Assessments of traffic pollutants along a rural highway roadside in southeastern Norway by quantifying lichen responses

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Preface

This thesis in General Ecology at the Department of Ecology and Natural Resource Management (INA), the Norwegian University of Life Sciences (UMB) has been carried out in cooperation with the Norwegian Public Roads Administration (Statens Vegvesen).

I wish to thank Professor Knut A. Solhaug and Professor Yngvar Gauslaa, from Ecology and Natural Resource Management Department of the Norwegian University of Life Sciences (UMB), for supervising my work, giving me a lot of useful advices and guidance throughout the project. I express my gratitude to Hans-Christian Teien, from Plant and Environmental Sciences Department of the university, who helped me with chemical analysis.

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I would also like to express much appreciation to my husband Kjell, for being my support and helping me with the field survey.

Finally, I desire to devote this work to my beloved grandfather and mother, teachers of botany Onischenko I. J. and Onischenko L. I., from whom I gained curiosity about the plant kingdom.

Ås, 10 February 2013
Olena A. Yemets

Abstract

The overall objective of this thesis was to investigate responses of epiphytic lichens to traffic related pollutants and to get new insight into accumulation and despersal of airborne pollutants. This was done in short term factorial studies under laboratory conditions and in long term studies of lichens transplants in the field.

For the laboratory experiment we selected three common foliose lichens, one sensitive to air pollution (*L. pulmonaria)* and two more resistant species with different salt tolerance (*P. sulcata* and the sea shore species *X. aureola).* Lichen thalli during lab conditions were soaked in 0.01, 0.2 and 0.6 M of de-icing salt (NaCl) and/or in 10, 100 and 500 μM of metal salts solutions ($CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$) for 24 hrs. To induce photoinhibition lichen thalli were exposed to high light – 600-700 μmol photons m^{-2} s^{-1} for 4 hrs.
In the field, lichen thalli (*L. pulmonaria*, *P. sulcata, U. filipendula, R. farinacea)* were placed on stands at 10, 15, 30, 50 and 100 m from both sides of the highway E6 in Ås and Vestby road at six separate gradients for 6 months. In order to understand how external factors influence internal responses of the lichens, chlorophyll fluorescence, conductivity, growth (dry weight), pigment content, visible damage and element content were quantified.

Paper I dealt with effect of external stress (salt, irradiance, heavy metals) on lichens in the lab on biont and species levels.
Lichen mycobionts responded on the exerted toxic effect of stress agents in the order: “Salt x Metal”>“Metal”>“Salt x Light”>“Salt”; the photobionts: “Metal”>“Salt x Metal”>“Salt x Light”>“Salt”. The results showed that photobiont viability (Fv/Fm) varied during the experiment from 30.3 to 66.3% of start levels, while the mycobiont exhibited conductivity levels 3.4 to 77.1 times higher than control values.
Studied lichen species showed tolerance to the lowest agent concentrations applied separately. Osmotic and metal stresses applied together significantly increased the sensitivity among lichen bionts, while combination of osmotic stress and irradiance only effected lichen photobionts.

Copper (Cu) showed higher impact on lichen viability than Zn (94.5% of cases against 5.5%), while the interactions between NaCl and Zn were stronger than with NaCl and Cu (41.6% of cases against 8.3%). Copper (Cu) negatively affected viability of *L. pulmonaria* and *X. aureola*, whereas *P. sulcata* was more damaged by Zn.
Studied species can be placed in the order of sensitivity to external stresses:
L. pulmonaria >P. sulcata >X. aureola.

In **Paper II,** the changes in lichen viability and elemental composition of lichen thalli transplanted along E6 highway were assessed.
Paper II Part I, showed that Ca and Na represented up to 44-54%, while P and K – up to 11-26% of the total accumulated elemental pool of harvested lichens, whereas Ba and Cu together constituted up to 59-74% of the total trace elemental deposition. The EC class "severe accumulation" was evident for Na, Fe, Al, Ni, Cr, V, Co, Mo, As, Sn, Sb, from "normal" to "severe accumulation" for Cu and Zn, while up to "severe loss" for K compared to background values.
There were high correlations within groups of elements related to road dust (Ca, Al, Fe), exhaust emissions (Ni, V, As, Cr, Cu, Zn, Pb). Sodium (Na) correlated positively with heavy metals (r= 0.41 to 0.85) and negatively with K (r= -0.51 to -0.86).
Exceeded concentrations of Al, Fe, Na, Co, Mo, Sb, Ba, V, Pb were found even at 100 m from the road, while 30 m was sufficient to reduce by 60-70% of the majority of elemental content of lichens compared with background values.
External factors influenced the elemental accumulation in lichens in the order: "Species">"Distance">"Side".
The accumulation capacity of lichen species increased in the order: *R. farinacea* < *L. pulmonaria* <*U. filipendula* <*P. sulcata.*
Paper II Part II, showed that trace heavy metals (Co, Ni, Cu, Fe, Cr, Zn, Mg, V, As, Al) and micronutrients (Na and Ca) are were related to reductions in Fv/Fm_{end} and DM growth of lichens, while K positively correlated with the parameters. There were more significant correlations between elemental content and Fv/Fm_{end} than with DM growth.
The decrease of DM growth, $Chl\ (a+b)_{end}$, Fv/Fm_{end} and $Chl\ a/b_{end}$ averaged among lichen species between 10 and 100 m from the road was 55.0%, 25.0%, 14.8% and

7.5%, respectively. There were found that viability parameters of *L. pulmonaria* highly responded to visible damage.

The field and lab studies confirmed that lichens are useful in biomonitoring surveys for atmospheric pollution. Chlorophyll degradation and membrane damage in lichens can be used as viability measurements after exposure to air pollution stress. The results of this study contribute to the knowledge about roadside abiotic impacts and can be used for management of roadside sites.

Keywords: *lichens, lichen bionts, viability,traffic related pollution, heavy metals, salt, biomonitoring, elemental accumulation capacity, highway*

Contents

Part I. The influence of roadside factors (salinity, irradiance and heavy metals) on lichen viability under controlled conditions

1.1. Introduction

Lichens are symbiotic organisms that consist of a fungal "mycobiont" and a green algal (and/or cyanobacterial) "photobiont". These organisms can cope with perturbations of temperature, radiation, and water availability, in particular by repetitive dehydration and consequent rehydration (Ahmadjian 1993; Alpert 2000; Kranner & Birtic 2005; Kranner et al. 2005; Nash 2008).

Sodium (Na), chloride (Cl), copper (Cu) and zinc (Zn) are essential micro nutrients/trace elements for plants including algae. Thus, ions of Na^+ and Cl^- regulate osmotic equilibrium, extracellular fluids and pH, involved in photosynthesis and cell division, accordingly to Epstein (2004). The most abundant Cu protein in plants is plastocyanin. Copper is also used for proper carbohydrate metabolism, formation of phenolics in response to pathogen attack and an important element for the signaling function of the ethylene receptors. Whereas Zn is required as a co-factor in over 300 enzymes, maintains the integrity of ribosomes and biological membranes, required for the formation of chlorophyll and involves in water uptake (Palmer & Guerinot 2009). Nevertheless, if their concentrations exceed the critical thresholds it may seriously alter the biota.

The literature mainly focused on the impact of the salt ions regarding to plants, soils and other organisms with the lack of knowledge about the lichens. Regarding to Munns & Passioura (1984) and Munns (2002) salinity stress reduces not only the ability of plants to take up water, but causes other reductions, along with metabolic changes identical to those caused by water stress. However, existing data indicate a relationship between these stresses. In summary, an overlap in the response to osmotic and salt stress and the ability to survive desiccation exists, as is depicted in Fig. 1.

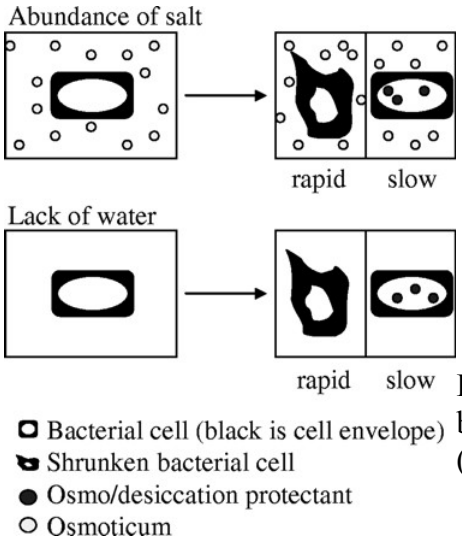

Figure 1. – Hypothetical physiological responses bacterial cell to desiccation and to osmotic stress (adopted from Vriezen et al. 2007)

Reduced photosynthesis is the common phenomenon for lichens because of their poikilohydric nature, whereas salinity stress can cause lichen dehydration, ionic unbalance and, eventually, inhibit photosynthetic apparatus. Thus, as has been shown by Hajek et al. (2006), in lichens, ongoing dehydration leads to gradual loss of photosynthetic activity, which is manifested as a decrease in the efficiency of absorbed energy transfer through photosystem II (PSII) and net CO_2 fixation. Matos et al. (2011) found that the incubation of the lichen *Ramalina* spp. in an artificial sea water solution reduced the maximum photochemical efficiency of PSII by 17% after 5 min, and this inhibition increased with incubation time. Also, with the increasing intracellular Na^+, there was a loss of K^+ from the lichen thalli interior, indicating on the membrane permeability damage.

Experiments of Bates et al. (2009) showed that osmotic stress posed by sea water could increase the susceptibility to photo- injury even in halophytic species. Thus, in *E. prunastri* salt stress combined with high light resulted in a much more pronounced decrease in the rate of photosynthesis than either salt stress in low light, or high light alone (Nash et al. 1990). According to Vaczi & Bartak (2006), osmotic stress in the lichen symbiotic alga *Trebouxia erici* did lead to the reduction of photosynthetic capacity as did high irradiance stress (500 μ mol m^{-2} s^{-1}). However, the response to the salinity stress varies substantially among lichen species (Mackay et al. 1984; Hajek et

al. 2006).

Increased amounts of micro elements, such as Copper and Zinc, can be highly toxic for plants including lichens. Thus because of their high redox properties, Cu can induce oxidative stress by generating reactive oxygen species (ROS), that directly damage proteins, amino acids, nucleic acids and membrane lipids. Over dosage of the element might decrease biomass production, affect plasma membrane functions, as well as photosynthesis and respiration (Meharg 1993; Jentschke & Godbold 2000; Küpper et al. 2009). In turn, high concentration of Zn can affect ionic homeostatic systems of organism, decrease the stability and forming complexes with DNA and RNA (Broadley et al. 2007), that can results in disruption of metabolic processes such as transpiration (Rout & Das 2003) and eventually decreases photosynthesis (Plechanov & Chemeris 2003).

Previous studies showed that Copper is more toxic element for lichens than Zinc. Thus, potassium ions (K^+) efflux from *Umbilicaria* spp. was not affected by the uptake of <20 μmol g^{-1} of Zn^{2+} as was in a case of Cu^{2+} (Nieboer et al. 1979). The influence of lichens to Cu pollution has often been studied and mainly focused on the survival capacity of lichen photobiont. Thus, an addition of 4 mM $CuCl_2$ reduced growth rates, inhibited dehydrogenase activity, altered pigment composition and reduced photosynthetic activity of *Trebouxia media* (Backor et al. 2003). Pawlik-Skowronska (2006) mentioned that even the short-term exposure to Cu^{2+} (40 and 400 μ mol g^{-1}) under non-complexing conditions caused a dose-dependent decrease in chlorophylls and total carotenoid contents in *Lecanora* spp. Kupper et al. (2009) found that Cu-induced inhibition of photosynthesis mainly affected PSII reaction center. Investigation on Zn toxicity are few for lichens. Thus we know that the lowest dose of Zn^{2+} that has been found toxic to the Fungi and Bacteria was 10 mM as reported Babich & Stotzky (1978). Regarding to Lanfranco et al. (2002), Zn^{2+} affect the cellular mechanisms of fungal growth in millimolar concentrations, resulting in hyphal morphology changes which did lead to an increased branching in the subapical parts and increase in chitin in metal-treated hyphae. Bačkor et al. (2006) and Pawlik-

Skowronska et al. (2008) reported that zinc ions negatively influenced on some physiological parameters of lichens.

The results of investigations indicating on the differences in the lichens responses are associated with the different sensitivity of biont partners to the heavy metal stress. Thus, in *Bryoria* spp., treated in the field experiment with Cu^{2+} solutions over two months, degeneration of fungal biont started when thallus metal concentrations exceeded >400 μg g^{-1} Cu, whereas for algae, critical metal concentrations in the thallus were only >50 μg g^{-1} (Tarhanen et al. 1999). Copper negatively affected vitality of the aquatic algae *Scenedesmus* much more compared to the lichen photobiont *Trebouxia* spp. (Piovár et al. 2011). Previously, Branquinho et al. (1997) indicated on species-specific sensitivity of lichens to a certain metal pollutant, by founding that *Usnea* spp. were the most sensitive to Cu uptake compare to *Ramalina* spp.

Because of heavy metals deposited into a specific environment, their mobility and availability for uptake by the biota can depend on complex physico-chemical factors of that environment, for example salinity. The literature review shows that deicing salt applications may affect heavy metal uptake and mobilisation. Amrhein et al. (1992) showed that trace metal concentrations were higher in soils treated with deicing salts than untreated. Lofgren (2001) found that winter applications of deicing salt on roads increased the mobility of H^+ ions and trace metals such as Zn and Cd in the streams. According to Bäckström et al. (2004), the mobilisation of metals in the roadside environment take place during the winter, which is contrary to most natural systems in boreal regions. Reinosdotter & Viklander (2007) simulated the process of melting snow near roadside by using the piles of snow with and without salt. The experiment showed that significant amounts of Cu and Zn were released with the melted water from the pile with salt in comparison with the no-salt pile. Because of this, the use of road salt may increase the dissolved metal phase in the urban snow melt water and sequentially increases the risk of toxicity for the biota (Warren & Zimmerman 1994; Reinosdotter & Viklander 2007). Thus, experiment conducted by Mahrosh et al. (2011) revealed, the sensitivity of early stages of salmon increases

when were applied multiple stressors as road salt (≥5000 mg L^{-1}) and Copper (10 μg L^{-1}) which simulated the road runoff.

Bauske & Goetz (1993) and Bäckström et al. (2004) found that the major mobilisation mechanisms of heavy metals due to deicing salt applications were ion exchange, lowering pH, chloride complex formation or association with organic matter. Furthermore, indicated that the increase of heavy metal concentrations during the winter can happen through different mechanisms for each element. Thus, during the period of deicing the redistribution of Zn in the roadside soils solutions occurs as 50% organic complexes, 50% free Zn^{2+} ions and 5% of chloride complexes (Bäckström et al. 2004). On the way how interact salinity and heavy metals and eventually make harm to the biota point of view among scientists is different. Previously demonstrated, the anionic Me-Cl species (chloride ions complex with heavy metals) can be apparently more or less toxic than Me alone (Babich & Stotzky 1978; Allen et al. 1980). Hasegawa et al. (1986) indicated that overall specific ion toxicities and osmotic stress may act synergistically, while Bauske & Goetz (1993), the major harm occurs due to chloro- complexes formation.

By summarizing above discussed, there are many studies have looked at effects of sodium chloride (NaCl), the most commonly used deicing agent, on the roadside environment, less known about whether comparable effects are caused through exposure lichens to the osmotic stress. In addition, there are not enough studies focused on the impact of deicer concentration, duration and type of exposure, additional stress factors (irradiance) with their possible interaction on lichen species sensitivity. The influence of Cu and Zn on lichens have been extensively investigated, limited information is available about the interaction of fungal and algal partners involved in conferring heavy metal stress tolerance. Since the literature results clearly demonstrate a strong relationship between metal mobilisation and deicing, the main aim of the experiment was to study possible additive (indifferent) or synergistic effects of heavy metals (Cu, Zn) and osmotic stress on lichen viability.

The laboratory experiments directed to answer to the following questions:

Chapter 1.3.1. Response of lichens to osmotic stress induced by deicing salt and high light exposure

- how does induced stress influences the lichens bionts?
- Is there an interaction between osmotic stress and high light?
- which concentration of NaCl is toxic to the lichens?
- are there strong species-specific tolerance of lichens to osmotic stress?

Chapter 1.3.2. Comparative effects of zinc and copper toxicity on lichens viability

- how does Cu and Zn influence lichen viability parameters?
- does the response of lichens to Cu stress differ from Zn?
- what are the Cu and Zn thresholds for lichen bionts?
- are there any differences between species-specific features of tolerance to the metal stress exposure?

Chapter 1.3.3. Interaction of deicing salt and metals, and their combined effect on lichen species

- how does induced stress influences each of the lichens bionts?
- is there a significant salt-heavy metal influence on biont viability?
- which concentration of applied stresses is toxic to the lichens?
- is the tolerance to the stresses exposure species-specific?

1.2. Materials and methods

1.2.1. Study species

For the laboratory experiment we selected three common foliose lichens, one is sensitive to air pollution and two are more resistant species with different salt tolerance.

