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Heavy metal sorption in the lichen cationactive layer

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Abstract

Results of copper ion sorption in lichens owing to the ion exchange between the surroundings (aqueous solution) and the lichen cationactive layer have been presented. It indicates that the course of sorption of these ions, similarly as in the case of cations of other heavy metals, depends on the concentration and type of cations naturally found in lichen surroundings: H^+, Na^+, K^+, Mg^{2+} and Ca^{2+}. A determination method of heavy metal concentration in lichen surroundings has been proposed. It consists in exposure of transplanted lichens in the presence of salts that provide precisely determined, artificial salinity of precipitation with which the lichens are in contact. The studies were conducted on *Hypogymnia physodes* lichens.

Keywords: Lichens; Lichenoindication; Heavy metals

1. Introduction

The possibilities of using lichens as bioindicators and biomonitors of atmospheric air pollution have been known for many years. The precursor of such studies was William Nylander (1822–1899) who published in 1866 an article (*Les lichens du Jardin du Luxembourg*) in which he indicated mutual relations between environment pollution and disappearance of less resistant species of lichens.

Lichens show significant anatomical, morphological and physiological changes resulting from pollution influence; they are also good sorbents. They accumulate e.g. heavy metals and radionuclides.

A qualitative and quantitative description of mutual relations between the concentration of trace elements in lichens and in their environment enables us to, for example, determine pollution of a given area [1] and determine the origin and directions of pollution spreading [2,3]. Modern analytical methods, e.g. INAA (*Instrumental Neutron Activation Analysis*), enable to identify elements accumulated in lichens on the ppb level and determine their locations e.g. by the PIXE (*Proton Induced X-ray Emission*) method [4].

The problem of research procedure validation arises from the multi-dimensionality of interaction between the surroundings and lichens. Abiotic factors, which affect mutual relations between the analyte concentration in the surroundings and in lichens, are usually different for diverse research areas and their time and area variability often precludes from obtaining a reliable comparison of obtained results. This problem does not solely relate to lichens. It is discussed in many publications related to environment biomonitoring [e.g. 5,6]. The authors also indicate complications related to synergic or antisynergic nature of these interactions [7]. To interpret the data, statistical methods are used, e.g. determining linear regression coefficients [8] or using factor analysis [9]. Furthermore, differences in dependences between analyte concentration in lichens and in their surroundings are used for that purpose, for instance determination of the enrichment factor indicating e.g. pollution origin [10].

Other important information is provided by studies related to effects of individual abiotic factors on sorption intensity. They concern e.g.: influence of climatic conditions: the direction and intensity of wind [11], precipitation intensity [12], and influence of the exposure time [13] and mutual relations between the concentration of the cation accumulated in lichens and that

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found in their surroundings. Studies included e.g. the effect of precipitation salinity on the macroelements accumulated in lichens: K, Mg and Ca [14]; the effect of precipitation salinity on heavy metal sorption e.g.: nickel [15] and uranium [16]; and the effect of magnesium and calcium ions on manganese ion sorption by lichens [17].

Results presented in this paper are related to changes of copper ion sorption parameters by lichens resulting from the presence of the following cations: H^+, Na^+, K^+, Mg^{2+} and Ca^{2+}, naturally found in the lichen environment. Copper, similarly as most of heavy metals, accumulates well in lichens owing to its chemical properties. The results presented describe conditions of the equilibrium between the amount of analysed cations accumulated by lichens and their concentrations in the aqueous solution in which the lichens were dipped. The experiments simulate natural conditions in which the equilibriums between the anthropogenic substance content, in atmospheric precipitation, including heavy metals and in the lichen structure depend on the concentration and type of cations found in the lichen aqueous surroundings with which lichens are in contact. The cations come from a lichen substratum and are components of dry and wet precipitation. One can assume that the kind and content of cations naturally occurring in lichen surroundings influence the heavy metal sorption. Therefore, the aim of the piece of research was to tackle the problem by determining the effect of calcium cation concentration in the (artificial) atmospheric wet precipitation.

