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# Physiological responses of *Xanthoria parietina* to long-term copper excess: role of the extracellular secondary metabolite parietin

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**ABSTRACT:** *Xanthoria parietina* is a common lichen that is widespread around the world. Tolerance of heavy metal pollution in this lichen is well known. *Xanthoria parietina* contains the secondary metabolite parietin, which protects the photobiont from high UV- radiation. Secondary metabolites of lichens have been found to form complexes with metal cations. In a long-term experiment (lasting 8 weeks), we tested the resistance of *X. parietina* with and without the secondary metabolite parietin against two concentrations of Cu (50 μM and 500 μM). Removal of parietin did not affect the measured physiological parameters. However, it caused higher accumulation of intracellular Cu. *De novo* synthesis of parietin in the lichen thallus after removal of the metabolite was not observed. Physiological parameters (chlorophyll *a* fluorescence, chlorophyll *a* integrity, content of soluble proteins, ergosterol levels, TBARS production) were affected by the higher dose of Cu during the long-term experiment. It seems that the secondary metabolite parietin does not protect the lichen against Cu excess.

**Keywords**: parietin, lichens, heavy metals, accumulation, tolerance

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## INTRODUCTION

Lichens represent a typical example of mutualistic symbiosis, where symbionts need each other in order to benefit. It has been known for almost 150 years that lichens consist of a photobiont (green algae and/or cyanobacteria) and a mycobiont (lichenised fungi). Recently, a third partner of symbiosis was discovered, namely yeast in the lichen cortex (Spribille *et al*. 2016). Lichens are dominant vegetation of approximately 8% of terrestrial ecosystems, including habitats with extreme environmental conditions of temperature, desiccation or nutrient levels (Larson 1987; Ahmadjian 1993).

Lichens are frequently exposed to excesses of metals in their natural habitats. Heavy metals like copper, zinc, cadmium, lead, chromium or mercury are major environmental pollutants that accumulate in areas under anthropogenic pressure of industries and agriculture. Plants growing at metal-polluted sites exhibit altered metabolism, growth reduction, lower biomass and metal accumulation (Nagajyoti *et al*. 2010). Metals affect physiological and biochemical processes in living organisms.

The primary source of elements in lichens is the atmosphere (Garty 2002). Polluting particles from the air are accumulated by lichen thallus surfaces exposed to external influence. Entry from the environment into the lichen depends on the concentration and chemical character of the elements and also on attributes of the lichen surface (Wittig 1993).

Lichens do not have a root system, and their mineral nutrition depends on intake from the atmosphere. Due

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to insufficient protection (e.g., absence of cuticle and stomata), the lichen thallus may accumulate mineral substances (including heavy metals) in extremely high concentrations (Bačkor & Loppi 2009). For instance, *Acarospora rubulosa* can accumulate up to 16% of copper per unit of dry weight (Chisholm *et al*. 1987). *Lecidea lactea* and *Acarospora rugulosa*, which grow on rocks with copper in central Scandinavia, contain more than 5% copper per unit of dry weight (Purvis 1984). Apotecia of the lichen *Lecanora polytropa* may accumulate up to 1,6% copper per unit of dry weight (Pawlik-Skowrońska *et al*. 2006). Some lichens were considered as new ecotypes on the basis of colour. It turned out that the characteristic colour was a particular result of the presence of a copper complex with the secondary metabolite norstictic acid (Purvis *et al*. 1987).

Secondary metabolites of lichens, also known as lichen acids, are typically produced by the fungal partner and stored extracellularly on hyphae as crystals. Lichen substances have several biological and ecological roles, including antiherbivory and allelopathy (Goga *et al*. 2016, 2017). They are also known as chelators of cations, including heavy metals. The chelating of heavy metals by secondary metabolites was studied by Takani *et al*. (2002). Lichens that grew on cupriferous substrata showed the copper-complexing ability of lichen secondary metabolites (Purvis *et al*. 1985, 1987).

*Xanthoria parietina* is an epiphytic nitrophilous lichen that grows worldwide. The main secondary metabolite of this lichen is the yellow anthraquinone parietin, which is distributed within the upper cortex of the lichen thallus. The role of parietin is, for example, to protect the lichen from UV-radiation (Solhaug & Gauslaa 1996). Also, secondary metabolites such as norstictic, psoromic and usnic acids are involved in metal detoxification by forming complexes with copper (Purvis *et al*. 1987; Takani *et al*. 2002; Bačkor *et al*. 2010).

The aim of the present study was to assess the role played by the lichen secondary metabolite parietin in a long-term (8-week) copper stress experiment and show any influence on the viability of either symbiotic partner in *X. parietina*. Several physiological parameters (Cu bioaccumulation, chlorophyll degradation, content of soluble proteins, parietin content, ergosterol levels and membrane lipid peroxidation) were chosen to show toxicity to the lichen.

