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RESEARCH ARTICLE

Response of secondary metabolites to Cu in the Cu-hyperaccumulator lichen *Stereocaulon japonicum*

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Abstract

Lichen secondary metabolites are known to be associated with heavy metal uptake and tolerance in lichens. Understanding the relationship between their secondary metabolites and heavy metals in them is important for clarifying the mechanisms of their heavy metal accumulation and tolerance. To determine the relationships between the concentrations of secondary metabolites and Cu in the Cu-hyperaccumulator lichen *Stereocaulon japonicum* and to clarify its response to Cu, we collected Cu-contaminated and uncontaminated samples of the lichen and determined relative concentrations of secondary metabolites and concentrations of Cu, K, glucose, and sugar alcohols in them. We found significant negative correlations between the relative concentrations of secondary metabolites—atranorin and stictic acid—and the concentration of Cu. These negative correlations can be interpreted in one of two ways: (a) *S. japonicum* itself reduced the relative concentrations of secondary metabolites in response to the increase of Cu concentration or (b) its carbon and energy metabolism was damaged by Cu stress, resulting in the reduction of the relative concentrations of secondary metabolites. The analysis of K, glucose, and sugar alcohols showed no effect of Cu on these concentrations, which means that the carbon and energy metabolism was not damaged by Cu stress. Therefore, the negative correlations can be interpreted that *S. japonicum* itself reduced the relative concentrations of secondary metabolites with the increase of Cu concentration. These findings provide a deeper understanding of the response of secondary metabolites to Cu in the lichen.

Keywords *Stereocaulon japonicum* · Copper hyperaccumulator · Lichen secondary metabolite · Atranorin · Stictic acid · Glucose · Sugar alcohol · Carbon and energy metabolism

Introduction

Lichen secondary metabolites are important factors in metal homeostasis and pollution tolerance in lichens; moreover, some of the metabolites are associated with heavy metal uptake and tolerance (Purvis et al. 1987; Molnár and Farkas 2010). Hence, by examining these abiotic roles of the metabolites, the mechanisms of heavy metal accumulation and tolerance in lichens must be understood, which will provide useful information to use lichens for monitoring of heavy metal pollution. These abiotic roles, however, have not been entirely explored (Molnár and Farkas 2010).

Results of relationships between concentrations of secondary metabolites and heavy metals in lichens remain controversial (Pawlik-Skowrońska and Bačkor 2011; Hauck et al. 2013; Nakajima et al. 2015; Kalinowska et al. 2015; Gauslaa et al. 2016); in particular, concentrations of lichen secondary metabolites have been positively (Pawlik-Skowrońska and Bačkor 2011; Hauck et al. 2013; Gauslaa et al. 2016) or negatively (Gauslaa et al. 2016) correlated with those of heavy metals in lichens. This contradiction indicates that the relationships between concentrations of secondary metabolites and heavy metals in lichens are not fully understood. To clarify

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the relationships, further study is needed. The understanding of the relationships will help to reveal the mechanisms of heavy metal accumulation and tolerance in lichens.

Stereocaulon japonicum Th. Fr. is a suitable species to investigate the relationships between lichen secondary metabolites and Cu as follows. *Stereocaulon* species commonly occur in heavy metal-polluted areas (Nash 1990; Dobson 2005); furthermore, they are known to be a heavy metal indicator (Smith 2013). *S. japonicum* has been recently found to be a Cu-hyperaccumulator lichen (Nakajima et al. 2013, 2015), since the highest concentration of Cu in the lichen samples was about 200 times that in the control samples (6.6 ± 1.6 mg/kg dry weight) (Nakajima et al. 2015). *S. japonicum* is endemic to East Asia (Yoshimura 1974; Aptroot and Seaward 1999; Huang 2010) and is common in Japan and South Korea (Nakajima et al. 2015; Park et al. 2018). Major secondary metabolites of the lichen are atranorin, stictic acid, and norstictic acid (Yoshimura 1974; Park et al. 2018) (Fig. 1). Atranorin is one of the most common secondary metabolites, and it is contained in almost all species of *Stereocaulon* genus (Ismed et al. 2018). These three metabolites are reported to be associated with heavy metal adsorption: atranorin in a lichen (*Hypogymnia physodes* (L.) Nyl.) has been decreased after transplantation to a heavy metal-contaminated site (Białońska and Dayan 2005); stictic acid in another lichen (*Lobaria pulmonaria* (L.) Hoffm.) has been correlated with heavy metals (Gauslaa et al. 2016); norstictic acid in lichens (*Acarospora smaragdula* (Wahlenb.) Massal. and *Lecidea lactea* Flörke ex Schaerer (synonym of *Lecidea lapicida* (Ach.) Ach. var. *pantherina* Ach.)) can form a complex with Cu (Purvis et al. 1987), suggesting that the complex can also be formed in *S. japonicum*. Thus, it is a suitable species to investigate the relationships between lichen secondary metabolites and Cu. In a previous study of *S. japonicum*, the relative concentration of total secondary metabolites in Cu-contaminated samples of the lichen was lower than that in uncontaminated samples (Nakajima et al. 2015). However, carbohydrates such as ribitol and mannitol, which are the basic source for carbon and energy metabolism in lichens (Ahmadjian 1993; Eisenreich et al. 2011), were not investigated, and the relative concentration of each secondary metabolite was not determined. Hence, the relationship between secondary metabolites and Cu in *S. japonicum* has not been clarified.

