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# Assessment of air pollution at the indoor environment of a shooting range using lichens as biomonitors

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**ABSTRACT**

The aim of the study was to examine the changes in ecophysiological parameters and accumulation of heavy metals in lichens transplanted to a shooting-range environment. Thalli of the epiphytic lichen were transplanted from an unpolluted site to a shooting range. Chlorophyll a fluorescence, thiobarbituric acid reactive substances (TBARS), and damage to cell membranes in the lichen *Evernia prunastri* and *Ramalina farinacea* thalli were determined after 3 months exposure period indoors in a shooting range. The concentrations of some heavy metals including cadmium, copper, iron, manganese, nickel, lead, antimony, and zinc were measured in lichens as indicators of the levels of air pollution in the studied environment. Thalli of the lichens transplanted to the shooting-range environment showed stress symptoms where the presence of metal pollutants produced a loss of integrity of lichen cell membranes and induced oxidative stress as evidenced by increased levels of TBARS. The response of lichens transplanted to indoors of shooting range demonstrated a significant accumulation of Pb indicating potential increased metal exposure and consequent adverse health effects.

**KEYWORDS**

Shooting range; trace metals; lichens; indoor pollution; Pb

## Introduction

Trace elements are widely found in our environment from numerous sources. Shooting ranges are of increasing environmental concern in some countries (Sorvari 2007; Štěpánek et al. 2020). The negative environmental impact of the shooting activity has been related to the dispersal of shot and bullets that contains trace elements (Hui 2002). Shooting emits residues of trace elements into the air, posing a risk to the biota (Olson et al. 2018; Pineau et al. 2017) and human health (Grandahl, Suadicani, and Jacobsen 2012; Lach et al. 2015; Orru et al. 2018).

Shooting practices conducted at indoor shooting ranges are a source of potential health hazards. The indoor range air contains various gaseous components and trace elements including lead (Pb), copper (Cu), zinc (Zn), and antimony (Sb). In particular adverse health effects in the form of Pb poisoning were reported (Orru et al. 2018; Vandebroek et al. 2019). Lead exposure in indoor shooting ranges is a significant problem due to improper ventilation, ammunition type, and other factors (George et al. 1993; Orru et al. 2018). Workers and visitors in the shooting ranges may be exposed to hazardous Pb concentrations due to the release of this metal from the lead dust which is released into the air when the gun is fired (Demmeler, Nowak, and Schierl 2009; Valway et al. 1989). The employees and frequent shooters are subject to Pb exposure in indoor firing ranges when there are no adequate environmental controls to effectively protect the health of these individuals.

Lichens are useful to assess environmental contamination (Canha et al. 2014; Conti and Cecchetti 2001). Lichens lacking roots, possess large surface area and relatively low growth rate, associated with adsorption of substances from the atmosphere through the entire surface indicating that these organisms are largely dependent upon atmospheric deposition and are considered as effective biomonitoring specimens. Biomonitoring studies using lichens showed that different sources of air pollution induced a decrease in photosynthesis and damaged cell membranes (Zambrano and Nash 2000) enhancing oxidative stress (Carreras et al. 2009; Oztetik and Cicek 2011).

Lichens were used in the identification of air quality studies. Despite that lichens are extensively used in outdoor studies, in recent years there were

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few studies, which were conducted to assess the indoor air quality, such as school environment (Canha et al. 2014, 2012; Paoli et al. 2019a; Protano et al. 2017) or inside smoker's cars (Paoli et al. 2019b). Based upon these observations, the use of lichen biomonitoring was found as a suitable method for assessing indoor air quality. Thus, the aim of the study was to determine the effects of trace elements on the vitality and accumulation capacities of lichens transplanted indoor in shooting ranges.

## Materials and methods

### Sampling site characterization

The site of the study was an indoor shooting range that is located in Alytus recreational and sport center (54°24′15.2"N 24°01′21.2"E). The shooting range is 10 m long with nine firing lines (area of range approx. 100 $m^2$). Shooting range is designed only for shooting with air rifles and pistols and only 4.5 mm caliber plain Pb bullets (pellets) are used. The range is relatively new – established in 2013, modern with a ventilation system. The range was utilized by shooters constantly throughout the transplantation experiment, roughly 30 shooters per day.

### Transplantation of lichens

Thalli of lichens *Evernia prunastri* (L.) Ach. and *Ramalina farinacea* (L.) Ach. were collected from a relatively clean area, located 15 km from Kaunas. Prior to exposure, lichens thallus were cleaned from extraneous materials and placed in net bags (mesh size 0.5 cm). Lichen bags were placed indoors of the shooting range at different distances from the firing line – 0, 5, and 10 m. At each distance two different bags of each species were fixed at the height of 2 m from the floor of the range. The control set was transplanted to trees in the same location – outside the shooting range. Bags were exposed for 3 months (October – December 2019).

