulata*. Very high correlations ($p<0.001$) between these three elements were confirmed. The concentrations of water-soluble copper and zinc in the substrates of *S. ligulata* were much lower than those of water-soluble iron. There was no significant difference in the water-soluble copper and zinc concentrations between the substrates of *S. ligulata* and those of the other non-copper mosses.

Chemical Elements in the Substrates of *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch.

Genera *Scopelophila* and *Mielichhoferia* include some classical "copper mosses". Authors focused on the water-soluble elements found in the substrates of *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. The following results were obtained. The *t*-tests for the pH value, and the water-soluble iron, copper, zinc and sulfate concentrations showed significant differences between *S. cataractae* and *M. japonica*. With respect to the water-soluble elements in the substrates of *S. cataractae* and *M. japonica*, high correlations were observed between each combination of two components. The iron and the zinc concentrations ($p<0.001$), the iron and the sulfate concentrations ($p<0.001$), the copper and the zinc concentrations ($p<0.01$), the copper and the sulfate concentrations ($p<0.001$), and the zinc and the sulfate concentrations ($p<0.001$) were directly proportional. The pH value and the iron concentration ($p<0.001$), the pH and the copper concentration ($p<0.05$), the pH and the zinc concentration ($p<0.05$), and the pH and the sulfate concentration ($p<0.01$) were inversely proportional.

Contents of Heavy Metal Elements in Copper Mosses: *Scopelophila ligulata* (Spruce) Spruce, *Scopelophila cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. and their Substrates.

Scopelophila ligulata (Spruce) Spruce, *S. cataractae* (Mitt.) Broth. and *Mielichhoferia japonica* Besch. (*Mielichhoferia mielichhoferi* (Hook.) Wijk et Marg. var. *japonica* (Besch.) Wijk et Marg.) are well known "copper mosses" which show peculiar ecological characteristics consisting of a preference for metal-rich soils, lithosols, rocks, etc. as their substrates. The total and water-soluble element contents in the substrates and heavy metal contents in the moss body were analyzed in this study. The results of these chemical analyses were as follows: The iron contents in the moss body and substrate of *S. ligulata* were higher than those of *S. cataractae* and *M. japonica*. On the contrary the copper contents of the former were lower than those of the latter. The pH values of substrate of *S. ligulata* were lower than those of *S. cataractae* and *M. japonica*. The concentrations of water-soluble iron and sulfate in the substrate of *S. ligulata* were higher than those of *S. cataractae* and *M. japonica*. On the contrary, the water-soluble copper concentrations in the substrate of *S. ligulata* were lower than those of the others. Although in the substrate of *S. cataractae* the iron content was several times higher than the copper content, in the moss body the copper content was higher than iron content.

Chemical species of iron in the bryophyte substrates and their pH values, and efficiency of cleaning methods for contaminant of bryophytes

The results of the chemical analyses in this chapter revealed the following. It seemed that Fe^{3+} and SO_4^{2-} ions were most important factors to give a definite pH value of the bryophyte substrates. *S. ligulata, Campylopus* sp. and *J. vulcanicola* accumulated a huge iron in their body in due consideration of their contaminant from substrate particles. Ignition loss and the concentration of titanium in the bryophyte may be a good indicator of contaminant in certain cases. The concentration of copper in all three bryophytes washed with 2% EDTA solution was higher than those without washing. These indicated the accumulation of copper into the cell of bryophyte. The solution of 2% EDTA was a favorable agent to eliminate the bryophyte contaminant from substrate particles.

Copper Mosses and Their Substrates in Mineralized Areas

Scopelophila ligulata (Spruce) Spruce, *S. cataractae* (Mitt.) Broth., and *Mielichhoferia japonica* Besch. are famous "copper mosses". In addition, *Jungermannia vulcanicola* Steph. and *Campylopus sp.*, which is a close relative of *C. schwarzii* Schimp., seem to be related to the heavy metal in their rock and soil hosts. In this chapter, the study was focused on the relationships between the above-mentioned mosses and their substrates in several mineralized areas in Japan. The water-soluble elemental concentrations and total elemental contents in the substrate and the elemental contents in the bryophyte bodies were analyzed. The following results were obtained. The results of the chemical analyses in this chapter revealed the following. A very high correlation was found among the pH, the EC and the water-soluble iron in the substrate. Also a very high correlation was found between the EC and the water-soluble sulfate. Typically, a high correlation was observed between the water-soluble iron concentration in the substrates and the total iron content in the bryophyte bodies, and the water-soluble copper concentration in the substrates and total copper content in the bryophyte bodies. *S. ligulata* contained higher iron contents in its moss body, while *S. cataractae* contained higher copper in its moss body than the other bryophytes. *S. ligulata* seems to prefer an iron-sulfate environment, while *S. cataractae* does a copper-sulfate environment regardless of location.

