E3S Web of Conferences **1**, 29007 (2013)
DOI: 10.1051/e3sconf/20130129007

Effects of Cadmium on Physiological Parameters of the Lichen *Evernia Prunastri* and *Ramalina Fastigiata*

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Abstract. The aim of the study was to investigate in short term experiment the physiological effects and accumulation of cadmium in the lichens *Evernia prunastri* (L.) Ach. and *Ramalina fastigiata* (Pers.) Ach. Treatment of lichen thalli with 5, 25, 50 and 500 μM Cd solutions caused a significant decrease in chlorophyll *a* and *b* contents and production of membrane lipid peroxidation, expressed as MDA content. Severe negative physiological effects were observed at the highest Cd concentration (500 μM Cd). Cd content in treated lichens thalli increased gradually as Cd concentration increased in treatment solutions. It was concluded that Cd exposure causes physiological and oxidative stress, with higher damage to *E. prunastri* due to higher Cd content bounded at intracellular sites.

Key words: cadmium, lichens, toxicity, oxidative stress.

Introduction

Elevated levels of heavy metals are toxic for the biota. The increase in metal working industries or urban traffic, also release by power stations and heating systems leads to intensified environmental pollution by these chemical elements.

Hundreds of studies carried out over the last 40 years confirmed that lichens were among the most reliable accumulators of inorganic contaminants (Bargagli and Mikhailova, 2002; Wolterbeek et al., 2003). Lichens could accumulate and retain many heavy metals in quantities that vastly exceed their physiological requirements and tolerate these high concentrations by binding metals extracellularly and intracellularly (Bačkor and Loppi, 2009).

As cadmium is a non-essential element it negatively affects plant growth and development (Benavides et al., 2005). It has no known metabolic function in plants, including lichens. Cadmium is recognized as a significant pollutant because of it high toxicity and large solubility in water. Cadmium inside cell directly affect physiological and metabolic processes related to toxicity - induced ultrastructural changes (Sorbo et al., 2011), changes in respiratory and photosynthetic rates (Bačkor et al., 2007; 2010), decrease in chlorophyll content (Bačkor et al., 2010) or cause oxidative stress (Sanitá di Toppi et al., 2005).

Different species respond differently to heavy metals stress. The relationship between morphology and acquisition of elements is still poorly understood (Bačkor and Loppi, 2009). The aim of the study was to investigate the effect of different Cd^{2+} concentrations on assimilation pigment composition and oxidative stress in lichens *Evernia prunastri* (L.) Ach. and *Ramalina fastigiata* (Pers.) Ach. and to compare the accumulation capacity of treated lichens.

Materials and Methods

Evernia prunastri (L.) Ach. and *Ramalina fastigiata* (Pers.) Ach. are fruticose green algae lichens with *Trebouxia* sp. as the photobiont. The lichen thalli were collected from Dubrava forest, an area 12km south-west of Kaunas city. Great care was taken to collect material under similarly exposed conditions. The basal part of the lichen thalli was detached together with the adhering pieces of wood substrate with a ceramic scissor. The thalli were stored in the laboratory at 25˚C under conditions of constant humidity (60%).

The experiments were undertaken 2–3 days after collection. The solutions were prepared using deionized

water. All the reagents used were of analytical grade (Merck). In order to study the impact of different concentrations of Cd^{2+} on some physiological parameters, 2 g of fresh thalli were soaked for 30 min in 100 mL solutions containing such Cd concentrations: 5, 25, 50 and 500 μM. Cadmium was supplied as $CdCl_2$. Control samples of lichen thalli were soaked for 30 min in deionized water. After the treatments, thalli were carefully washed with deionized water.

