

# Content and Localisation of Heavy Metals in Thalli of Hemerophilous Lichens

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## Abstract

For the purpose of this work an epiphytic lichen species, *Hypogymnia physodes* (L.) NyL, was used. Thalli of the lichen were treated with solutions of nitrate salts of heavy metals, including Zn(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, and Cd(NO<sub>3</sub>)<sub>2</sub>. To simulate the acid rain impact, pH of the metal mixture was lowered to pH 4, using Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>. Content of heavy metals was determined after a standard UV mineralization method (water solution) and burning method (in temperature 450°C- thalli), using a Microcomputer Voltage-ammeter Analyser (MAW). After two months exposure of the lichen to the mixture of heavy metal nitrates it was ascertained that there was an increase of accumulation of Zn, Pb, Cu, and Cd in the thalli; a decrease of thalli pH caused increased uptake of toxic elements by the lichen; Pb and Cd remained mainly on the surface of the thalli and in the cell walls, whilst Zn and Cu penetrated to the protoplast.

**Keywords:** hemerophilous lichens, heavy metals, "acid rain"

## Introduction

Lichens are regarded as organisms particularly sensitive to anthropogenic environmental changes. Their reaction to pollution amongst other things arises from lack of covering tissue, which facilitates direct penetration by gases, dusts and solutions inside the thalli. This unfavourable effect is additionally amplified by water absorption directly from the precipitation, and not after partial filtration through the soil, as is the case in higher plants. The thalli also have very low content of chlorophyll per unit of dry mass. Destruction of the chlorophyll to the photosynthetically inactive pheophytin causes much larger damage than in higher plants. The degree of reaction of lichens to harmful factors is diverse and depends in part on thalli morphology, physiological state, and properties of the site [1, 3, 4, 9, 16].

There is, however, a comparatively small group of hemerophilous lichens, whose occurrence depends on anthropogenic transformation of the environment [7, 8]. The biological and biochemical bases for expansion of hemerophilous lichens are not completely understood. Therefore, they deserve particular attention and further research. As a result, it may be possible to explain the causes of the tolerance of those organisms to changes resulting from human activities [1, 2, 12, 13, 14, 15].

## Materials And Methods

For the purpose of this work an epiphytic lichen species, *Hypogymnia physodes* (L.) NyL., was used. This species occurs both in environments free from anthropogenic pressure as well as in transformed areas, with average air pollution (IV, middle sphere of weakened vegetation, distinguished in licheno-indication) [6, 7, 8, 9]. The experiments were conducted during a two-month period

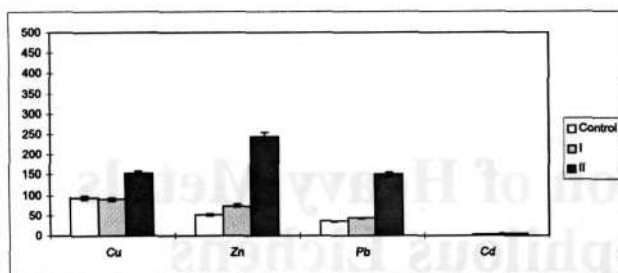


Fig. 1. Total amount of extracted metals in thalli of the lichens treated with solutions I and II with a concentration of  $10^{-2}$  M/dm<sup>3</sup>.

(August-September), in natural surroundings (coastal pine forest). Thalli of the lichens were treated with heavy metal solutions, using 10 cm<sup>3</sup> per day for a thallus with 5 cm diameter. Solutions of nitrate salts Zn(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>, were used with concentrations of 10<sup>-1</sup>M/dm<sup>3</sup>, 10<sup>-2</sup> M/dm<sup>3</sup>, at pH 6.5. The pH of the metal mixture was lowered to pH 4 (to simulate the effect of acid rain) using 10<sup>-1</sup>M/dm<sup>3</sup> Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>.

The following combinations of solutions were used:

- MI - mixture of Zn(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>
- MII - mixture of Zn(NO<sub>3</sub>)<sub>2</sub>, Cu(NO<sub>3</sub>)<sub>2</sub>, Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>

The extraction of heavy metals from the lichens was conducted using Brown's method [1]. Samples of lichens (0.1 g) were washed three times in 10 ml deionised water for 0.5 h; treated twice, for 1 h, with 20 ml 1000 ppm strontium chloride solution; washed for 20 min in 20 ml deionised water; boiled for 20 min in 5 ml deionized water and washed in 20 ml deionized water; treated twice with 20 ml 1 M HNO<sub>3</sub> for 1 h and washed as above; and finally the lichens were totally digested by heating with 2 ml concentrated 65% HNO<sub>3</sub>. Determinations were made in triplicate.