## MATERIAL AND METHODS

### Lichen material and acetone treatment

Thalli of the lichen *Xanthoria parietina* (L.) Th. Fr. (40-60 mm in diameter) were collected randomly from approximately 20-cm-long aspen twigs (*Populus tremula* L.) as their substrate during October of 2017 in the village of Zemplínsky Branč at an elevation of 110 m a. s. l., latitude of 48° 32′ 12.52″ N and longitude of 21° 43′ 27.71″ E (eastern Slovakia, EU). Macroscopic foreign material adhering to lichen surfaces was removed with forceps and specimens were stored in the dark at room temperature for 48 h.

Dry lichen thalli were then randomly divided into two equal groups. One group was chosen as a control variant of the experiment with the lichen secondary metabolite parietin present (P+), while the other group of lichen samples was subjected to extraction of parietin (P-). Following the procedure of Solhaug & Gauslaa (1996), thalli were rinsed three times sequentially with dry acetone at room temperature for 5 minutes each time (approximately 100 ml of acetone for 1 g of air-dry thallus in measuring cylinders). It was demonstrated by Solhaug & Gauslaa (1996), as well as in a pilot study of ours, that acetone treatment does not affect the viability of dry thalli of *X. parietina*. Acetone was allowed to evaporate completely from the lichen thalli for 24 h. Similarly, untreated thalli were left during this period at room temperature.

**Cu treatment of samples.** Thalli of both P+ and P- were divided into three equal groups in each P variant and placed in six plastic containers. Containers were stored in the greenhouse of the botanical garden of P. J. Šafárik University in Košice, Slovakia (at day/night temperatures of 22-25/16-22°C and average daily irradiation of ~ 350 μmol $m^{-2}$ $s^{-1}$ PAR). Lichens were watered using distilled water (control P+, control P-), a solution of 50 μM Cu in distilled water (variants 50 μM Cu P+ and 50 μM Cu P-) and a solution of 500 μM Cu in distilled water (variants 500 μM Cu P+ and 500 μM Cu P-). Copper was added as $CuCl_2 \cdot 2H_2O$, and the pH of all solutions was adjusted to a value of 6.5. Lichens were irrigated at 48-h intervals (approximately 10 ml of solution for 1 g of air-dry weight of thalli; solutions were applied in two doses within a period of one hour in order to keep the lichens wet for a long time). Solutions were applied using a hand sprayer, and excessive amounts flowed out from the containers through pores in their bottoms.

The dry mass of lichens was determined by weighing of sub-samples dried in an oven overnight at 90°C. Sub-samples of lichens for analyses were taken from each container at 0, 2, 4 and 8 weeks after the start of the experiment. For physiological measurements, sub-samples were sprayed with the respective solutions 24 h before measurements and stored in plastic Petri dishes in order to be metabolically active.

**Chlorophyll degradation.** Lichen sub-samples (DW) were extracted in the dark for 1 h at 65°C in 5 ml of dimethyl sulphoxide (DMSO). Extracts were allowed to cool to ambient temperature, and absorbance was checked at 750 nm with a UVI Light XTD 2 spectrophotometer (Secomam, France) to be certain that absorbance was always less than 0.01. The ratio of optical densities at 435

and 415 nm (OD 435/OD 415), termed the phaeophytinisation quotient, was assumed to reflect the ratio of chlorophyll a to phaeophytin a. This parameter indicated integrity of the photobiont´s chlorophyll (Ronen & Galun 1984). Three replicates were used for each time and treatment.

**Photosynthetic efficiency .** The maximum efficiency of PSII was assessed from the Fv/Fm ratio (Fv/Fm = Fm – Fo/Fm), which interprets the difference between maximal (Fm) and minimal (Fo) fluorescence divided by the Fm fluorescence of dark-adapted lichens. Chlorophyll *a* fluorescence was measured with a FluorCam 800 MF instrument (Photon Systems Instruments Ltd., Brno, Czech Republic) on well wetted and dark-adapted samples (10 min) using a saturating flash of light (2000 μmol $s^{-1}$ $m^{-2}$) for 1 s.

**Soluble proteins analysis.** Approximately 100 mg (DW) of lichen material was homogenised on an ice-cold mortar with 50 mM potassium phosphate buffer. Homogenised material was centrifuged at 15 000 rpm at 4°C for 15 min. A measured volume (100 μl) of supernatant was taken, and absorbance (at 595 nm) was measured using the method of Bradford (1976) with bovine serum albumin as a calibration standard.

**Content of parietin.** Parietin content was analysed using high-performance liquid chromatography (HPLC). Lichen sub-samples (15-20 mg of dry weight) were extracted in cooled acetone for 60 min (Feige *et al.* 1993). The extraction was repeated at least three times. Acetone extracts were collected and evaporated. The residues were dissolved with a fresh 1.5-ml batch of acetone. Filtered acetone extracts were analysed by gradient HPLC (Feige *et al.* 1993) on a Tessek SGX C18 column at a flow rate of 0.7 ml × $min^{-1}$ [mobile phase: A = $H_2O$-acetonitrile-$H_3PO_4$ (80:19:1); and B = 95% acetonitrile]. The gradient program was as follows: 0 min 25% B, 5 min 50% B, 20 min 100% B, 25 min 25% B. Detection was performed at a wavelength of 245 nm (with an Ecom LCD 2084 detector). The standard of parietin was prepared from crystallised acetone extracts obtained from the lichen *X. parietina* (93% purity). Three replicates were used for each time and variant of the experiment.

