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Biomonitoring of urban air quality in Kaunas City (Lithuania) using transplanted lichens

Gintarė Sujetovienė*

Department of Environmental Sciences, Vytautas Magnus University, Vileikos 8, LT-44404 Kaunas, Lithuania

The aim of the study was to evaluate air quality using an active biomonitoring method. The lichens *Ramalina farinacea* and *Evernia prunastri* were transplanted to urban sites in Kaunas City, Lithuania. Changes in chlorophyll *a*, chlorophyll *b*, phaeophytization ratio, electrical conductivity were determined after one, two and three months. After stimulation of chlorophyll synthesis the increase in chlorophyll content was observed after one month with its decrease during the next months. No significant variation in content of chlorophyll was observed in comparison with control and this could be attributed to variables of air pollution which did not exceed threshold levels. There was no interspecific difference in the response of the two lichen species in the physiological parameters, but *Ramalina farinecea* was more sensitive to cell membrane damage than *Evernia prunastri* in the studied urban environment. The study provides information that one month of transplantation is not enough in order to determine air quality status in urban environment.

Key words: lichen transplants, urban environment, air pollution

INTRODUCTION

Air pollution has been reported as an extensive cause of damage to the vegetation of urban environment. The main pollutants of concern are emitted into the atmosphere by traffic and industry – nitrogen oxides, sulphur dioxide, particulate matter, trace elements, etc.

In order to evaluate the effects of the airborne pollutants many organisms are used as bioindicators of changes in such environment. Such technique is an effective tool detecting the impact on environment and human health in

* Corresponding author. E-mail: g.sujetoviene@gmf.vdu.lt

the surroundings of the emission sources. Lichens are one of the most extensively studied biomonitors using active and passive lichenology (Conti, Cecchetti, 2001).

The amount of pollutants has considerably increased in cities because of increasing urbanization. To find naturally growing species as biomonitors is difficult in polluted or urbanized environment. In these cases lichen transplantation technique has been used. Studies involving the physiological and chemical analyses of transplanted lichens for biomonitoring of environmental pollution have been extensively used. These applications have proved the sensitivity of lichens to a variety of air pollution and the ability to indicate polluted environment (Boonpragob, Nash III, 1991; Carreras et al., 2009; Chaparro et al., 2013). Analysis of their chemistry and physiological characteristics is a suitable and inexpensive tool for air quality evaluation. Some pollutants in urban environment are known to destroy cell membranes (Munzi et al., 2009), induce oxidative stress (Paoli et al., 2011), disrupt photosynthetic apparatus (Tretiach et al., 2012) and bring change in photosynthetic pigments (Carreras et al., 1998; Bačkor et al., 2010).

The use of biological indicators is very important for the assessment of environmental quality and human health risk (Carreras et al., 2009). The aim of the present study was to evaluate the physiological response of *Evernia prunastri* and *Ramalina farinacea* under the influence of urban environment.

MATERIAL AND METHODS

Study area

Kaunas City is located in the centre of Lithuania (54° 54' N, 23° 54'). The climate is continental with mean temperature of 16.9 °C in July and −5.5 °C in January. Average annual rainfall is 627 mm. Prevailing winds blow from the south east. A detailed description of the study area and sources of atmospheric pollution is given in Sujetoviene, Sliumpaite (2013).

Sample collection and exposure

Two lichen species were chosen to evaluate air quality of the city – *Ramalina farinacea* (L.) Ach. and *Evernia prunastri* (L.). These species are quite common in Lithuania, except in the cities and around industrial complexes. Thalli of lichens were collected from an area with a low pollution level, Birštonas suburbs, 35 km south from Kaunas City. All lichen material was obtained and transported to the laboratory until the exposure in clean plastic bags. Material was cleaned from extraneous substances using ceramic tweezers.

The lichen material was packed loosely in nylon netting bags (1 × 1 mm) with a nylon rope and suspended at 2–3 m above the ground. The control set was transplanted into lichen native unpolluted habitat (Birštonas). The lichen thalli were transplanted to nine study sites in Kaunas City.

The transplantation period was from October to December, 2011. At the end of every month part of transplanted lichens was taken, the remaining part was left for further exposure (after 30, 60, 90 days). Part of the picked material was subjected to the analysis after each transplantation period. At the end of the exposure period, lichen bags were collected and lichen material was placed in clean plastic bags in order to avoid contamination.