In order to assess the quantity of uptake and distribution of the ions by the thallus surface, cell wall and the protoplast, varied extraction methods were used, i.e. (successively) distilled water, strontium nitrate, boiling distilled water, 1M nitric acid, and 65% nitric acid. Swilling with distilled water allows the extraction of unbound metals from the surface of the thallus. Ions of Sr<sup>2+</sup>, on the basis of ion exchange or complex connections, remove the metals immobilized in the cell wall, enabling at the same time (through blocking of the ion-exchange groups) secondary binding of the elements in further stages of the extraction process. Boiling water can be used to extract the elements accumulated in cytoplasm, and 1 M HNO<sub>3</sub> can be used to detect metals located in the cell wall, but not undergoing the exchange with strontium ions [1, 5].

Content of heavy metals was determined after a standard UV mineralization method (water solution) and burning method (at 450°C - thalli), using a Microcomputer Voltage-ammeter Analyser (MAW). For statistical comparisons, the data were analysed by one-way analysis of variance (ANOVA). Results of these calculations were accepted as statistically significant at a level of  $p < 0.005$ .

The purposes of experiments with *Hypogymnia physodes* were to explain the influence of two months

treatment with the mixture of nitrates of Zn, Pb, Cu, and Cd on:

1. accumulation of Zn, Pb, Cu, Cd in *Hypogymnia physodes* thalli;
2. uptake and distribution of metal ions in the thallus surface, cell wall and protoplast;
3. effect of lowering the pH on accumulation of the above-mentioned elements in the thalli.

## Results and Discussion

A significant increase in the content of heavy metals in *Hypogymnia physodes* thalli after 2 months exposure to mixtures I and II was ascertained. Average contents of heavy metals in thalli followed the series: Cu > Zn > Pb > Cd, except for pH 4 where the series was as follows: Zn > Cu > Pb > Cd (Figs. 1-2). This selectivity seems to be independent of concentration, since it was similar at 10<sup>-2</sup> and 10<sup>-1</sup>M, which shows that *Hypogymnia physodes* has a selective ability for uptake of cations, which has also been found by Fabiszewski and Brown [1, 2, 5]. It was observed that the dependence of metal accumulation on concentration is not proportional to concentration. Between 10<sup>-2</sup> to 10<sup>-1</sup>M/dm<sup>3</sup>, i.e. with a tenfold difference in concentration the contents of Zn, Pb, Cd in the thallus did not increase significantly. A statistically significant increase was ascertained only for copper. Brown [1] under similar experimental conditions observed doubling of all metal contents in the thallus.

It appears from the research of many authors [2,5,10, 11] that the accumulation of heavy metals increases with the acidity of the environment. In our experiments aqueous solutions of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> were used to acidify the medium, since earlier experiments of other authors [e.g. 5] revealed that this compound interacts with thalli similarly to sulphur dioxide. The SO<sub>2</sub> present in the atmosphere dissolves in water to create sulphurous acid, which is passively absorbed by the thallus and causes defects of metabolism in contaminated lichens. Therefore, studying the effect of SO<sub>2</sub> in the laboratory lichen thalli are exposed to SO<sub>2</sub>-contaminated air and aqueous solutions of SO<sub>2</sub> [10]. Other researchers recommend using Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> solutions, because of the predominance of the HSO<sub>3</sub><sup>-1</sup> ion in the dissociation of the H<sub>2</sub>SO<sub>3</sub> [5]. In Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> solutions used in this research the anion HSO<sub>3</sub><sup>-1</sup> predominates, whereas N<sub>2</sub>SO<sub>3</sub>, SO<sub>3</sub><sup>-2</sup> and S<sub>2</sub>O<sub>5</sub><sup>-2</sup> are present in small quantities [5].

Lowering the pH of the mixture, Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> caused increases of heavy metal concentrations in the thallus of

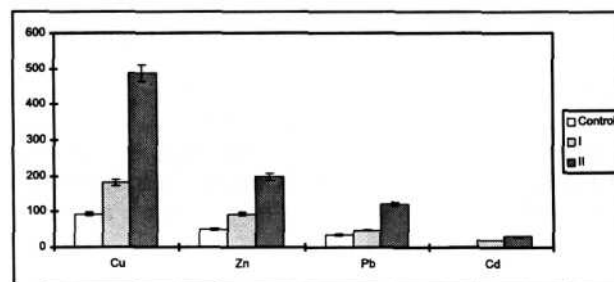


Fig. 2. Total amount of extracted metals in thalli of the lichens treated with solutions I and II with a concentration of  $10^{-1}$  M/dm<sup>3</sup>.

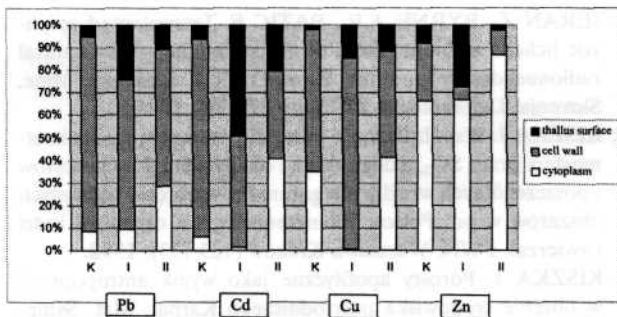


Fig. 3. Percentage share of metals in thalli of the lichens treated with solutions I and II with a concentration of  $10^{-1}$  M/dm<sup>3</sup>.