Comprehensive Discussion

Multiple regression analysis according to established methods was applied to calculate the relationships between the water-soluble elements in all 116 substrate samples in these studies. When the iron, copper, zinc and sulfate concentrations were taken as the independent variables and the pH value as the dependent variable, high multiple correlation coefficient (R=0.653, p<0.001) was determined. The multiple linear regression analysis was also used to calculate the relationships between the water-soluble elements and the iron and copper contents in the bryophyte bodies. The samples were 31 of *S. ligulata*, *S. cataractae*, *M. japonica*, *Campylopus* sp. and *J. vulcanicola*, which were analyzed both water-soluble elements and elemental contents in these studies. When the iron, copper, zinc and sulfate concentrations, and the pH value were taken as the independent variables and the iron content in the bryophytes as the dependent variable, a high multiple correlation coefficient (R=0.722, p<0.001) was obtained. Also, when the same five elements above were taken as the independent variables and the copper content in the bryophytes as the dependent variable, high multiple correlation coefficient (R=0.777, p<0.001) was obtained. In case of the zinc content and the lead content in the bryophytes as the dependent variable, multiple correlation coefficient was R=0.550 (p<0.01) and R=0.409 (p<0.05), respectively. These results were better than those by simple bivariate regression analysis. Especially, the iron and copper content in the bryophytes was well predicted by each multiple regression analysis. These water-soluble heavy metal ions, in conjunction with the sulfate ions, appear to be a major factor in fixing the pH value of each bryophyte substrate, and these water-soluble elements and pH value could affect the amount of heavy metals accumulated by each bryophyte. The habitat segregation between *S. ligulata* and *S. cataractae* was discerned. *S. ligulata* would prefer an iron-sulfate environment, while *S. cataractae* would prefer a copper-sulfate environment, which include a case of suppliance as each metal rich solution from certain headwaters. "Copper mosses" have long been considered to be related to their substrates, which contain large amounts of heavy metals such as copper, zinc, iron and lead. The reason why "copper mosses" grow well in such conditions, which are generally harmful to plants, is not well understood. Further investigation of the ecology and environment of "copper mosses" is useful in the application to environmental issues on heavy metals.

APPENDIXES

Photo 1. Abandoned coal mine in Santa Catalina, Brazil. The basin has been formed by rainwater in the open pit digging area. The pond water had very strong acidity caused by sulfate.

Photo 2. Drainage in the coal mine area.

Photo 3. Drained water from abandoned coal mine.

Photo 4. Drained water from abandoned coal mine. The bryophyte was spontaneously growing around the drainage area.

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Photo 5. Environmental monitoring effort around the coal mine area. Effluent water was strongly acidic and contained much iron, sulfate and BOD substance.

6'93

Photo 6. Effluent water monitoring house, which consisted of much monitoring equipments, by the technical cooperation of JICA for Brazilian Government in Santa Catalina.

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論文の内容の要旨
(SUMMARY *in Japanese*)

論文の内容の要旨

応用生命化学専攻

平成9年度博士課程（社会人特別選抜）入学

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論文題目 STUDY ON THE RELATIONSHIP BETWEEN "COPPER MOSSES" AND THEIR SUBSTRATES.