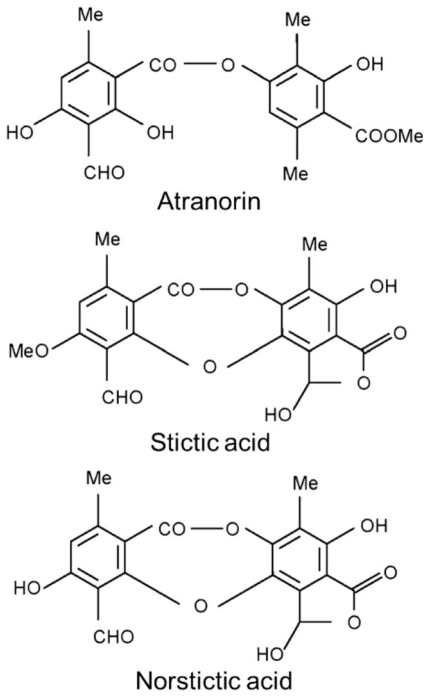

Fig. 1 Structures of major lichen secondary metabolites in *S. japonicum*

Concentrations of glucose and sugar alcohols in lichens can be used as indicators of heavy metal stress (Roser et al. 1992; Huang et al. 2017). The basic metabolic network of lichens is characterized by CO_2 fixation (photosynthesis) by the green algae, cyanobacteria, or both affording carbohydrates—ribitol and glucose—that transferred to the mycobionts, which convert those carbohydrates to mannitol and arabitol, as the basic source for carbon and energy metabolism (Ahmadjian 1993; Eisenreich et al. 2011). *S. japonicum* has phyllocladia and cephalodia (Yoshimura 1974; Park et al. 2018), in which green algae and cyanobacteria produce ribitol and glucose, respectively (Ahmadjian 1993; Eisenreich et al. 2011). Heavy metal stress on a lichen may cause the reduction of its carbon and energy metabolism, resulting in decreases in carbohydrates. This is consistent with the fact that the concentration of total reducing sugars in a lichen *Evernia prunastri* (L.) Ach. was decreased by Cu treatments (Vantová et al. 2013). Hence, concentrations of glucose and sugar alcohols (ribitol, mannitol, and arabitol) in lichens can be used as indicators of heavy metal stress.

Moreover, the concentration of K in lichens can be another indicator of Cu stress (Branquinho et al. 2011). The concentration of K in experimentally Cu-contaminated lichen samples has been negatively correlated with the concentration of Cu (Cabral 2002; Branquinho et al. 2011), indicating the membrane damage due to Cu stress (Tarhanen et al. 1999). Thus, the concentration of K in Cu-contaminated lichen samples can be a useful indicator of Cu stress.

The purpose of this study is to determine the relationships between the concentrations of secondary metabolites and Cu in the Cu-hyperaccumulator lichen *S. japonicum* and to clarify its response to Cu, providing a key to understanding the mechanisms of Cu accumulation and Cu tolerance in the lichen. To achieve this purpose, we collected Cu-contaminated and uncontaminated samples of *S. japonicum* in Japan and determined relative concentrations of secondary metabolites (atranorin, stictic acid, and norstictic acid) and concentrations of metals (Cu and K), glucose, and sugar alcohols (ribitol, mannitol, and arabitol). Based on these results, we examined

the relationships between the concentrations of secondary metabolites and Cu in natural samples of *S. japonicum*.