2. Physico-chemical processes

Our earlier studies demonstrated that the cation sorption mechanism in lichens consists in the ion exchange [18]. This mechanism is also suggested by other authors [e.g.: 19–22] who distinguish the extracellular and intracellular structures responsible for cation exchange with the surroundings. It was also determined that the extracellular layer is the buffer between the surroundings and the internal lichen structure [17,23]. The ion exchange process precedes or is parallel to the process of labile extracellular cation bonding, which can be followed by cations irreversibly embedding into the intracellular lichen structure. For example, radiocaesium studies in *Cetraria islandica* lichen structure showed presence of ^{137}Cs proteins, polypeptides and saccharides extracted from the lichens [24]. In lipids, it was found the location of Cu and Pd that indicated the method of metal cations complexing in lichens [25].

In these studies, we assumed that the ion exchange occurs between the surroundings (aqueous solution) and the lichen cationactive layer defined as the part of the lichen structure that reversibly bonds cations found in the lichen surroundings. In the experiments conducted with hydrogen ions, the course of the ion exchange was followed by the measurement of conductivity changes and pH of the solution in which the lichens were dipped. As a result of the ion exchange, mobile hydrogen ions bonded with the lichen cationactive layer are released into salt solutions in which the lichens were dipped: $M^{z+}+H_zR \leftrightarrows zH^+ + MR$ (R — anion in the cationactive layer). The course of the process can be described by a linear function of conductivity changes depending on hydrogen ion concentration changes in the solution. Our earlier studies of its kinetics demonstrate that the equilibrium between the surroundings and the lichen cationactive layer is settled after approximately 30 min [26].

The determined sorption capacity of the cationactive layer for *Hypogymnia physodes* lichens, is $c^*=0.145$ mol/g d.m. ($c^*=10^3 \cdot c \cdot z$, where z — ion valence, c — concentration mol/g, d.m. — dry mass of sample) [18]. Concentrations expressed as c^* (mol/g d.m. or M) and other values calculated in this way allow to compare mutual relations between cations of different valences.

Mutual relations of cation pairs can be described by the expression for the equilibrium constant of the heterophase double displacement exchange process: $y^*=(K^*_{A/B} \cdot x^*) \cdot [1+(K^*_{A/B}-1) \cdot x^*]^{-1}$, where: K^* — is the equilibrium constant, y^*, x^* — are mole fractions, respectively, in lichens and in the solution, A, B — are cations [18], and the expression describing the rate constant of the second-order reaction, for $c^*_A \neq c^*_B$: $\log (x_A/y_B)=2.303^{-1} \cdot k^* \cdot t \cdot (c^*_{A,0}-c^*_{B,0})$, where: k^* — reaction rate constant, t — time, $c^*_{A,0}$, $c^*_{B,0}$ — initial A and B ion concentrations, respectively, in the solution and in the lichen cationactive layer [26]. This description was used by the authors to develop the assessment method of precipitation pH value based on the analysis of the lichen cationactive layer composition [27–31].

Results of the copper ion sorption studies presented in this work are discussed by comparing the investigated ion distribution in the state of equilibrium between the solution and the lichen cationactive layer. To assess calcium ion effect on copper ion sorption efficiency, the results are interpreted according to the Langmuir isotherm model: $1/c^*_{Cu(i,1)}=1/c^*_{Cu,m}+1/(c^*_{Cu,m} \cdot b \cdot c^*_{Cu(s,1)})$, where: $c^*_{Cu(i,1)}$/mol/g d.m. — concentration of copper accumulated in lichens, remaining in the equilibrium with the copper concentration in the solution: $c^*_{Cu(s,1)}$/M, $c^*_{Cu,m}$ — copper concentration in lichens at $c^*_{Cu(s,1)} \rightarrow \infty$ (sorption capacity), b — constant.