Physiological measurements

The following parameters were measured: chlorophyll *a* and *b*, chlorophyll degradation and cell membrane permeability. Chlorophyll content and chlorophyll degradation were determined according to Boonpragob (2002). As solvent for extraction of chlorophylls and phaeophytins dimethyl sulfoxide (DMSO) was used. The ratio of optical densities at 435 and 415 nm (OD 435/OD 415) was interpreted as the phaeophytization quotient, which reflects the ratio of chlorophyll *a* to phaeophytin *a*.

To check the integrity of the plasma membrane enclosing cells a difference in electrical conductivity measured by placing the thalli in distilled water was used (Munzi et al., 2009). Each sample was rinsed several times with deionised water, until stable conductivity values were obtained. The electrical conductivity of water was measured before and after lichen immersion using conductivity meter. Then samples were put in the oven at 105 °C for 24 h to obtain dry weight. Conductivity values were expressed in μS cm^{-1} ml mg^{-1} dry weight.

Statistics

As the number of study sites was low, non-parametric statistics was used for statistical data tests. The Mann-Whitney test was used to determine the significant differences between physiological parameters of samples collected during different periods and comparing the difference between exposed and control samples.

RESULTS

Chlorophyll *a* concentrations significantly increased in lichens *Ramalina farinacea* during the first month after transplantation (by 75.8%) in comparison with control ($p < 0.05$, Figure a). Over time chlorophyll *a* concentration decreased in *Ramalina farinacea* but after Day 90 it still remained significantly higher (9.6%) in the urban environment than under the background conditions ($p < 0.001$). The increase in chlorophyll content in *Evernia prunastri* was not so prominent after the first study month (17.7%) as in *Ramalina farinacea*. After Day 60 and Day 90 chlorophyll *a* content in *Evernia prunastri* gradually decreased and was not significantly different from that of the reference ($p > 0.05$, Figure).

A similar tendency of changes during the study period was observed for chlorophyll *b* in