Materials and methods

Sampling and sample identification

Fifteen lichen samples were collected from March 2014 to March 2015 at seven sites in Japan (Table 1). At each site, the lichen formed roughly circular colonies of more than 0.15 m in diameter on rocks or stone walls. The lichen samples of nos. 4–6, 8–11, 14, and 15 were collected at Cu-polluted sites: nos. 4–6 and 11, adjacent to Cu-roofed buildings of shrines or temples; nos. 8–10, 14, and 15, near abandoned metal mines. All the samples were dried under normal laboratory conditions. These samples were identified as *S. japonicum* Th. Fr. by their morphology and secondary metabolites.

Analysis of lichen secondary metabolites

The secondary metabolites in the lichen samples were analyzed as described by Nakajima et al. (2012). Shortly, the secondary metabolites were extracted from dried samples overnight in analytical grade acetone in which the sample concentration was 5.0 mg/mL. The acetone solution was analyzed by high-performance liquid chromatography (HPLC) with a photodiode array detector. The HPLC conditions were as follows: column, YMC-Pack ODS-A (150 mm × 4.6 mm id, 5 μm) at 40 °C; solvent, MeOH:H_2O:H_3PO_4 = 80:20:1 at 1 mL/min; the chromatogram detected at 254 nm; and the wavelength range of the UV spectrum, 200–800 nm. Peaks were detected at retention times corresponding to secondary metabolites—atranorin, stictic acid, and norstictic acid—for *S. japonicum* (Yoshimura 1974). Each secondary metabolite was identified by retention time and UV spectrum. The relative concentrations of these secondary metabolites were represented by peak areas at the specific retention times.

Analysis of glucose and sugar alcohols

Glucose and sugar alcohols—ribitol, mannitol, and arabitol—in 12 of 15 samples of *S. japonicum*, because of sample shortage, were analyzed as follows. Glucose and sugar alcohols were extracted from the samples in Milli-Q water at 65 °C for 1.0 h, and were analyzed by HPLC with an electrochemical detector (GL Science ED723). The anion exchange column packed with styrene-divinylbenzene copolymer (150 mm × 4.6 mm id, 5 μm) (GL Science InertSphere Sugar-1) was used. The eluent was 0.02 mol/L NaOH solution flowing at 0.4 mL/min.

Analysis of Cu and K

The Cu and K concentrations in pseudopodetia of the *S. japonicum* samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x) as described in a previous study (Nakajima et al. 2013). The pseudopodetia were dried at 90 °C for 24 h and were dissolved in 65% HNO_3 and 30% H_2O_2 (2:1, *v*/*v*) for 24 h. The volumes of the dissolved solutions were made up to 20 mL with ultrapure water, and Cu and K in the solutions were analyzed by ICP-MS.

Correlation analysis

A correlation test was performed using the R software, and Pearson's correlation coefficients (R) and p values (p) were calculated. The correlation was considered to be significant at the 0.05 level.

Results

Cu analysis

The Cu concentrations in the *S. japonicum* samples are listed in Table 1, confirming that the lichen is a Cu-hyperaccumulator lichen, widely distributed in Japan. The highest concentration of Cu in the lichen samples (2000.1 mg/kg dry weight) was about 260 times that in the control samples (7.6 ± 3.6 mg/kg dry weight) (Table 1). Lichen sample nos. 4, 6, and 11 with the three highest concentrations of Cu (2000.1 mg/kg, 768.5 mg/kg, and 124.3 mg/kg, respectively) and no. 5 with Cu (82.8 mg/kg) were collected adjacent to Cu-roofed buildings of shrines or temples; lichen sample nos. 9, 10, 14, and 15 with high concentrations of Cu (99.2 mg/kg, 29.0 mg/kg, 13.5 mg/kg, and 30.3 mg/kg, respectively) were collected near abandoned metal mines. Hence, the Cu concentrations in *S. japonicum* samples collected adjacent to the Cu-roofed buildings were higher than those collected near the metal mines.

Relationships between the concentrations of Cu and the secondary metabolites

We found significant negative correlations between the logarithm of Cu concentration and the relative concentrations of major secondary metabolites—atranorin ($R = -0.574$, $p = 0.025$) and stictic acid ($R = -0.682$, $p = 0.005$)—in the *S. japonicum* samples (Fig. 2a, b). We observed a slight, but not significant, negative correlation between the logarithm of Cu concentration and the relative concentration of the other major secondary metabolites—norstictic acid ($R = -0.493$, $p = 0.073$)—in the lichen samples (Fig. 2c).