**Cu uptake, total and intracellular Cu accumulation.** Sub-samples of lichen from each variant of the experiment were removed from the containers and subsequently rinsed with 10 ml of deionised water for analyses of total Cu content. Another set of sub-samples was washed for 20 min in 10 ml of 20 mM $Na_2$-EDTA (analytical grade) to remove surface-adsorbed Cu, and then rinsed with 10 ml of deionised water (for analyses of intracellular Cu content). Thalli were dried at 90°C for 24 h, weighed and subsequently digested for 48 h in 3 mL of 65% $HNO_3$ (Suprapur, Merck, Darmstadt, Germany) and 30% $H_2O_2$ (2:1, v/v) at room temperature with the volume brought to 10 mL with deionised water (n = 3) (Bačkor *et al.* 2009).

Copper concentration of the solution was determined by flame atomic absorption spectroscopy (FAAS). The analysis was performed using a Perkin-Elmer 3030B spectrometer (Perkin-Elmer Corp., Norwalk, CT, USA). Detection was at Cu $\lambda_{max}$ = 324.8 nm. Three replicates for each time and treatment were analysed. Assurance of quality and quality control were achieved by using ACS or better grade chemicals, analysis of all reagent blanks and calibration with standards.

**Ergosterol.** Ergosterol is the principal compound of the fungal plasma membrane and was used as a marker of fungal metabolic activity (Dahlman *et al.* 2002). All steps of sample preparation for HPLC analysis were conducted almost in the dark, since ergosterol is sensitive to light. Samples of lichen thalli (100 mg) were homogenised with 99% ethanol for 10 min. Extracts were transferred to 1.5 mL-Eppendorf tubes, shaken in the dark at 25°C for 30 min, then vortexed and centrifuged at 10,000×g for 15 min. The supernatant was immediately analysed by HPLC with a Kromasil 100 C18 column (150 × 4.6 mm, particle size 7 μm) as the separator at a flow rate of 0.8 mL $min^{-1}$, and isocratic elution was conducted with methanol as the mobile phase (Dahlman *et al.* 2002). Total time of analysis was 15 min. Ergosterol absorption was measured with a UV detector (Ecom LCD 2084) at 280 nm. A standard curve was prepared for a range of from 1 to 200 μg of ergosterol (Sigma-Aldrich, USA) dissolved in 1 mL of ethanol.

**Membrane lipid peroxidation.** Membrane lipid peroxidation was estimated using the thiobarbituric acid reactive substances (TBARS) assay, as described by Bačkor *et al.* (2009). Lichen thalli (200 mg) were homogenised with 0.1% (w/v) trichloracetic acid (TCA). The homogenate (1.5 mL) was centrifuged at 15,000×g for 15 min. The supernatant (0.3 mL) was collected and added to 1.2 mL of 0.5% thiobarbituric acid dissolved in 20% TCA. Eppendorf tubes were put in an oven at 90°C for 30 min and cooled in an ice bath (which gives a typical colour reaction), after which the solutions were then centrifuged again. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific absorption at 600 nm. The concentration of TBARS was calculated using the extinction coefficient for the TBA – MDA complex (155 $mM^{-1}$ $cm^{-1}$), and the results were expressed as μmol $g^{-1}$ of DW. Production of TBARS describes the membrane damage.

**Statistical analysis.** For statistical analysis, one-way analysis of variance and Tukey's pairwise comparison (MINITAB Release 16) were used at < 0.05. The number