***Lobaria pulmonaria* (L.) Hoffm**. is a lichen with a distinct upper and lower side. The upper side is bright green when wet and the lower side is dull green-brown. It is a cephalodial lichen with the green alga *Dictyochloropsis reticulata* as a primary

photobiont and the nitrogen fixing cyanobacterium *Nostoc sp*. as a secondary. It grows in old open-shaded forests (Fig.2).

Figure 2. – *Lobaria pulmonaria* (L.) collected and used in laboratory experiment

Lichens with cyanobacteria are particularly susceptible to the effects of acid rain, because the subsequent decrease in pH reduces nitrogen fixation through inhibition of the algal nitrogenase enzyme (Sigal & Johnson 1986). This species are very sensitive species to air pollution (Hallingbäck 1989**;** Ahmadjian 1993).
Thalli of the lichen were collected in September 2011 on a beech (*Fagus sylvatica*) close to Farrisvatnet in Larvik, Norway (59°05'31"N, 9°56'52"E; 200 m above sea level).

Parmelia sulcata **Taylor** has a silver-grey color at the upper side of the thallus. Recognized as chlorolichen with the green algae *Trebouxia.* It is one of the most common epiphytic species in the northern hemisphere on the bark of coniferous and deciduous trees in open habitats, but also occurs on rocks at all elevations (Fig. 3). This lichen was considered to be pollution resistant (Von Arb et al. 1990, Seaward 1993, and Bennett, 2002).

Figure 3. – *Parmelia sulcata* Taylor collected and used in laboratory experiment

Thalli of the lichen were collected in September 2011 on stems of *Tilia cordata* in an alley along a local farm road in Ås, Akershus, Norway (59°40'N and 10°45'E; 100-150 m.a.s.l).

***Xanthoria aureola* (Ach.) Erichsen** has a golden-yellow to orange thallus. It is chlorolichen with the green algae *Trebouxia arboricola* as a photobiont (Fig. 4).

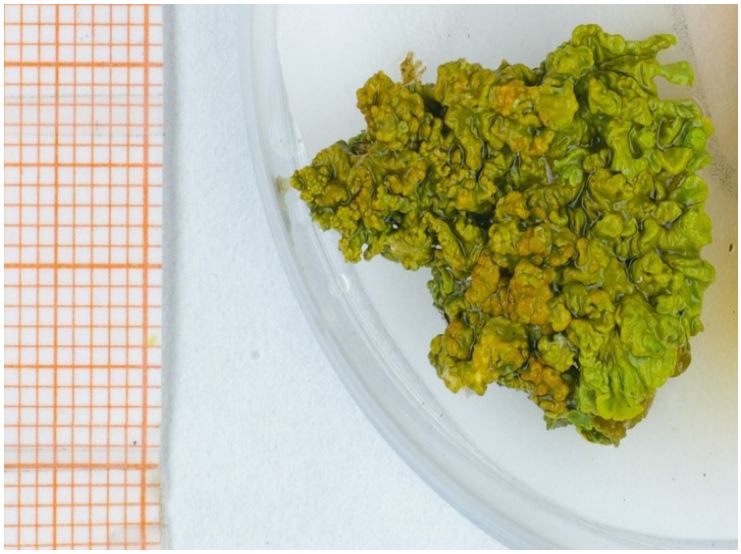

Figure 4. – *Xanthoria aureola* (Ach.) collected and used in laboratory experiment

These species prefers a nutrient rich substrate. Usually they can be found on maritime cliffs (salt tolerant), on old buildings, walls and areas of animal droppings.
The lichen marked by Gaio-Oliveira et al. (2005) and Johansson et al. (2011) as tolerant to nitrogen pollution, while relatively pollution-tolerant to air pollution by Silberstein et al. (1996).
Samples of the lichen thalli were collected in October 2011 on sun-exposed rocks by the seashore in Asmaløy, Hvaler, Norway (59°02'46"N and 10°55'45"E; 2 m.a.s.l).

1.2.2. Laboratory measurements

After collection, all lichen thalli were air-dried for 24 hours and stored at -18 ºC until the start of the laboratory experiments. Prior to the run of laboratory experiments, each thallus was hydrated and kept at laboratory room temperature (18-20 ºC) at low light levels (10 μmol photons m^{-2} s^{-1}) in order to be physiologically active during 24 h as recommended by Honegger (2003).

Salt treatment. Lichen thalli were treated by soaking in 25 ml of de-icing salt (NaCl) solutions: 0.01, 0.2 and 0.6 M. For the laboratory experiments were used snow and ice melting "Sjøsalt" de-icer with composition: NaCl – 96,476%; $CaSO_4$ – 0,413%; MgSO4 – 0,181%; $MgCl_2$ – 0,062%; MgO – 0,053%; H_2O – 2,775% and 0,034% of unsolvable components. The duration of incubating period was 24 h at 20 ºC under low light (10 μmol photons m^{-2} s^{-1}). The control samples were soaked in deionized water.

Metal treatment. Lichen thalli were treated by soaking in 25 ml of metal salts solutions: $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$. The metal concentrations were used: 10, 100 and 500 μM. The duration of incubating period was 24 h at 20 ºC under low light (10 μmol photons m^{-2} s^{-1}). The control samples were soaked in deionized water.

Chlorophyll fluorescence. Photosynthetic performance of lichens was measured with a portable chlorophyll fluorometer (Plant Efficiency Analyser, PEA, Hansatech

Instruments Ltd., King's Lynn, Norfolk PE32 1JL, UK) before and after the treatments. Thalli were dark adapted for 15 min before measurements. Maximal quantum yield of PSII (Fv/Fm) was then determined as the ratio of the maximal fluorescence (Fm) minus the minimal fluorescence (F_o) and divided on a measurement of dark-adapted status: $Fv/Fm = (Fm - F_o)/Fm$.

Photoinhibition. To induce photoinhibition lichen thalli were exposed to high light (HL) under LED lamp – 600-700 μmol photons m^{-2} s^{-1} at 20 °C during 4 hours. After light treatment thalli were hydrated and dark-adapted for 15 min before measurements.

Electrical conductivity. Electrolyte leakage in lichens was determined by Mettler Toledo SevenGo Portable Routine Conductivity Meter. The initial electrical conductivity (Ci) of the deionized water was measured in advance as a blank. After the salt treatment the thalli were 3 times rinsed in deionized water in order to remove unbounded sodium and potassium ions. The variable electrical conductivity of the solution (Cv) was measured after 12 h of shaking the lichen thalli in deionized water. Finally, the thalli were boiled at 100 °C for 15 min in water-bath to cause total rupture of cell membranes and release all electrolytes; cooled to 25 °C and the final electrical conductivity (Cf) was measured. Five replicates were measured for each treatment and for the controls control. Conductivity measurements were taken as μS cm^{-1}. The degree of electrical leakage (ET) was calculated from the following equation: $ET = ((Cv – Ci)/Cf) \times 100$, %.

1.2.3. Statistics

Different experimental analyzes were applied in order to test the influence of salinity, irradiance and heavy metals on lichen viability under controlled conditions. Thus, summary statistics were used to obtain the means, standard deviations and standard errors, which were used for the systematizing data in Appendix and building graphics (Veusz plotting and graphing package). Beside that, data distribution were examined

with the application of the Shapiro-Wilk test for the normality. All statistical analyses were performed using the statistical package R (version 2.15.1., R Development Core Team 2012).

In **Chapter 1.3.1.**, two-way ANOVA was performed for Fv/Fm in order to find the significance of differences between the samples of lichens thalli treated with factors "Salt" (0.01, 0.2 and 0.6 M NaCl), "Light" and "Salt x Light".

In **Chapter 1.3.2.**, one-way ANOVA was performed for Fv/Fm and conductivity in order to analyse how changes of the parameters can be explained by metal applications (Cu or Zn). Two-way ANOVA was used to find the significance of differences between the samples of lichen thalli before and after applied metal treatments (Cu or Zn) and their concentration (10 μM, 100 μM and 500 μM). For this analysis were chosen factors "Concentration", "Metal" and "Concentration x Metal".

In **Chapter 1.3.3**., two-way ANOVA was used to test the hypothesis the significance of differences for the lichens viability parameters due to applied salt and metal treatments together and their possible interaction. For this analysis were chosen factors "Salt", "MetCon" and "Salt x Metcon".

1.3. Results

1.3.1. Responses of lichens to osmotic stress induced by deicing salt and high light exposure

The results of deicer agent applications are presented in Tables 1-3 (Appx. I) and showed the chlorophyll fluorescence parameter response depended on the concentration, irradiance and species-specific features (Fig. 5).
The Figure shows that the photoinhibitory effect of high light was exaggerated by salt, evidenced as a strong reduction of Fv/Fm (especially for *L. pulmonaria),* whereas thalli treated with salt alone showed almost no reduction in Fv/Fm after recovery in low light.

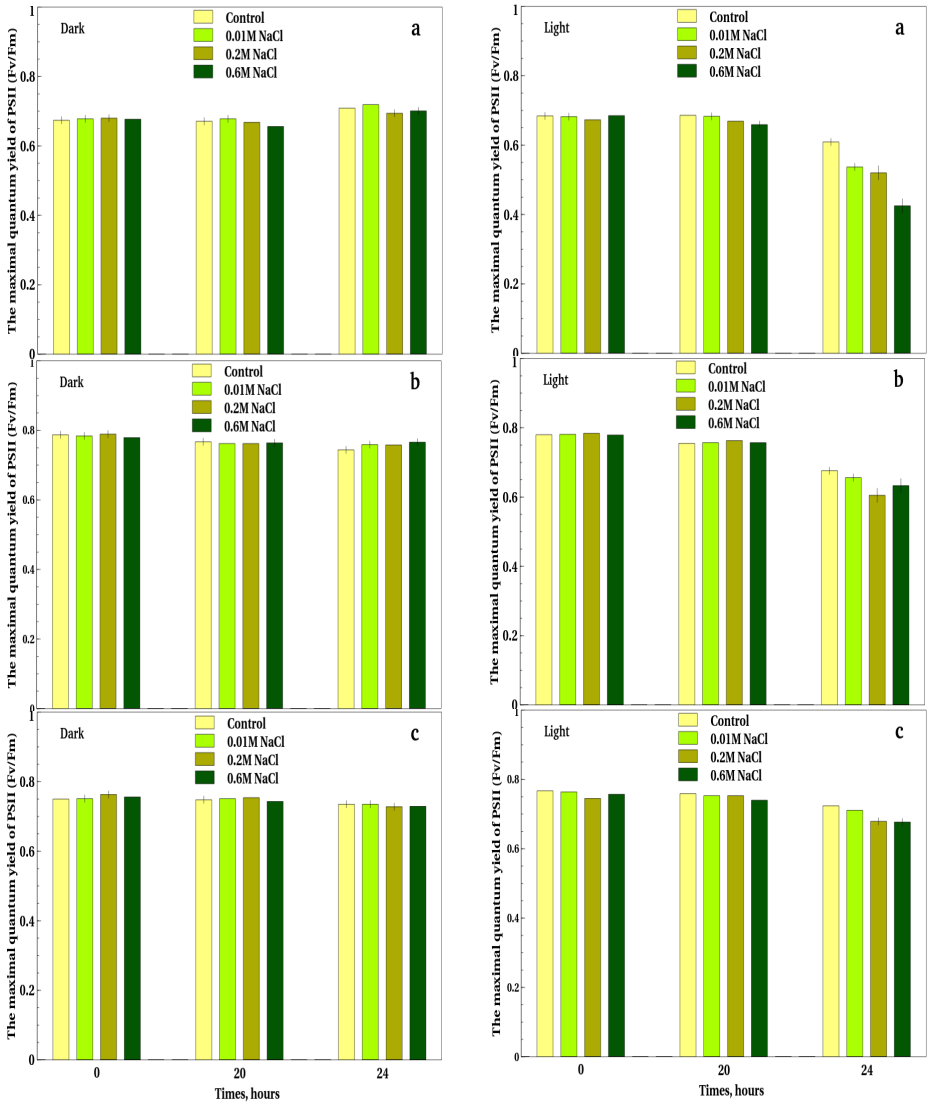

Fig. 5. – Photoinhibition measured as Fv/Fm for the lichens treated with deicing salt and high light in: a) *L. pulmonaria,* b) *P. sulcata* and c) *X. aureola*. Fv/Fm measured at the start of experiment, after NaCl application (20 h) and after irradiance with prolonged salt stress (24 h). High light treatment was 600-700 µmol photons m^{-2} s^{-1} at 20° C during 4 hours. Mean ± SE is shown for all experiments, n=5

The analysis of two-way ANOVA (Tab. 5) showed variety in significance effects of NaCl applications and irradiance on the chlorophyll fluorescence among the lichen species. Thus, the influence of the factor "Salt" was highly significant in a case of *L. pulmonaria* ($F=25.58$; $P<0.000$), less significant for *X. aureola* and with no difference for *P. sulcata.* The major effects of the factor "Light" were observed for all studied

lichen species and characterized as very high significant differences (F=48.06 to 523.06; P=0.000). The interaction between the factors ("Salt x Light") as highly significant obtained only for *L. pulmonaria* (F=20.27; P=0.000).

Table 5. – Summary of two-way ANOVA of effects of light ("Light"), salt ("Salt") and interaction ("Salt x Light") as fixed factors on the Fv/Fm

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Salt"	3	**25.58**	0.000	1.85	0.16	**7.85**	<0.001
"Light"	1	**523.06**	0.000	**144.21**	0.000	**48.06**	0.000
"Salt x Light"	3	**20.27**	0.000	**3.79**	<0.02	**4.15**	<0.05
Error	32						
Total	39						

Significant values marked in bold*

The effect of induced stresses on the mycobiont of the lichens was assessed from electrical conductivity parameter in the lichens (Fig. 6).

The results showed that lichen mycobionts were quite tolerant to the lowest concentration of NaCl (0.01 M) and the conductivity parameter increase started from the middle concentration (0.2 M) and up to the highest. The range of lichens response on the highest concentration of NaCl (0.6M) varied from 10.7 to 38.5%, whereas the influence of prolonged salt stress and irradiance was similar compare to previous data and varied from 10.4 to 42.9%.

There were found species-specific responses of lichens to the induced stresses. Thus, the figure shows that the response of *L. pulmonaria* to the increase of NaCl concentration and irradiance grows quite rapidly, while in a case of other species it is not so clear, especially in a case of *X. aureola.*

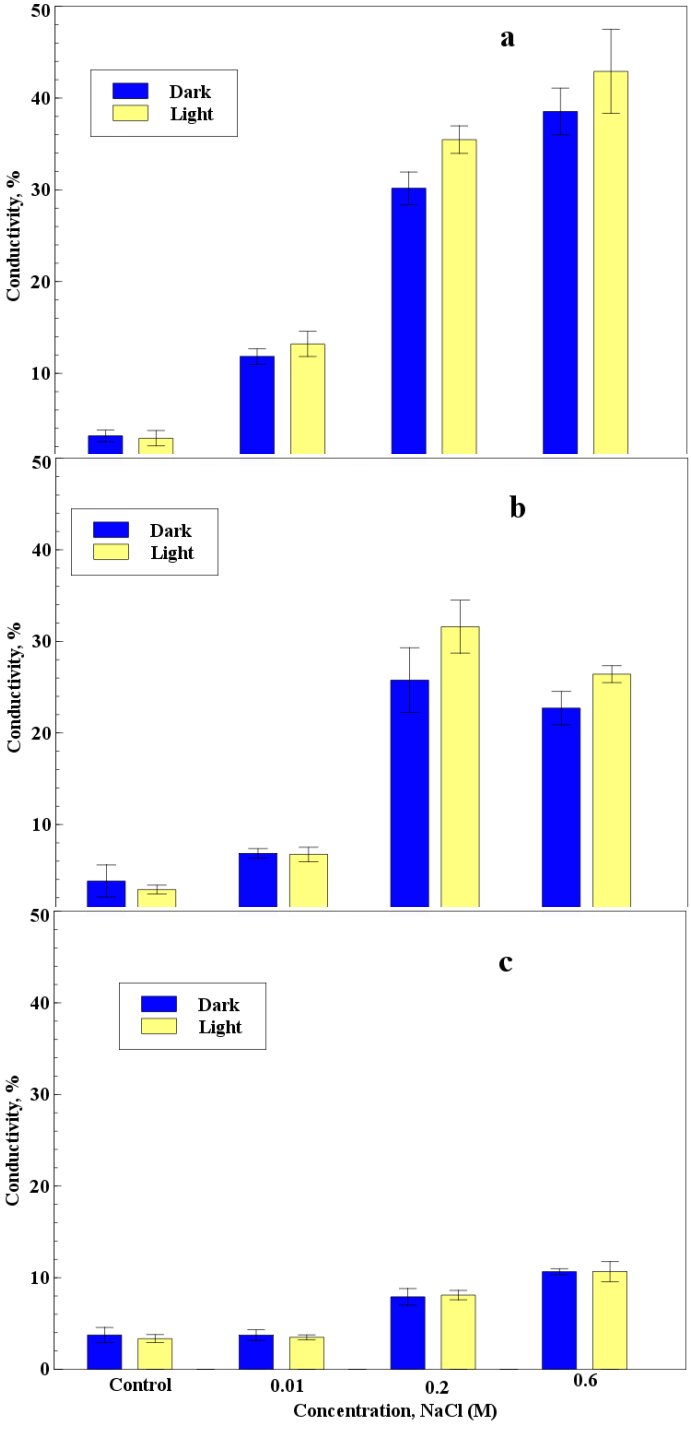

Fig. 6. – Electrical conductivity among studied lichen species as response on NaCl and high light treatments: a) *L. pulmonaria,* b) *P. sulcata* and c) *X. aureola*. Parameter "Conductivity" measured as percent value of total ions leakage. High light treatment was 600-700 µmol photons m^{-2} s^{-1} at 20° C during 4 hours. Mean ± SE is shown for all experiments, n=5

The testing of changes in the electrical conductivity among the lichens species due to applied external factors showed in the Table 6.