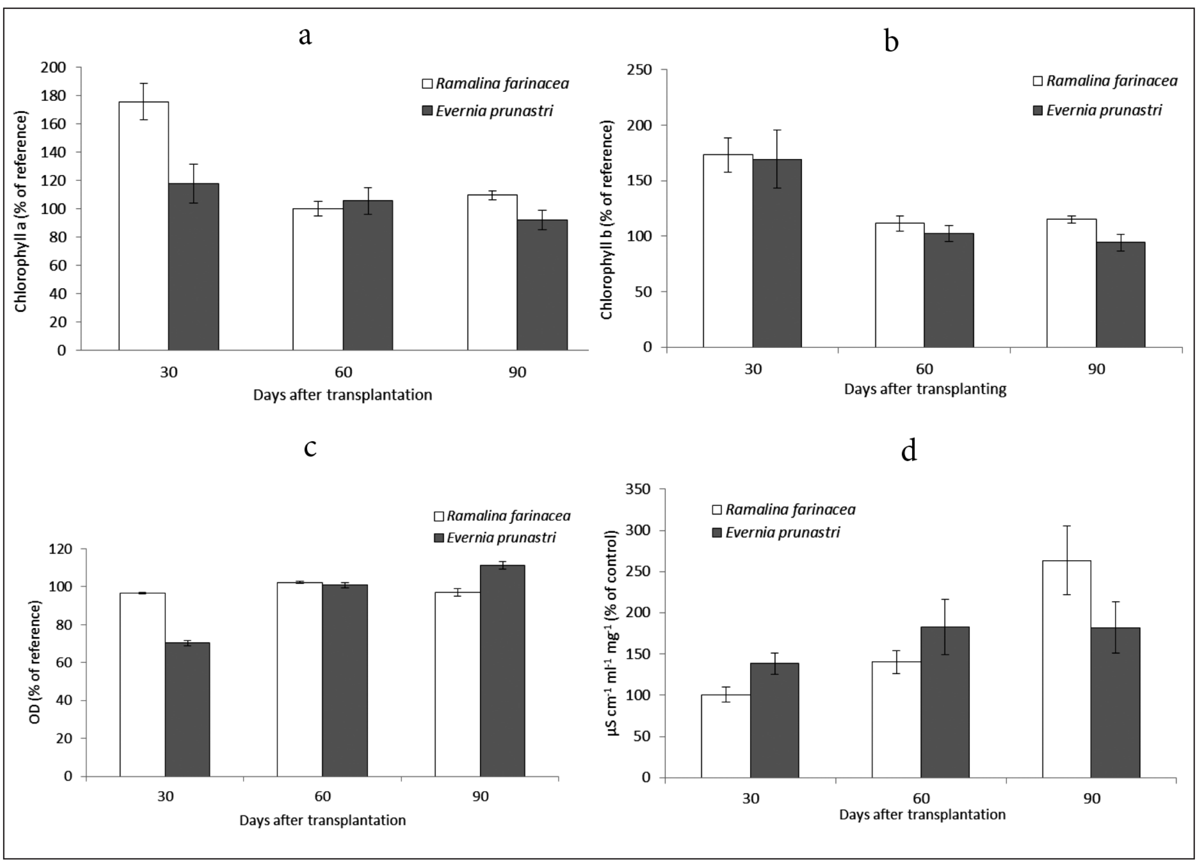

Figure. Content of chlorophyll *a* (a), chlorophyll *b* (b), OD_{435}/OD_{415} (c) and membrane permeability (d) of the transplanted lichens over time (% of the reference content) the transplanted lichens (Figure b). After Day 30 chlorophyll content was significantly higher than in control – 69.4% in transplants of *Evernia prunastri* and 48.2% in *Ramalina farinacea*. At the end of the transplantation period, decrease in chlorophyll *b* content was observed and its content in *Evernia prunastri* was not significantly different from control ($p > 0.05$). Chlorophyll *b* content in *Ramalina farinacea* was significantly higher in urban environment than under the background conditions ($p < 0.05$).

The ratio of optical densities at 435 and 415 nm (OD 435/OD 415), interpreted as the phaeophytinization quotient, significantly decreased in *Evernia prunastri* after the first month of the transplantation (Figure c). However, during the next study month the ratio remained relatively stable compared with the reference. In contrast, in *Ramalina farinacea* the degradation of chlorophyll still persisted in the urban environment during the study period.

Another parameter used in order to assess the response of transplanted lichens to urban environment was determined according to the measurements of the leachate electrical conductivity. The values of the conductivity increased significantly in lichen *Evernia prunastri* (Day 30 – 38.3%, Day 60 and Day 90 – more than 70%) transplanted to the urban area in comparison with control ($p < 0.05$, Figure d). After 30 days the membrane permeability in *Ramalina farinacea* was not affected, but after Day 60 and Day 90 the damage to membranes was significantly higher (163.0%) under the influence of environmental conditions than in control ($p < 0.05$, Figure d).

DISCUSSION

Physiological response of lichens transplanted to polluted environment is very useful as a stress indicator. The results give additional knowledge on the effects of pollutants on organisms in the urban environment and may be used detecting symptoms of affected environmental conditions. Chlorophyll content of both transplanted lichen species increased in the beginning of the transplantation period (Day 30). Such phenomenon could be explained as the effect of pollutants – compounds of nitrogen and sulphur in urban environment could act as nutrients for lichens and as a result their vitality increased compared with control. A similar response of transplanted lichens was observed in Biel City – transplanted *Parmelia sulcata* also contained higher amount of chlorophyll (von Arb, Brunold, 1990). Samples of transplanted lichens in the polluted urban environment also had higher chlorophyll content than in remote areas in northern Switzerland (von Arb et al., 1990). Higher nutrient availability resulted in higher concentrations of photosynthetic pigments in *Platismatica glauca* under the moderately enhanced nitrogen and sulphur deposition (Ra et al., 2005). Such phenomenon was attributed to nitrogen pollution from the traffic emissions. Some treatments with solutions containing ammonium and nitrate nitrogen also showed positive increase in chlorophyll content in lichens along with higher concentrations of nitrogen (Sanchez-Hoyos, Manrique, 1995). However, a decrease in total chlorophylls was observed in the sites of Bratislava with a greater proportion of nitrophilous species (Lackovičová et al., 2013).

A similar tendency of changes during the study period was observed for chlorophyll *b* in the transplanted lichens. The highest content of chlorophyll *b* was also measured at the sites of the city centre (Bačkor et al., 2003), on the contrary, chlorophyll *b* content decreased in polluted urban environment (Zambrano et al., 1999; Zambrano, Nash III, 2000).

Results of experiments showed that during the long-term exposure the chlorophyll content decreased in some lichens species (Boonpragob, Nash III, 1991; Canas, Pignata, 1998). This suggests that the duration of the exposure can influence the photosynthetic pigment concentrations and this is in accordance with our results – chlorophyll *a* and *b* content decreased over the study period. The content of photosynthetic pigments in the transplants gradually decreased over the exposure period. One month period of transplantation was not enough in order to determine the effects of atmospheric pollutants on the transplanted lichens. This was possibly caused by chlorophyll degradation. It is assumed that changes in chlorophyll content can be used as an estimate of environmental quality: low level would indicate poor quality. Some authors found some relationship between transplants lichen's responses to air pollution – photosynthetic pigments content was related with atmospheric pollution (Canas, Pignata, 1998; Carreras et al., 1998; Carreras, Pignata, 2001; Bačkor et al., 2003). In our study, the level of chlorophyll degradation was higher in *Evernia prunastri* after transplantation to urban environment but after 90 days transplantation chlorophyll degradation decreased and at the end of transplantation it was not significantly different from control. Based on these results it was concluded that biomonitors could favour the moderate levels of fertilizing pollutants under the inconsiderably altered air quality status.