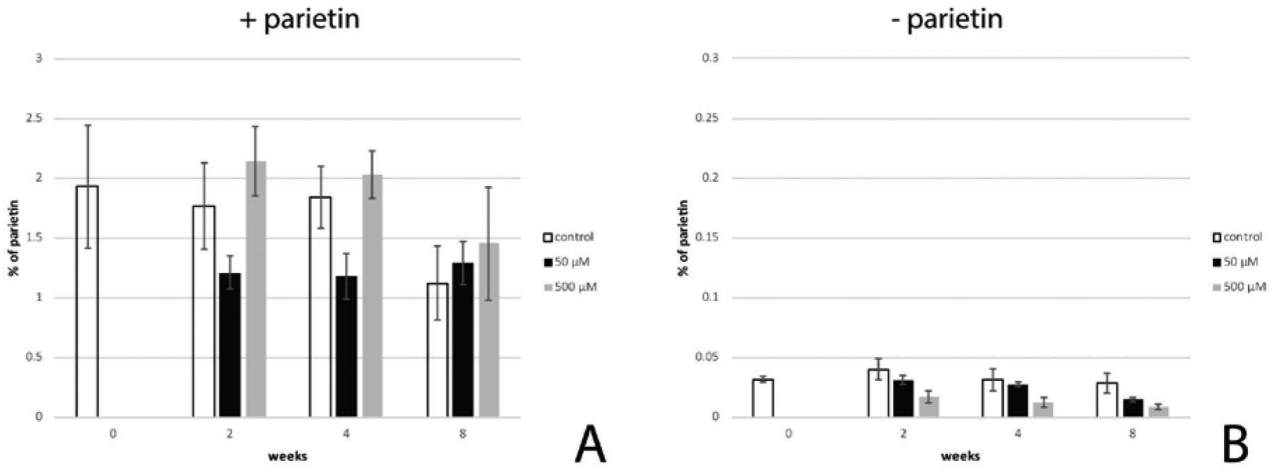


**Fig. 1.** Parietin content (% of DW) in thalli of *Xanthoria parietina* treated by exposure to Cu excess (µM) during 8 weeks of experiment (A) P+ = natural content of parietin, (B) P- = experimentally removed parietin, n = 3). The scale of Fig. 1B is reduced 10 times.

of replicates (n) denotes the number of individual measurements for each parameter.

## RESULTS

**Copper uptake and parietin content.** Removal of parietin from the lichen *X. parietina* did not significantly affect total and intracellular content of Cu in thalli in either the P+ or the P- variant at the beginning of the experiments.

Removal of parietin led to its significant decrease in the P- variant of the experiment. However, the content of parietin in *X. parietina* in individual test variants was stable during the whole experiment. No additional parietin synthesis was observed, either related to the excess of Cu or to prolongation of the experiment (Fig. 1A-B).

Application of Cu led to an increase of Cu accumulation in both variants (P+ and P-) in a concentration- and dose-dependent manner. While total Cu content was comparable in both variants of the experiment, after 8 weeks of prolonged exposure we observed increased accumulation of intracellular Cu in the P+ variant of the experiment when compared to the P- variant (Fig. 2A-D).

**Effect of Cu excess on physiological parameters in *X. parietina*.** Removal of parietin from dry thalli of *X. parietina* did not affect metabolic activity of the lichen. We did not observe significant changes in the measured photosynthetic parameters (chlorophyll fluorescence and integrity of chlorophyll *a*), symbiont membrane integrity (assessed as the content of MDA and ergosterol) or protein content (Figs. 3-4).

Fluorescence of chlorophyll *a* ($F_V/F_M$) decreased significantly in a dose-dependent manner at doses of 50 and 500 µM Cu, this decrease being most pronounced after 8 weeks of the experiment (Fig. 3A-B). The integrity of chlorophyll *a* decreased significantly as a result of exposure to a dose of 500 µM Cu after 2 weeks and more prolonged exposure, doing so similarly in both the P+ and the P- variants of the experiment (Fig. 3C-D).

The content of soluble proteins decreased significantly after 2-week and longer application of 500 µM Cu doses in both of the tested variants of the experiment (P+ and P-) (Fig. 4A-B).

Production of TBARS or MDA in *X. parietina* was not affected by the removal of parietin. TBARS increased significantly as a result of 2-week and longer application of 500 µM Cu doses in both variants of the experiment (P+ and P-) (Fig. 4C-D).

The content of ergosterol in the lichen *X. parietina* decreased significantly after 2-week and longer application of 500 µM Cu in both variants (P+ and P-) (Fig. 4E-F).

## DISCUSSION

Copper is an essential element that plays a crucial role in physiological processes of plants as well as lichens. Normal growth and development require quantities of Cu in the tissue between 5 and 20 µg $g^{-1}$ (Rhoads *et al.* 1989), while in lichens it can be even more. Copper is a constituent of the enzymes superoxide dismutase and cytochrome c oxidase, in addition to which it is a necessary co-factor of plastocyanin in the transmission of electrons in the lumen of thylakoids (Eriksson *et al.* 2004).

Responses of lichens to environmental stress induced by heavy metal excesses include many physiological adaptations. Some of them prevent entry of heavy metals into the thalli, while others are involved in intracellu-