Table 6. – Summary of two-way ANOVA of effects of light ("Light"), salt ("Salt") and interaction ("Salt x Light") as fixed factors on the conductivity

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Salt"	3	**133.24**	0.000	**87.36**	0.000	**51.95**	0.000
"Light"	1	3.17	0.080	2.45	0.13	0.15	0.7
"Salt x Light"	3	0.73	0.540	**1.39**	<0.02	0.07	0.97
Error	32						
Total	39						

Significant values marked in bold*

The results of the table showed that the factor "Salt" was depicted at all lichen species and characterized as very highly significant for the mycobiont viability, especially for *L. pulmonaria* (F=133.24; P=0.000), whereas for the factor "Light" and the interaction of factors "Salt x Light" there were found any highly significant values.

1.3.2. Comparative effects of zinc and copper toxicity on lichens vitality

The effect of Cu and Zn on the chlorophyll fluorescence of the lichens were examined and the results presented in the Tables 2-4 (Appx. I).

The results showed that lichens photobionts exhibited varied response on the metal applications (Fig. 7). The inhibitory effect on Fv/Fm of lichens started to grow in the presence middle concentration of the both metals (100 μM) and up to the highest. Thus, the highest inhibitory affect was recoded for *L. pulmonaria* due to 500 μM of Cu treatment (up to 66.3% from the control value).

There were found species-specific influence of type of metal on the parameter. Applications of Cu inhibited photobionts of *L.pulmonaria* and *X. aureola*, while Zn mainly affected the photosynthetic activity of *P. sulcata.*

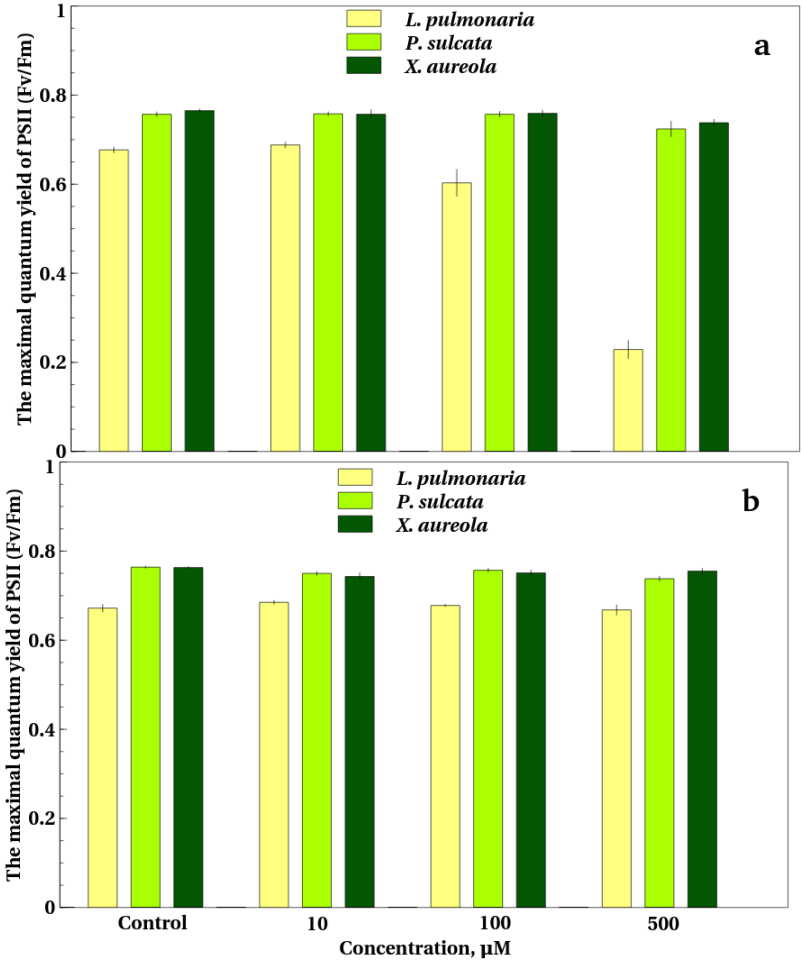

Fig. 7. – Photoinhibition measured as Fv/Fm for the lichens treated with heavy metals: a) Copper and b) Zinc. Parameter Fv/Fm presented as percent of the start value measured at the start of experiment. Mean ± SE is shown for all experiments, n=5

The results of one-way ANOVA (Tab. 7) showed that concentration of Cu was highly significant for *L. pulmonaria* (F=138.8; P=0.000) and not significant for *P. sulcata* and *X. aureola*. Affect of Zn concentration on the parameter was significant for *P. sulcata* (F=9.85; P <0.001), while no significance depicted for *L. pulmonaria* and *X. aureola*.

Table 7. – Summary of one-way ANOVA of effects of Cu ("Copper") and Zn ("Zinc") treatments as fixed factors on Fv/Fm

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Copper"	3	**138.8**	0.000	3.03	0.06	2.62	0.09
"Zinc"	3	1.01	0.41	**9.75**	<0.001	1.85	0.18
Error	16						
Total	25						

Significant values marked in bold*

The influence of concentration, type of metal and their interaction on the Fv/Fm performance by using two-way ANOVA showed in the Table 8.

Table 8. – Summary of two-way ANOVA of effects of concentration ("Cont"), metal ("Metal") and interaction ("Con x Met") as fixed factors on Fv/Fm

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Con"	3	**125.3**	0.000	**6.89**	<0.01	2.63	0.06
"Met"	1	**163.4**	0.000	0.43	0.52	0.13	0.72
"Con x Met"	3	**113.5**	0.000	0.88	0.46	1.97	0.14
Error	32						
Total	39						

Significant values marked in bold*

From the data above we can assume that all studied factors expressed highest values of significance on Fv/Fm of *L. pulmonaria* (F=125.3, 163,4 and 113.5; P=0.000), while factor "Concentration" showed significant influence on Fv/Fm of *P. sulcata* (F=6.89; P<0.01).

Mycobiont responses among the lichens on the metal stress were assessed from the electrical conductivity and showed strong negative influence on their viability (Fig. 8).

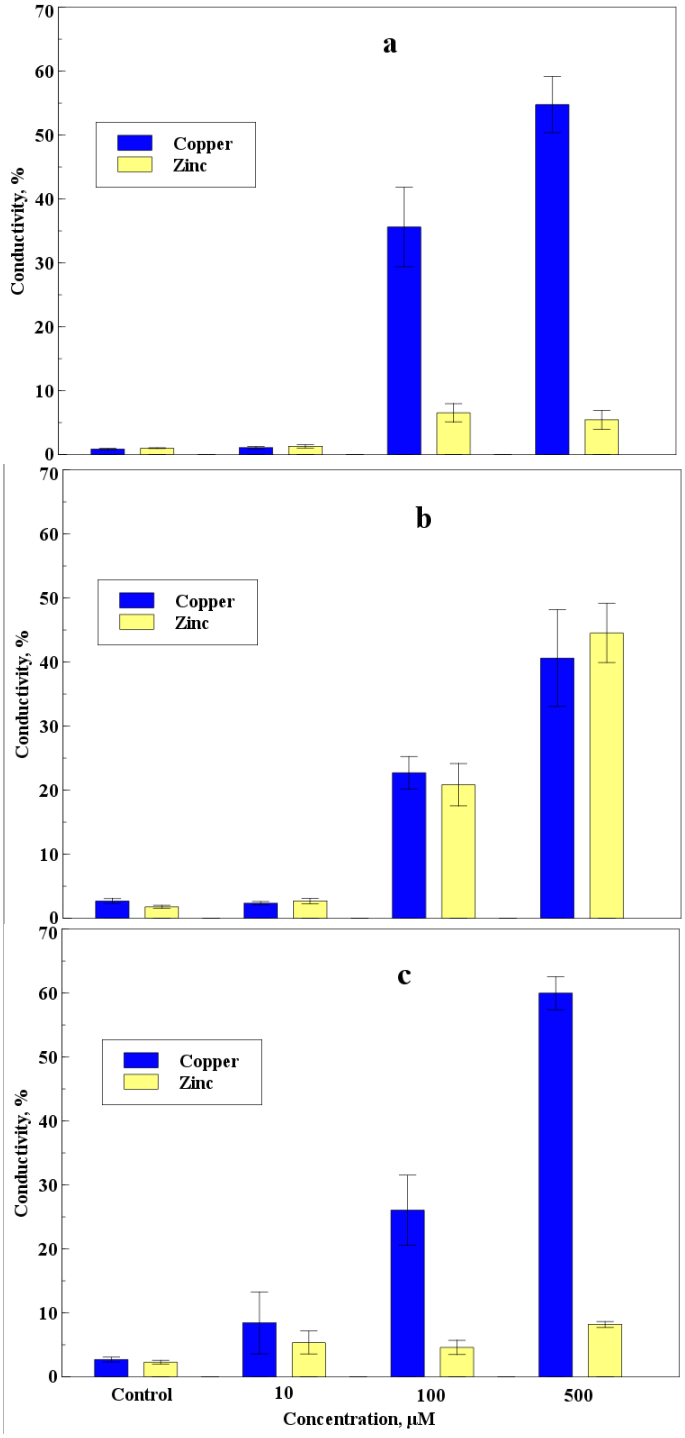

Fig. 8. – Electrical conductivity among studied lichen species as response on heavy metal treatments: a) *L. pulmonaria,* b) *P. sulcata* and c) *X. aureola*. Parameter "Conductivity" measured as percent value of total ions leakage. Mean ± SE is shown for all experiments, n=5

The figure shows that lichen mycobionts were quite tolerant to the lower concentration of metals (10 μM) and the conductivity parameter increase started from the middle concentration (100 μM) and up to highest for the both metals.
The range of lichens response on the highest concentration of Cu (500 μM) varied from 40.6 to 60.0%, whereas influence of the same amount of Zn was from 5.4 to 44.5%. There were found species-specific responses to the type of metal. The results evidently show that Cu higher influenced the conductivity of *L. pulmonaria* and *X. aureola,* whereas Zn had more impact on conductivity of *P. sulcata.*

The results of one-way ANOVA analysis (Tab. 9) indicated the influence of "Copper" on the conductivity parameter was highly significant for among the linen species (F=21.17 to 49.03; P=0.000), whereas effect of "Zinc" had stronger influence on the parameter of *P. sulcata* (F=49.52; P=0.000).

Table 9. – Summary of one-way ANOVA of effects of Cu ("Copper") and Zn ("Zinc") treatments as fixed factors on the conductivity

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Copper"	3	**49.03**	0.000	**21.17**	0.000	**44.14**	0.000
"Zinc"	3	**7.54**	<0.01	**49.52**	0.000	**4.9**	<0.05
Error	16						
Total	25						

Significant values marked in bold*

The main results about the influence of concentration, type of metal and their interaction on the electrical conductivity by using two-way ANOVA can be found in the Table 10.

Table 10. – Summary of two-way ANOVA of effects of concentration ("Cont"), metal ("Metal") and interaction ("Con x Met") as fixed factors on the conductivity

Source		*L. pulmonaria*		*P. sulcata*		*X. aureola*	
of variation	df	F	P-value	F	P-value	F	P-value
"Con"	3	**54.91**	0.000	**61.28**	0.000	**48.01**	0.000
"Met"	1	**97.65**	0.000	0.02	0.88	**90.52**	0.000
"Con x Met"	3	**37.49**	0.000	0.27	0.84	**34.47**	0.000
Error	32						
Total	39						

Significant values marked in bold*

The data above indicate that the major effect of the factor "Concentration" was detected in all lichen species and characterized as very significantly high (F=48.01 to 61.28; P=0.000). For the factor "Metal" and interactions between the factors there were obtained highly significant differences only for *L. pulmonaria* (F=97.65 and 37.49; P=0.000) and *X. aureola* (F=90.52 and 34.47; P=0.000), respectively.

1.3.3. Interaction of deicing salt and metals, and their combined effect on lichen species

The results of common stress agents applications on photosynthetic activity of the lichen species are showed in the Tables 11-13 (Appx. I).

The effect of applications was quite different among the lichens, but mostly attributed to photobionts of *L. pulmonaria*. The damage of *L pulmonaria* mainly occurred due to joint effects of 0.01 M NaCl and 500 μM of Cu compare to an effect of Zn and salt applications (Fig. 9).

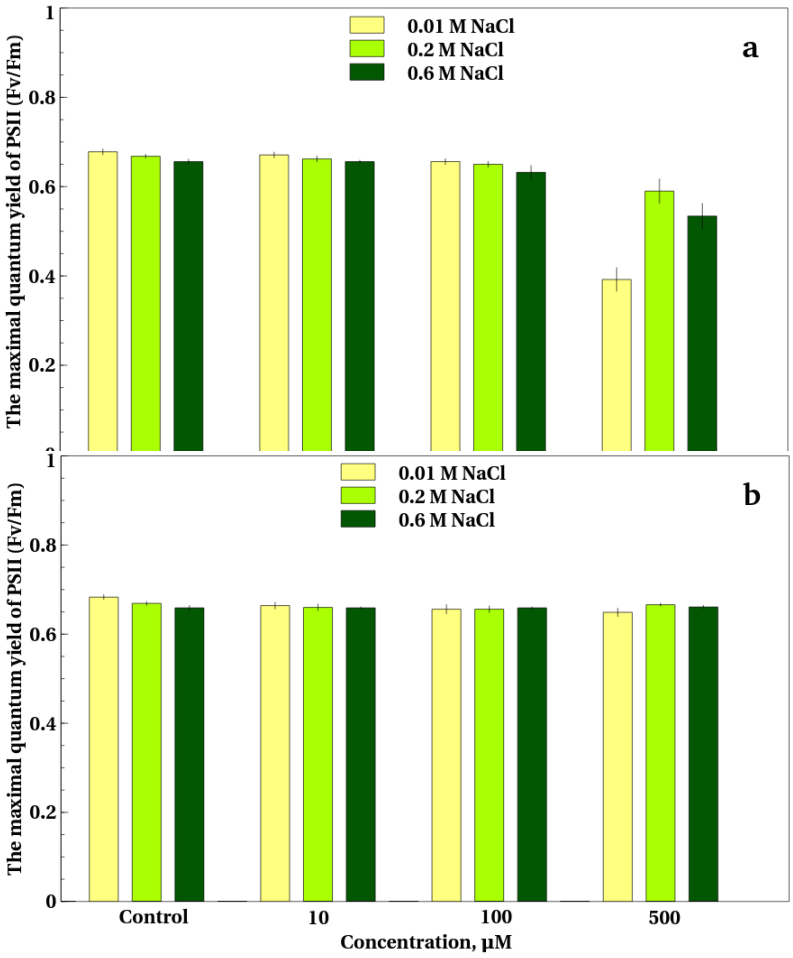

Fig. 9. – Photoinhibition measured as Fv/Fm for *L. pulmonaria* treated with NaCl and a) Copper or b) Zinc. Parameter Fv/Fm presented as percent of the start value measured at the start of experiment. Mean ± SE is shown for all experiments, n=5

The results of two-way ANOVA analysis (Tab. 14) showed that affect of studied factors along with heir interaction on the Fv/Fm in the presence of Cu was highly significant only for *L. pulmonaria* (F=26.95 to 204.1; P=0.000). In the presence of Zn the affect of factor "Salt" was highly significant for all lichen species (F=6.47 to 6.8; P<0.001), while factor "MetCon" showed significance only for *P. sulcata* (F=9.81; P=0.000). Additionally, the interaction of factors found to be significant when Zn and NaCl applied together for *P. sulcata* and *X. aureola* (F=3.84 and 3.75; P<0.001), respectively.

