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Road traffic pollution effects on epiphytic lichens

Gintarė Sujetovienė

Department of Environmental Sciences,
Vytautas Magnus University,
Vileikos 8-223,
LT-44404 Kaunas,
Lithuania

The content of the major elements – nitrogen, sulphur and carbon – in *Physcia tenella* and *Parmelia sulcata* thalli exposed to the influence of traffic pollution was investigated. The distance from the Kaunas–Vilnius highway (Lithuania) was regarded as the main parameter to check this effect. Results of the present study indicated that NO_2 levels were not correlated with the distance from the road. The relatively low NO_2 values may be due to the effects of rains. A significant relation between NO_2 levels and N accumulation was found in lichen thalli. The study lichens, both with rough (*Ph. tenella*) and smooth (*P. sulcata*) thalli, showed a similar capacity to retain nitrogen. Sulphur and carbon content in the lichens did not change along the transect of the highway, either.

Key words: bark pH, biomonitoring, carbon, lichen, nitrogen, *Parmelia sulcata*, *Physcia tenella*, traffic pollution, sulphur

INTRODUCTION

The global cycle of nitrogen is changing due to nitrogen emissions from human activities – mainly fossil fuel burning and livestock farming. The increasing number of vehicles produces large emissions of pollutants. Nitrogen oxides, mainly nitrogen dioxide (NO_2) and nitric oxide (NO), are now among the most important atmospheric pollutants of roadside environments (Rodes, Holland, 1981; Roorda-Knape et al., 1998; Cape et al., 2004; Pleijel et al., 2004; Zou et al., 2006; Gilbert et al., 2007). Nitrogen oxides are important air pollutants because they contribute to the formation of photochemical smog which can have significant impacts on human health (Latza et al., 2009).

Instrumental monitoring provides data on pollutant concentrations in the air for short and fixed periods of time, whereas biomonitors are a very useful additional tool in evaluating the degree and extent of environmental contamination as time integrators of atmospheric pollutants (Vingiani et al., 2004). Lichens are generally considered as useful indicators to monitor air quality because many of their species are sensitive to air-borne gaseous and depositional pollutants, especially nitrogen- and sulphur- based compounds. This sensitivity of lichens to a variety of air pollutants is abundantly documented (e. g., Conti, Cecchetti, 2001). Nitrogen is an important factor affecting epiphytic lichen vegetation. The main effect is an increase in nitrophytic species with a decrease in acidophytic or neutro-nitrophytic ones (Frati et al., 2008). Numerous studies have been undertaken where lichen diversity or the presence / absence of indicator species was used as a measure of air quality. NO_2 from traffic emissions limited lichen diversity in Seville, Spain (Fuentes, Rowe, 1998) and Tuscany, Italy (Lorenzini et al., 2003). Also, in London the diversity of epiphytic species was increasing during 1970–2004; species present in areas of highest NO_x were considered pollution-tolerant and were mainly associated with eutrophication (Davies et al., 2007).

Beside indicating air quality by their presence / absence, lichens have also been used as accumulator organisms in studies. In pollution-enhanced environments, lichens can accumulate nitrogen, sulphur, metals and other pollutants well in excess of their nutritional needs (Søchting, 1995; Glavich, Geiser, 2008). Nitrogen accumulation was reported by S. Vingiani et al. (2004) in the urban area of Naples (Italy) suffering from NO_x pollution. Nitrogen concentrations in *Physcia adscendens* growing adjacent to roads were found to increase with proximity to roads of a high traffic flow, but this was not the case in *Hypogymnia physodes* (Gombert et al., 2003). In general, the impact of NO_x on lichen flora remains poorly understood, although there is increasing evidence that traffic emissions influence lichen growth, diversity and abundance (Purvis et al., 2003).