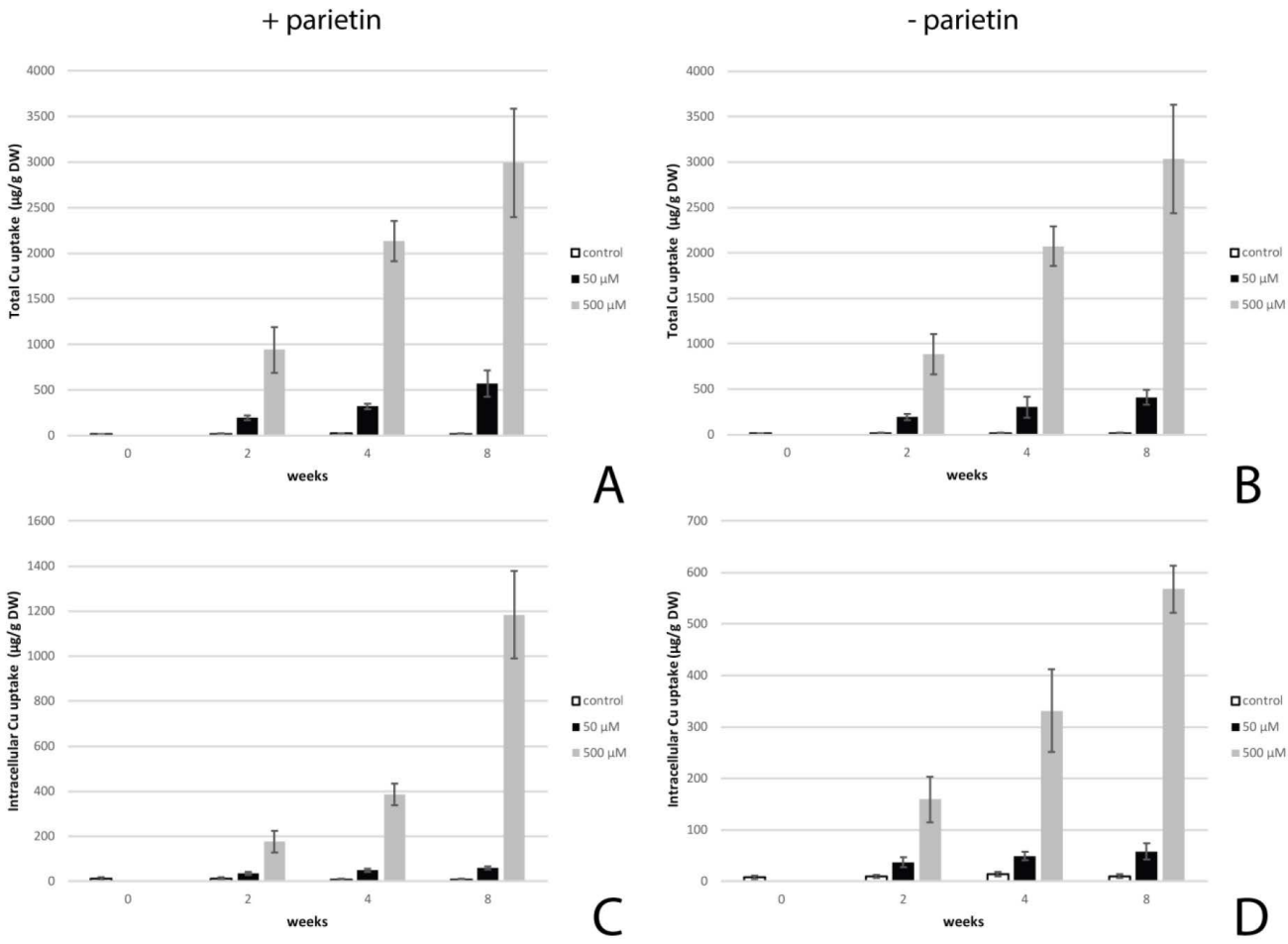


**Fig. 2.** Lichen *X. parietina* treated with distilled water (white bars), 50 µM Cu (black bars) and 500 µM Cu (grey bars) during prolonged exposure (0, 2, 4 and 8 weeks). The first row is the lichen *X. parietina* with the secondary metabolite parietin (+ parietin), the second row is the lichen without parietin (- parietin). (A, B) total Cu content in µg/g of DW; (C, D) intracellular Cu content in µg/g of DW, n = 3.

lar mechanisms of detoxification. In our experiment, we studied physiological adaptations of both P+ and P- thalli of *X. parietina* to excessive (50 µM and 500 µM) doses of Cu tested over 8 weeks of experimentation. To assess the role of the extracellular metabolite parietin, several physiological parameters (chlorophyll degradation, photosynthetic efficiency, the content of soluble proteins, Cu uptake, ergosterol content and membrane lipid peroxidation) were monitored in both variants of the experiment (P+ and P-).

Photosynthetic efficiency is defined as the potential quantum yield of electron transfer through photosystem II (Bačkorová *et al*. 2015). This parameter is expressed as the $F_V/F_M$ ratio, which in healthy lichen thalli is in the range of 0.6-0.7 (Kappen *et al*. 1998). Chlorophyll *a* fluorescence is an important parameter that reflects physiological conditions of lichen thalli (Boonpragob 2002). A decrease of photosynthetic efficiency was observed in the lichen *Ramalina lacera* after short-term exposure to Cu sulphate (Garty *et al*. 2002). In our experiment, we observed a decrease of fluorescence after 8 weeks, and vitality of the lichen photobiont was more influenced by doses of 500 µM. No differences were observed between thalli with parietin and ones without parietin.

High content of Cu can cause a decrease of chlorophyll *a* in lichens (Puckett 1976; Bačkor *et al*. 2003), which leads to disturbed integrity of the photosynthetic apparatus. Bačkorová *et al*. (2015) tested the effect of Cu excess in a long-term experiment and, as in the present study, observed a decrease of chlorophyll *a* integrity and other photosynthetic parameters during the 8 weeks of their experiment. Values between 1.2 and 1.4 are typical for healthy lichen thalli, and a gradual decrease was observed in our experiment after exposure to the higher tested Cu dose in both thallus variants over the course of 8 weeks.