Table 14. – Summary of two-way ANOVA of effects of NaCl concentration ("Salt"), metal concentration ("MetCon") and interaction ("Salt x MetCon") as fixed factors on the Fv/Fm

Source of variation	df	*L. pulmonaria*		*P. sulcata*		*X. aureola*	
		F	P-value	F	P-value	F	P-value
Copper							
"Salt"	3	**26.95**	0.000	**3.26**	<0.05	0.85	0.47
"MetCon"	3	**204.1**	0.000	**3.16**	<0.05	**2.81**	<0.05
"Salt x MetCon"	9	**27.61**	0.000	**2.13**	<0.05	1.62	0.13
Error	64						
Total	79						
Zinc							
"Salt"	3	**6.8**	<0.001	**5.2**	<0.01	**6.47**	<0.001
"MetCon"	3	1.72	0.17	**9.81**	0.000	0.26	0.85
"Salt x MetCon"	9	1.42	0.2	**3.84**	<0.001	**3.75**	<0.001
Error	64						
Total	79						

Significant values marked in bold*

The effects of increasing metal concentrations combined with increasing salt concentrations on the electrical conductivity of the lichens visually presented into the Figure 10.

The figure showed that samples of lichen thalli treated with Cu in the presence of NaCl were much more damaged by the concentration increase than those with Zn in the presence of NaCl. Besides that, from 100 μM of Zn accompanied with 0.2 and 0.6 M of NaCl was detected even the decrease in the conductivity among the lichen species. There were found similarities and species-specific differences in response to different combinations of the agents.

Additionally, the results showed that lichens species started respond to induced stress from the lowest concentration of the agents (0.01 NaCl and 10 μM). Applications of 0.6 NaCl and 500 μM Cu showed the highest values of the parameter among the species, especially in a case of *L. pulmonaria* (77.1%). The concentration response of *P. sulcata* and *X. aureola* was highest due to combination of 0.01 M NaCl and 500 μM Zn, while in a case of *L. pulmonaria* – 0.6 NaCl and 10 μM Zn.

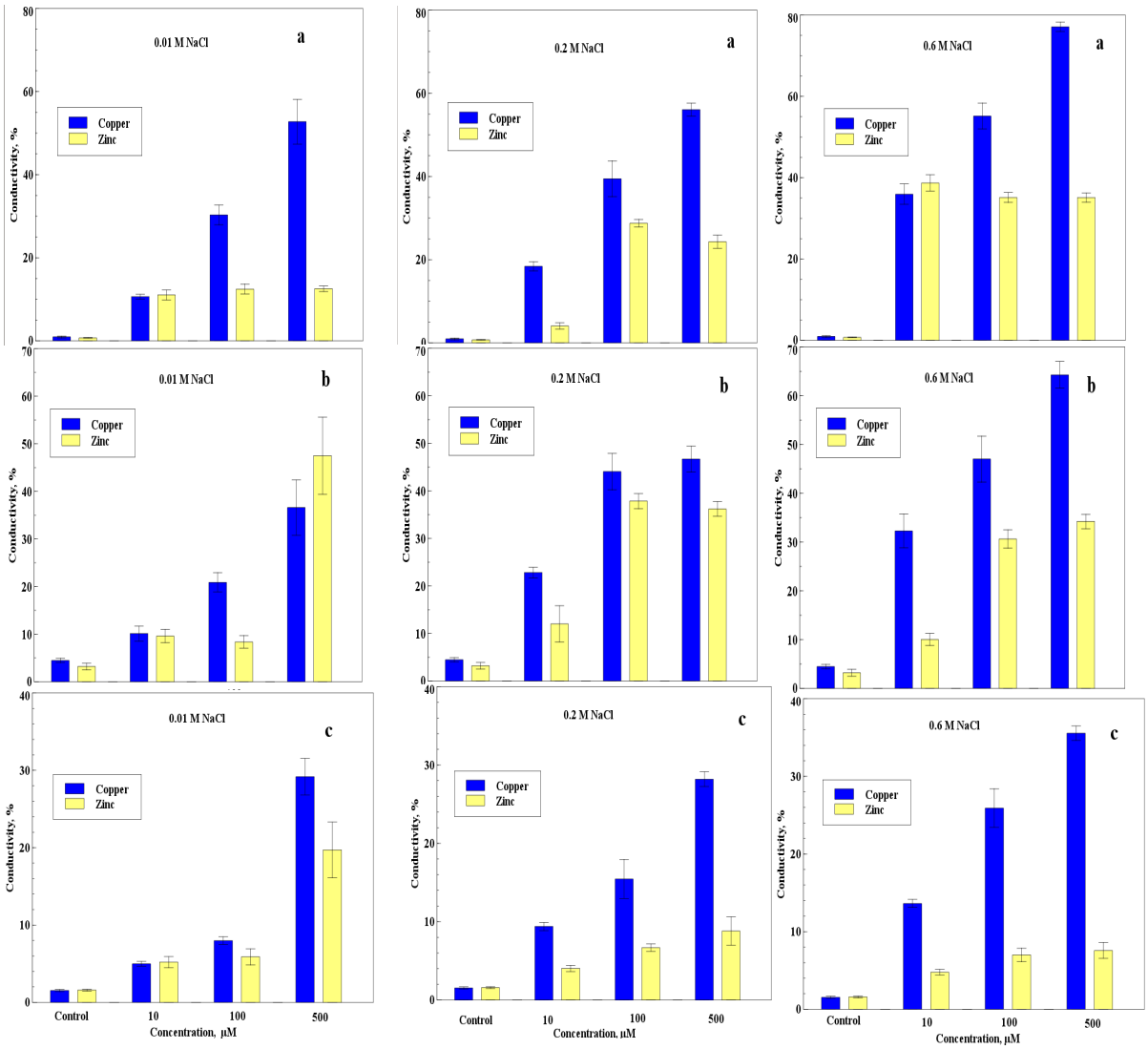

Figure 10. – Electrical conductivity among studied lichen species as response on heavy metal treatments in salt solutions: a) *L. pulmonaria,* b) *P. sulcata* and c) *X. aureola*. Parameter "Conductivity" measured as percent value of total ions leakage. Mean ± SE is shown for all experiments, n=5

To test the hypothesis the significance of differences for the conductivity parameter due to mixed treatments and their possible interaction was performed two-way ANOVA analysis (Tab. 15).

Table 15. – Summary of two-way ANOVA of effects of NaCl concentration ("Salt"), metal concentration ("MetCon") and interaction ("Salt x MetCon") as fixed factors on the conductivity

Source of variation	df	*L. pulmonaria*		*P. sulcata*		*X. aureola*	
		F	P-value	F	P-value	F	P-value
				Copper			
"Salt"	3	**73.41**	0.000	**49.34**	0.000	**17.07**	0.000
"MetCon"	3	**180.52**	0.000	**79.23**	0.000	**108.96**	0.000
"Salt x MetCon"	9	**3.56**	<0.01	1.78	0.09	**7.41**	0.000
Error	64						
Total	79						
				Zinc			
"Salt"	3	**456.93**	0.000	**10.93**	0.000	**5.89**	<0.01
"MetCon"	3	**23.19**	0.000	**86.25**	0.000	**18.84**	0.000
"Salt x MetCon"	9	**21.82**	0.000	**9.14**	0.000	**8.53**	0.000
Error	64						
Total	79						

Significant values marked in bold*

The data from the table indicated that significant effect of all studies factors on the conductivity was detected among the lichens. Thus, in a case of Cu, the factor "MetCon" was the most highly significant for *L. pulmonaria* and *X. aureola* (F=180.52 and 108.98; P=0.000), whereas in a case of Zn, there were discovered more species-specific responses of the lichens. The highest values of significance for the parameter were detected for *L. pulmonaria* due to the factor "Salt"(F=456.93; P=0.000) and for *P. sulcata* due to the factor "MetCon" (F=86.25; P=0.000). The affect of interaction of the factors on the conductivity among the lichens was significantly higher in a case of Zn (F=9.14 to 21.82; P=0.000) compare to Cu (F=3.56 to 7.41; P<0.01 to P=0.000).

1.4. Discussion

Lichen organism consists of a myco- and a photobiont, therefore they differ in their sensitivity to the environmental stresses. Regarding to Beltman et al. (1980), the sensitivity of lichens to pollution related to the degree of dependency of the mycobiont on the photobiont metabolic energy. Besides that, Kranner & Birtić (2005) found that

symbionts of lichen may induce up-regulation of protective systems in each other in stress tolerance.

In the lab experiment the photobiont viability of lichen we evaluated by measuring chlorophyll fluorescence (Fv/Fm), while the mycobiont – the electrical conductivity parameter. Several authors mentioned Fv/Fm as a useful parameter in determining the sensitivity of lichens (photobiont) to pollution (Branquinho et al. 1997, 1999; Tarhanen et al. 1999; Küpper et al. 2009). Whereas, Gauslaa & Solhaug (2000) and Garty et al. (2002) mentioned that chlorophyll fluorescence methods are not always sufficient during a chronic long-term damage in lichens. Regarding to Meharg (1993) the plasma membrane is the primary "living" target for external toxicity, therefore electrolyte leakage is widely used as an indicator for membrane damages inducing by various stresses (Backor et al. 2003). Since the mycobiont represents most of a lichen biomass, it is reasonable to assume that ion leakage due to pollution mainly occurred from fungal cells (Munzi et al. 2009).

The road maintenance in winter time is a major source of chlorinated deicers particularly along the busy highways (Hahne & Kroontje 1973; Blomqvist 200; Wike et al. 200; Sivertsen 2010). The results of incubating the thalli with NaCl along with high light treatment (Chapter 1.3.1.) showed a significant negative effect on the Fv/Fm of lichens in following order: "Salt"<"Light"<"Salt x Light", while on the lichens membrane integrity parameter: "Light"<"Salt x Light"<"Salt". Thereby, lichen mycobionts are mainly affected by salt stress, whereas lichen photobionts – combination of high light and salt stress. It agrees with investigations of Kranner et al. (2005), where isolated fungus of *Cladonia vulcani,* due to increasing oxidative stress, was more susceptible to desiccation than the alga. In turn, the alga of *C. vulcani* tolerated a very dim light without the fungal contact. Also among the reviews available an opposite experimental results. Thus, Takahagi et al. (2002) showed the growth of mycobionts from the thallus fragments of *Ramalina* spp. was affected higher level of NaCl (0.8 M) compare their photobionts (0.4 M). Experiment of Wieners et al. (2012) illustrated the considerable potential of the photoprotective mechanisms in the

desiccated alga *Trebouxia asymmetrica.*

The results of lab studies showed that lichen species exhibited varied responses to salinity and irridiance, where *L. pulmonaria* was the most sensitive to the stresses. Our findings are supported by Gauslaa & Solhaug (1996), who found that *Lobarion* lichens were most susceptible to high light. A strong negative effect of NaCl on the recovery of *L. pulmonaria* has been showed by Chakir & Jensen (1999). Regarding to Kopecky et al. (2005), the high irridiance sensitivity of cephalodial lichens comparing to chlorolichens may occurs due to vulnerability of cyanobacterial photobiont. Since the ability to recover after combined drying-light stress in lichens positively correlates with species-specific water holding capacity (Gauslaa et al. 2012), it may be an important factor for the species faster recovery from the abiotic stress. Instead, *X. aureola* demonstrated high salt stress tolerance that might be explained by a higher amount of stored carbohydrate in the lichen fungus, which may increase the internal osmotic pressure sufficiently that absorb moisture from the atmosphere under conditions of moisture stress (Brock 1975), either by sufficient buffering effect of hyphal cortex layers of a thallus (Bartak et al. 2005).

Traffic is a major contributor to Cu and Zn emissions. Thus, Pacyna & Pacyna (2001), found these heavy metals are emitted through the abrasion of tires, lubricating oils, brake pads or fuel additives. The results of our investigation (Chapter 1.3.2.) showed a high tolerance of lichens to lower dose of Cu or Zn (10 μM) by using both viability parameters, while the influence on the conductivity was much stronger than on the Fv/Fm. It can be explained by studies of Goyal & Seaward (1982), demonstrated that most of the metal ions are sequestered by the fungus, due to the greater contribution of fungal symbionts to total thallus weight. Also, it agrees with studies of Pawlik-Skowronska et al. (2008), where found that metal accumulation in mycobiont layers of lichen prevails. Instead, some authors indicated that lichen algal partners were more sensitive to heavy metals than mycobionts (Tarhanen et al. 1999; Backor & Loppi 2009).

Regarding the experimental data, *L. pulmonaria* showed a very high sensitivity to

copper toxicity compare to other species, especially on photobiont level. Similarly, Brown & Beckett (1983) recorded that heavy metals inhibited photosynthesis in cephalolichens at substantially lower concentrations than those causing decreased photosynthesis in chlorolichens. Zinc in the experiment had a minor influence on lichen photobionts, nevertheless it significantly affected *P. sulcata* mycobiont. Regarding to Branguinho et al. (1997) lichen sensitivity response to the metal pollution can be explained by species various thallus morphology and chemistry, different metal binding capacities of various genera (Branquinho 2001), in which higher chitin content in mycobiont cell walls may plays a role (Palmquist et al. 1998).

The increase of heavy metals realize from the roadside environment and the negative impact of deicing salt due to road winter maintenance are often discussed as separate ecological problems. However, a relationship between them which leads to increasing heavy metal mobility has supported by field and laboratory experiments (Granato et al. 1995; Bäckström et al. 2004). The results of our experiment (Chapter 1.3.3.) showed that desiccation and metal treatments applied together seriously damaged lichen viability and led to several responses: extensive growth of electrical conductivity compare with the decrease of Fv/Fm; higher interactions between NaCl and Zn than with Cu; increase the sensitivity to stress among species *(L. pulmonaria> P. sulcata>X. aureola)*. Besides, we found that lichens photobionts, showed the lowering toxicity of the agents that indicate on their effective system against the stress. Regarding to Pawlik-Skowronska et al. (2006) and Backor et al. (2007), lichens in a response to increasing level of stress may enhance their phtoprotective capacities by synthesizes phytochelatins (mechanism of metals detoxication by complexation). Also, the hypothesis that lowering effect of increasing NaCl concentration on metal toxicity due to Me-Cl species forming and have much lover toxicity than free metal ions as pointed by Babich & Stotzky (1978), may be implicated.

Part II. Quantitative and qualitative changes in transplanted lichen samples as surrogate receptors for roadside atmospheric depositions

Part II. Paper I. Spatial patterns of airborne pollutant and their accumulation in lichen transplants along a rural highway

2.1. Introduction

Roads and their associated vehicular traffic have a major and increasing impact upon the environment. The effect of a road upon the environment is complex and includes disturbance pollution from road maintenance and the pollution from the traffic of an established road (Antgold 1997).

According to Klos et al. (2009) and Amato et al. (2011), vehicular traffic emissions consist of gaseous pollution, fine and coarse particulate matter, airborne particulate-bound trace metals and metals. Along with that, during a winter time salt aerosols can also be produced by vehicular traffic from dried salt deposits on the road surface (Williams et al. 2000). A considerable hazard to the environment is associated with the emission of heavy metals. Some of them are emitted through the abrasion of tires (Cu, Zn, Cd) and brake pads (Sb, Cu), corrosion (V, Fe, Ni, Cu, Zn, Cd), lubricating oils (V, Cu, Zn, Mo, Cd) and fuel additives (V, Zn,Cd, Pb) (Pacyna & Pacyna 2001). Once released, pollutants are transported into the local roadside environment either by runoff or short distance aerial dispersion followed by deposition. The deposition of heavy metals emitted due to motor traffic can be determined in soil and plant material, highway runoff and ground waters. Thus, in tunnel wash water runoffs, originating from the nearby roads and tunnels (Meland et al. 2010) detected traffic related metals like Al, Cd, Cr, Cu, Fe and Pb in particles and colloids, whereas As, Ca, K, Mg, Mo, Ni, Sb and Zn were more associated with low molecular mass species (<10 kDa).

The motor traffic related factors that affect the volume and dispersion of air pollution from a roadway sources may include: traffic volume, vehicle speeds, vehicle mix, technical condition of vehicles, roadway topography and surrounding terrain. Most

investigations were carried out to estimate the deposition roadway pollution changes with the increase of distance away from the road. Regarding to Angold (1997), the effect of traffic pollutants on vegetation was detectable up to 80 m away from the road with a maximum edge effect of 200 m. Viskari et al. (1997) showed a similar decline of the local inorganic and organic pollutant load deposited in snow samples: 30 m and up to 60 m from the road. Pedersen & Fostad (1996) and Blomqvist & Johansson (1999) found that the spreading of deicer salt to soil via splash and spray caused a deposition very close to the road (2-8 m). However, some part of chlorides can be still transported as aerosols over quite long distances (Lundmark 2003). The direction of prevailing winds is a major factor in the distance-dependent distribution of heavy metals off roadsides as showed Blomqvist & Johansson (1999). Also the wind speed was an important factor that influenced the distance to which the salt was dispersed (Blomqvist 2001). Other factors, like weather and traffic characteristics, are also likely essential. Blomqvist (1998) recorded that traffic speed at 80-90 km/h resulted in damage to vegetation up to 5-8 m from road, but up to 2-3 m at lower speed. A strong correlation between atmospheric pollution dispersion and meteorological parameters (temperature, relative humidity and wind speed) in the road vicinity was found by Tasic et al. (2004).