（和訳　いわゆる銅ごけと呼ばれる蘚苔類と基物土壌との関係についての研究）

欧米では，重金属類を含んだ鉱化地帯を好んで生育の場としているように思える，ある特定の蘚苔類が古くから知られている。これらは，通称「copper mosses（銅ごけ）」と呼ばれ，その特異な生態学的特性について研究がなされてきた。そして，我が国においても，*Scopelophila ligulata*（イワマセンボンゴケ），*Scopelophila cataractae*（ホンモンジゴケ）などについて研究がなされている。「銅ごけ」が着生する基物としては重金属類に富む土壌，岩屑土，岩石などがあるが，「銅ごけ」とこれら基物中の重金属類との関係についての系統的な研究は少ない。その理由として次の様な事項があげられる。①かつて分析技術が未発達であったこと，②学際領域の研究分野であること，③蘚苔類が飼料植物でないため研究そのものに対して多くの興味が持たれなかったこと，④蘚苔類の同定が難しいこと，⑤「銅ごけ」は不連続な生育分布をしており希少であること，⑥重金属を多く含む基物に由来する粒子による植物体へのコンタミネーションへの対処が，湿式分析で行う場合，非常に難しかったこと。この研究では，今まで実施された「銅ごけ」に関する研究を追跡するとともに，今まで注目されなかった基物の水溶性成分と「銅ごけ」生育の関係，及び植物体の洗浄に関する事項，また一概に「銅ごけ」とされる蘚苔類の種間での違いなどについて追求し，「銅ごけ」の重金属を濃縮する能力に着目して，環