### Assessment of physiological and biochemical changes in lichens

For the assessment of lichen vitality, chlorophyll fluorescence of samples ($F_V/F_M$) was measured in three replicates as described by Sujetovienė and Galinytė (2016). For the assessment of the integrity of cell membranes, electrical conductivity (EC) was measured in triplicates before and after the samples were soaked and shaken for 1 hr (Marques et al. 2005). For the assessment of oxidative stress, membrane lipid peroxidation was determined with the thiobarbituric acid reactive substances (TBARS) assay (Heath and Packer 1968). Before centrifugation, samples were homogenized using trichloracetic acid (TCA). The supernatant was added to 0.5% thiobarbituric acid in 20% TCA and placed in the oven at 95°C for 30 min. The absorbance of the centrifuged supernatant was measured at 532 nm using microplate spectrophotometer SPECTROstar Nano (three replicates).

### Trace elements analysis

In order to detect the presence of trace elements in the samples, dried and homogenized lichen samples (approximately 100 mg of lichen powder) were digested (4:1 v/v mixture of nitric acid and hydrogen peroxide) using a microwave oven (Milestone Ethos One). The concentrations of trace elements (Cd, Cu, Fe, Mn, Ni, Pb, Sb, Zn) were determined using ICP-OES (Perkin-Elmer, Optima 8000). The calibration of trace elements was performed with the Quality Control Standard 21 (Perkin Elmer). The accuracy of the analysis was estimated by the coefficient of correlation (>0.99 for all measured elements).

### Data analysis

The exposed to control ratio (EC ratio) was employed to determine the levels of accumulated trace elements by lichens. This is the difference between the values of the content after and prior to exposure. The obtained values of the EC ratio were grouped according to the scale proposed by Frati, Brunialti, and Loppi (2005). For the comparison of the significant difference between means, the Tukey HSD test was used. The criterion for significance was set at $p < 0.05$. Statistical analyses were performed using STATISTICA 7 software.

## Results

During the exposure to shooting-range emissions, a decrease in lichen vitality was detected expressed

as fluorescence intensity ($F_V/F_M$) as presented in Table 1. Data in Table 1 also demonstrate a significant increase content of TBARS levels in *E. prunastri* and *R. farinacea* indicative of oxidative stress after shooting-range emission exposure. Damage to cell membranes as evidenced by electrolyte leakage (EC) was markedly higher only in thalli of *E. prunastri* (Table 1)

The response of lichens transplanted to indoors of shooting range for 3 months indicated a significant accumulation of Pb (Figure 1). The bioaccumulation of Pb ranged from 4.88 to 36.54 µg/g in *E. prunastri* and from 1.65 to 7.89 µg/g in *R. farinacea*. Although *E.prunastri* accumulated higher amounts of the element, there was no significant difference in the accumulation capacity of treated lichen species. In the case of other trace elements, normal EC levels ranging from 0.53 to 1.12 were detected in lichens transplanted indoors of the shooting range (Figure 1). A significantly higher amount of Fe, Sb, Zn was bioaccumulated in *R.farinacea* as compared to *E.prunastri*.

**Table 1.** The ecophysiological parameters ($F_V/F_M$, EC (µS $cm^{-1}$ ml $mg^{-1}$), TBARS (µmol $g^{-1}$) of exposed lichens *Evernia prunastri* and *Ramalina farinacea*.

| | *Evernia prunastri* | | *Ramalina farinacea* | |
|---|---|---|---|---|
| Parameter | Control | Shooting range | Control | Shooting range |
| Fv/Fm | 0.78 ± 0.05 | **0.15 ± 0.01** | 0.73 ± 0.01 | **0.23 ± 0.04** |
| EC | 6.80 ± 0.53 | **10.17 ± 1.28** | 6.40 ± 0.67 | 7.99 ± 1.03 |
| TBARS | 5.91 ± 0.14 | **3.94 ± 0.13** | 7.03 ± 0.52 | **3.96 ± 0.19** |

Bold values indicate significant from control ($p < 0.05$).

## Discussion

Shooting ranges are places of concern because of Pb in ammunition fired at the ranges and potential adverse consequences. Various investigators try to verify design criteria of ranges and to collect chemical and physical data that might help define the nature and extent of problems (Schaeffer, Deem, and Novak 1990). The users of indoor ranges may be at risk from Pb exposure and protecting them from the inhalation of shooting related contaminates is the key objective (Morgenthaler and Shumway 2002). It is estimated that concentrations of airborne particulate Pb in indoor shooting range far exceeded the standards (Jackson and Dell 1992; Olmez et al. 1985) leading to increase blood metal levels of trainers (Abudhaise et al. 1996; Grandahl, Suadicani, and Jacobsen 2012; Valway et al. 1989). Exposure to airborne Pb in indoor firing ranges is a documented occupational health hazard (Tripathi et al. 1990).