Table 1 Date and site of collection; concentrations of Cu, K, ribitol, glucose, arabitol, and mannitol; and peak areas in the HPLC chromatograms ($\times 10^3$) (i.e., relative concentrations) of atranorin, stictic acid, and norstictic acid in the *S. japonicum* samples

No.	Collection date	Collection site	Cu (mg/kg)	K (mg/kg)	Ribitol (wt%)	Glucose (wt%)	Arabitol (wt%)	Mannitol (wt%)	Atranorin	Stictic acid	Norstictic acid
1*	2 March 2014	Yakushima, Kagoshima (30° 19′ N, 130° 38′ E)	12.7	2098.8	–	–	–	–	121.7	120.3	21.27
2	2 March 2014	Yakushima, Kagoshima (30° 18′ N, 130° 38′ E)	54.5	2589.5	–	–	–	–	169.2	110.3	83.69
3*	22 March 2014	Ito, Shizuoka (34° 53′ N, 139° 06′ E)	10.0	1770.8	–	–	–	–	201.0	201.0	23.98
4	18 May 2014	Hakone, Kanagawa (35° 12′ N, 139° 02′ E)	2000.1	2789.0	0.37	0.21	2.09	0.20	126.1	82.0	10.52
5	1 June 2014	Izu, Shizuoka (34° 57′ N, 139° 00′ E)	82.8	2263.2	0.31	0.21	1.86	0.18	190.4	198.7	11.05
6	1 June 2014	Izu, Shizuoka (34° 57′ N, 139° 00′ E)	786.5	1829.9	0.50	0.32	2.50	0.16	161.3	123.6	9.88
7*	1 June 2014	Izu, Shizuoka (34° 57′ N, 139° 00′ E)	4.4	1722.9	0.48	0.18	1.98	0.16	102.8	98.4	9.47
8	19 September 2014	Inagawa, Hyogo (34° 54′ N, 135° 21′ E)	5.2	1751.5	0.31	0.38	2.25	0.69	38.9	56.7	3.58
9	19 September 2014	Inagawa, Hyogo (34° 54′ N, 135° 21′ E)	99.2	2352.4	0.32	0.24	2.13	0.57	51.1	27.5	4.33
10	19 September 2014	Inagawa, Hyogo (34° 54′ N, 135° 21′ E)	29.0	2063.8	0.28	0.37	1.97	0.63	138.9	124.2	9.06
11	20 September 2014	Nose, Osaka (34° 56′ N, 135° 28′ E)	124.3	2079.5	0.32	0.35	2.72	0.43	116.5	111.1	13.15
12*	20 September 2014	Nose, Osaka (34° 55′ N, 135° 27′ E)	5.6	2810.2	0.23	0.45	2.65	0.88	148.0	112.6	8.57
13*	29 March 2015	Iwakuni, Yamaguchi (34° 10′ N, 132° 11′ E)	5.1	2308.6	0.63	0.24	2.52	0.33	53.5	89.3	4.86
14	30 March 2015	Iwakuni, Yamaguchi (34° 09′ N, 132° 03′ E)	13.5	1728.6	0.56	0.17	2.59	0.32	52.7	65.6	4.11
15	30 March 2015	Iwakuni, Yamaguchi (34° 09′ N, 132° 03′ E)	30.3	1749.6	0.36	0.25	1.99	0.34	95.9	114.0	9.96

Concentrations of ribitol, glucose, arabitol, and mannitol in sample nos. 1–3 were not measured because of sample shortage

*The control sample

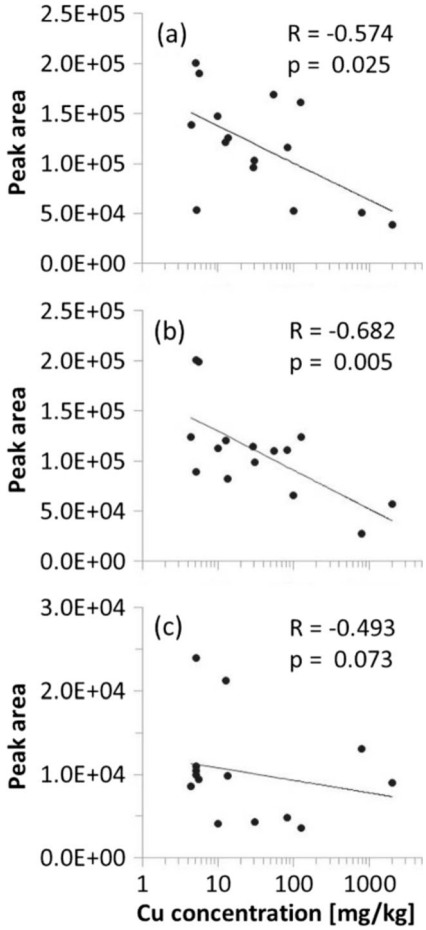

Fig. 2 Relationships between Cu concentration and peak area, corresponding to relative concentration, of atranorin (**a**), stictic acid (**b**), and norstictic acid (**c**) in the *S. japonicum* samples. Solid lines are regression lines

