Physiological Response of the Lichen *Evernia prunastri* Transplanted near a Landfill in Central Lithuania

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Abstract. Landfilling is the most widely used method for disposal of municipal solid waste. The waste disposal sites are a matter of concern due to their adverse effects on the environment and health. Lichen *Evernia prunastri* were used for biomonitoring the effect of air pollution in the vicinity of one of the largest solid waste landfill in Lithuania. Lichen transplants were exposed for 3.5 months. Potential quantum yield expressed as Fv/Fm in thalli was affected at the closest to the pollution source sites and the values were lower in comparison with the control. The conductivity of leachate and content of malondialdehyde content (MDA) increased in lichen material transplanted at sites facing the landfill.

Keywords: atmospheric pollution; biomonitoring; lichen; landfill.

1. Introduction

Worldwide waste production is growing in volume and in toxicity. According to the Environmental Protection Agency (EPA), the average person dumps almost 4.5 pounds of waste into landfills every single day. Products contain toxic chemicals, such as heavy metals and these toxic products are combined with other chemicals, which eventually impact public health and the environment (Lindberg and Price, 1999, Dincer *et al*., 2006).

The environmental problems caused by landfills are numerous. There are many environmental problems related with landfills. The mixture of chemicals in landfills can produce toxic gases that can significantly impact the quality of air in the surrounding of the landfill (Mendes *et al*., 2004). Environmental concerns include such gases as methane, carbon dioxide, nitrogen oxides, volatile organic compounds and others (Bogner *et al*., 2008, Protano *et al*., 2014). Continuously monitoring of these compounds is important in order to prevent harmful effects on environment and human health.

Epiphytic lichens are used as indicators of environmental quality. Transplantation studies highlighted the biological effects of air pollution around the landfill – discoloration, necrosis, membrane lipid peroxidation, lower ergosterol content (Paoli *et al*., 2015a). Similar effects were found around a municipal solid waste – increased cell membrane damage and reduced vitality were observed in some of the transplanted lichens (Paoli *et al*., 2015b). İn addition, lichen diversity was reduced along with increase in heavy metals concentrations in the surroundings of the landfill. Particular matter is also generated in the landfill which are enriched in heavy metals and accumulated in transplanted lichens (Paoli *et al*., 2012, Nannoni *et al*., 2015).

The aim of the study to determine physiological response of lichen *Evernia prunastri* (L.) Ach. tranpslanted nera a landfill in central Lithuania.

2. Material and methods

2.1. Study area and experimental design

The study area is located near a landfill which situated near the second largest cities – Kaunas (20 km), in central part of Lithuania. The landfill was established in 1973 and is operating till now. The territory of the landfill covers an area of 374 000 m^3. The annual volume of deposable waste is 0.12 tones. The daily amount of composed filtrate is 80-100 m^3. The chemical characteristics of landfill leachate determined were high in nutrients concentrations – 50 mg NO_3^--N/l, 256 mg NH_4^+-N/l, 30 mg PO_4^{3-}-P/l with pH 8.5 (Žaltauskaitė and Čypaitė, 2008). Elevated nutrients concentrations were found in groundwater of surrounding area (Juodkazis and Urbanavičiūtė, 2002).

2.2. Experimental design

Thalli of *Evernia prunastri* were collected in a remote area. The fruticose lichen *E. prunastri* was chosen because such species is easily to collect, transplant and observe physiological changes.

The lichen material were packed loosely in nylon netting bags (1 × 1 mm) with a nylon rope and hanged at the height of 2 m above the ground. The control set was transplanted into lichen native unpolluted habitat. The study area was divided into 3 zones: 1) sites facing the landfill (0 m), 2) sites located up to about 200 m and 3) 400 m from the landfill. Lichen samples were exposed for 14 weeks months – from December, 2016 to the middle of March, 2017. At the end of the exposure period, lichen bags were collected and lichen material were placed in clean plastic bags in order to avoid contamination.

2.3. Physiological parameters

To check the integrity of the plasma membrane enclosing cells the difference in electrical conductivity measured by placing the thallus in distilled water was used. Each sample (100 mg) was several times rinsed with deionised water for 3–5 s, until stable conductivity values were obtained. The electrical conductivity of the water was measured before and after lichen immersion using conductivity meter (inoLAB Multi 720). 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.

Chlorophyll *a* fluorescence of samples was measured with a plant efficiency analyser (Handy PEA, Hansatech). Before measurement of chlorophyll fluorescence, thalli were dark-adapted for 15 min. Fluorescence was measured on well-wet samples, applying a saturating flash of light of 2 400 µmol s^{-1} m^{-2} for 1 s. The Fv/Fm parameter (maximum quantum yield efficiency of PSII) was used as a stress indicator.

Malondialdehyde (MDA) was measured by a colorimetric method (Heath, Packer, 1968). Lichen samples were rinsed with distilled water and homogenized in a mortar using 0.1% (w/v) trichloracetic acid (TCA) with the addition of sand. The homogenate (1,5 mL) were put in Eppendorf tubes and centrifuged at 12 000g for 20 min. Supernatant (0,5 mL) was collected and added to 1,5 mL of 0,6% thiobarbituric acid (TBA) 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 again at 12 000g for 10 min. The absorbance of the supernatant was measured at 532 nm and corrected for non-specific absorption at 600 nm. Concentration of MDA was calculated using the extinction coefficient 155 mM-1 cm-1and expressed as µmol g-1 dry weight.