Soluble proteins also play an important role in lichen tolerance against heavy metals, as do amino acids, the content of which can vary after heavy metal stress (Kong

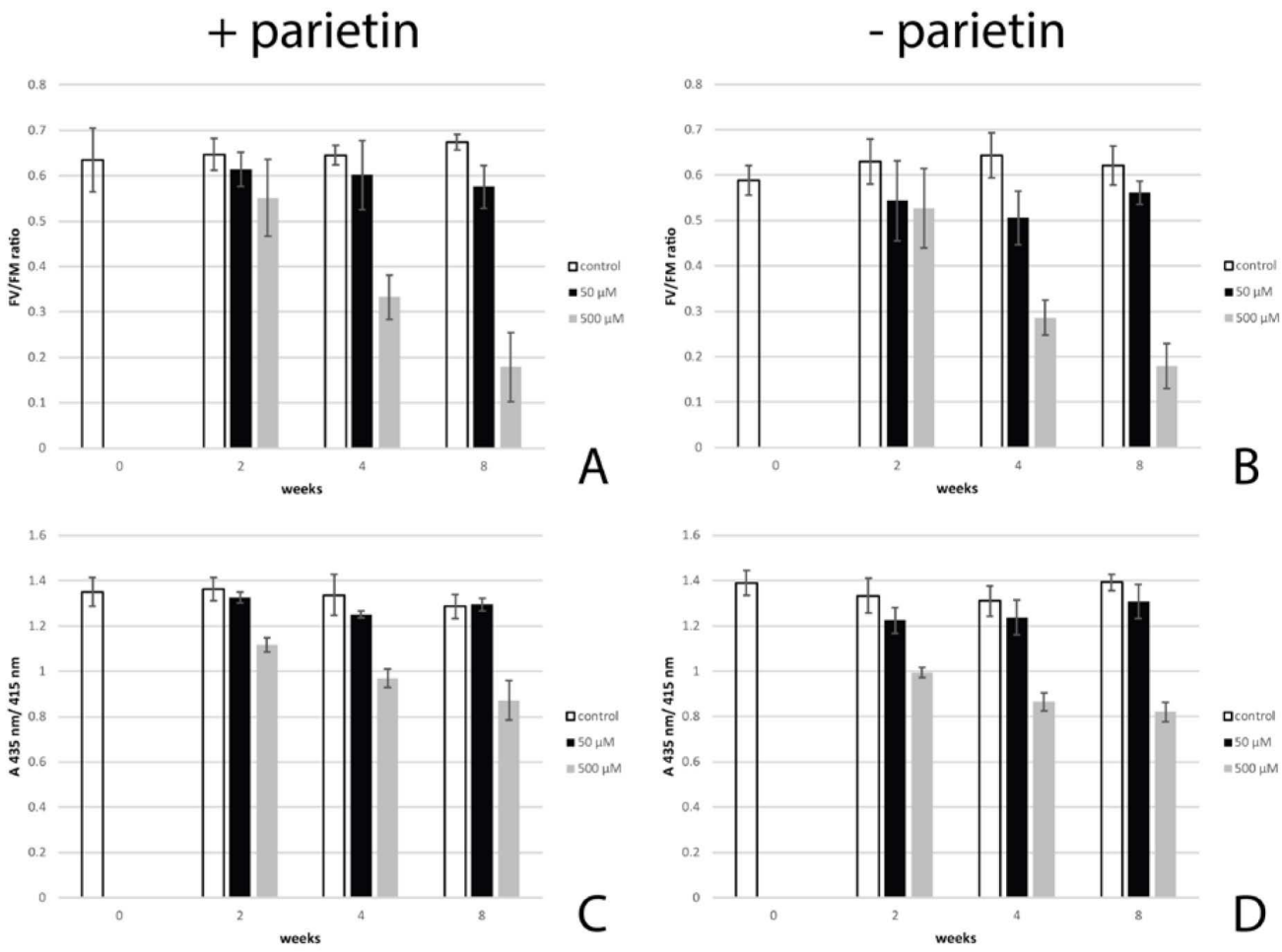


**Fig .3.** Lichen *X. parietina* treated with distilled water (white bars), 50 µM Cu (black bars) and 500 µM Cu (grey bars) during prolonged exposure (0, 2, 4 and 8 weeks). The first row is the lichen *X. parietina* with the secondary metabolite parietin (+ parietin), the second row is the lichen without parietin (- parietin). (A, B) Chlorophyll *a* florescence calculated as Fv/Fm; (C, D) Chlorophyll *a* integrity calculated as 435nm/415nm, n = 3.

*et al*. 1999; Monnet *et al*. 2006). Soluble proteins in the lichen *D. luridum* decreased after Cu excess (Monnet *et al*. 2006). Bačkor & Fahselt (2008) demonstrated Cu excess in axenic cultures of lichen photobionts. The sensitivity of photobionts to Cu doses is different in short-term experiments and appears to also depend on the type of photobiont. The lichens *C. arbuscula* subsp. *mitis*, C. *furcata* and *P. rufescens* with different photobionts were treated with 500 µM doses of Cu and a decrease of protein content was observed after 24 hours in *C. arbuscula* subsp. *mitis* and *P. rufescens* (Bačkor *et al*. 2009, 2011). A time- and dose-dependent decrease in the content of soluble proteins was demonstrated in an experiment with Cu excess lasting 8 weeks (Bačkorová *et al*. 2015). In our experiment, we obtained similar results: the content of soluble proteins decreased during 2-8 weeks of exposure to conditions of excessive Cu.