3. Method of sample preparation and measurements

The studies used *Hypogymnia physodes* lichen that grow on coniferous trees. Lichens, collected and cleaned from mechanical impurities (bark, sand), were kept in paper bags. The samples were rinsed in demineralised water for several times (conductivity $\kappa=0.1$ µS/cm). Then, they were dipped in solutions ($c^*=2$ M) of chlorides of the following cations, Ct: Na^+, K^+, Mg^{2+}, Ca^{2+} (pH = 4.9 to 5.4, the values are characteristic for the 2 M salt solutions) or H^+ (pH = 3.5, for pH < 3.5 lichen thallus degrades quickly). This action was to saturate the lichen cationactive layer with one type of cations, different for each series of measurements on Cu/Ct equilibria. Lichen samples for studies of ion exchange $Cu^{2+} \leftrightarrows Ca^{2+}$ were saturated with Ca^{2+} solutions. In this way, we eliminated the effect of other cations, naturally bonded in the cationactive layer, on the course of investigated copper sorption processes with solutions of Cu/Ct cation pairs. The saturated lichens were rinsed in demineralised water and dried at 303 K temperature.

In the studies were used 1 g samples of prepared and dried lichens that were dipped in solutions (200 cm^3) of different

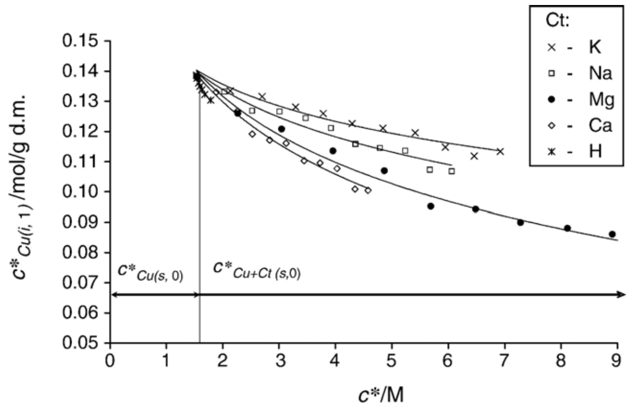

Fig. 1. Changes in the equilibrium state of the copper ion sorption after *Hypogymnia physodes* lichen dipping in solutions of constant initial copper concentration $c^*_{Cu(s,0)}$ and different initial concentration $c^*_{Ct(s,0)}$ of cations: H^+, K^+, Na^+, Mg^{2+} and Ca^{2+} ($c^* = 10^3 \cdot c \cdot z$).

concentrations of Cu/Ct cation pairs. Copper ion concentrations in solutions were examined before and after sorption. Copper content in samples was determined by atomic absorption spectrometry using UNICAM SOLAAR 969 apparatus.

The stable solution conductivity and pH values (after approx. 30 min) demonstrated attainment of the state of equilibrium. The ion exchange process: $M^{z+} + H_zR \leftrightarrows zH^+ + MR$ was examined by measuring and analysing the solution conductivity κ and pH values. The detailed procedures of κ and pH measurements have been given elsewhere [26].

4. Results

Previously conducted studies indicated that cation affinity (after recalculation for unit electrical charge, i.e. for Ca^{2+} by dividing measured value by 2) to the lichen cationactive layer increases in the series: $K^+ < Na^+ < Mg^{2+} \approx Ca^{2+} < H^+ < Zn^{2+} < Ni^{2+} < Cu^{2+} < Pb^{2+}$ [32]. This series in the case of heavy metals is partly in accordance with data by Conti and Cecchetti [5]. Copper ions, similarly as most of heavy metal ions, undergo sorption well in the lichen cationactive layer. The present study results are limited to copper ions. In the case of other heavy metal cations the process should also take place promptly. The numerical values describing the state of equilibrium will depend on the affinity of these ions to the lichen cationactive layer. Our results describe ion exchange equilibria between the lichen surrounding solution and the lichen extracellular cationactive layer. In this case the equilibrium is attained in 30 min or even earlier; Branquinho et al. observed it [33] after 10–30 min. However for the intracellular equilibria the time is 60–90 min [33] or even 4 days [34].