Less attention has been devoted to the possibility of using native lichens to monitor air pollution by S, N and C pollutants (Conti, Cecchetti, 2001, Vingiani et al., 2004). Because S, C and N accumulation in lichens is based on the active

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

process of intake of pollutants in gaseous forms, lichens are more effective in accumulating these elements compared with mosses. The aim of this study was to investigate whether NO_2 emitted by traffic can influence the content of nitrogen in lichen thalli, and to monitor the content of sulphur and carbon. For this purpose, the distance from a highway was regarded as the main parameter to prove this effect.

MATERIALS AND METHODS

Site description and sampling

The study was carried out in a site (54°54'49"N, 24°7'23" E) located along the Kaunas–Vilnius highway (central Lithuania). This road connects two largest cities of Lithuania. The climate here is continental with a mean annual temperature of 6.2 °C. A mean annual rainfall is 780 mm and prevailing winds are from SW. The traffic flow of the highway at the site ranged between 10,000 and 20,000 vehicles day^{-1}.

On the north-eastern side of the highway (down the prevailing wind), 12 sampling plots 1×50 m each were selected at an increasing distance (8, 10, 15, 48 m) from the road, the longest side being parallel to the highway. The study sites were located within 50 m from the highway because there were no naturally growing oak trees and lichens at a greater distance from the road. Most of lichen and bark samples were collected from oak trunks (*Quercus robur* L.) faced towards the road. Lichen thalli were collected with a knife at the hight of 1 m to about 2 m.

Nitrogen dioxide monitoring

A central tree of each sampling plot was selected along the transect. On each tree passive samplers were placed for two weeks (29 April – 6 May 2008) on the side facing the road at the height of 2 m from the ground. The sampling, analysis and calculation were performed according to D. Krochmal and A. Kalina (1997). For each tree, three samplers were placed. Samplers contained a filter impregnated with a triethanolamine which chemio-adsorbs gas-phase NO_2 as nitrite ions which were measured spectrophotometrically by the sulphanilamide and N-1-napthylethylene-diamine-dihydrochloride method. The detection limit was 4.5 µg/m^3, the uncertainty being 11.6%.

Bark analysis

Bark samples were collected beneath the sampled lichens and were used for pH measurements; 0.2 g of the surface 2 mm bark was ground and soaked with 10 ml deionized water and shaken for 1 h. Then the samples were centrifuged for 10 min at 4000 rpm, and the clear fluid fraction was filtered and used for analysis. Bark pH was measured with a pH-meter (inoLab pH 720).

Bioaccumulation of nitrogen, sulphur and carbon

During April 2008, thalli of the foliose lichen *Parmelia sulcata* Taylor and *Physcia tenella* (Scop.) DC were collected from oak trees (*Quercus robur* L.) where passive samplers were exposed. Samples were taken form tree trunks with an inclination not higher than 10°, without signs of damage. In the laboratory, lichen samples were immediately analyzed for their N, S and C content. Samples were not washed since there is evidence that washing can remove the nitrogen deposited on the lichen surface (Gombert et al., 2003). The samples were air-dried to constant weight and carefully cleaned under a binocular microscope to remove as much extraneous material as possible. Unwashed samples were ground, and the element content of three sub-samples, expressed on a dry weight basis, was determined with a nitrogen-carbon-sulphur analyzer (Leco CNS 2000).

Statistical analysis

The non-parametric Kruskal–Wallis test was used for testing variable differences among different sampling sites. The Sign test was used to test the relationship between nitrogen dioxide concentration and nitrogen content of samples collected along the transect. The influence of the road on lichen element concentration was tested for statistical significance, using a single factor one-way analysis of variance (ANOVA).

RESULTS

Nitrogen dioxide monitoring

Atmospheric NO_2 levels measured by passive samplers are shown in Fig. 1. The mean level was 11.4 µg m^{-3}, with a range 9.3–15.5 µg m^{-3}. A decline of NO_2 levels with the distance from the highway was found.