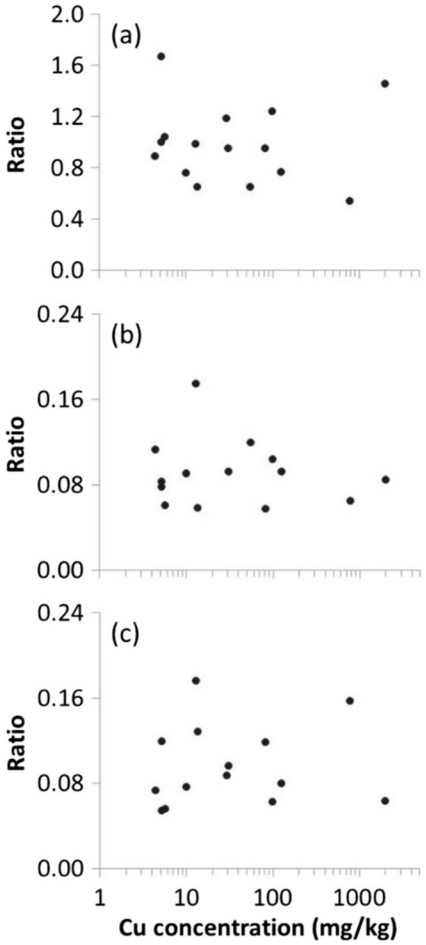

Fig. 3 Ratios of peak areas, corresponding to relative concentrations, (stictic acid)/(atranorin) (**a**), (norstictic acid)/(atranorin) (**b**), and (norstictic acid)/(stictic acid) (**c**) in the *S. japonicum* samples

To determine whether there are differences between the decreases in the relative concentrations of the major secondary metabolites with increasing Cu concentration in *S. japonicum* samples, the ratios of these relative concentrations, (stictic acid)/(atranorin), (norstictic acid)/(atranorin), and (norstictic acid)/(stictic acid), were calculated (Fig. 3). Figure 3 shows that the three ratios varied independent of Cu concentration, demonstrating no significant differences between the decreases in the relative concentrations of atranorin, stictic acid, and norstictic acid with increasing Cu concentration.

Glucose and sugar alcohol analysis

The concentration of arabitol in *S. japonicum* samples was three to four times the highest concentration of glucose, ribitol, or mannitol in them (Fig. 4), indicating that arabitol was a major carbohydrate in the lichen samples. We observed that the concentration of arabitol in *S. japonicum* samples was almost constant (2.27 ± 0.31 wt% (average ± standard deviation)) and independent of Cu concentration (Fig. 4c) and also that the concentrations of glucose, ribitol, and mannitol in them varied independent of Cu concentration (Fig. 4a, b, d).

Relationship between the concentrations of Cu and K

Figure 5 presents the relationship between the concentrations of Cu and K in the *S. japonicum* samples, showing that the concentration of K in them was almost constant (2092 ± 339 mg/kg) and independent of Cu concentration.

Discussion

The negative correlations between the relative concentrations of secondary metabolites and the concentration of Cu (Fig. 2) can be interpreted in one of two ways: (a) *S. japonicum* itself reduced the relative concentrations of secondary metabolites in response to the increase of Cu concentration or (b) its carbon and energy metabolism was damaged by Cu stress, resulting in the reduction of the relative concentrations of secondary metabolites. If the metabolism is damaged by Cu