Lichens are extremely sensitive to environmental stress, especially atmospheric pollution, eutrophication, and climate change (Galun 1988; Richardson 1992; Nash 1996; Nimis et al. 2002). Herzig et al. (1989) noted that lichens do not specially react to single toxic components in the air, but rather indicate the integrative toxic effect of a combination of different pollutants. Kovács (1992) summarized the physiological and morphological features of lichens, which make them more sensitive to air pollutants than higher plants. These features include the absence of a cuticle, lack of excretion, long-living and with low chlorophyll content. However, according to Van Dobben et al. (2001), lichens are generally insensitive to toxic effects of trace elements, and can therefore be used as accumulator organisms to estimate concentrations of these elements in the environment. Similarly, Loppi & Pirintsos (2003) showed that epiphytic lichens proved to be very effective as an early warning system to detect signs of a changing environment as well as sentinel organisms for a

heavy metal deposition. Regarding to Figueira et al. (2002), lichens can be used as biomonitors to indicate the dry deposition of sea salt as well. The experiments which had been conducted with lichens as biomonitors of airborne pollution near the highways showed, that the distribution of accumulated pollution over the exposition period depended on various environmental factors such as direction of the winds (Bari et al. 2001), tree cover and topographic level (Gonzalez et al. 2003) as well as distance from the road (Bignal et al. 2008; Jozic et al. 2009; Klos 2009).

Many have tried to study the mechanisms by which lichens accumulate and interact with pollutants. Reis et al. (1999) pointed that accumulation of trace elements by lichens can be passive over the long term, dynamic or immediate involving uptake and release to the point of equilibrium. They introduced the concept of "remembrance time", the time over which lichen functioning reflects recently-experienced environmental availability. Sarret et al. (1998) working with lichen-metal interaction, discovered that hyperaccumulation of metals in lichens might be the result from a reactive mechanism of organic acid production. However immobilization of metals by lichens is still not well known.

Lichens may accumulate mineral elements from aerial sources, from their substrata or solutions. According to Brown & Brown (1991), mineral elements may be found in three major cellular locations of the lichen thalli: deposited at the surface or trapped in the intercellular spaces (surface fraction); bound to exchange sites on the cell wall (cell wall fraction); and accumulated in cytoplasm (intracellular fraction). They pointed that the intracellular fraction is most important for studying the impact of element accumulation on physiological parameters of lichen. In a case of metal-rich particulate entrapment, Honegger (1991) added to the surface fraction the entrapment in intercellular spaces and divided the extracellular complexation into two levels (Fig. 1).

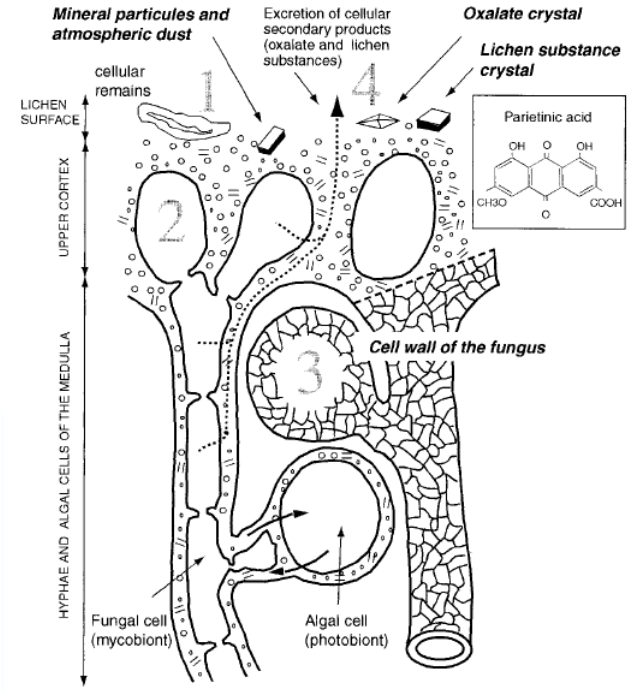

Figure 1. – Possible locations and uptake mechanisms of metals in the lichen thallus: (1) metal-rich particulate entrapment on the lichen surface and in intercellular spaces of fungal biont; (2) intracellular complexation to metallothioneins; (3) extracellular complexation to functional groups of fungal macromolecules from the cell walls; (4) extracellular complexation to organic acids, such as oxalate, or lichen substances, such as parietinic acid (adopted from Honegger 1991)

Furthermore, Pawlik-Skowronska et al. (2008) showed that metal containing particles accumulated primarily on the surface of lichens or trapped within intercellular spaces may become solubilised. Metal ions will either be adsorbed onto fungal cell walls where they form precipitates or will be taken up intracellularly both by myco- and photobionts. In order to tolerate high concentrations exceeding their physiological requirements of trace elements, lichens can retain many of them by sequestering elements extracellularly as oxalate crystals or lichen acidcomplexes (Nieboer et al. 1978). According to Hale (1983), intercellular spaces of the lichen thallus can accumulate and retain heavy metals in particulate form or bound to cation exchange sites.

Many scientists have concluded that accumulation of heavy metals by lichens are complex and affected by many factors, such as time of exposure, age and size of thallus, species-specific features, environmental and physiochemical factors (Baranowska-Bosiacka et al. 2001; Garty 2001; Nimis et al. 2001; Pawlik-Skowrońska et al. 2002; Loppi & Pirintsos 2003; Hauck 2007, 2008). According to Nieboer et al. (1972), thin, flat surfaces in lichens are ideal for intercepting particulate

matter. Puckett and Finegan (1980) found that finely branched form of thalli may assist in a process of accumulation. Garty (2001) found that combination of two growth forms and curled flaps on the branches of lichen may secure particulate matter to its surface and reduce wind or water erosion. Garty et al. (2001) as well pointed on more efficient uptake and adsorption salt originated from sea spray by thinner thallus. Such intrinsic features of some lichens as higher chitin content in mycobiont cell walls may give a base for higher metal-resistance that relays on metal avoidance or immobilization in external layers, accordingly to Palmqvist et al. (1998). Branquinho et al. (1997) reported that even at similar extracellular metal accumulation, lichen species of various thallus chemistry might accumulate intracellularly different amounts of metals, which can be explained by their different cation-exchange capacity (Branquinho, 2001). Garty (2002) found that metal accumulation by lichens can depend on the metal speciation features. For instance, thus nonparticulate elements (S and K) are not as affected by these structural differences compared to metals (Pb, Ni, Cr, and Al) which adhere to particles.

As a summary an increasing demand for transportation and traffic volumes makes roads and traffic major non-point sources of pollutants. Much effort has been directed towards what concentrations and type of pollutants are released into the atmosphere through vehicular traffic emission and road maintenance. Besides, a lot of attention has been paid to understanding the process of pollutants distribution along the roadside environment. There has been not enough comprehensive studies which have looked at the quantities of compounds that are released and how far they disperse from the local roadside environment.

The main task of this work was to study distribution, accumulation and proportion of traffic related pollutants from lichens transplanted along the highway E6 in Akershus fylke, southern Norway, in order to develop biomonitoring technigues.

The study aims to answer to the following questions:

- which elements are the most abundant deposited in lichen thalli?

- are there any correlations between individual element concentrations?
- will lichen transplants displayed at different distances from the road change their elemental composition?
- what factors influence the element accumulation in lichens due to field conditions?
- does the accumulation capacity of elements vary between the lichen species?

2.2. Methods

2.2.1. Study area and supplementary conditions

The field study was performed at Støkken, in Ås commune, Akershus fylke, south-eastern part of Norway (59°64'N and 10°74'E; 100-150 m.a.s.l) (Fig. 2).

Lichens in transects were installed with increasing distances from the highway E6 in Ås and Vestby from 25th of September 2011 to March 26th 2012. This season is a period with much road dust and salt-containing aerosols. We believe that winter is a critical period as this is normally the season with the highest levels of pollutants. Furthermore, during this part of the year we will have easy access to the open agricultural fields that are common at Ås and Vestby, where distance from the road is not confounded by factors as forest density, tree composition, vegetation cover etc.

Three lines perpendicularly to E6 road were selected. There were Line A (Row 1 and Row 5) placed 200 meters north of the Støkken bridge, Line B (Row 2 and Row 6) with 80 meter spacing from Line A and Line C (Row 3 and Row 4) placed 220 meters south of the bridge. Close to the east end of Lines A and B there was a small local road. The area did not have any woody or shrubby vegetation. There was 2% inclination in the terrain from E6 towards the east. On the west side of E6 road the terrain was more flat except for Line C (Row 4) where 2-3 meters depth valley crossed it (Fig. 2).

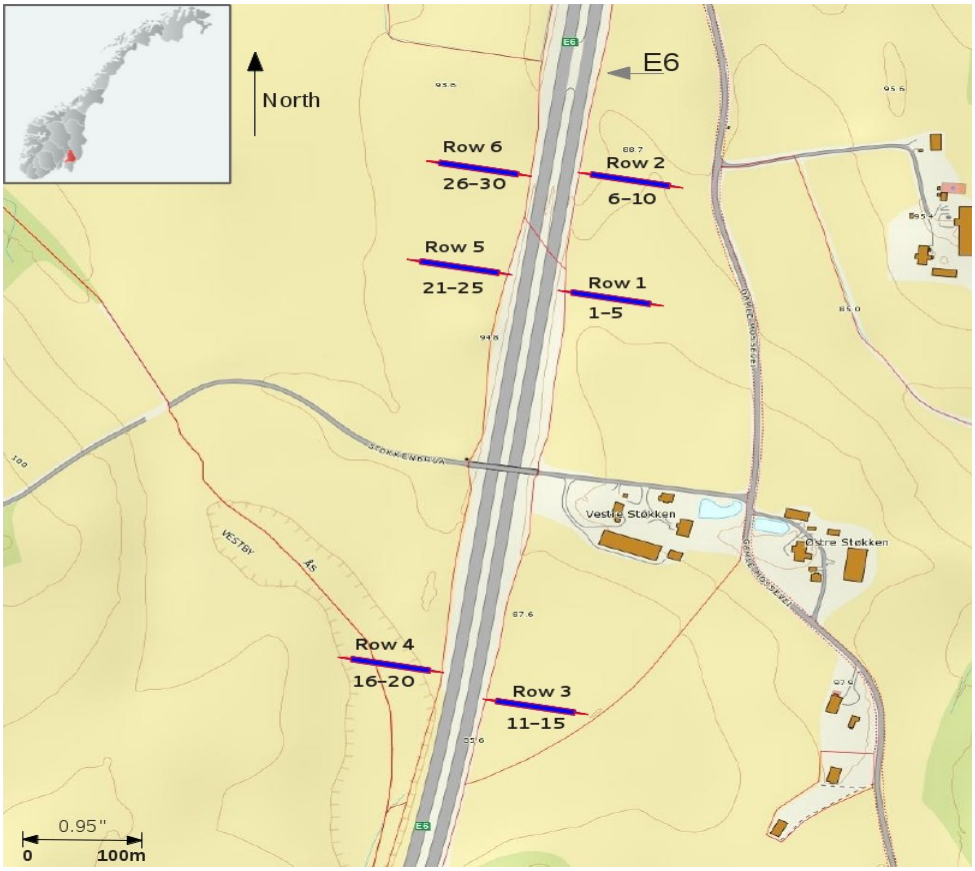

Figure 2. – Study area and location of transects with lichens near E6 highway

Lichen thalli were placed on stands at five different distances from both sides of the road (10, 15, 30, 50 and 100 m) at six separate gradients. In the experiment, four replicates of each of four species were randomly placed on each stand made of wood (Fig. 3). The lichen thalli were fastened to a mosquito net made of nylon by a thread. Thus the thalli were exposed in a vertical position mimicking their natural location on tree trunks. Each stand was 2 m long with a wood board 20 x 30 cm, attached to1.5 m pole inserted to 20 cm depth into soil. The nets with lichens faced the road. In total 30 stands were installed.

Figure 3. – Stands with four replicates of each of lichen species lichens in the field

As supplementary conditions of the study area we also considered climatic data, traffic characteristics and quantities of deicing salt applications.
The climatic data for the field experiment obtained from the eKlima data base and presented as mean values in Table 1.

Table 1. – Average local climatological data during the field experiment, September 2011- March 2012 (Rygge airport, eKlima data base)

Variable/Month	September	October	November	December	January	February	March
Precipitation, mm (24 hours)	0.3	3.8	5.7	1.9	8.9	1.1	0
Air tempetarure, ^{o}C	13.6	8.8	4.9	1.8	-1.7	-1.9	2.2
Wind speed, m/s	3.6	3.8	2.9	4.1	3	3.2	3.1
Wind direction, degrees	189	191	160	204	138	182	175

The maximum precipitation (8.9 mm) was observed in January, while the minimum in September and March (0-0.3 mm). The results showed that an average air temperature varied from 13.6 to -1.9 °C (September and February) which are warmer than normal found for the region.

The data of wind speed and wind direction were transformed into the Figure 4. The results showed that during the field experiment were prevailing southern winds, with southeast and southwest directions during the winter season.

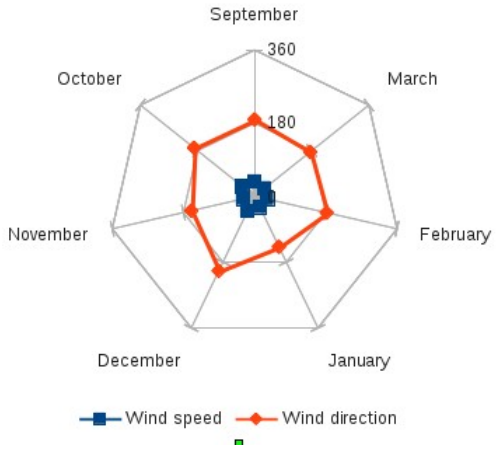

Figure 4. – Wind speed and wind direction according to eKlima data base

The study was conducted along E6 road, which is the main north-south road in Norway (3.140 km) (http://en.wikipedia.org/wiki/European_route_E06).

The total amount of vehicles per 24 h and averaged over the year during the period 2006-2010 in both direction of E6 road, close to Korsegården has been calculated. It showed that the total amount of vehicles per 24 hours and averaged over the year increased by 6 461 items due to light models, whereas the percentage of heavy vehicles decreased in the total amount by 0.8%. At Smihagen tunnel direction, the total number of vehicles during the period 2006-2011 also increased by 7 102 items that was due to mostly light models and slightly growth of heavy vehicles by 1.3%. Tsubota & Kawashima (2002) found that total traffic volume mainly consisted of small cars, but air pollution along the roadside depended mainly on the number of heavy goods vehicles. By summarizing the data from Table 2 (Appx. II), possible to result that the study area characterizes with a high traffic density (HTD) of 15 759 cars per day in single direction in the nearby Korsegården and

16 552 cars in the nearby Smihagen tunnel. The percentage of heavy vehicles for the site varied from 11.9 to 14.0.
The road E6 is periodically treated with deicing salts (particularly by sodium chloride) during each winter season. Thus, during 2011-2012 winter season applied 14.5 tonnes of deicers per kilometer of E6 road in this region (60 km), which gives 880 tonnes due to data of the local Vegvesen (Ås kommune, Akershus fylke).

2.2.2. Study species

In addition to *Lobaria pulmonaria* and *Parmelia sulcata*, which were used in the laboratory experiment, we selected the two lichens species which in most cases evaluated by the majority of biomonitoring studies.

***Ramalina farinacea* (L.) Ach**. is a fruticose lichen with green-grey to grey thalli. It is chlorolichen with a green alga *Trebouxia* as photobiont.
The lichen grows in tufts on tree trunks and twigs, usually on the side of the tree exposed to strong sunlight. It is nitrophilous, likes nutrient rich bark and hedges (near farms, for example) (Fig. 5).

Figure 5. – *Ramalina farinacea* (L.) collected and used in laboratory experiment

Van Dobben & Ter Braak (1999) found that *Ramalina* spp. were very sensitive to air pollution showing a monotonous response. Accordingly to Branquinho et al. (1997) the lichen photobiont was highly sensitive to Copper and particularly under acidic conditions (Garty et al. 2007).
Thalli of the lichen were collected in September 2011 in young and open *Populus tremula* stands on former pastures in Skipstad, Hvaler, Østfold, Norway (59°03′13-14″N and 10°56′27-30″E; 5-10 m.a.s.l).

***Usnea filipendula* Stirt**. is a fruticose, pendulous (alectorioid) lichen with green alga as primary photobiont *Trebouxia* (division Chlorophyta).
The lichen has grey-green to yellow-green range of color and black at base of main stem. Main branches are rather few, arising close to the base and hanging vertically, with abundant, short, fine side-branches (fibrils) that project horizontally in a «fish-bone» pattern. A widespread species on birch (*Betula sp.)* and conifers (Fig. 6).