NO_2 levels were not correlated with the distance from the road ($R^2 = 0.18$, $p > 0.05$). The results indicated that the road had an influence on NO_2 levels at the roadside ($F = 15.1$, $p < 0.05$): the plot closest to the highway (8 m) showed significantly higher ($p < 0.05$) levels (14.8 µg m^{-3}) compared with those at a longer distance (9.9–10.5 µg m^{-3}). These results are in agreement with data on air pollution obtained near highways (Rodes, Holland, 1981; Roorda-Knape et al., 1998; Gilbert et al., 2003; Cape et al., 2004; Kirchner et al., 2005;

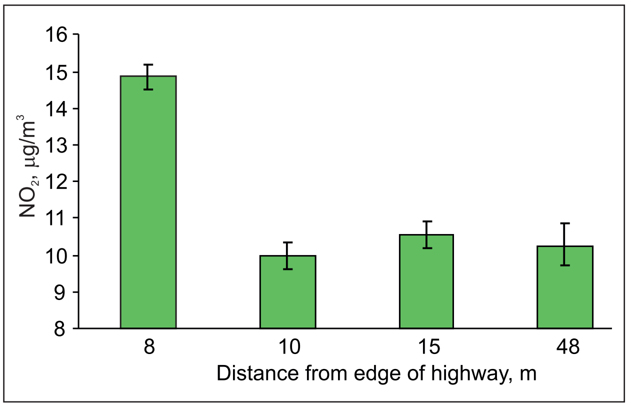

Fig. 1. Atmospheric NO_2 concentrations (µg m^{-3}) measured by passive samplers near the road Kaunas–Vilnius (bars indicate standard error of mean)

Frati et al., 2006) where NO_2 concentration was negatively correlated with the distance. It was verified that nitrogen dioxide levels positively correlated with traffic density (Roorda-Knape et al., 1998; Gilbert et al., 2007).

Nitrogen, sulphur, and carbon content in *Physcia tenella* and *Parmelia sulcata*

Nitrogen levels in *Ph. tenella* thalli ranged within 0.32–0.58%, the mean content being 0.48 ± 0.01% (Fig. 2). The range of nitrogen levels in *P. sulcata* was 0.32–0.53%, the mean level 0.45 ± 0.02. The results suggest that the study lichens with both rough (*Ph. tenella*) and smooth (*P. sulcata*) thalli had the same capacity to retain nitrogen ($p > 0.05$).

Total nitrogen content in lichen thalli decreased only at a distance of 10–15 m from the road; here, the N values were lower than in the closest and the farthest plots (Fig. 2). A reduced N content in *Ph. tenella* thalli was observed at a distance of 15 m; it was significantly lower than in other sample plots ($p < 0.05$). A similar trend was found in changes of N content in *P. sulcata* thalli. The N content in *P. sulcata* thalli was highest 8–10 m from the highway; N content also decreased and was statistically lower at a distance of 15 m ($p < 0.05$). At higher distances from the highway (48 m), N content in sampled lichen thalli increased, and there were no significant differences from plots near the roadside (8–10 m). The effect of the highway on N content in lichen *Ph. tenella* and *P. sulcata* thalli was significantly related to NO_2 levels in the atmosphere ($Z = 2.84$, $p < 0.05$ and $Z = 2.47$, $p < 0.05$, respectively).

The mean S content measured in *Ph. tenella* and *P. sulcata* samples was 0.014%. S content in *Ph. tenella* tallum (0.02 ± 0.003%) was higher than in *P. sulcata* thallum (0.01 ± 0.005%), but the difference was not statistically significant ($p > 0.05$). No significant differences among the sample plots were obtained (Fig. 3). Only for *Ph. tenella* an increase in S content (361.9%) was observed in a sample plot at a distance of 10 m from the highway, and it was significantly higher than in any other sampling plot ($p < 0.05$). A threefold increase in S content in *P. sulcata* thalli was found in the farthest sampling plot (48 m) from the highway, and it was significantly higher than in lichen thalli near the roadside (8–15 m, $p < 0.05$).