For the MDA analysis, fragments of lichen thalli were homogenized in a mortar using 0.1% (w/v) trichloracetic acid (TCA) with the addition of sand. The homogenate were centrifuged at 12 000g for 20 min. Supernatant (0.5 mL) was collected and added to 1.5 mL of 0.6% thiobarbituric acid in 10% TCA and put in glass tubes. Tubes were put in the oven at 95˚C for 30 min, cooled in an ice bath and then solutions were centrifuged. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific absorption at 600 nm.

Pigment analysis was performed according to Boonpragob (2002). Samples were subjected to washings with $CaCO_3$ saturated acetone. Lichens were then air-dried at room temperature to allow complete acetone evaporation and dry thalli were immersed in DMSO, with the addition of polyvinylpyrrolidone (PVP). Thalli were incubated at 65 ˚C for 45 min each in the dark to allow chlorophyll and phaeophytin to be extracted. Extracts were centrifuged and optical density was measure at 665 and 648 nm.

Thalli removed from metal solution were subsequently rinsed with 10 ml dH_2O (total metal content). Thalli were washed for 20 min in 10 ml of 20 mM Na_2-EDTA – to remove unspecifically cell-wall bound metals and then rinsed with 10 ml of deionized H_2O (intracellular metal content) (Ba2kor et al., 2010). Lichen samples were dried at 60˚C until constant weight and then digested using a mixture of 65% HNO_3 and 30% H_2O_2 (3:1, v/v). The extract was filtered through qualitative filter (Windaus-Labortechnik) and the filtrate volume was adjusted to 25 mL with deionised water. Cd content in lichen thallus was measured by flame atomic absorption spectroscopy (AA-6800, Schimadzu, Japan).

In order to evaluate the effect of different metals concentrations on physiological parameters of the lichen thalli, the results were analyzed using one-way ANOVA. For the significance ($p < 0.05$) of differences between control and Cd treated samples were calculated using nonparametric Kruskal-Wallis test.

Results and Discussion

Because the lichen photobiot is considered to be as a key element of lichen sensitivity, the content of photosynthetic pigment was regarded in this study. The concentration of Chl *a* significantly decreased as the concentration of Cd^{2+} in the solutions increased (Fig. 1a). The highest concentration of chlorophyll was detected in solution without cadmium (control) and the lowest levels corresponded to the highest metal concentration. This suggest that Cd has a harmful effect namely to the molecules of Chl *a*, particularly in *R. fastigiata*. Increased Cd (500 μM) also caused significant decrease on chlorophyll *a* in the lichens *Peltigera rufescens* (Bačkor et al., 2010) while there was no significant difference in the content of chlorophyll *a* of the lichen photobiont *Trebouxia erici* (Bačkor et al., 2007) the same photobiont as have in this study treated lichens.

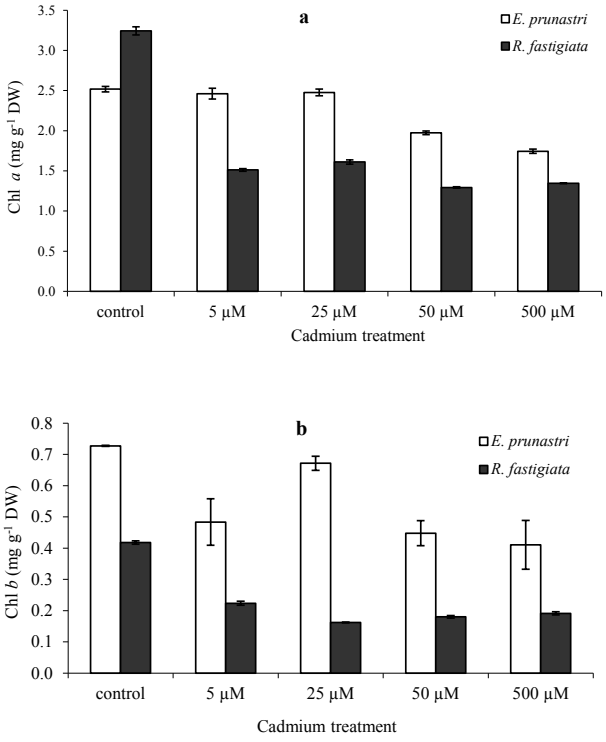

Fig. 1. Chlorophyll *a* (a) and *b* (b) content in the lichen *Evernia prunastri* and *Ramalina fastigiata* treated for 1 h with different Cd concentrations.