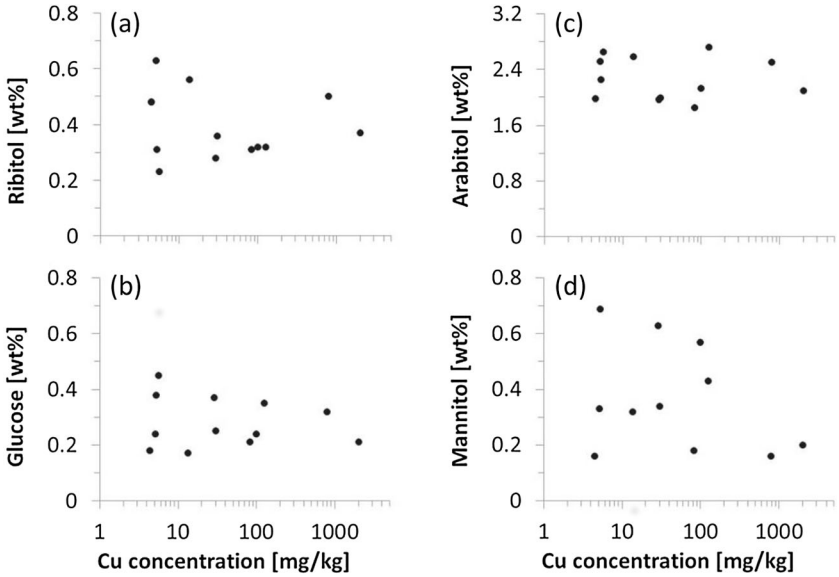

Fig. 4 Relationships between the concentrations of Cu and ribitol (**a**), glucose (**b**), arabitol (**c**), or mannitol (**d**) in the *S. japonicum* samples

stress, the concentrations of K, glucose, or sugar alcohols in the lichen would decrease as the Cu concentration increases. It is reported that the exposure of a lichen *E. prunastri* to Cu reduced photosynthesis and thus decreased its glucose concentration (Vantová et al. 2013). In contrast to the previous study, the analysis of glucose and sugar alcohols, varied independent of Cu concentration (Fig. 4), and of K, being almost constant and independent of Cu concentration (Fig. 5), shows no effects of Cu on these concentrations in *S. japonicum* samples, meaning that the carbon and energy metabolism of the lichen was not affected by Cu stress. Therefore, the negative correlations between the relative concentrations of secondary metabolites and the concentration of Cu can be interpreted that (a) *S. japonicum* itself reduced the relative concentrations of secondary metabolites in response to the increase of Cu concentration.

A possible reason for the decreases in the relative concentrations of the three secondary metabolites with the increase in the Cu concentration (Fig. 2) is the maintenance of carbon and energy metabolism in compensation for the decreases in the secondary metabolites. It can be explained that in response to excess Cu, *S. japonicum* reduced the allocation of carbohydrates to produce its secondary metabolites and, accordingly, increased the allocation to detoxify Cu, which resulted in the maintenance of carbon and energy metabolism against Cu pollution. This is supported by the fact that the concentrations of chlorophyll *a* and *b* in natural samples of *S. japonicum* did not decrease as that of Cu increased (Nakajima et al. 2015). Another possible reason for the decreases in the relative concentrations of secondary metabolites as shown in Fig. 2 is the substitution of Cu, which is toxic to invertebrates and microbes (Flemming and Trevors 1989; Malaj et al. 2012; Ingle et al. 2014), for these metabolites, having antiherbivore and antimicrobial activities (Gauslaa 2005; Ranković and Mišić 2008; Ranković et al. 2008; Vatne et al. 2011; Gauslaa et al. 2016; Ismed et al. 2018). It can be explained that Cu accumulated in *S. japonicum* functions as an antiherbivore and antimicrobial agent, instead of its secondary metabolites, allowing the lichen to reduce its secondary metabolites with the increase of Cu concentration.

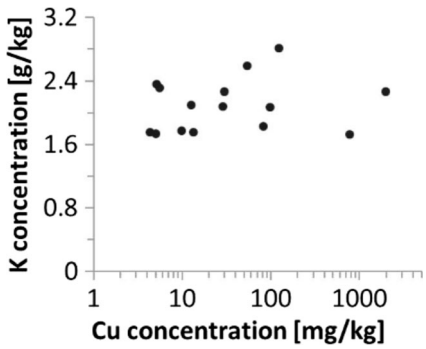

Fig. 5 Relationship between the concentrations of Cu and K in the *S. japonicum* samples