The mean C content in lichen thalli was 8.921%. The C content measured in samples of *Ph. tenella* and *P. sulcata* was higher (9.21%) in sampling plots closest to the highway (8–10 m) but it was not significantly different ($p > 0.05$) from that found in other plots (15–48 m) where the mean C content was 8.71% (Fig. 4). There were no significant differences between C content in different sampling plots ($p > 0.05$).

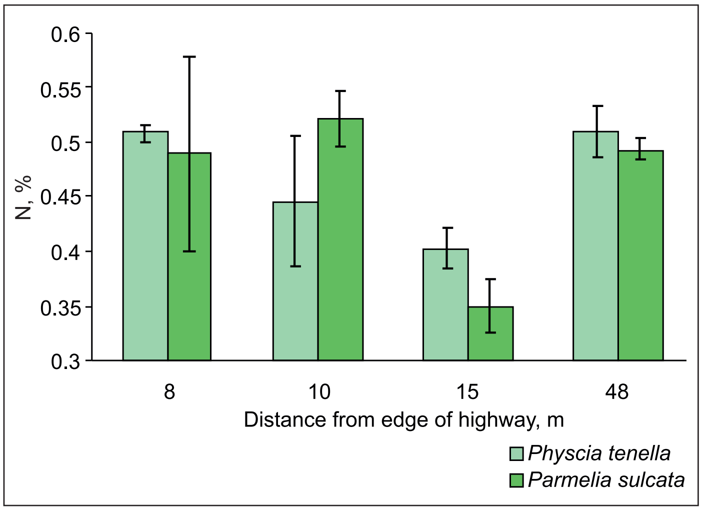

Fig. 2. Mean concentration of total nitrogen (% dry weight) measured in thalli of the *P. tenella* and *P. sulcata* in samples at increasing distances from the road

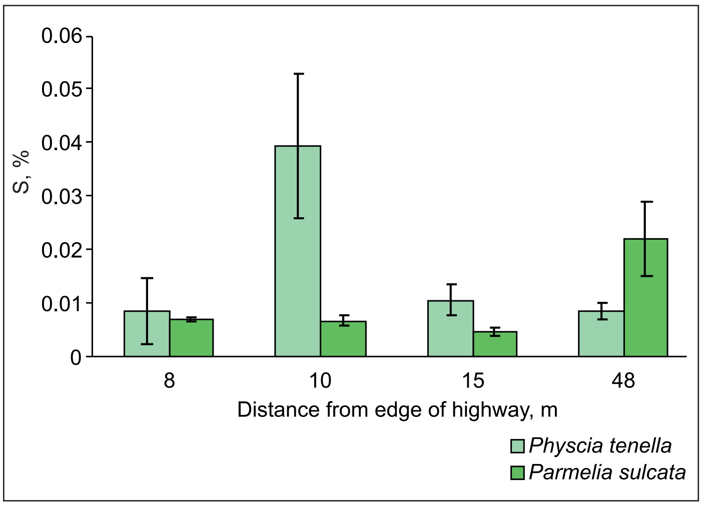

Fig. 3. Mean concentration of sulphur (% dry weight) measured in thalli of the *P. tenella* and *P. sulcata* in samples at increasing distances from the road