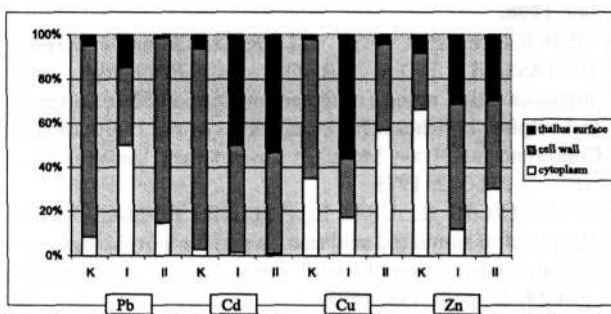


Fig. 4. Percentage share of metals in thalli of the lichens treated with solutions I and II with a concentration of  $10^{-2}$  M/dm<sup>3</sup>.

the lichen, at the same time changing the sequence of the metals in the accumulation series. The partitioning of the metals in various compartments of the thalli is shown in Figs 3 and 4.

The extraction of considerable amounts of lead from *Hypogymnia physodes* by strontium nitrate and 1 M HNO<sub>3</sub> shows that the lichen accumulates this element mainly in the cell wall, but at  $10^{-2}$  M more Pb is found in the cytoplasm and acidification of both solutions, resulting in an increase of lead content in the cell wall.

The largest quantity of cadmium was extracted from the surface of the thalli. As compared with the previous elements, copper and zinc were found in the controls to a much greater extent in the cytoplasm. A very significant increase of percentage share of cadmium in the cytoplasm took place as a result of the use of the metal mixture with the higher concentration and acidification of the medium, whereas without acidification copper stayed mainly on the surface of the thallus. In the case of zinc a very significant increase in the share of this metal in the cell wall was found using the mixture with the higher concentration.

The results of the experiments showing penetration into the protoplast by copper and zinc partially verify the findings of Brown [1]. In similar research by other authors [5, 18, 23], using metal carrying dusts, the percentage share of elements deposited on the surface of the thallus was considerably higher, which is associated with low solvability of heavy metal compounds as part of dusts.

Trace elements can be taken up by the lichens passively or actively [16,18]. In the active process the amount of an element penetrating the cell depends on propinquity of respective ions with specific carriers in plasmatic membranes [20, 21, 22]. In the passive process the main role is being played by physico-chemical properties of the elements and centres of ion adsorption (complex structures, ion exchange groups) in the plasmalemma. Earlier work [11, 18, 24] suggests, that functional groups actively participating in accumulating processes are phosphate, carboxyl, phenolic, amino and sulphhydryl groups of cell wall proteins. Some role in passive uptake of cations is attributed to the presence of galacturonic acid [2, 20]. Recent reports [17] point to melanin present in apothecia of *Trapelia involuta* and its high adsorption capacity for uranium. The authors suggest that it can be involved in a mechanism allowing for protection of ascospores against the toxic influence of heavy metals. Other researchers [18, 20] attribute a great role to lichen acids in accumulation of metals. However, Brown [2] states, that abstraction of fumarprotocetraric acid from *Cladonia rangiformis* did not cause a decrease of heavy metal uptake, but even an increase of their accumulation. McLean [17] ascertained the presence of gyrophoric acid in *Trapelia involuta* thallus, but it is lacking in apothecial walls, where accumulation of uranium was highest.

In the experiment conducted the presence of Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> in mixtures of nitrates of Zn, Pb, Cu, Cd caused increased levels of the elements in the lichen. Abstraction of SO<sub>2</sub> from the solution by the lichen thallus is, like in the case of metal ions, both a passive and an active process [10]. The greater role, however, is played by the passive mechanism. It is presumed that the locations of SO<sub>2</sub> absorption in the cell are cell walls and membrane proteins [10, 19]. SO<sub>2</sub> as a result of reaction with cell sap creates sulphuric acid, which damages plasmatic membranes, causing quicker penetration of heavy metal ions into the protoplasts of the cells [19, 24], at the same time hydrogen ions lead to dissociation of Mg from chlorophyll molecules, degrading it to phaeophytin [5].

## Conclusions

After two months of exposure of *Hypogymnia physodes* lichen to the mixture of nitrates of heavy metals it was ascertained that:

- there was an increase of accumulation of Zn, Pb, Cu, Cd in the thalli,
- a pH decrease of the thallus caused increases of toxic element uptake by the lichen,
- Cd remained mainly on the surface of the thallus in the cell wall, whereas Zn and Cu penetrated into the protoplast,
- Pb can penetrate into the protoplast depending on the concentration of solutions.

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