Ergosterol is a sterol of the fungal plasma membrane, and it is also used as a physiological parameter of stress. The level of ergosterol correlates with basal respiration rates of lichens (Sundberg *et al*. 1999). Bačkor *et al*. (2006) demonstrated a decrease of ergosterol content after short-term exposure to different Cu doses in the aposymbiotically grown *Cladonia cristatella* mycobiont. A decrease of ergosterol was also confirmed in a long-term experiment with Cu excess on *C. arbuscula* subsp. *mitis* (Bačkorová *et al*. 2015). We observed continuous decreases in the level of ergosterol as a function of the time of exposure to excessive Cu doses.

In the stress physiology of lichens, products of lipid peroxidation (e.g., malondialdehyde- MDA) are used as markers of membrane damage. Malondialdehyde is the main compound which reacts with thiobarbituric acid (TBARS). The content of TBARS is sensitive to Cu excess (Bačkorová *et al*. 2015). Copper is a redox-active metal, and a positive correlation between increase of ion permeability and the degree of membrane lipid peroxidation was observed previously (Vavilin *et al*. 1998). A

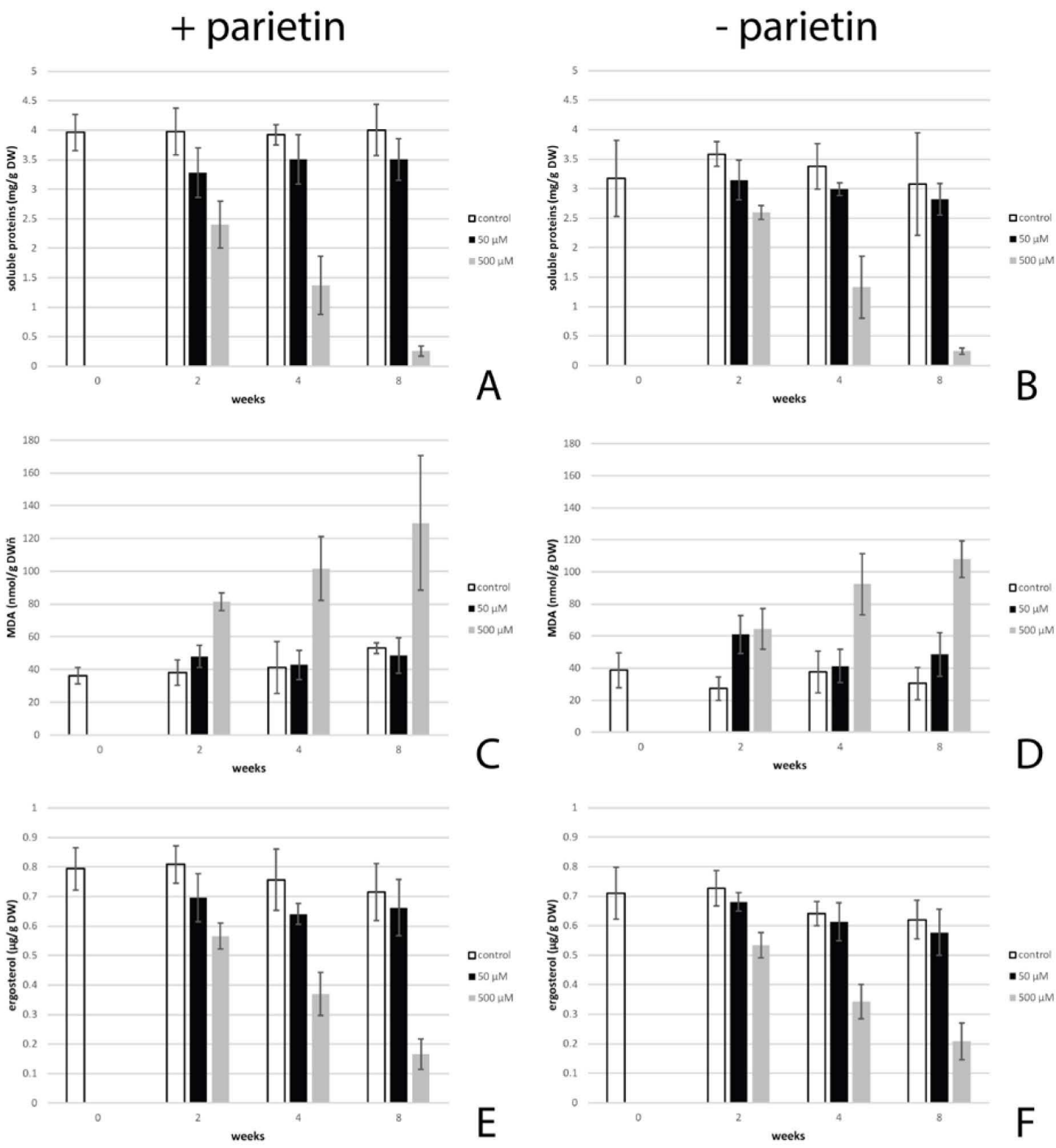


**Fig. 4.** Lichen *X. parietina* treated with distilled water (white bars), 50 µM Cu (black bars) and 500 µM Cu (cross-hatched bars) during prolonged exposure (0, 2, 4 and 8 weeks). The first row is the lichen *X. parietina* with the secondary metabolite parietin (+ parietin), the second row is the lichen without parietin (- parietin). (A, B) content of soluble proteins in mg/g of DW; (C, D) content of TBARS in nmol/g of DW; (E, F) content of ergosterol in mg/g of DW, n = 3.

negative impact of Cu excess on the MDA parameter in lichens was demonstrated in several studies (Monnet *et al*. 2006; Bačkor *et al*. 2011; Bačkorová *et al*. 2015). In our experiment, 500 µM Cu doses increased the production of MDA during 8 weeks of exposure.

Parietin is an orange-yellow anthraquinone pigment in lichens growing in habitats exposed to sunlight (Goga *et al*. 2018). This secondary metabolite is mainly localised in the upper cortex of the lichen thallus in the genera *Xanthoria*, *Teloschistes* and *Caloplaca*. Parietin

protects the photobiont against harmful UV radiation. It is deposited as an extracellular crystal on the surface of fungal hyphae. Metal complexation is very commonly recorded in the study of lichen substances (Hauck *et al.* 2008). There is still a lack of knowledge about Cu binding on parietin. Engstrom *et al.* (1980) studied complexes of $Fe^{3+}$ and parietin, and 20 years later Arakawa *et al.* (2001) demonstrated the formation of complexes of the anthraquinone chrysophanol with cations of Al, Ni and Zn.