The optimal value of OD_{435}/OD_{415} is considered to be about 1.4 and it is characteristic for healthy lichen (Ronen, Galun, 1984). The study results showed that such phaeophytization quotient was characteristic for the transplanted lichens in control sites. Despite of a small decrease in the beginning of the study the ratio in the thalli of *Evernia prunastri* remains quite stable over the study period under relatively unpolluted conditions. Contrariwise, chlorophyll degradation in the thalli of *Ramalina farinacea* transplanted in the city was very low over the study period. It could be concluded that chlorophyll degradation was not induced under the urban environment. Such a tendency of changes was also observed in lichens treated with nitrate solutions (Balaguer, Manrique, 1991). The results of the other study showed no significant difference in the ratio between exposed to nitrate and ammonium samples of lichens (Munzi et al., 2009).

The damage to cell membrane is proved to be one of the best physiological indicators consistent with air quality status (Paoli et al., 2011). The electrical conductivity was higher in the samples transplanted to the urban environment. A higher degree of cell membrane damage was observed in *Evernia prunastri* than in *Ramalina farinacea* during the first month but changes occurred in reverse order at the end of the study period – higher values of conductivity were characteristic for *Evernia prunastri*. These results are consistent with the experiment treating lichens with different nitrogen concentrations (Munzi et al., 2009). The injuries to cell membrane were also positively correlated with the concentration of trace elements in the urban and industrial sites (Garty et al., 1998). When lichens were transplanted in and around an industrial area of Italy the increase in cell membrane damage was consistent with the lower air quality depicted by the lichen diversity (Paoli et al., 2011).

CONCLUSIONS

The transplanted lichens to Kaunas City did not experience a physiological decline as content of chlorophyll increased and phaeophytization quotient was relatively stable under the urban environment. After stimulation of chlorophyll synthesis the increase in chlorophyll content was observed after one month with its decrease during next months. Over the transplantation period chlorophyll gradually decreased but it was not significantly different in comparison with control. The effects of gaseous pollutants could be the cause of contribution to lichen injury – damage to cell membranes of the transplanted lichens was observed after three-month transplantation period. There was no interspecific difference in the response of the two lichen species in the physiological parameters but *Ramalina farinecea* was more sensitive to cell membrane damage than *Evernia prunastri* in the studied urban environment. Based on these results it was concluded that biomonitors could favour the moderate levels of fertilizing pollutants under inconsiderably altered air quality status.

The duration of exposure could have a significant effect on the vitality of lichens in the transplantation studies. The results of the study demonstrated that one month exposure of urban pollutants did not indicate the real situation of environment quality.

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Gintarė Sujetovienė

KAUNO MIESTO URBANIZUOTOS APLINKOS KOKYBĖS BIOMONITORINGAS

Santrauka

Tyrimų tikslas – įvertinti oro kokybę aktyviosios lichenoindikacijos metodu. Kerpės *Evernia prunastri* ir *Ramalina farinacea* iš santykinai švarios aplinkos buvo transplantuotos į urbanizuotą – Kauno miestą (Lietuva). Chlorofilo *a* ir *b* kiekis, feofitino santykis ir elektrinis laidumas kerpėse buvo vertintas po vieno, dviejų ir trijų ekspozicijos mėnesių. Nors fotosintezės pigmentų koncentracija praėjus mėnesiui padidėjo, vėliau jų kiekis urbanizuotoje aplinkoje sumažėjo, bet statistiškai patikimo skirtumo su kontrole nenustatyta. Remiantis gautais rezultatais, galima teigti, kad oro kokybė neviršija leistinų lygių. Ryškių tarprūšinių skirtumų nenustatyta, tik *Ramalina farinacea* buvo jautresnė ląstelių membranų pažeidimams nei *Evernia prunastri* tirtoje urbanizuotoje aplinkoje. Tyrimo rezultatai rodo, kad, siekiant nustatyti oro kokybę urbanizuotoje aplinkoje, vieno transplantacijos mėnesio nepakanka.

Raktažodžiai: kerpės, urbanizuota aplinka, oro tarša