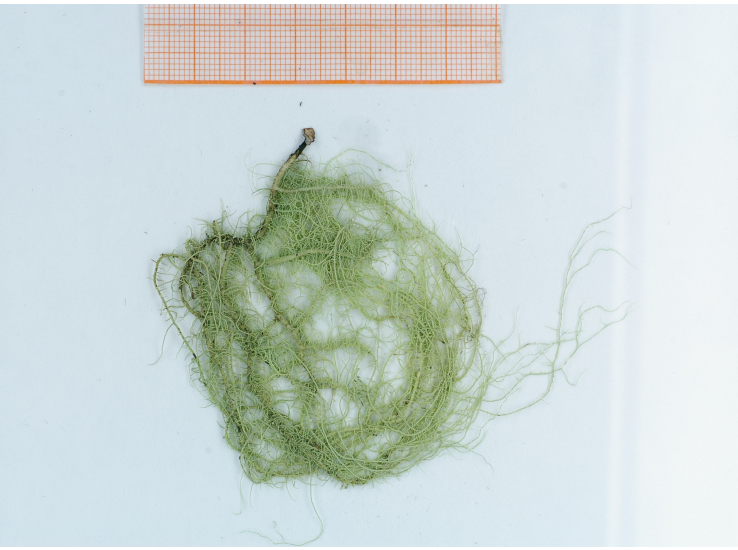

Figure 6. – *Usnea filipendula* Stirt. collected and used in laboratory experiment

Member of the genus *Usnea* are recognized as highly pollution-sensitive, especially to SO_2 (Conti & Cecchetti 2001; Carreras et al. 2005). Hauck et al. (2008) suggest that usnic acid influence the acidity tolerance of the lichens.
Thalli of the lichen were collected in September 2011 and taken from open, western-

facing rocky slope with scattered Betula spp. and Pinus sylvestris in Intaket, Töckfors, Sweden (59°29'16-19"N and 11°52'13-16"E; 120-135 m.a.s.l).

2.2.3. Laboratory analysis

After the transplantation period along the highway, nets with air dry lichen thalli were carefully collected from the field stands and temporary stored at room temperature in darkness until they were removed from the nets and put into paper envelopes. The control samples have been kept air dry in the freezer since the transplantation period started. Freezing is the recommended way for long-term storage of lichen thalli for later physiological experiments (Honegger 2003).

Elemental analysis. Lichen samples collected from the transects processed and analyzed for elements such as calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), aluminium (Al), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) through inductively coupled plasma optical emission spectrometer. Whereas, elements as phosphorous (P), barium (Ba), arsenic (As), cadmium (Cd), cobalt (Co), molybdenum (Mo), lead (Pb), tin (Sn), antimony (Sb) and vanadium (V) were determined using inductively coupled plasma mass spectrometer.

Powdered and homogenized dried lichen thalli, (0.2 g DM), were transferred into Teflon tubes. Then, the samples were mineralized with concentrated HNO_3 (5 ml) with an addition 1 ml Milli-Q water (MQ H_2O) and 250 l µL Internal standard (IS). The tubes with samples were transferred to ultraCLAVE III Microwave Digestor (MLS GmbH Mikrowellen-Labor-Systeme, Milestone S.r.l.) under 50 bar pressure and 260°C for 2.5-3 hours. The extract, as clear liquid, from each sample was passed to plastic tubes with addition in volume up to 50 ml by MQ H_2O. Total concentration of trace elements, expressed on a dry weight basis, were determined by Plasma Optical Emission Spectrometer Perkin-Elmer Optima 5300 D (ICP-OES) and Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Analytical quality was checked by analyzing Standard Reference Materials (SRM) No. NCS DC 73348 “Bush

Branches and Leaves" and No. NCS ZC 73013 "Spinach". The "Bush Branches and Leaves"certified reference material had an average recovery of 93.5%, while the "Spinach" certified reference material had 106.3%.

The elements were measured on one pooled sample for each lichen species from each stand. The analyses were performed at the Department of Plant and Environmental Sciences (IPM), UMB, Ås.

2.2.4. Statistics

For the statistical analysis data distribution were examined with the application of the normality test. The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics. Graphics were performed by using Veusz plotting and graphing package. All statistical analyses were performed using the statistical package R (version 2.15.1., R Development Core Team 2012).

Three way ANOVA and two-way ANOVA were performed to check if the any significant differences between the changes of elemental content in lichens and performance of the factors "Distance", "Side", "Species" and their interactions.

A Pearson correlation tests were applied to obtain correlation coefficients between elemental concentration for each of lichen species and between the concentrations of elements in total atmospheric deposition of lichens.

Distributions of the total elemental accumulation among the lichen species and the stands were illustrated as Box-plots by using single linear regression and descriptive statistics.

2.3. Results

The element concentrations accumulated in thalli of *L. pulmonaria*, *P. sulcata*, *R. farinacea* and *U. filipendula* after a 6-month exposure in the vicinity of heavy traffic

road as well as background level values (as controls) are presented in Tables 3-10 (Appx. II). The data elemental content in lichens varied between stands, species, directions and distances. Also the proportion of elements in the total accumulation content varied between species.

The elements from the lichen content were analysed as a whole elemental chemical composition of lichens or was separated into two groups. Thus, the group of major elements includes macronutrients (K, P, Ca, Mg) and microelements with concentration more than 100 μg g^{-1} DM and, the second group was conducted on a base of the trace elements from the lichen content (<100 μg g^{-1} DM).

In order to compare the level of element variability among lichen species their Coefficients of Variance (CV) were calculated (Tab. 11-12; Appx. II). The study reveals that the maximum range of variation was recorded for Na (75-81.7%), Co (55.1-71.8%), Fe (51.9-69.2%), Al (45.0-58.7%) and V (50.6-66.2%), whereas the minimum for P (14.0-24.1), As (17.1-24.7%) and Pb (16.9-27.9%) among the lichen species analyzed by two groups.

The next task was to determine how many times the element content in transplanted lichen species to exceeded the background value (Tab. 13-14; Appx. II).

To compare the results elements were classified in three categories. Thus, to the category of elements with the highest value of times exceeded the control, were belonged V and Na (22 and 35 times over), to the medium – Al, Cu, Fe, Sn, Mo, Sb and Co (9-17 times over) and with the minimum of changes – Ca, Zn, Ni, Ba, Pb, As, P (2-5 times over). Potassium (K) and Cd showed even reduction in the concentration compare to the background value (0.5 and 0.7 times less).

We determined that Na (25-54%) and Ca (16-44%) consume a highest percentage of the major element content in harvested transplants of lichens (Fig.7).

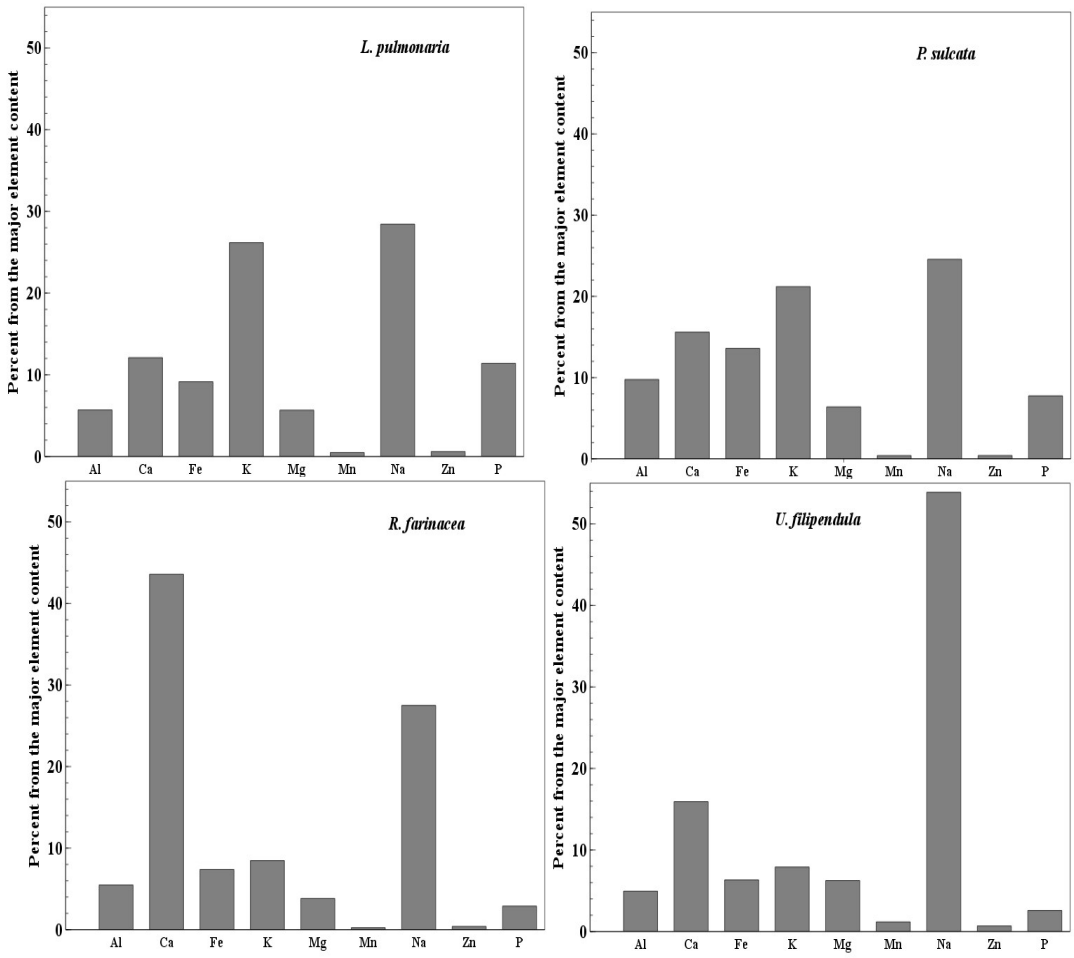

Figure 7. – The distribution patterns of the group of major elements accumulated by lichen species. Presented as percent (%) from the total major element content

Besides, the abundance of elements accounted for the bulk of accumulation content in lichen species followed the order K>Fe>Al>Mg>P, with the rest of analyzed elements less than 1% (Mn, Zn). Likewise, the trace elemental content of lichen species are shown in the Figure 8.

The distribution patterns showed that Cu and Ba together are the main elements of the total trace element content in lichen tissue (59-74%). Sequentially, the abundance of elements accounted for the bulk of accumulation content in lichen species followed the order Pb>V>Cr>Ni >Sn with the rest of determined elements less than 1-2%.

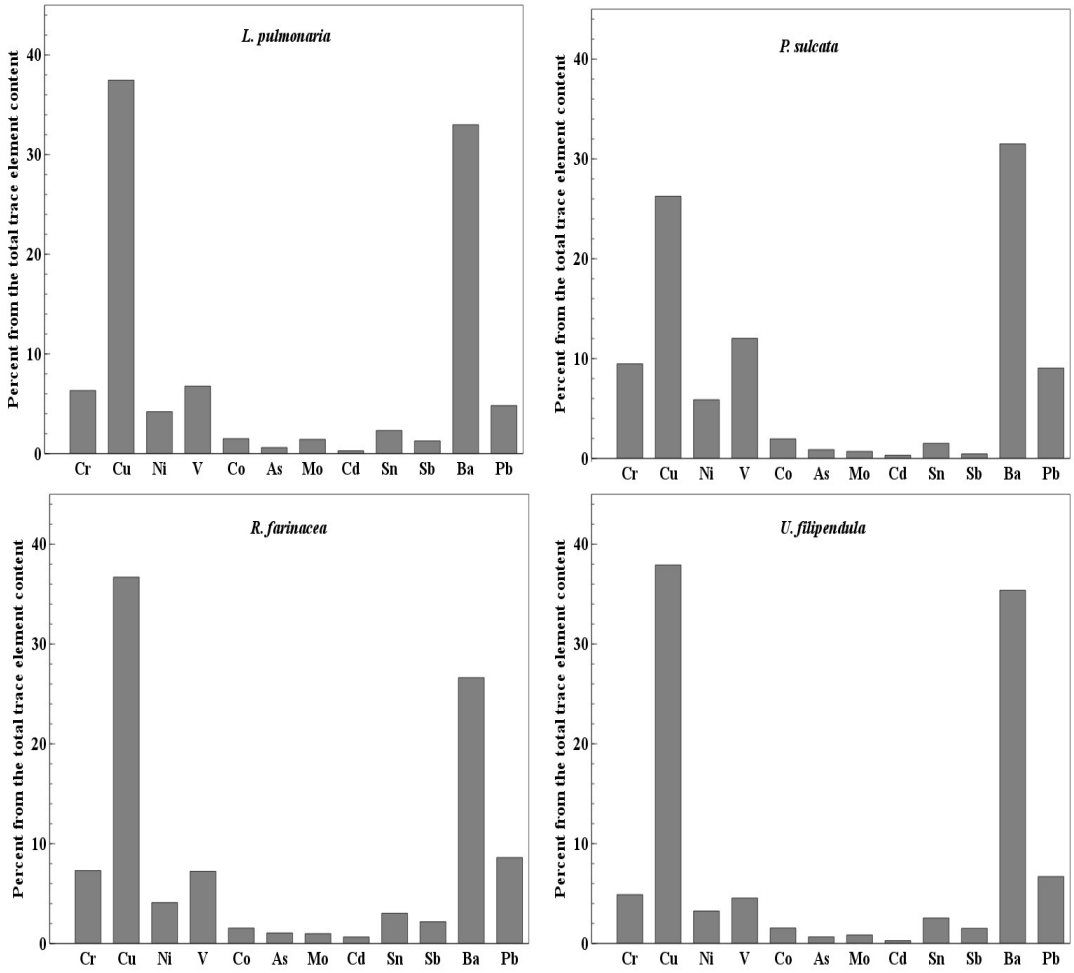

Figure 8. – The distribution patterns of the group of trace elements accumulated by lichen species. Presented as percent (%) from the total trace element content

In order to investigate the extent to which elements accumulated in lichen tissue related, Pearson's correlation coefficients between the element concentrations among the species were calculated and presented in Table 15 (Appx. II). Pearson's correlation coefficients between the elements in the total accumulation content showed differences between the elements as well as between the lichen species. The highest positive ($r = 0.89$ to 0.99) significant correlations ($P<0.001$) were between the elements in the group of Al, Cr, Cu, Fe, Mg, Ni and Zn. The same tendencies were found for the elements within the group of As, V, Co, Mo, Ba and Sn ($r = 0.72$ to 0.99).

Sodium (Na) showed high positive association with heavy metals within the species (r= 0.41 to 0.85). In most cases, this element had strong negative correlation with K (r= -0.51 to -0.86), Cd (r= -0.50 to -0.70), while with P (r= -0.63) for *L. pulmonaria.*

Potassium (K) had positive association with P (r= 0.47 to 0.65), but exhibited from moderate till a very strong negative relationships with the majority of metal elements (r= -0.37 to -0.88), including Pb (r= -0.40) for *P. sulcata*, Sb (r= -0.88) and Ca (r= -0.84) for *L. pulmonaria.* While Ca showed a strong positive correlation with the majority of metals and correlation coefficients varied from 0.37 to 0.92. Only for *U. filipendula* there was a strong positive correlation between Mn and K (r= 0.68) and negative correlations of Mn with the rest of analyzed elements (r= -0.36 to -0.65).

The macronutrient P was lacked strong and high negative correlations with the rest of elements, but showed them for *L. pulmonaria* (r= -0.36 to -0.65) along with moderate positive correlation to Cd (r= 0.45). Whereas Cd had from moderate till strong negative relationship with other metals (r= -0.37 to -0.69). We also found species-specific response with accumulated Sb. This element had strongly positive correlations with the majority of elements (r= 0.51 to 0.86), except of *P. sulcata* where correlation coefficients varied from -0.33 to -0.68.

Element like Pb showed varied positive correlation with metal elements, especially strong they were for *P. sulcata* (r= 0.64 to 0.85). This element also found in close association to Ca (r= 0.40 to 0.70).

Manganese (Mn) characterized a strong positive correlation with the majority of metals (r= 0 .53 to 0.86), while it did not show significance for *L. pulmonaria* and strong negative correlations for *U. filipendula* (r= -0.61to -0.67) along with positive correlation to K (r= 0.68).

Pearson's correlation coefficients between the element concentrations were recalculated in order to investigate the common for all lichen species element relations due to the total elemental content in their tissue after the exposure (Tab.16; Appx. II). The highest

correlations were found inside the group of elements: Al, Cr, Fe and Ni (r= 0.97 to 0.99); Cu, Zn and Ba (r= 0.80 to 0.90); As, V, Co and Ba (r= 0.52 to 0.90). Also, the analysis showed K negatively correlated with Cu, Na, Ca, Sn and Sb (r= -0.34 to -0.63) along with strong positive correlation to P (r= 0.83). Elements P and Ca showed moderate negative relation (r= -0.45) and any other significant correlations with the rest of elements with exception of Ca to Cd (r= 0.77). Regarding correlation, Na was positively correlated with Ba, Mg, Co, Mo (r= 0.34 to 0.48) along with higher values for Sn, Zn and Cu (r= 0.53 to 0.63). We found a high positive correlation between Sb and Sn (r= 0.65) along with Sn to Cu (r= 0.85). For Mn was found high positive relations with Ba, Zn and Mg (r= 0.47-0.48), while Pb had much higher relation to Al, Cr, Mg, Ni and As (r= -0.70 to -0.84).