境修復や環境モニタリングへの応用の可能性について検討した。試料としては蘚苔類の生育直下の土壌部分を用い，本研究ではこれを基物とする。

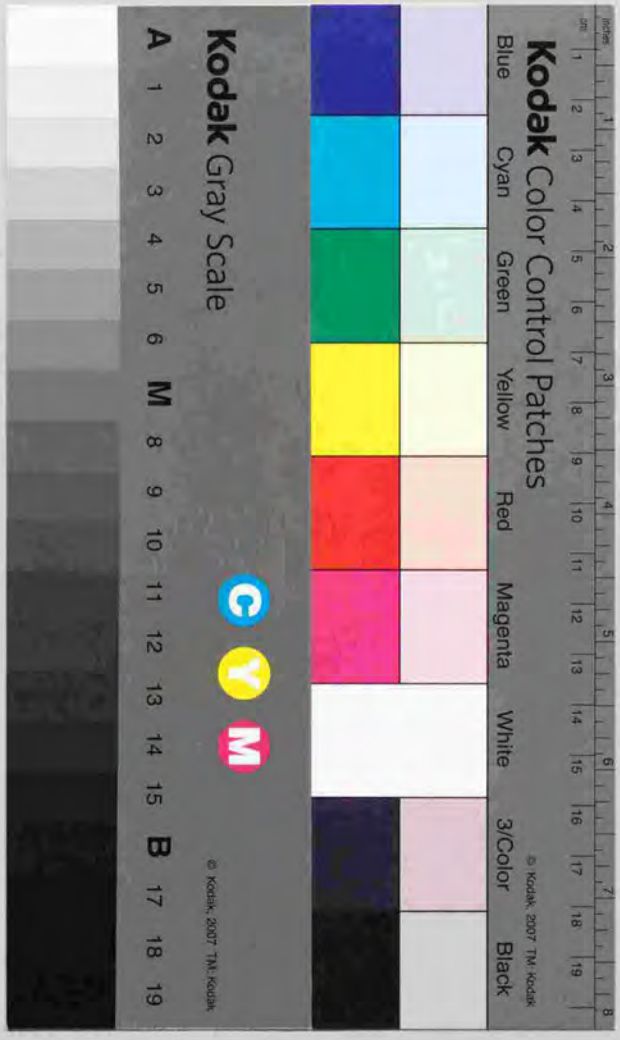
1．「銅ごけ」と基物の水溶性成分

「銅ごけ」と呼ばれる蘚苔類の中で，イワマセンボンゴケ（*Scopelophila ligulata* (Spruce) Spruce）は強酸性の基物を好むことが知られている。*S. ligulata* が生育している黒色粘板岩上の基物の水溶性成分について研究するとともに，*S. ligulata* の群落と同じ岩面で相接して生育しているが，「銅ごけ」ではない *Campylopus umbellatus* (Arm.) Par., *Dicranella heteromalla* (Hedw.) Schimp., *Leucobryum juniperioideum* (Brid.) C. Muell., *Pogonatum nipponicum* Nog. et Osada など４種の蘚類の基物についても比較，検討した。基物の pH 及び水溶性の Fe，SO_4^{2-}濃度に関しては，*S. ligulata* とその他の蘚類では，有意な違いが見られた（$p<0.01$）。基物の pH，水溶性の Fe 及び SO_4^{2-}の３成分の間で，それぞれ非常に強い相関関係（$p<0.001$）が確認された。*S. ligulata* の基物において水溶性の Cu と Zn が検出されたが，それらはいずれも Fe 濃度に比較して１桁低い濃度であった。一方，*S. ligulata* 以外の４種の蘚類の基物では，ほとんど Cu と Zn の溶出は見られなかった。次に，*S. ligulata* は垂直岩面に良く生育することが知られているが，*S. ligulata* が生育する岩面とその直上で *S. ligulata* 以外の蘚苔類が生育する岩棚の条件の違いを把握するため，調査・研究を実施した。岩面に生育する *S. ligulata* の基物と岩棚上に生育するその他の蘚苔類の基物の pH，水溶性 Fe 濃度，及び水溶性 SO_4^{2-}濃度については有意な違い（$p<0.01$）が認められた。そして基物の pH，水溶性 Fe 濃度，及び水溶性 SO_4^{2-}濃度の３成分の間で，それぞれ非常に強い相関関係（$p<0.001$）が確認された。また，*S. ligulata* の基物の水溶性 Cu 濃度と水溶性 Zn 濃度は，同じ基物の水溶性 Fe 濃度に比較して非常に低い値であった。この水溶性 Cu 濃度と水溶性 Zn 濃度については，*S. ligulata* の基物とその他の蘚苔類の基物との間には有意な違いは見られなかった。更に，*S. ligulata* と同じく「銅ごけ」に含められるホンモンジゴケ（*Scopelophila cataractae* (Mitt.) Broth.）とホソバゴケ（*Mielichhoferia japonica* Besch.）を対象として，基物水溶性成分の濃度の違い，そして各水溶性成分間の関係を研究した。pH 及び水溶性 Fe，Cu，Zn，SO_4^{2-}濃度に関する t 検定の結果は，*S. cataractae* と *M. japonica* の基物の間に有意な違いがあることを示した。*S. cataractae* と *M. japonica* の基物の pH と各水溶性成分については，0.1％の有意水準で Fe 濃度と Zn 濃度，Fe 濃度と SO_4^{2-}濃度，Cu 濃度と SO_4^{2-}濃度，Zn 濃度と SO_4^{2-}濃度の間に，1％の有意水準で Cu 濃度と Zn 濃度の間に，それぞれ正の相関関係が見られた。また，0.1％の有意水準で pH と Fe 濃度の間に，1％の有意水準で pH と SO_4^{2-}濃度の間に，そして５％の有意水準で pH と Cu 濃度，pH と Zn 濃度の間に，それぞれ負の相関関係が見られた。「銅ごけ」の間でも pH に有意な違いが見られるので，この原因を追求するため，*S. ligulata*，*Campylopus* sp.， *Jungermannia vulcanicola* Steph.を用いて，基物中の Fe の形態について研究した。その結果，基物中の水溶性成分における Fe^{3+} と SO_4^{2-} 及びそれらの結びつきが基物 pH を決定するのに重要な要因である事が推察された。以上，この一連の研究で実施した１１６試料の水溶性成分の分析結果を重回帰分析により解析した。Fe，Cu，Zn を独立変数，SO_4^{2-} を従属変数とした場合，非常に強い相関（$p<0.001$）が得られた。これら水溶性４成分を独立変数とし，pH を従属変数とした場合も強い相関（$p<0.001$）が得られた。基物の pH 値はこれら水溶性重金属と SO_4^{2-} に影響を受けていると考えられ，特に Fe の影響が強いと判断された。