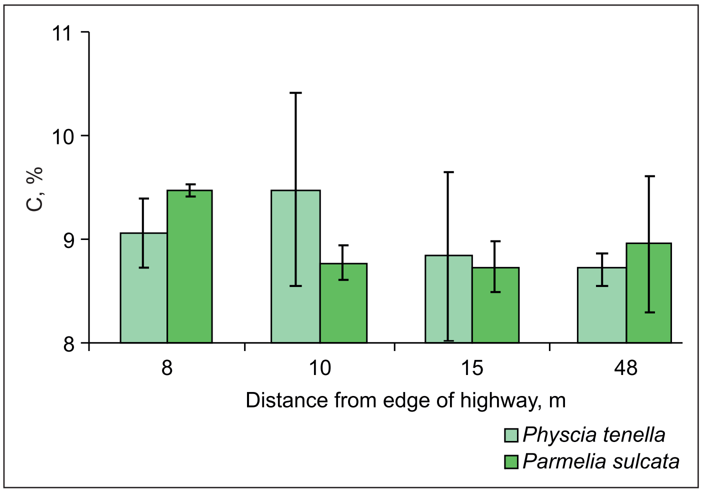

Fig. 4. Mean concentration of carbon (% dry weight) measured in thalli of the *P. tenella* and *P. sulcata* in samples at increasing distances from the road

Bark pH

Bark pH ranged between 5.67 and 7.14 (Fig. 5); the highest values (pH 7.1) in the closest to highway plots (8 m). A slight increase in bark acidity was observed with increasing the distance from the highway: it decreased by 11.34% and 15.91% at a distance of 10 and 15 m from the highway compared to the plot located near the highway (8 m). Thus, the distance from the highway significantly influenced bark acidity ($F = 18.59$, $p < 0.05$).

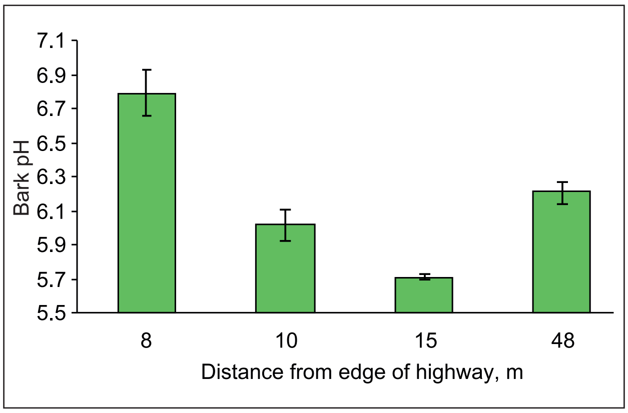

Fig. 5. Bark pH values measured at different distances from the pollution source – road Kaunas–Vilnius

DISCUSSION

Nitrogen dioxide monitoring

The study results showed, in general, an exponential decrease of NO_2 level with the distance from the highway. Also results from other studies established such a decreasing profile for NO_2 (Rodes, Holland, 1981; Roorda-Knape et al., 1999; Pleijel et al., 2004; Zou et al., 2006; Bignal et al., 2008; Gilbert et al., 2003, 2007). NO_2 was also significantly associated with traffic count (Gilbert et al., 2007).

Surveying the effects of traffic-born pollution on plants, it was hypothesized that motor vehicle pollution was responsible for the effects observed, and that nitrogen oxides had a key influence (Angold, 1997; Bernhardt-Römermann et al., 2006; Bignal et al., 2008). The study sites were located on the northeast side of highway, i. e. downwind. Gilbert et al. (2003) assumed that NO_2 concentrations were higher downwind than upwind, and the major decrease occurred within 200 m of the highway. With reference to the data on nitrogen dioxide levels at the roadside and their contribution to nitrogen deposition, it was estimated that the rate of decrease in gas concentration was rapid away from the edge of the roads, their levels falling by 90% within the first 15 m for NO_2 (Cape et al., 2004). The same pattern of NO_2 levels across the sites was confirmed. The studies have provided evidence that the strongest effects may be found within 50–100 m (Bignal et al., 2008) or even up to 230 m from major roads (Angold, 1997; Bernhardt-Römermann et al., 2006).

The threshold value for NO_2 (40 $\mu g\ m^{-3}$), which appeared to have a phytotoxic effect on epiphytic species in London (Davies et al., 2007), was not exceeded in the study roadside ecosystem.