Elemental concentrations in the lichen species have varied with the distance to the road (Fig. 9 and 10). The impact of the road on the lichen elemental contents was detectable up to a distance of 10-100 m. The distribution of the element in the total accumulated lichen content varied depending on the field condition as well as the species.

From the spatial patterns (Fig. 9), it is possible to see that concentration of the major elements in lichens tissues were much higher close to the road (10 m) and gradually or sharply reduced to the distance of 100 m. Calcium (Ca), Na, K; Al and Fe showed the highest concentrations in the lichens, and were therefore subjected to detailed analysis. Thus, on the change from 10 to 15 m in the vicinity of road Ca reflected a reduction of 20-30%, Na: 20-40%, Al: 25-30% and Fe: 30-45% decreases among the lichens species. Moving further, from 15 to 30 m, resulted in a lower reduction of Ca: 5-15%, Na: 20-30%, Al: 15-25% and Fe: 20-25%. This mean that approximately 25-70% reduction of these element concentrations take place with increasing the distance from the road (from 10-30 m). From 30 to 50 m the amount of elements in lichen tissue did continue to decrease and their amount reduced from 5 up to 15%.

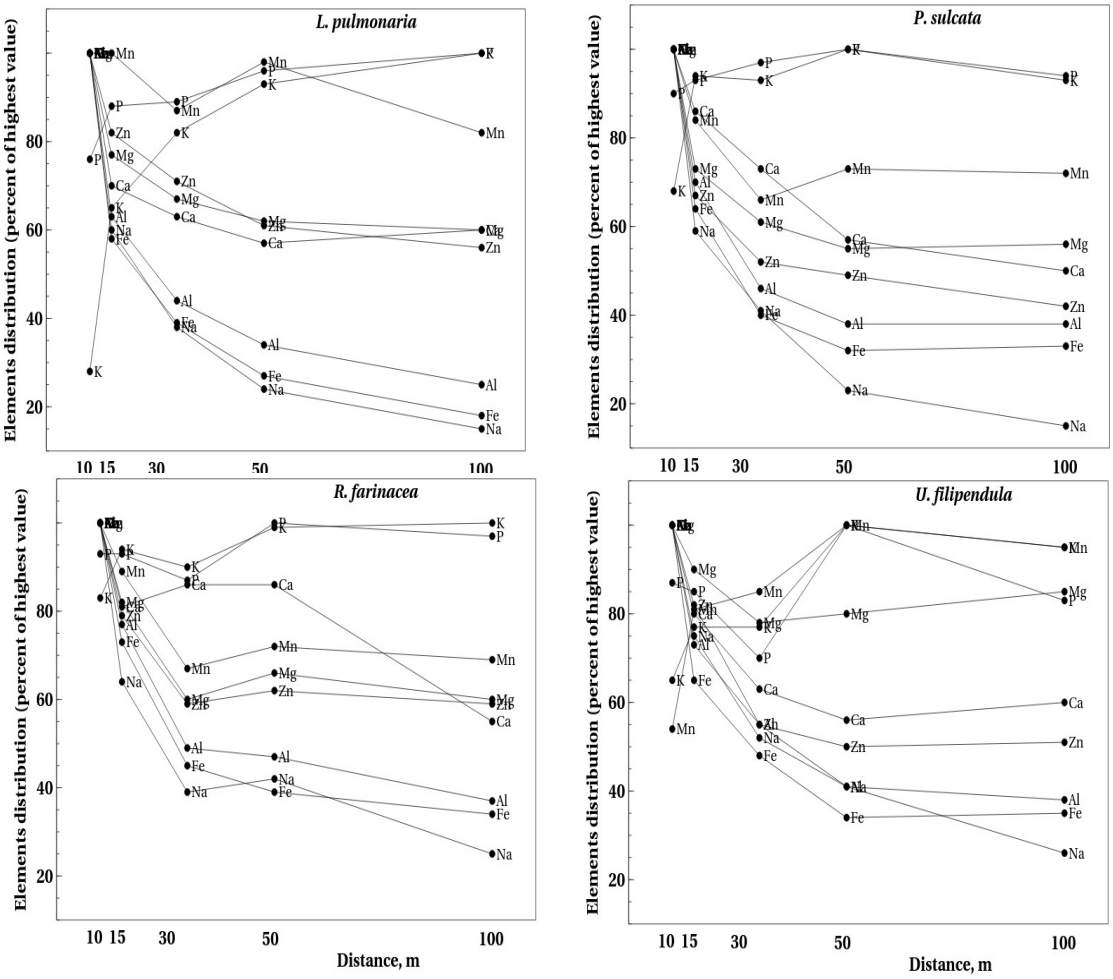

Fig. 9. – Effect of distance (m) from the road on the major elements distribution among the lichen species. Presented as percent (%) of the highest value

The most distant stands, which were located in 100 m from the road, showed hardly any change in the elemental content of lichen tissue up to 0-5% for Al and Fe or the decrease up to 15% for Ca and Na. Therefore, with a distance of 50 m from the road the amount of studied elements has been additionally reduced by 5-15%, whereas with distance of 100 m – 0-15%.

Potassium (K), contrastingly showed a unique pattern of distribution with increasing distance from the road in all lichen species was found. Thus, the amount of K was

lowest close to the road (10 m), and it increases at 15 m by 10-40%, at 30 m by 0-15%, at 50 m by 5-10% and at final distance, 100 m by 0-5%. Within the distance 10-30 m from the road the amount of K in the lichen tissue increased up to 55%.

The concentration of trace elements in the lichen tissue decreased in a majority of cases with the distance (Fig. 10) as was for the previous group of elements.

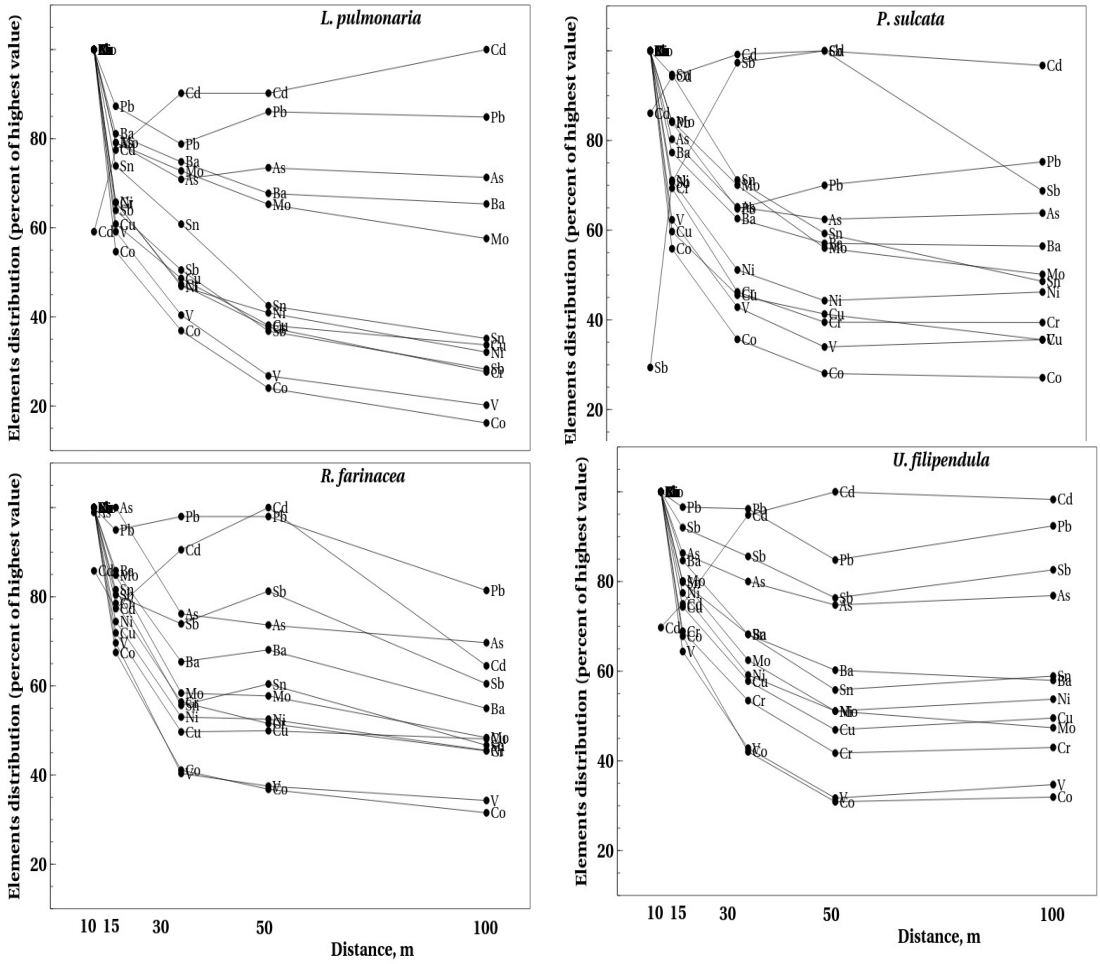

Figure 10. – Effect of distance (m) from the road on the trace elements distribution among the lichen species. Presented as percent (%) of the highest value

Changes with the distance from the road will be described in detail for elements as Cu, Ba, V and Pb. For an increase from 10 to 15 m, the amounts of accumulated Cu decreased by 25 to 40%, Ba: 15-25%, V: 30-40% and Pb: up to 15% in studied lichens

species. The increase in location up to 30 m showed a reduction in the element accumulation by 10-25%, 10-20%, 15-30% and 0-10%, respectively. Thereby, 25-65% of element concentration at 10 m can be lost at 30 m from the road. With increase of distance from 30 to 50 m and from 50 to100 m, the amount of the elements has been continuously did reduced as well. Thus, in a case of Cu it decreased up to 5%, Ba and V–15%, whereas for Pb – showed any changes even at 100 m. Cadmium (Cd), showed contrastingly different pattern of distribution. Thus, with increasing distance from the road increased concentrations of this element in all lichen species with the exception of *R. farinacea*. The amount of Cd was lowest close to the road (10 m), and it increased at 15 and 30 m by 5-20%, at 50 m by 3-5% and at final distance, 100 m by 0-10%. While, for *R. farinacea,* the concentration of Cd was not constant and eventually decreased within the distance 10-100 m by 20%.

The developmental design with lichen transplants in two directions of the road (west and east) and at different distances from the road, allowed us to studying the influence factors as “Distance”, “Side”, “Species” and variety of their interaction on element content in 3-way ANOVA (Tab. 17).

The results showed that factor “Species” had much more significant influence ($P=0.000$) on the most of elemental content in tissue among the lichens than the other factors. Distance showed also a very high significant influence ($P<0.01$ to $P=0.000$) with the exception of Mn and Cd, whereas side of the road and the interactions of factors were much weaker. Thus, factor “Side” showed a significant influence ($P<0.05$ to $P=0.000$) on the distribution of Na, Sb (east side) and K, Pb, Ba, As (west side). The interaction of factors “Side x Distance” was higher in influence ($P<0.01$ to $P=0.000$) than other type of interactions on the differences in the elemental content accumulated by lichens. We found that the common elements that influence “Side x Species” and “Distance x Species” were Mg, K, Al, Ba and Sb. Interaction of factors “Species x Side x Distance” was very weak ($P<0.05$) with an exception of Mg, Zn, Ba, Sn.

Table 17. – The developmental design, elemental content of lichen transplants along with statistic results presented as three-way ANOVA

Var.	Lichen species (Sp)					Distance from the highway (D)						Side (S)	Sp*S	Sp*D	S*D	Sp*S*D	
Element	*Parmelia sulcata*	*Lobaria pulmonaria*	*Ramalina farinacea*	*Usnea filipendula*	*P* (Sp)	10 m	15 m	30 m	50 m	100 m	*P* (D)	*P*	*P*	*P*	*P*	*P*	r^2_{adj}
Ca*	3.31±0.21	2.03±0.11	**6.98±0.78**	3.32±0.20	0.000	**5.15±0.54**	4.12±0.67	3.85±0.63	3.55±0.71	2.87±0.30	0.019	ns	ns	ns	ns	ns	37.5
Mg*	**1.36±0.73**	0.95±0.44	0.61±0.30	1.31±0.44	0.000	**1.40±0.10**	1.12±0.72	0.94±0.63	0.91±0.56	0.92±0.70	0.000	ns	0.000	0.000	0.000	0.007	87.3
Na*	5.21±0.77	4.77±0.68	4.40±0.60	**11.2±0.2**	0.000	**12.1±1.7**	8.01±1.22	5.35±0.91	4.05±0.80	2.53±0.44	0.000	0.000	0.000	ns	0.000	ns	80.4
K*	**4.50±0.13**	**4.39±0.31**	1.36±0.35	1.65±0.65	0.000	1.89±0.21	2.88±0.33	3.10±0.36	**3.51±0.38**	**3.49±0.40**	0.000	0.008	0.000	0.000	ns	ns	94.6
P*	**1.91±0.07**	1.65±0.04	0.46±0.01	0.54±0.02	0.000	1.05±0.12	1.21±0.14	1.11±0.15	1.23±0.15	1.19±0.16	0.029	ns	0.000	ns	ns	ns	91.3
Fe*	**2.88±0.29**	1.54±0.19	1.18±0.11	1.32±0.13	0.000	**3.22±0.33**	2.07±0.19	1.36±0.11	1.05±.09	0.95±0.13	0.000	ns	ns	0.000	0.000	ns	84.1
Al*	0.96±0.10	**2.07±0.17**	0.88±0.07	1.03±0.08	0.000	**2.11±0.20**	1.48±0.13	1.01±0.08	0.83±0.07	0.74±0.09	0.000	ns	0.009	0.000	0.008	ns	87.2
Mn	85.5±4.3	80.1±3.8	39.0±1.8	**246±14**	0.000	108±10	115±18	108±19	124±22	116±22	ns	ns	ns	0.000	ns	ns	83
Zn	87.4±6.0	100±4	64.5±3.3	**141±9**	0.000	**143±10**	112±8	84.3±5.2	78.2±4.5	73.9±6.2	0.000	ns	ns	0.000	ns	0.018	86.5
Cu	16.0±1.4	18.7±1.7	13.4±0.9	**23.3±1.6**	0.000	**29.5±7.8**	19.6±1.2	15.0±1.0	12.9±0.7	12.2±1.2	0.000	ns	ns	ns	0.007	ns	76.4
Ba	19.2±1.0	16.5±0.7	9.7±0.5	**21.8±1.1**	0.000	**22.7±1.6**	18.5±1.2	15.3±1.0	14.1±0.8	13.3±1.0	0.000	0.015	0.003	0.023	ns	0.005	83.4
V	**7.33±0.68**	3.38±0.41	2.63±0.26	2.79±0.28	0.000	**7.49±0.83**	4.73±0.48	3.14±0.34	2.43±0.27	2.38±0.34	0.000	ns	ns	0.000	0.000	ns	89.1
Pb	**5.52±0.24**	2.40±0.09	3.13±0.16	4.12±0.13	0.000	4.36±0.38	3.92±0.32	3.54±0.25	3.56±0.23	3.59±0.29	0.001	0.001	ns	0.029	ns	ns	72.8
Cr	**5.77±0.48**	3.16±0.32	2.66±0.19	3.01±0.24	0.000	**6.09±0.56**	4.26±0.35	3.02±0.20	2.53±0.18	2.34±0.25	0.000	ns	ns	0.000	0.000	ns	83.9
Ni	**3.58±0.25**	2.10±0.19	1.49±0.11	2.00±0.13	0.000	**3.65±0.32**	2.62±0.21	1.90±0.14	1.68±0.12	1.61±0.16	0.000	ns	ns	0.000	0.000	ns	85.7
Sn	0.92±0.06	1.16±0.09	1.10±0.07	**1.56±0.08**	0.000	**1.71±0.10**	1.39±0.06	1.10±0.07	0.92±0.05	0.82±0.07	0.000	ns	0.017	ns	ns	0.015	76.6
Co	**1.19±0.13**	0.75±0.13	0.56±0.06	0.95±0.10	0.000	**1.69±0.14**	1.02±0.06	0.65±0.04	0.49±0.03	0.45±0.06	0.000	ns	ns	0.000	0.000	ns	84.5
Sb	0.27±0.02	0.63±0.06	0.79±0.06	**0.93±0.06**	0.000	**0.83±0.10**	0.69±0.06	0.65±0.05	0.60±0.07	0.52±0.08	0.001	0.016	0.027	0.001	ns	ns	60
Mo	0.43±0.02	**0.72±0.03**	0.36±0.02	0.52±0.03	0.000	**0.71±0.04**	0.58±0.03	0.47±0.04	0.41±0.03	0.37±0.03	0.000	ns	0.000	ns	ns	ns	82.6
As	**0.53±0.02**	0.30±0.01	0.39±0.02	0.40±0.01	0.000	**0.51±0.03**	0.44±0.02	0.37±0.02	0.36±0.01	0.35±0.02	0.000	0.019	ns	0.010	0.012	ns	76.2
Cd	0.19±0.01	0.14±0.01	**0.24±0.02**	0.17±0.01	0.000	0.16±0.01	0.17±0.01	0.20±0.02	0.21±0.02	0.18±0.01	ns	ns	ns	ns	ns	ns	21.7

Note. Contents of analyzed elements in each of the four studied lichen species (n=30; all distances combined), and at each of five distances from the highway (n=24; all species combined). The highest value in each group is marked in bold. Elements marked with * are given in mg g^{-1}, remaining elements in µg g^{-1}. From Fe and downwards, elements are ranked after decreasing total concentration. *P*-values are given according to a 3-way ANOVA with species (Sp), distance from road (D))

Additionally, the influence of "Distance", "Side" and their interaction on element content was investigated among the lichen species (Tab.18; Appx. II). The results showed that "Distance" had much more significant influence on the concentration of elements among the species than the side of the road (P<0.01 to P=0.000) with the exception of phosphorus (P). The influence of "Side" was not permanently significant across elements and showed only high significant influence (P=0.000) on the distribution of Na with higher values on the east side. Also, "Side" significantly influenced the accumulation of Pb (P=0.000), As and Cd (P<0.001) in *U. filipendula*, and Ba (P<0.01) in *P. sulcata* and *R. farinacea* with highest values on the west side. The interaction of factors was generally weak with an exception of Na (P=0.000) for *L. pulmonaria, P. sulcata* and *R. farinacea*, along with Cu (P=0.000), V (P<0.001) and Ni (P<0.01) for *P. sulcata.* There were found some species-specific differences in the element accumulation. Thus, *P. sulcata* showed more significant influence of the factor interaction due to Zn, Mg and Ca accumulation, whereas *U. filipendula* – detected more side dependence, specially for Ca, Mg and K (west side).