Similar to the negative correlation of atranorin with Cu (Fig. 2a), a significant negative correlation between the concentrations of atranorin and Cu was observed ($R = -0.425$, $p < 0.05$) in a foliose lichen *Parmelia sulcata* Taylor (Gauslaa et al. 2016). These negative correlations mean that atranorin has no protective role against heavy metal damage, which is compatible with the fact that atranorin did not affect the adsorption of transition metals including Cu (Hauck and Huneck 2007). In contrast to the negative correlation of stictic acid with Cu (Fig. 2b), strong and positive correlations of

stictic acid with heavy metals including Cu in a lichen *L. pulmonaria* were reported (Gauslaa et al. 2016), indicating that stictic acid plays a role in heavy metal accumulation in the lichen; however, its Cu accumulation capacity did not prevent Cu-induced membrane damage (Cabral 2002). These results show that stictic acid has no function in heavy metal protection. The negative correlation of Cu with norstictic acid was not significant (Fig. 2c) whereas those with atranorin and stictic acid were significant (Fig. 2a, b). This may be because of the low concentration and the large coefficient of variation of norstictic acid; the average relative concentration of norstictic acid was 13% or 14% of that of atranorin or stictic acid, and the coefficient of variation of norstictic acid was 3.0 or 3.1 times that of atranorin or stictic acid.

The result of the negative correlations between the relative concentrations of secondary metabolites and the concentration of Cu (Fig. 2), combined with the result that the ratios of the three secondary metabolites were independent of Cu concentration (Fig. 3), indicates that the relative concentrations of the three secondary metabolites decreased proportionately to each other as the Cu concentration increased.

Conclusions

We found significant negative correlations between the relative concentrations of secondary metabolites—atranorin and stictic acid—and the concentration of Cu in natural samples of the Cu-hyperaccumulator lichen *S. japonicum* (Fig. 2). These negative correlations can be interpreted in one of two ways: (a) *S. japonicum* itself reduced the relative concentrations of secondary metabolites in response to the increase of Cu concentration or (b) its carbon and energy metabolism was damaged by Cu stress, resulting in the reduction of the relative concentrations of secondary metabolites. The analysis of K, glucose, and sugar alcohols showed no effect of Cu on those concentrations, meaning that the carbon and energy metabolism in the lichen was not damaged by Cu stress. Therefore, the negative correlations can be interpreted that (a) *S. japonicum* itself reduced the relative concentrations of secondary metabolites with the increase of Cu concentration. Possible reasons for the decreases in the relative concentrations of secondary metabolites with the increase in the Cu concentration are (i) the maintenance of carbon and energy metabolism in compensation for the decreases in the secondary metabolites and (ii) the substitution of Cu, which is toxic to invertebrates and microbes, for the secondary metabolites, having antiherbivore and antimicrobial activities. These findings provide a deeper understanding of the response of secondary metabolites to Cu in the lichen.

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References

Ahmadjian V (1993) The lichen symbiosis. John Wiley & Sons, Inc, New York

Aptroot A, Seaward MRD (1999) Annotated checklist of Hongkong lichens. Trop Bryol 17:57–101

Białońska D, Dayan FE (2005) Chemistry of the lichen *Hypogymnia physodes* transplanted to an industrial region. J Chem Ecol 31: 2975–2991

Branquinho C, Matos P, Vieira AR, Ramos MMP (2011) The relative impact of lichen symbiotic partners to repeated copper uptake. Environ Exp Bot 72:84–92

Cabral JP (2002) Differential sensitivity of four *Lobaria* lichens to copper in vitro. Environ Toxicol Chem 21:2468–2476

Dobson FS (2005) Lichens. The Richmond Publishing Co Ltd, Slough

Eisenreich W, Knispel N, Beck A (2011) Advanced methods for the study of the chemistry and the metabolism of lichens. Phytochem Rev 10: 445–456

Flemming CA, Trevors JT (1989) Copper toxicity and chemistry in the environment: a review. Water Air Soil Pollut 44:143–158

Gauslaa Y (2005) Lichen palatability depends on investments in herbivore defence. Oecologia 143:94–105

Gauslaa Y, Yemets OA, Asplund J, Solhaug KA (2016) Carbon based secondary compounds do not provide protection against heavy metal road pollutants in epiphytic macrolichens. Sci Total Environ 541: 795–801

Hauck M, Huneck S (2007) Lichen substances affect metal adsorption in *Hypogymnia physodes*. J Chem Ecol 33:219–223

Hauck M, Böning J, Jacob M, Dittrich S, Feussner I, Leuschner C (2013) Lichen substance concentrations in the lichen *Hypogymnia physodes* are correlated with heavy metal concentrations in the substratum. Environ Exp Bot 85:58–63

Huang MR (2010) Altitudinal patterns of *Stereocaulon* (lichenized Ascomycota) in China. Acta Oecol 36:173–178

Huang X, Wang L, Laserna AKC, Li SFY (2017) Correlations in the elemental and metabolic profiles of the lichen *Dirinaria picta* after road traffic exposure. Metallomics 9:1610–1621