The decrease in lichen vitality indicated lowered indoor air quality due to emitted trace elements. The exposed lichens in the shooting range indicated higher conductivity values than the reference level. This tendency is observed in other indoor studies (Canha et al. 2014, 2012). Exposure of shooting-range users to high levels of indoor air pollutants such as trace elements was confirmed in our biomonitoring study using lichens. Elevated levels of Pb in the shooting ranges atmosphere are may be attributed to Pb dust particles when bullets containing this metal are fired. The bioaccumulated levels of Pb in our study were higher than those noted in primary schools where the concentration of Pb in indoor air was in the range of 5.18–7.01 µg/g (Awang and Jamaluddin 2014).

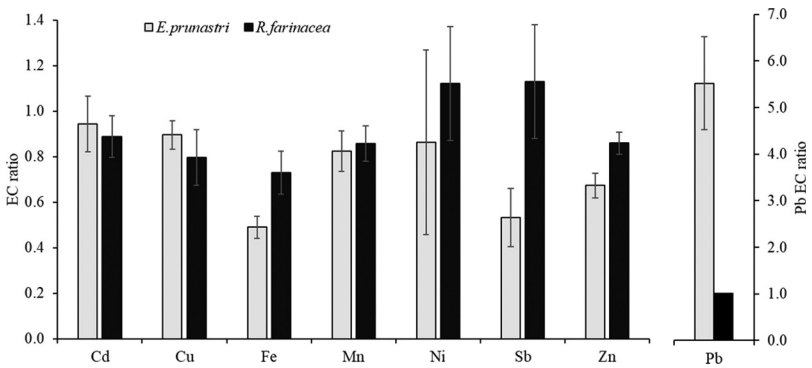


**Figure 1.** Element accumulation as exposed-to-control ratio (EC, mean ± SE) of samples transplanted indoors in the shooting range.

An accumulation for several other trace elements by lichens was found indoors by other investigators. Canha et al. (2012, 2014) detected Ca as the sole element in indoor origin in school environments due to the use of chalks. Other studies showed Cd accumulation in transplanted *Pseudevernia furfuracea* at the urban school and Hg in the rural area (Protano et al. 2017). Paoli et al. (2019a) reported accumulation of Cd, Cu, and Pb in urban and rural areas of Italy. While in most studies bioaccumulated elements were attributed to vehicular traffic emissions, in our case the origin of Pb significant accumulation was associated with indoor air shooting activity. Despite the fact that there are only a few studies using lichens (Canha et al. 2012, 2014; Canha, Do C. Freitas, and Almeida 2019; Protano et al. 2017; Paoli et al. 2019a, 2019b), mosses (Capozzi et al. 2019) and plants (Rzepka et al. 2010) for assessment of indoor air quality, this is the first investigation focusing on risk assessment in shooting ranges. It is conceivable our data might help define the nature and extent of the problem in shooting ranges. Our findings support the postulation for use of lichens in biomonitoring studies assessing indoor air quality (Paoli et al. 2019). The high levels of Pb observed indicate potentially exposure and risk for adverse consequences for users and personnel in these shooting ranges.

Comparing two transplanted lichens, *E. prunastri* accumulated higher levels of Pb than *R. farinacea*. Nevertheless, for the other elements including Fe, Sb, and Zn the accumulation was significantly higher in *R.farinacea*. The variation in accumulation capacity of different species was also noted in other studies. The content of toxic elements (Ni, Pb, Cd, Zn) was detected to be higher in *Ramalina pollinaria* than in *E. prunastri* (Cansaran-Duman, Altunkaynak, and Aras 2014; Cansaran-Duman, Atakol, and Aras 2011). However, Cercasov et al. (2002) investigating the suitability of lichen species as transplants to trace-element air biomonitoring found that *E.prunastri* was more sensitive followed by *R. farinacea* as evidenced by the correlation between bioaccumulation and bulk deposition measurements. The differences might be related to the morphology of transplanted lichen species where *Evernia* has dorsiventral thalli while *Ramalina* exhibits radial thalli (Jahns 1973). *Ramalina* despite displaying a flat lobe has algal cells in a flattened circle while in *Evernia* is just a single upper algal layer. Because climatic and environmental conditions were equal for both transplants higher concentrations of trace elements in *R. farinacea* confirm that it appears to be more useful to identify contamination sources in the indoor environment by bioaccumulation of transplanted lichens.

In conclusion, data demonstrated the importance of heavy metal accumulation in lichens. The transplanted lichens *Evernia prunastri* and *Ramalina farinacea* exhibited bioaccumulation of element capacity for assessment of indoor air quality in shooting ranges. Exposure to control ratios showed higher trace element deposition under the shooting activities. The response of lichens transplanted indoors to shooting ranges indicated a significant accumulation of Pb. Data demonstrate that good air quality during shooting activities may be a concern for health.

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