Indexes used in the table and figure descriptions mean: i — lichen cationactive layer, s — solution, 0 — initial state, 1 — final state, the state of equilibrium.

4.1. The effect of H^+, Na^+, K^+, Mg^{2+} and Ca^{2+} cations on copper ion sorption in the lichen cationactive layer

The objective of this stage of studies was to determine the effect of the following cations present in the environment: H^+, Na^+, K^+, Mg^{2+} and Ca^{2+} on copper ion sorption in the lichen cationactive layer. The results are illustrated in Fig. 1. In a series of research on the effect of hydrogen ions on copper sorption, hydrogen ion concentration was changed in a narrow pH range from 5.4 (demineralised water pH) to 3.5; it was observed that thallus destruction of the examined lichen occurs at pH <3.9. Sorption studies above the value of distilled water pH require solution alkalisation i.e. introduction of metal cations, which affect the copper ion solution, together with the OH^- anions.

Table 1
Dependence of changes in the equilibrium state of the copper ion sorption on initial copper ion concentration $c^*_{Cu(s,0)}$/M and sum of metal cation ($Cu^{2+} + Ca^{2+}$) concentration $c^*_{Ct(s)}$ in solution in which the *Hypogymnia physodes* have been dipped

$c^*_{Cu(s,0)}$/M	$c^*_{Ct(s)}$/M	3.02	2.04	1.49	1.20	0.96	0.84	0.73
0.65	$c^*_{Cu(i,1)}$/mol/g d.m.	0.077	0.081	0.085	0.087	0.091	0.094	0.097
	$c^*_{Cu(s,1)}$/M	0.265	0.245	0.225	0.215	0.195	0.180	0.165
0.45	$c^*_{Ct(s)}$/M	3.18	2.21	1.69	0.89	0.78	0.66	0.54
	$c^*_{Cu(i,1)}$/mol/g d.m.	0.059	0.066	0.069	0.076	0.077	0.078	0.079
	$c^*_{Cu(s,1)}$/M	0.157	0.120	0.105	0.072	0.069	0.061	0.057
0.35	$c^*_{Ct(s)}$/M	3.17	2.17	1.60	0.81	0.67	0.56	0.43
	$c^*_{Cu(i,1)}$/mol/g d.m.	0.051	0.054	0.057	0.063	0.064	0.065	0.065
	$c^*_{Cu(s,1)}$/M	0.098	0.083	0.064	0.035	0.03	0.028	0.028
0.20	$c^*_{Ct(s)}$/M	3.08	2.03	1.46	0.63	0.49	0.36	0.24
	$c^*_{Cu(i,1)}$/mol/g d.m.	0.030	0.032	0.035	0.037	0.037	0.038	0.038
	$c^*_{Cu(s,1)}$/M	0.047	0.034	0.025	0.015	0.015	0.010	0.010
0.10	$c^*_{Ct(s)}$/M	3.05	1.99	1.40	0.53	0.41	0.26	0.14
	$c^*_{Cu(i,1)}$/mol/g d.m.	0.016	0.017	0.0185	0.0193	–	–	–
	$c^*_{Cu(s,1)}$/M	0.020	0.015	0.0075	0.0035	–	–	–
0.05	$c^*_{Ct(s)}$/M	3.01	1.94	1.35	0.53	0.34	0.25	0.09
	$c^*_{Cu(i,1)}$/mol/g d.m.	0.0082	0.0086	–	–	–	–	–
	$c^*_{Cu(s,1)}$/M	0.0089	0.0072	–	–	–	–	–
$c^*_{Cu(s,0)}$/M $= c^*_{Ct(s,0)}$		1.04	0.65	0.45	0.35	0.19	0.11	0.04
$c^*_{Cu(i,1)}$/mol/g d.m.		0.121	0.100	0.079	0.065	0.037	0.021	0.008
$c^*_{Cu(s,1)}$/M		0.441	0.148	0.056	0.028	0.008	0.002	0.000

– below the determination limit.