The lower chlorophyll *b* concentrations resulted in the thalli of treated lichens used in Cd treatments (Fig. 1b). *E. prunastri* had higher chlorophyll content than *R. fastigiata*. Over the treatment concentrations, the decrease in chlorophyll b concentration was more significant for *R. fastigiata* with lower Chl *b* content (54%) in comparison with *E. prunastri* (43%). In general, when compared with results of other studies (Bačkor et al., 2010), the present study results showed higher cadmium toxicity to treated lichens.

The levels of MDA were similar in control samples for both species (Fig. 2). Negative effect as a result of the increase in cadmium concentration was mostly pronounced following treatment with 500 μM Cd. Concentrations of MDA were significantly modified by any Cd treatment in lichen *R. fastigiata* ($p < 0.05$). This suggested that this cation is harmful even in the lowest concentrations.

Treatments with similar concentrations of Cd had no significant effect on TBAS levels of *Peltigera rufescens* and *Cladina arbuscula* subsp. *mitis* (Bačkor et

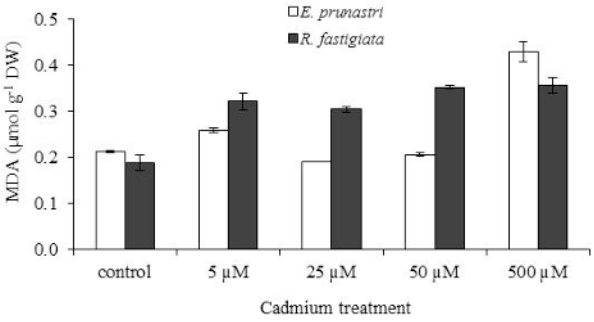

Fig. 2. Mean membrane lipid peroxidation (MDA content) in the lichen *E. prunastri* and *R. fastigiata* treated with different Cd concentrations.

al., 2010). Cd excess did not induced production of MDA also in lichen photobiont (Bačkor et al., 2007). Dzubaj et al. (2008) showed that MDA content was positively correlated with the presence of metal content in stress tolerant lichen *Xanthoria parietina* thalli but cadmium was unable induce severe lipid peroxidation. Cadmium as non-redox active metal is considered to be unable to stimulate formation of ROS directly. Correlation between MDA and extracellular potassium tended to prove that potassium leakage could be partly attributed to a loss of membrane integrity by lipid peroxidation as a result of extracellular Cd concentration (Cuny et al., 2004).

Baseline total concentrations of Cd in treated lichen were similar for both species - 0.5 μg g^{-1} DW ($p > 0.05$). Cd concentration increased with increasing concentrations of metal in solution (Fig. 3a; ANOVA, $P < 0.001$). Total cadmium concentration in *E. prunastri* and *R. fastigiata* reached 624 and 648 μg g^{-1} DW, respectively, when supplied cadmium concentration was 500 μM. There was no difference in the two lichen species capacity to accumulate Cd because the total Cd content was similar in both species at all tested concentrations ($p > 0.05$; Fig. 3a).

Intracellular Cd concentrations were significantly higher in *E. prunastri* than in *R. fastigiata* following 25 μM Cd treatment and higher (Fig. 3b). In *R. fastigiata* only concentration of 500 μM Cd caused significant increase in intracellular Cd accumulation.