Sulphur, nitrogen and carbon content in *Physcia tenella* and *Parmelia sulcata*

Vehicle exhaust emissions are a dominant feature of urban environments and have detrimental effects on plants (Angold, 1997; Bernhardt-Römermann et al., 2006; Bignal et al., 2008; Honour et al., 2009). The effect of nitrogen oxides on lichens remains poorly understood because of their conflicting influence on bark characteristics. Nitrogen oxides may stimulate plant growth, including lichen photobionts, but may be toxic at high levels (Mansfield, 2002). Nitrogen oxides (i) may cause lichen injury (chlorophyll reduction) or damages (Zambrano, Nash, 2000; Purvis et al., 2003), (ii) can decrease growth rate (von Arb, Brunold, 1990), (iii) can accumulate in some species (Hyvarinen, Crittenden, 1998; Gombert et al., 2003; Frati et al., 2007), (iv) limit lichen frequencies and diversity (Loppi et al., 1996 a, b; van Dobben et al., 2001; Loppi, Corsini, 2003; Purvis et al., 2003; Davies et al., 2007).

NO_x effects on epiphytic lichens are not known very well. Many lichen species are known to be sensitive to SO_2 while, on the other hand, only few could be used to predict NO_2 concentrations (Bates, 2002). Another problem is that epiphytic lichen floras are poorer on trees near major roads than elsewhere, which also indicates a possible negative impact of NO_x derived from traffic.

The influence of NO_x on particular lichen species is difficult to determine, even if a general influence on lichen nitrophytic communities seems to be established (Gombert et al., 2003). One reason is the numerous environmental factors other than air pollution levels, which play a role in the location, frequency and nutrition of a species. Hence, it is very important that lichen species were collected from the same tree species. Because the sampling sites were characterized by minimal variations of climate and topographic factors, these sampling conditions give a unique opportunity to elucidate the tendency of changing nitrogen concentrations in lichens, when the main source of pollution is road traffic.

Lichen nitrogen levels vary depending on the species; different morphological and/or physiological properties of the lichen species might be responsible (Gombert et al., 2003). The mean total nitrogen content in *Ph. tenella* and *P. sulcata* thalli was 0.46%, a value which is quite low in comparison with the values of nitrogen content reported by other authors. The range of nitrogen content in *Evernia prunastri* thallus was 0.8–1.0% (Palmqvist et al., 2002) and 1.3–1.6% after transplantation near a highway in Italy (Frati et al., 2006). Higher $\delta^{15}N$ values in healthy *Parmelia sulcata* samples near roads suggested a local NO_x accumulation (Purvis et al., 2005). Nitrogen levels in lichens, found in the Grenoble urban area (France), showed the mean value of 2.41% for *Hypogymnia physodes* and 2.97% for *Physcia adscendens* (Gombert et al., 2003).

Pollution from pig farms was found to impact nitrogen content (1.5–2.56%) in nitrophytic lichen species such as *Xantoria parietina*, *Flavoparmelia caperata* (Frati et al., 2007). Road size (i. e. also traffic density) and proximity influenced N concentrations in the lichen *Physcia adscendens* but not in *Hypogymnia physodes* (Gombert et al., 2003). It was suggested that the rough thallus of *Physcia adscendens* may permit better entrapment of nitrogen particulates than the smooth thallus of *Hypogymnia physodes* (Gombert et al., 2003). The results of our study have shown that lichens with both rough (*Ph. tenella*) and smooth (*P. sulcata*) thalli have a similar capacity to retain nitrogen.

Several studies recognized a different nitrogen accumulation tendency (Frati et al., 2006) than that observed in our study. With the distance from the road, the N accumulation tendency was a concave function, suggesting that NO_2 emitted by road traffic was not the main source of nitrogen in the study area. Nitrogen accumulated by lichen transplants originated from N-fertilizer. In our study, N content tendency was a convex function. Nitrogen increase in lichen thallus growing on trees close to the roadside could be an "edge effect". The highest N levels in lichen thalli near the highway could be due to boundary layer where gaseous compounds were retained.