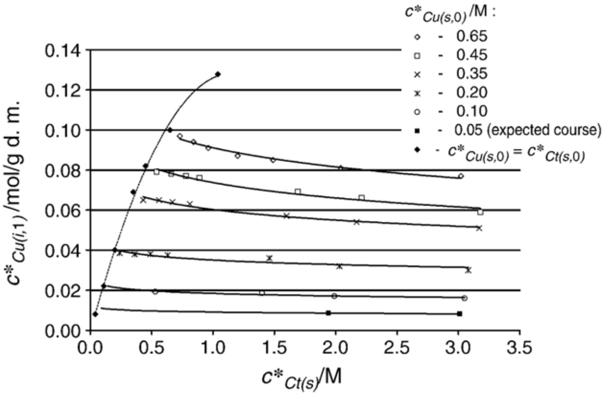

Fig. 2. Dependence of changes in the equilibrium state of the copper ion sorption on initial copper ion concentration $c^*_{Cu(s,0)}$/M and sum of cation ($Cu^{2+}+Ca^{2+}$) concentration $c^*_{Ct(s)}$ in solution in which the lichens have been dipped.

The initial copper ion concentration in solutions, much higher than the recorded copper concentrations in the environment, was selected in such a way to record significant changes of sorption efficiency.

According to the above-presented series of cation affinity to the lichen cationactive layer, hydrogen ions have a greater effect on limitation of copper ion sorption; their concentration in the natural lichen environment is much lower in comparison with the calcium ion concentration expressed as c^*.

Taking into account the highest, relative to the K^+, Na^+ and Mg^{2+} cations, calcium ion affinity to the lichen cationactive layer and its more frequent appearance in nature, also in the lichen surroundings, detailed studies of the heterophase equilibrium related to the ion exchange of Cu/Ca cation pair were conducted.

4.2. The effect of calcium ions on copper ion sorption in the lichen cationactive layer

The studies of the effect of calcium ion concentration on the copper ion sorption, from the solution in which the lichen were dipped, were conducted for different initial ion concentrations of calcium. The results are presented in Table 1 and illustrated in Fig. 2. The limitation in the lower range of Cu/Ca ratio is the case for which $c^*_{Cu(s,0)}/M = c^*_{Ct(s,0)}$. In this case, the equilibrium settles between copper ions in the initial solution and other ions released from the cationactive layer as a result of the ion exchange.

Table 2
Coefficients of the function: $c^*_{Cu(i,1)} = a \cdot \ln(c^*_{Ct(s)}) + b$, determined from Fig. 2 for different values $c^*_{Cu(s,0)}$ and coefficients of the function: $c^*_{Cu(s,1)} = a' \cdot \exp(b' \cdot c^*_{Cu(i,1)})$ with the mean standard deviation determined for different values $c^*_{Ca(s,0)}$ (Fig. 5)

$c^*_{Cu(i,1)} = a \cdot \ln(c^*_{Ct(s)}/M) + b$				$c^*_{Cu(s,1)} = a' \cdot \exp(b' \cdot c^*_{Cu(i,1)})$			
$c^*_{Cu(s,0)}$/M	a	b	R^2	$c^*_{Ca(s,0)}$/M	a'	b'	R^2
0.65	−0.0138	0.0911	0.9757	1.0	0.0037	43.58	0.9814
0.45	−0.0111	0.0737	0.9715	2.0	0.0062	45.59	0.9848
0.35	−0.0076	0.0603	0.9742	3.0	0.0076	47.99	0.9856
0.20	−0.0033	0.0351	0.8513	4.0	0.0085	50.16	0.9862
0.10	−0.0019	0.0184	0.8897	5.0	0.0091	52.13	0.9866

The results presented in Table 1 can be interpreted through comparison of the effect of the calcium ion concentration on copper cation distribution in the state of equilibrium between the lichen cationactive layer (1 g d.m.) and the solution (200 cm^3) in which the lichens were dipped: $c^*_{Cu(i,1)}/c^*_{Cu(s,1)}$. It is illustrated in Fig. 3.