Results of the present study indicated that increased Cd concentrations resulted in the increasing metal uptake for two lichen species. Cd was mainly accumulated in extracellular sites and this is in accordance with the results of other authors where cell walls were considered to be the primary targets of trace metals (Gonzalez et al., 1998). Sanitá di Toppi et al. (2005) observed that more than half of the total Cd was immobilized by the cell wall in *X. parietina*. Such accumulation tendency was in accordance with the results of the studies with other heavy metals - Cd, Ni (Bačkor et al., 2010), Cu (Branquinho et al., 1999; Bačkor et al., 2009). On the other hand, Cd was accumulated mainly in intracellular sites in *Hypogymnia physodes* thalli transplanted under conditions of chronic pollution (Mikhailova and Sharunova, 2008). Cd showed the highest affinity

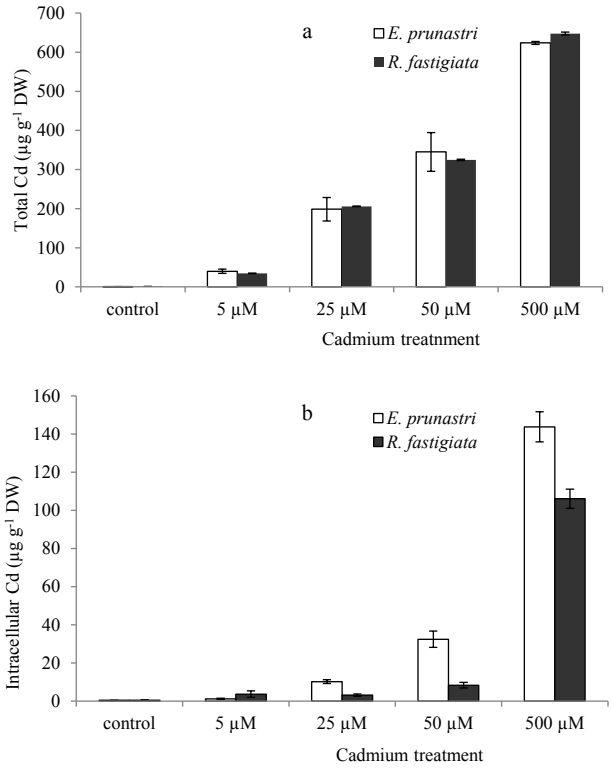

Fig. 3. Total (a) and intracellular (b) Cd content in the lichen *E. prunastri* and *R. fastigiata* treated with different Cd concentrations.

towards intracellular fraction also in moss *Pleurozium schreberi* samples (Virkutytė et al., 2008).

According to this mechanism, cadmium bounded to cell wall was unable to cause oxidative stress. In the present study, despite high binding of Cd in extracellular sites, this did not protect the cells from intracellular Cd accumulation in *E. prunastri* thalli. It is likely that cadmium has been fixed on intracellular sites, thus enhancing the peroxidation of membrane lipids (Fig. 2). It could be concluded that higher sensitivity of *E. prunastri* to metal stress was due to higher intracellular accumulation of metal.

Both particle trapping and ion uptake from solution depend on morphological features (branching, wrinkling, roughness) and anatomical features (size of pores, density of hyphae in the medulla) (Bargagli and Mikhailova, 2002). Since comparison of element uptake by morphologically similar species of *Parmelia* did not reveal any clear trend (Bargagli et al., 1987). Treated *E. prunastri* possesses pseudocyphellae in both lower and upper cortex as the main pore structures (Legaz et al., 1985) and such pores structure allows to higher element uptake. In *Ramalina* sp. the cortex is two layered, composed of an external and inner layers (Bowler, 1981). Such morphological characters of *R. fastigiata* may allow it to accumulate more Cd on intracellular sites.

Conclusion

Treatment of lichens with different Cd solutions caused a

significant decrease in chlorophyll *a* and *b* content and increase in MDA. Severe negative physiological effects were observed at the highest Cd concentration (500 μM Cd). Higher external cadmium concentration caused increase in total and intracellular cadmium uptake in treated samples. The increase in MDA levels suggests that Cd^{2+} has a damaging effect on the cellular membranes.

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