Another explanation may be that N is accumulated not only from NO_2 but also from NH_3. Effects from NH_3 emitted by cars with catalytic converters (Baum et al., 2001) are moderate near the roadside due to the high deposition rate of NH_3 (Cape et al., 2004). Its higher content in lichen could be attributed not only to NO_2 but also to NH_3. Therefore, the increase in N content may be related to other N gaseous compounds, such as NO_x and NH_3.

The effect of sulphur compounds on lichens has been extensively studied (Conti, Cecchetti, 2001) in relation to combustion of sulphur-containing fossil fuel. These studies were carried out to evaluate the effects of sulphur compounds on the physiology of lichen thalli and / or on the integrity of photobiont chlorophyll. Assessment of S content in lichens provides a good estimation of the atmospheric SO_2 concentration (Garty et al., 1988). The high levels of sulphur, found in lichens transplanted to the far surroundings of the industrial areas indicate the transport of sulphur-containing particles derived from the combustion of heavy fuel oil (Carreras, Pignata, 2002). The relation among sulphur content in *Ramalina celastri* thalli, traffic and tree cover indicated that the accumulated sulphur reflected not only traffic emissions, but also that low ventilation conditions affected its accumulation (González et al., 2003). In the sample plots, road traffic is the main source of atmospheric pollution. As A. Kabata-Pendias and H. Pendias (1985) concluded, lead-containing sulphate particles entrapped by lichens most probably result from the conversion of Pb halide salts present in automobile exhausts. Thus, S content in sampled lichen thalli was not high as compared with the data of other studies: the mean sulphur level in *R. celastri* was 0.63 mg/g under low traffic conditions in 1992 (González et al., 1996).

Very few studies have been carried out on the carbon content in lichens in urban areas. Carbon monoxide is one of the major pollutants in exhaust gases, and its presence in urban air is largely due to traffic (Capilla, 2007). Körner and Miglietta (1994) found that under an elevated carbon supply, the mean concentration of total non-structural carbohydrates (TNC, sugars and starch) was higher in leaves of herbaceous and ruderal plants, and of sucrose content in sugarcane was increased (De Souza et al., 2008). The increased air CO_2 concentrations can strongly influence the element contents in mosses, as heavy metal concentrations can be relatively lower and "diluted" because of the intensified dry matter production (Peli et al., 2008). The decrease of C content in thalli of *Pseudevernia furfuracea* in an urban area suggested a lower accumulation of the element through absorbtion processes along with oxidative phenomena of the tissues with CO_2 release (Vingiani et al., 2004).

Bark pH

Tree bark is recommended as a sensitive and simple indicator of air pollution (Santamaría, Martín, 1997; van Herk, 2001). The pH of bark is a sensitive index responding to relatively small changes in the habitat acidification (Bienkowski, 1984).

Trees near the highway have exhibited the "alkaline dust effect" which may be caused by an increase in bark pH. The high bark pH of trees in the closest edge to the highway may be due to calcareous dusts from a gravelly roadside contributing to the alkaline dust effect. Gilbert (1976) has shown that alkaline dusts increase bark pH and can cause hypertrophication. Besides, dusts coat the trees, and the bark desiccates independently of the dust chemistry (Loppi, Pirintsos, 2000). The estimated bark pH values (6.0–6.8) near the pollution source (8–10 m) were very high for oak trees. The higher *Quercus* sp. bark acidity (pH 5.1) was recorded at the innermost London's zone where the levels of NO_2 and other pollutants were high (Larsen et al., 2007).

According to van Herk (2001), pH values of the bark on wayside *Quercus robur* were lower in forests (3.65–4.40) than in towns (3.80–4.95). Such low pH values could be partly explained by the nature of acid sandy soils. Studies in Norway and Scotland showed that the pH values of *Quercus* sp. bark was also dependent on the calcium content in bark and soil (Gauslaa, 1985; Bates, 1992). Bark pH was positively correlated with bark Ca in *Quercus* sp. bark (Bates, 1992).