２．「銅ごけ」の生育環境と環境修復，環境モニタリングへの応用について

S. ligulata，*S. cataractae* 及び *M. japonica* を用いて，その基物と植物体中の重金属含有量の他，基物の水溶性成分を測定し，比較検討を行った。*S. ligulata* の植物体中及び基物中の Fe 含有量は *S. cataractae* や *M. japonica* に比べて高い値（$p<0.01$）であった。一方で，前者の Cu 含有量は後者に比べ低い値であった。*S. ligulata* の基物 pH は，*S. cataractae* や *M. japonica* に比較して有意に低かった（$p<0.01$）。*S. ligulata* の基物中の水溶性の Fe と SO_4^{2-} は，*S. cataractae* や *M. japonica* の基物中のそれらに比べ高かったが，水溶性の Cu は逆に後者に比べ低かった（$p<0.01$）。*S. cataractae* の基物中の Fe 含有量は Cu のそれに比べ数倍高い値であるが，植物体中では逆に Cu の含有量が Fe の含有量を上回っていた。この研究では，まず従来から行われている植物体の洗浄方法を前処理として３種の蘚類を分析したが明確な洗浄効果が得られなかったため，Fe の固着がかなり見られた *S. ligulata*，*Campylopus* sp.，*Jungermannia vulcanicola* Steph.を用いて，植物体外側に付着する汚染を取り除く洗浄方法について検討した。その結果，２％の EDTA 溶液で３０分間振とうすることが植物体に付着した汚染物質を取り除くのに有効である事が判明した。その結果，植物体には数％に及ぶ Fe が３種類の蘚苔類に含有されていた。また，Cu については EDTA 洗浄しない試料より洗浄した試料の方が含有量が高い傾向を示し，植物体によって濃縮されている事が示された。そして Ignition loss 及び Ti は植物体外側に付着している汚染物量の指標になる事を確認した。次に，日本の複数の鉱化地帯に生育する *S. ligulata*，*S. cataractae*，*M. japonica*，*Campylopus* sp.，*J. vulcanicola* を用いて，それらの生育基物中の水溶性成分，含有量及び植物体中の含有量成分について比較検討を行った。基物の水溶性成分間では，pH，Fe，EC の３者間に強い相関関係（$p<0.001$）が見られた。EC はまた，水溶性の SO_4^{2-} との間にも強い正の相関（$p<0.001$）が認められ，EC は水溶性 SO_4^{2-} の指標になり得ると考えられた。基物の水溶性成分，含有量成分及び植物体中の含有量成分を通じて，特徴的に Fe 濃度と Cu 濃度との間に負の関係が認められた。基物の水溶性 Fe 濃度と植物体中の Fe 含有量との間に，また水溶性 Cu 濃度と植物体中の Cu 含有量との間に非常に強い正の相関関係（$p<0.001$）が認められた。

「銅ごけ」の中でも，*S. ligulata* は，その植物体中の Fe 含有量は他と比較すると高く，一方で *S. cataractae* は Cu 含有量が他の蘚苔類よりも明らかに高かった。水溶性成分と蘚苔類の植物体分析を同時に実施している３１試料の結果を，水溶性 Fe，Cu，Zn，SO_4^{2-}及び pH を独立変数とし，植物体中の各重金属をそれぞれ従属変数として重回帰分析を実施したところ，これら水溶性５成分と Fe 及び Cu の間にそれぞれ強い相関（$p<0.001$）が得られた。蘚苔類の Fe と Cu の吸収にこれら水溶性成分と pH が因子として深く関わっていることが考えられた。*S. cataractae* は，直下の基物がこの蘚苔類の通常の生育 pH 域になくても，水溶性成分として Cu と SO_4^{2-}が供給される所で生育していることがある。一概に「銅ごけ」とされる蘚苔類でも，その生育環境や植物体中の重金属含有量に違いが見られ，*S. ligulata* は，水溶性成分として Fe と SO_4^{2-}が豊富に供給される環境に，また *S. cataractae* は水溶性成分として Cu と SO_4^{2-}が供給される環境に，それぞれ好んで生育している事が推測された。それぞれの水溶性成分の供給の如何がこれら蘚苔類の棲息を決定しているように考えられた。これら「銅ごけ」と呼ばれる蘚苔類は，その重金属を濃縮しうる能力を環境修復や環境モニタリングに応用することが考えられる。特に，排水管理への利用ついては，必要性はあるが物理的にモニタリング設備や排水処理プラントが設置できないような狭小な場所への適用や，あるいはコスト面や運転管理面で，現在の日本で一般的に利用されている設備が設置できないような発展途上国での，重金属類を含んだ排水の管理に応用されることが期待される。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