The dependences shown in Fig. 3 demonstrate that, together with the decrease of the initial copper ion concentration in the solution, even minor changes of calcium ion concentration result in significant variations of copper ion distribution between the solution and the lichen cationactive layer. This conclusion is very important in the case of searching for quantitative dependences between concentration of heavy metals accumulated in lichens and their concentration in the environment.

The dependences $c^*_{Cu(i,1)} = f(c^*_{Ct(s)})$ (in the course of the ion exchange process $c^*_{Ct(s,0)} = c^*_{Ct(s,1)}$), determined for different initial calcium ion concentrations in the solution (Fig. 2) are logarithmic dependences. On their basis, using the numerical data collected in Table 1, it is possible to determine coefficients of these functions (Table 2) and then determine the functions describing changes of copper ion concentrations in the lichen cationactive layer $c^*_{Cu(i,1)}$ and in the solution $c^*_{Cu(s,1)}$ in the state of equilibrium for different initial copper ion concentrations $c^*_{Cu(s,0)}$ and constant initial calcium ion concentrations $c^*_{Ca(s,0)}$ in the solution.

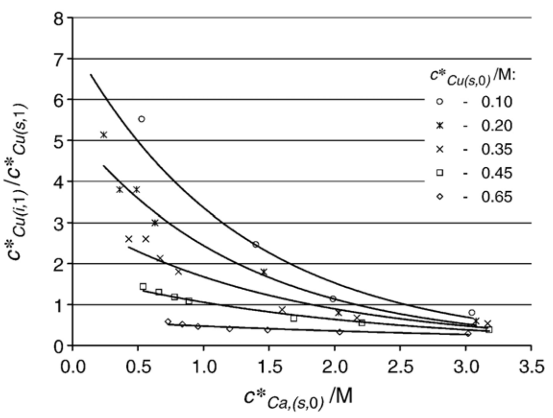

Fig. 3. The effect of calcium ion initial concentration $c^*_{Ca(s,0)}$ and that of copper $c^*_{Cu(s,0)}$ on copper cation distribution in the state of equilibrium between the lichen cationactive layer and the solution in which the lichens were dipped.

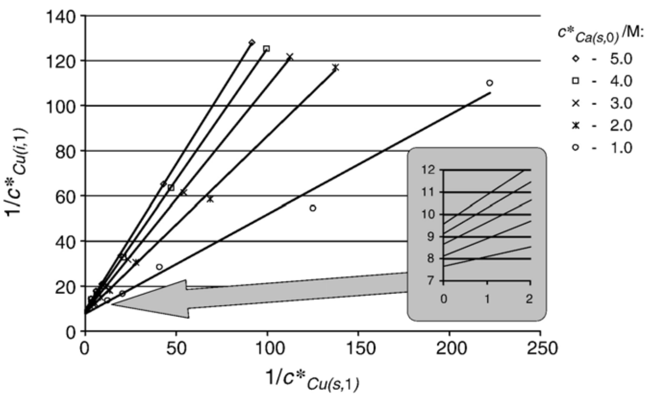

Fig. 4. Langmuir isotherms describing equilibriums of copper sorption from solutions by lichens, determined for different initial concentrations of calcium ions $c^*_{Ca(s,0)}$ and copper contained in the solution.