Some researchers found an appreciable correlation between bark acidity in deciduous trees and SO_2 levels in the atmosphere (Johnsen, Søchting, 1973; Yong et al., 2000). Bark pH is known to affect sulphur speciation which determines its toxicity, and may influence the speciation and bioavailability of other potentially toxic elements (Larsen et al., 2007).

N deposition alters bark pH which is known to influence epiphytic communities (Mitchell et al., 2005; Larsen et al., 2007). N deposited on the bark may act as a fertilizer or as an acidifier, depending on its form. The relationship between the lower plant frequency and bark pH confirmed that pH was an important driver for the distribution of lichens and bryophytes (Larsen et al., 2007). An increased bark pH appeared to be the primary cause of the enormous abundance of nitrophytic species in the Netherlands (van Herk, 2001), whereas oak (*Quercus* sp.) bark pH in an urban park in Italy (where air pollution was fairly low) did not emerge as a discriminate parameter affecting the floristic composition and diversity of epiphytic lichen (Loppi, Frati, 2004).

One of the reasons for these facts could be that nitrophytic species had a low sensitivity to toxic effects of SO_2, their only requirement being high bark pH (van Herk, 2001). These conditions prevail for lichen growth and give the possibility of investigations. The nitrophytic species *Physcia tenella* preferred a less acidic bark and was most abundant where bark pH was high, but it was less abundant in sites with the highest bark pH (Larsen et al., 2007). The possible explanation of such distribution may be high levels of NO_x and / or other transport-related pollutants. Bark pH below 3.0 may exert a negative effect similar to that of high atmospheric NO_2 concentrations (Gries, 1996).

CONCLUSIONS

The results of the present survey have indicated that the test parameters decline with the distance from the roadside. NO_2 concentrations, although rather low, were negatively correlated with the distance from the road according to a typical logarithmic function. The results indicate that the traffic significantly influences NO_2 concentrations and bark acidity at the roadside. The influence of traffic on N content in lichen thalli was significantly related with NO_2 levels in the atmosphere. The results suggest that the study species of lichens with a rough (*Ph. tenella*) and a smooth (*P. sulcata*) thallus have a similar capacity to retain nitrogen. Although it is not possible to trace which air pollutants are responsible for the observed effects, the evidence is consistent with the effects that are primarily due to NO_x concentrations closer to the road. S and C content in the lichens did not change along the transect of the road, either.

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Gintarė Sujetovienė

TRANSPORTO SUKELIAMOS ORO TARŠOS POVEIKIS EPIFITINĖMS KERPĖMS

Santrauka

Tirtas pagrindinių elementų (N, S, C) kiekis kerpių *Physcia tenella* ir *Parmelia sulcata*, augančių veikiant transporto sukeliamai taršai, gniužuluose. Darbo tikslas – ištirti šių pagrindinių elementų kaupimąsi ir azoto kiekio kerpėse priklausomumą nuo NO_2 koncentracijos ir žievės rūgštumo. NO_2 koncentracija nebuvo susijusi su atstumu nuo kelio pakraščio. Santykinai maža NO_2 koncentracija galėjo susidaryti dėl drėgno oro tyrimo metu. Nustatytas NO_2 koncentracijos ore ir N kiekio kerpių gniužule patikimas ryšys ($P < 0,05$). Tirtosios kerpės turėjo tokią pačią azoto akumuliacijos gebą kaupti azotą, nepaisant jų gniužulo paviršinių skirtumų. Sieros ir anglies kerpių gniužuluose kiekio, tolstant nuo greitkelio, patikimų kaitos dėsningumų nenustatyta.

Raktažodžiai: anglis, azotas, biomonitoringas, kerpės, *Parmelia sulcata*, *Physcia tenella*, siera, transporto tarša, žievės pH