Natural content of the secondary metabolite parietin in the lichen *X. parietina* is very high and may reach up to 2% of DW. Major secondary metabolites of *X. parietina* are parietin, emodin and parietinic acid. Parietin represents approximately 96-98% of secondary metabolites. In our experiment, the content of parietin in control thallus was 1.927 ± 0.514% of DW. The range of parietin in lichen thalli was 1.121 ± 0.314 – 2.143 ± 0.293% of DW. We did not observe any correlation with Cu treatment during the 8 weeks of our experiment. After removal of secondary metabolites from lichen thalli, the content of parietin was between 0.00863 ± 0.03947 and 0.3947 ± 0.00878%. Likewise, no correlation was observed between Cu treatment and *de novo* synthesis of parietin.

## CONCLUSION

*Xanthoria parietina* is a common lichen widespread around the world, the main secondary metabolite of which is parietin. There is evidence indicating that Cu chelates with secondary metabolites of lichens. Lichens treated with 500 μM Cu over a period of 8 weeks showed higher content of intracellular Cu in thalli in comparison with lichen thalli from which secondary metabolites were rinsed. It seems that the presence of parietin can promote bioaccumulation of Cu in the lichens. Removal of parietin from lichens did not influence their viability. *De novo* synthesis of parietin in Cu-treated thalli was not observed during the 8 weeks of our experiment. Physiological parameters measured after Cu stress did not differ in lichen thalli with and without the secondary metabolite parietin. *Xanthoria parietina* is sensitive to the higher dose of Cu during a long-term experiment, and it seems that the presence of parietin did not protect the studied metabolic processes against Cu excess.

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**REZIME**

# Fiziološki odgovor vrste *Xanthoria parietina* na dugotrajno izlaganje višku bakra: uloga ekstracelularnog sekundarnog metabolita parietina

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*Xanthoria parietina* je čest lišaj, rasprostranjen širom sveta. Tolerancija ovog lišaja na teške metale je dobro poznata. *Xanthoria parietina* sadrži sekundarni metabolit parietin koji štiti fotobiont od visokog UV zračenja. Utvrđeno je da sekundarni metaboliti lišajeva formiraju komplekse sa katjonima metala. U dugotrajnom eksperimentu (8 nedelja) smo testirali vijabilnost lišajeva sa i bez sekundarnog metabolita parietina, kada se izlažu dvema dozama bakra (50 μM i 500 μM). Uklanjanje parietina nije uticalo na merene fiziološke parametre, ali je uzrokovalo veću akumulaciju intracelularnog Cu. *De novo* sinteza parietina u talusima lišajeva, gde su metaboliti uklonjeni, nije istraživana. Visoke doze Cu su uticale na fiziološke parametre (fluorescencija hlorofila, integritet hlorofila *a*, sadržaj rastvorljivih proteina, ergosterol, proizvodnja TBARS) tokom dugotrajnog eksperimentalnog izlaganja. Izgleda da sekundarni metabolit parietin ne štiti lišaj od viška Cu.

**Ključne reči**: parietin, lišajevi, teški metali, akumulacija, tolerancija