Determined dependences: $1/c^*_{\mathrm{Cu(i,1)}}=f(1/c^*_{\mathrm{Cu(s,1)}})$ (Langmuir isotherm model; see Section 2 of the present paper) and $c^*_{\mathrm{Cu(s,1)}}=f(c^*_{\mathrm{Cu(i,1)}})$ are illustrated successively in Figs. 4 and 5.

Intersection of the straight lines with axis of ordinates ($c^*_{\mathrm{Cu(s,1)}}\rightarrow\infty$) shows the changes of sorption capacity to copper ions, caused by the presence of calcium ions in the solution: $c^*_{\mathrm{Cu,m}}=1/b$, where b — intercept of the straight line: $y=a\cdot x+b$. Values $c^*_{\mathrm{Cu,m}}$/mol/g d.m. depending on the initial calcium ion concentration in the solution ($c_{\mathrm{Ca(s,0)}}$/M) are as follows: 0.105 (5.0), 0.110 (4.0), 0.116 (3.0), 0.124 (2.0), 0.131 (1.0) and the previously determined sorption capacity of the cationactive layer is as follows: 0.145 (0.0) [18].

Another interpretation is presented in Fig. 5. Dependences $c^*_{\mathrm{Cu(s,1)}}=f(c^*_{\mathrm{Cu(i,1)}})$, determined for different initial calcium ion concentrations in the solution, show the minimum copper ion concentrations in the solution below which, for given concentrations $c^*_{\mathrm{Ca(s,0)}}$, there is no copper sorption in the lichen cationactive layer.

The values of correlation coefficients R^2 and standard deviations δ of function shown in Fig. 5 are gathered in Table 2. The dependences presented (Fig. 5) are the basis of the examined determination method of environment pollution with heavy metals.

4.3. The determination method of environment pollution with heavy metals

The core of the examined method is to determine increases of heavy metal concentrations in exposed lichens, transplanted from areas of low pollution, at known and artificially induced precipitation salinity. The presented example relates to the copper ions for which the sorption mechanism and parameters are discussed.

The chart (Fig. 5) demonstrates that, for a series of samples of lichens that are in contact with the precipitation, with controlled concentration, e.g. calcium ions, different for each of the samples, on the basis of the measurement, after the exposure time, copper concentration increase in lichens, the interval of calcium ion concentrations contained in the precipitation can be determined. An example of conducted studies is presented in Table 3.

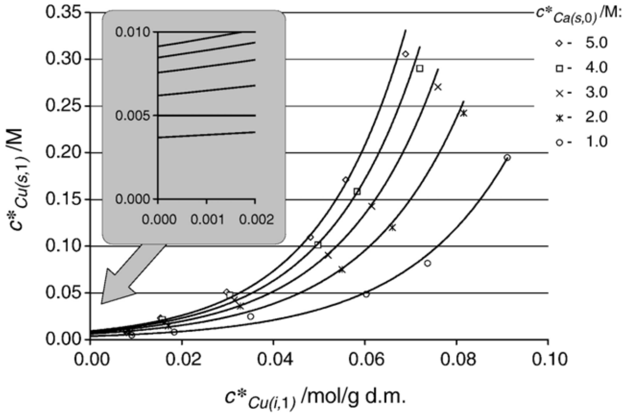

Fig. 5. The effect of calcium ions on copper ion distribution between the solution $c^*_{\mathrm{Cu(s,1)}}$ and the lichen cationactive layer $c^*_{\mathrm{Cu(i,1)}}$ in the state of equilibrium for different initial concentrations of copper ions $c^*_{\mathrm{Cu(s,0)}}$ and calcium ions $c^*_{\mathrm{Ca(s,0)}}$ in solution in which lichens were dipped.