2.3. Data analysis

The physiological parameters measured in the lichens and mosses were subjected to one-way analysis of variance (ANOVA) between the sampling sites ($P < 0.05$). In the case of a significant difference, a post-hoc comparison was run Least Significant Difference (LSD) method ($P < 0.05$). All calculations were performed with Statistica 6.0 and Microsoft Office Excell 2010.

3. Results and discussion

The photosynthetic efficiency expressed as Fv/Fm of transplanted lichens was the lowest of samples exposed in sites directly facing the landfill – just beyond the fence (Fig. 1a). The mean value was 12.8 % lower than that of the control samples. Farther from the landfill - at the distance of 400 m - the values of fluorescence did not differ significantly from the control ($p>0.05$).

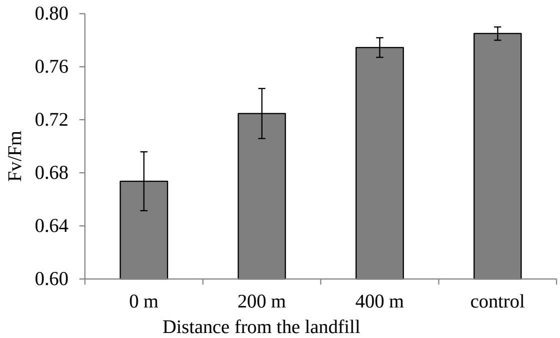

Figure 1. Values of photosynthetic efficiency in *E.prunastri* collected in control and transplanted in the surrounding of landfill.

The results are in accordance with other studies where frequency along with Fv/Fm of *E. prunastri* decreased abruptly above 3 µg/m^3 NH_3 suggesting direct adverse effects of NH_3 on its photosynthetic performance (Munzi *et al.*, 2014).

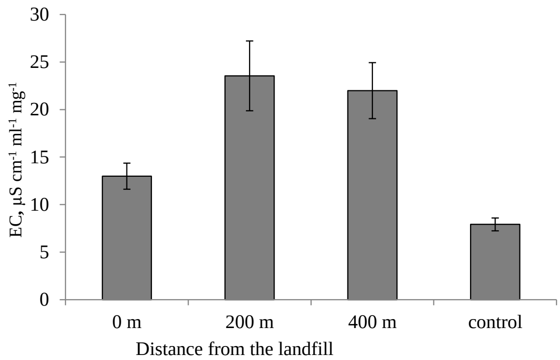

Figure 2. Values electrical conductivity in *E.prunastri* collected in control and transplanted in the surrounding of landfill.

The values of electrical conductivity in exposed lichen samples were significantly higher in the area of landfill comparing with the control ($p<0.05$, Fig. 1b). The mean values of this parameter was also significantly higher in two further located study sites ($p<0.05$). The integrity of cell membrane is considered to be a suitable parameter for monitoring biological effects of pollution (Munzi *et al.*, 2009). As gas emissions from solid waste landfills is complex mixture of contaminants and their effects could be harmful to living organisms. This could be as explanation of observed the cell membrane damage around the landfill.

The content of malondialdehyde (MDA) in lichen was significantly higher at the sites facing the landfill ($p < 0.05$). No signs of oxidative stress were detected at other sites. Dispersion analyses showed that values of oxidative stress along the study transect were not significantly correlated with the distance from the landfill ($F=1.28$, $p > 0.05$).

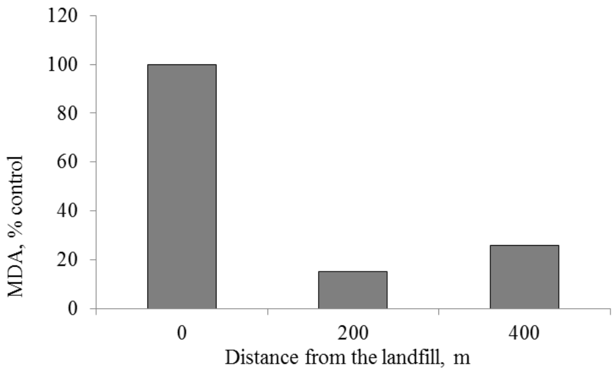

Figure 3. MDA concentration in the transplanted lichen near the landfill in comparison with the control (%)

MDA as an oxidative stress indicator showed that emissions from the landfill induced the membrane peroxidation in lichens. This is in accordance with the studies of lichens under the stress conditions such as heavy metals and excess of nitrogen (Gonzalez and Pignata, 1994, Cañas *et al*., 1997, Sujetoviene and Sliumpaite, 2013).

Assessing the results of the study it could be concluded that measured physiological parameters indicated stress conditions for lichens. During composting process the gaseous and particulate emissions may affect the surroundings. The accumulation in soil and surface water and groundwater could have a negative impacts and is a threat to all living organisms (El-Fadel *et al*., 1997, Mikac *et al*., 1998, Nannoni *et al*., 2015).

Conclusions

The study showed that the physiological status of transplanted *Evernia prunastri* samples was changed under the influence of the landfill. This indicated that used bioindicator can be applied to environmental quality evaluation.

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