Ingle AP, Duran N, Rai M (2014) Bioactivity mechanism of action, and cytotoxicity of copper-based nanoparticles: a review. Appl Microbiol Biotechnol 98:1001–1009

Ismed F, Devehat FL, Guiller A, Corlay N, Bakhtiar A, Boustie J (2018) Phytochemical review of the lichen genus Stereocaulon (Fam. Stereocaulaceae) and related pharmacological activities highlighted by a focus on nine species. Phytochem Rev 17:1165–1178. https://doi.org/10.1007/s11101-018-9576-y

Kalinowska R, Bačkor M, Pawlik-Skowrońska B (2015) Parietin in the tolerant lichen *Xanthoria parietina* (L.) Th. Fr. increases protection of *Trebouxia* photobionts from cadmium excess. Ecol Indic 58:132–138

Malaj E, Grote M, Schäfer RB, Brack W, von der Ohe PC (2012) Physiological sensitivity of freshwater macroinvertebrates to heavy metals. Environ Toxicol Chem 31:1754–1764

Molnár K, Farkas E (2010) Current results on biological activities of lichen secondary metabolites: a review. Z Naturforsch 65c:157–173

Nakajima H, Fujimoto K, Yoshitani A, Yamamoto Y, Sakurai H, Itoh K (2012) Effect of copper stress on cup lichens *Cladonia humilis* and *C. subconistea* growing on copper-hyperaccumulating moss *Scopelophila cataractae* at copper-polluted sites in Japan. Ecotoxicol Environ Saf 84:341–346

Nakajima H, Yamamoto Y, Yoshitani A, Itoh K (2013) Effect of metal stress on photosynthetic pigments in the Cu-hyperaccumulating lichens *Cladonia humilis* and *Stereocaulon japonicum* growing in Cu-polluted sites in Japan. Ecotoxicol Environ Saf 97:154–159

Nakajima H, Hara K, Yamamoto Y, Itoh K (2015) Effects of Cu on the content of chlorophylls and secondary metabolites in the Cu-

hyperaccumulator lichen *Stereocaulon japonicum*. Ecotoxicol Environ Saf 113:477–482

Nash IIITH (1990) Metal tolerance in lichens. In: Shaw AJ (ed) Heavy metal tolerance in plants: an evolutionary perspective. CRC, Boca Raton, pp 119–131

Park JS, Park CH, Park SY, Oh SO, Jayalal U, Hur JS (2018) Rivision of the lichen genus *Stereocaulon* (Stereocaulaceae, Ascomycota) in South Korea. Mycobiol 46:101–113

Pawlik-Skowrońska B, Bačkor M (2011) Zn/Pb-tolerant lichens with higher content of secondary metabolites produce less phytochelatins than specimens living in unpolluted habitats. Environ Exp Bot 72: 64–70

Purvis OW, Elix JA, Broomhead JA, Jones GC (1987) The occurrence of copper—norstictic acid in lichens from cupriferous substrata. Lichenologist 19:193–203

Ranković B, Mišić M (2008) The antimicrobial activity of the lichen substances of the lichens *Cladonia furcata*, *Ochrolechia androgyna*, *Parmelia caperata* and *Parmelia conspresa*. Biotechnol Biotechnol Equip 22:1013–1016

Ranković B, Mišić M, Sukdolak S (2008) The antimicrobial activity of substances derived from the lichens *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physodes*. World J Microbiol Biotechnol 24:1239–1242

Roser DJ, Melick DR, Seppelt RD (1992) Reductions in the polyhydric alcohol content of lichens as an indicator of environmental pollution. Antarct Sci 4:185–189

Smith PL (2013) Indicator plants: using plants to evaluate the environment. Wildtrack, Sheffield, p 58

Tarhanen S, Metsärinne S, Holopainen T, Oksanen J (1999) Membrane permeability response of lichen *Bryoria fuscescens* to wet deposited heavy metals and acid rain. Environ Pollut 104:121–129

Vantová I, Bačkor M, Klejdus B, Bačkorová M, Kováčik J (2013) Copper uptake and copper-induced physiological changes in the epiphytic lichen *Evernia prunastri*. Plant Growth Regul 69:1–9

Vatne S, Asplund J, Gauslaa Y (2011) Contents of carbon based defence compounds in the old forest lichen *Lobaria pulmonaria* vary along environmental gradients. Fungal Ecol 4:350–355

Yoshimura I (1974) Lichen flora of Japan in color. Hoikusha Publishing Co Ltd, Osaka in Japanese