Table 3

An example of determination of copper ion concentrations in prepared atmospheric precipitation on the basis of the studies of copper concentration increase in lichens

Type of sample:	Lichens	Lichens + CaF_2	Lichens + $CaSO_4$	Lichens + $CaCO_3$
$c^*_{\mathrm{Ca(s)}}$/M (*)	0.05–0.50	0.60–1.30	1.55–2.30	2.60–3.10
$c^*_{\mathrm{Cu(s)min}}$/M (**)	0.0012–0.0024	0.0026–0.0044	0.0050–0.0069	0.0076–0.089
Observed increase c_{Cu}	Yes	Yes	No	No
Conclusion:	$0.0026<c^*_{\mathrm{Cu(s)}}/\mathrm{M}<0.0050$			

Notes:

(*) — These calcium ion concentrations were obtained in the used test set. They depend on several factors e.g. the mass and the dosing method of calcium salt as well as precipitation flow rate, and should be determined individually in each case after changing parameters of the set.

(**) — Values determined from the function $c^*_{\mathrm{Cu(s,1)}}=f(c^*_{\mathrm{Ca(s,1)}})$, for $c^*_{\mathrm{Cu(i,1)}}=0$ (Fig. 5).

The principal advantage of this method is the fact that it does not require measurement of absolute values of the copper ion concentrations accumulated in lichens. To assess the copper ion content in precipitation, it is only necessary to determine in which samples exposed to precipitation with different artificial salinities was there an increase of copper concentration.

5. Summary and conclusions

Results of heavy metal cation sorption by lichens, the mutual equilibriums there and the effect of the cations, naturally occurring (H^+, Na^+, K^+, Mg^{2+} and Ca^{2+}) on the equilibriums have been presented. The data indicate one of the reasons that affect mutual relations between the analyte concentration in lichens and their surroundings. They must be taken into account while assessing the reliability of the lichenoindication methods.

Lichen surroundings salinity (and pollution concentration) changes together with the intensity (and sometimes duration) of precipitation and is higher at the initial precipitation stage and when there is fog or drizzle. It results from salts released from the substratum or accumulated on the surface of lichens in the form of dusts. Diagrams (Figs. 1 and 2) show that this variability of precipitation salinity type and quantity affects the intensity of heavy metal sorption from the surroundings. It can be assumed that the buffering action of the cationactive layer, which limits penetration of substances into the internal lichen structure, can be supported by its chemical composition, in particular by carbonates, contained in the substratum on which the lichens grow. Epiphytic lichens, which vegetate on areas polluted by e.g. sulphur dioxide, survive for the longest period of time on old trees with porous bark rich in mineral salts. Crustose lichens are considered to be the most pollution-resistant species, as they closely stick to the substratum, which also confirms the aforementioned thesis.

Monitoring makes use of lichens, mostly epiphytes, that grow in their natural surroundings or lichens transplanted from ecologically pure areas and exposed on urban and industrial areas. Measurement uncertainty of these two methods, used to determine environment pollution with heavy metals, can be assessed on the basis of diagrams shown in Figs. 3 and 4. They demonstrate that the copper distribution ratio $c^*_{\mathrm{Cu(i,1)}}/c^*_{\mathrm{Cu(s,1)}}$ and the maximum sorption capacity depend on the type and quantity of other cations contained in the precipitation with which the lichens are in contact. Therefore, we can assert that too excessive differences in natural substratum salinity in the place of lichen sampling or too excessive variability of precipitation salinity on the examined area might result in wrong interpretation of results. Diverse precipitation salinity is particularly important in the case of lichen exposure in a synthetic material when the precipitation with which the lichens are in contact is devoid of salinity originating from the substratum. Then, minute salinity differences result in great changes in the distribution ratio: $c_{\mathrm{M,lichen}}/c_{\mathrm{M,precipit}}$.

The suggested method, currently undergoing laboratory studies, does not require measuring and interpreting absolute values related to the increase of ion concentration of heavy metals accumulated in lichens. To assess the heavy metal ion content in precipitation, it is only necessary to determine in which samples exposed to precipitation with different artificial salinity was there an increase of concentration of these ions.

Further research on validation in biomonitoring of the presented method is in progress.

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