The comparison of lichen species due to the total elemental accumulation capacity showed as box plots (F= 4.23; df=116; P<0.01) (Fig. 11).

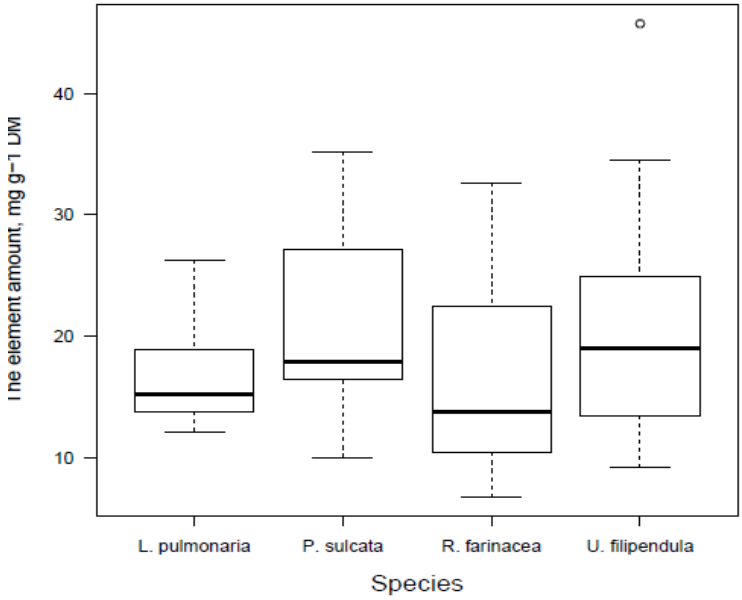

Figure 11. – Box plots of total element accumulation between the lichen species

The picture of elemental deposition by transplanted lichens in a vicinity of the road demonstrates that the total element amount (mg g^{-1} DM) ranges over species and shows significant variation. Thus, according to the simple linear regression analysis, *U. filipendula* (F= 2.92; P= 0.004) and *P. sulcata* (F= 2.51; P<0.01) showed significant increase in the total element accumulation content compare to *L. pulmonaria*, whereas *R. farinacea* showed any significance value (F= 0.36; P= 0.71).

Lichens were closely evaluated due to factor Stand by comparison the values of the total element accumulation (Fig. 12; Appx. II). The simple linear regression analysis showed significant differences (P<0.01 to P=0.000) between of stands located at 10 and 15 m from the road (1, 6, 7, 26, 11, 2, 12, 21, 3 and 16) with those located further away.

2.4. Discussion

Lichens can accumulate heavy metals and other elements of environmental concern to high levels that led to their widespread use as biomonitors (Garty 2001; Carreras, et al. 2005; Purvis & Pawlik-Skowronska 2008; Guttova et al. 2011). Yet, the mechanisms of metal accumulation in lichens and their tolerance to heavy metal are still not well understood (Hall 2002; Hauck 2008; Hauck et al. 2009).

Our study showed Ca and Na represented up to 44-54%, while macronutrients P and K – up to 11-26% of the total accumulated elemental pool of harvested lichens. There were found species specific features such as *L. pulmonaria* exceeded in accumulation of Mo and Ni, *P. sulcata* – V, Co, Pb, As and Mn, *R. farinacea* – Ca and Ni, while *U. filipendula* – Na, Zn, Cu, Al, Fe, Ba, Sn and Sb.

Ion of Ca^{2+} mentioned as a messenger in plant signal transduction pathways and usually its concentration increases in response to the stimuli including stress signals (Hepler 2005; Tuteja & Sopory 2008). However, Ca is often naturally deposited as Ca-oxalate in lichen cortices in Ca-rich environments (Prieto et al. 2000). Sarret et al.

(1998) pointed that lichens secondary components and oxalates form insoluble salts with Ca and with metal ions in the process of immobilization of metals. The formation of calcium-containing structures on the surface of the lichen *Ramalina lacera* was found *in situ* by Garty et al. (2002) to be a response to air pollution. Experiments conducted in the vicinity of roads showed that high concentration of Ca in lichen might result from road dust suspension due to calcite (Amato et al. 2001; Van Dobben et al. 2001) or come along with deicing salt that contain about 1% of $CaCl_2$ (Viskari et al. 1997). According to Monaci et al. (2000), positive correlation between Ca and metals indicates that air pollution caused by car traffic is one possible source of this elements.

Potassium (K) is an important element for membrane permeability in plants. Accordingly to Garty et al. 1998; Haffner et al. 2001 and Marques et al. (2005), the concentration of K in thalli correlated inversely with the electric conductivity of lichen, that suggested the leakage (loss) of this element as a result of air pollutants.

The increases of Sodium (Na) among lichen transplants along the road can be most likely explained by the use of deicing salt (mainly NaCl) as winter maintenance, in agreement with Viskari et al. (1997) and Blomquist (2001).

By using the interpretative scale of Frati et al. (2005), we determined the substantial increase in concentrations compare to background values for Na, Fe, Al, Ni, Cr, V, Co, Mo, As, Sn and Sb (the EC class "severe accumulation") in all lichen species. According to the results, increased values were recorded for Pb, Ba (the EC class "accumulation"), P, Cd and Mg were moderately changed (the EC class "normal"). The EC class "normal" comprises Ca for *L. pulmonaria* and *P. sulcata*, while "accumulation" and "severe accumulation" for *U. filipendula* and *R. farinacea* respectively. Concentration of K decreased in a case of *L. pulmonaria* and *U. filipendula* and was determined as "severe loss", while class "normal" for *P. sulcata* and *R. farinacea*. Class for Cu and Zn changed from "severe accumulation" to "normal" depending how close lichens located to the road. Since the cations Ca^{2+}, K^{+} Mg^{2+} and Na^{+} are found responsible for the interior ions balance and metal

homeostasis in photosynthetic organisms (Chow et al. 1990 and Essa 2002), a significant increase or decrease of these nutrients may indicate abiotic stress.

Trace metals that play significant roles in plants and were accumulated by studied lichen transplants are presented in Table 19.

Table 19. – The comparison of element concentrations among the natural objects and analysed lichen transplants from the field survey, data in μg g^{-1} DM

Element	Biologically relevant oxidation states	Lithosphere	Typical plant	Lichens	
				background value	after transplantation
Cu	Cu^{+}, Cu^{2+}	50	6	3 – 10	9.5 – 61.3
Fe	Fe^{2+}, Fe^{3+}	45 000	100	78 – 1 524	1 181 – 2 883
Mn	Mn^{2+}, Mn^{3+}, Mn^{4+}	950	50	22 – 705	39 – 246
Mo	Mo^{4+}, Mo^{6+}	1.5	0.1	0.1 – 0.4	0.4 – 0.7
Ni	Ni^{2+}	80	0.1	0.3 – 2.3	1.5 – 3.6
Zn	Zn^{2+}	75	20	28 – 86	64 – 141

Note. Some information adopted from Palmer & Guerinot (2009)

The number of trace elements we found in the lichen accumulated content are known to have no function in plant nutrition, instead they are highly poisonous and according to Monaci et al. (2000), Ozaki et al. (2004) and Amato et al. (2011) are typical pollutants by traffic.

Sources of heavy metals in roadside environment include traffic deposition (tire wear, brake linings leakage of oil and lubricants), dust from road pavement wear, maintenance operations on road (application of deicers), pesticides and herbicides application due to farming (Table 20).

Table 20. – Primary sources of heavy metals in roadside environment that were biomonitored with high plants, mosses and lichens

Element	Source	Reference
Aluminum (Al)	indicates on presence of industry, road dust	Tasic et al. 2004
Antimony (Sb)	used in many parts of a vehicle	Tasic et al. 2004
Arsenic (As)	emitted during combustion of fossil fuels	Tasic et al. 2004
	accumulated by soils from agro chemicals	Brus et al. 2002
Barium (Ba)	common component of combustion of heavy fuel	Monaci et al. 2000;
Copper (Cu)	related to vehicle circulation or local industry,	Tasic et al. 2004;
	results from use of diesel fuel	Garty 2001
Lead (Pb)	emitted from diesel and lubricant car oils	Garty et al. 2001;
		Jozic et al. 2009
Vanadium (V)	regarded as good tracer of fossil fuel combustion	Minganti et al. 2003
Nickel (Ni)	regarded as good tracer of fossil fuel combustion	Minganti et al. 2003
Cobalt (Co)	common component of heavy fuel combustion	Tasic et al. 2004
Chromium (Cr)	corrosion of welded metal plating	Tasic et al. 2004
Cadmium (Cd)	tire wear, brake pads, combustion of oils	Tasic et al. 2004
	accumulated by soils from agro chemicals	Brus et al. 2002
Iron (Fe)	related to traffic emission, corrosion of vehicles	Tasic et al. 2004
Zinc (Zn)	tire wear, grease, abrasion of metal parts of vehicles	Tasic et al. 2004
	tracer of unleaded fuel vs diesel powered vehicles	

We found among the species highly significant positive association of Na with heavy metals (Cu, Zn, Ba, Sn and Co), while negative with K. Additionally, the results showed that Na had a species-specific relationship between different heavy metals accumulated within the lichen tissue. Thus, there were found highly significant relations between Na with: Sb (r= 0.77) for *L. pulmonaria*; Sn (r= 0.76) for *P. sulcata*; Mg, Zn, Al, Ba (r= 0.77 to 0.87) for *R. farinacea* and Mo (r= 0.63) for *U. filipendula.* These findings are support our hypothesis about high impact of deicing salt on the viability of lichens.

Norrström (2005) pointed that corrosion of vehicles is a main source of heavy metal pollution (Zn, Co, Cu, Cr, Fe and Ni) and the high concentration of deicing salt is one of the most important factor causing it. According to Tasic et al. (2007), high and positive correlation coefficients indicate that elements either come from the same sources either have a strong interrelationship. During the study in a bulk of lichen chemical content we found high correlations inside the group of Ni, V and As (related to fuel combustion), Cr, Cu, Zn and Pb (associated with diesel engines and wearing of brakes), Ca, Al and Fe (originated from road and soil dust). That suggesting that elements accumulated by lichen tissue probably related to road dust (soil with trafic related particles) and exhaust emissions like oil burning.

The results of elemental contents of transplanted lichens showed that the highest values of heavy metals and Na were detected at 10 m from the road. Elements from the lichen accumulation content that showed the maximum value of variation responded on distance from the road by changing their concentration much faster than those with the minimum. Thus, our results showed that 30 m from the road was sufficient to reduce by 60-70% of Al, Fe, Ca, Na, Ba and V content in lichens tissue, while by 5-20% of Pb. Cadmium (Cd) found to be independent of distance from the road, indicating the other source of originating (Jozic et al. 2009), similar as Mn that partly associated with farming (fertilizers and manures) (De Vries et al. 2002). Instead, concentration of K was the lowest close to the road and increased with distance. Garty et al. (2000) showed that lowering K content of thalli provides additional evidence of injury caused to cell membranes in lichens. Our findings confirmed that exceeded concentrations of Al, Fe, Na, Co, Mo, Sb, Ba, V and Pb in the lichen tissues were found even at final point of transplantation, 100 m from the road. It agrees with Klos et al. (2009), where pollutants from highway was observed in lichens over the distance of 50-150 m.

The effect of sampling direction from the road significantly influenced on the accumulation capacity of lichen species. Thus, were found that concentrations of K, Pb, Ba and As Mg, Pb, As were higher in tissue by the road in the west side, compared to Na and Sb – in the east side. During the lichens exposure period were observed

southeast and southwest directions of winds, therefor we can not account significantly of it influence on the side distribution of emitted pollutants. Naeth & Wilkinson (2008) showed the effect of side on the lichen transplants varied depending on species and elements. They pointed that such distribution of pollutants could result from the physical similarities between the particles (state of aggregation, size, mass) characterize the physical and chemical nature of particles.

Were found that morphological and structural differences play a role in the process of accumulation of airborne elements. The results demonstrated that the accumulation capacity of lichen species collected at the same distances from the road was significantly different and increases in the following order: *R. farinacea* < *L. pulmonaria* <*U. filipendula* <*P. sulcata.* It is evidently that the most efficient in the total element amount were *U. filipendula* and *P. sulcata* – 20.9 mg g^{-1} and 21.2 mg g^{-1} compare to *R. farinacea* – 16 mg g^{-1} on average per stand.

The field experiment confirmed the ability of lichens for use in biomonitoring programs for detection and accumulation the atmospheric deposition. On the basis of the collected results it is possible to conclude that after six months of exposition in the vicinity of E6 road the considerable effect upon of the elemental content of the lichen transplants was assessed. The data of statistical analyses were highly significant, suggesting that changes in the lichen chemistry were mostly attributable to airborne pollution from vehicles and the road maintenance.

Part II. Paper II. How does accumulation of road pollutants affect lichen growth and viability?

3.1. Introduction

Nieboer & Richardson (1981), indicated that elements can be classified into three groups relative to their toxicity in lichens: a) K^+, Ca^{2+}, and Sr^{2+} are not toxic; b) Ag^+, Hg^+, Cu^+ tend to bind with N- and S-containing molecules, and are extremely toxic even at low levels; and c) Zn^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+} are intermediate, borderline ions. Furthermore, Schützendübel & Polle (2002) mentioned that heavy metals can be divided into two groups: redox active (Fe, Cu, Cr, Co) and redox inactive (Cd, Zn, Ni, Al, etc). Redox active metals participate in ROS formation and produce Fenton-type reactions with further plant cellular injury. Exposure of cell to redox inactive group results in oxidative stress through indirect mechanisms such as interaction with the antioxidant defense system, disruption of the electron transport chain, or induction of lipid peroxidation.

According to metal ions chemical and physical properties, three different molecular mechanisms of metal toxicity can be distinguished in lichens: a) blocking of essential functional groups in biomolecules, b) modification of essential biomolecules, and c) displacement of essential metal ions from biomolecules. For example, many enzymes contain metals in positions important for their activity. The displacement of one metal by another will normally lead to inhibition or loss of enzyme activities. Thus, Van Assche & Clijsters (1986) found the loss of ribulose-1,5- bisphosphate-carboxylase /oxygenase (RuBisCO) activity when cations like Co^{2+}, Ni^{2+}, and Zn^{2+} displace Mg^{2+} in that enzyme. Similarly, displacement of Ca^{2+} by Cd^{2+} in calmodulin, an important protein in plant cellular signaling, led to the inhibition of calmodulin-dependent phosphodiesterase activity (Rivetta et al. 1997).

Lichens generally, are exposed to a complex mixture of air pollutants making it difficult to identify the component(s) responsible for lichen damage (Caňas et al.

1997). Therefore the detecting of the metal speciation in biological systems remains challenging, and unprecedented in the case of lichens (Sarret et al. 1998). Nowadays the interactions between lichens and heavy metals at different levels are often reviewed. Thus, mechanisms of heavy metal accumulation and detoxification by lichens are discussed by Baranowska-Bosiacka et al. 2001; Garty 2001 and Backor & Loppi 2009. Regarding to Pawlik-Skowronska & Backor (2011), lichens have a variety of mechanisms to resist metallic pollution. The latest studies show that lichen substances might play a role in the metal homeostasis in lichens (Hauck 2007; Bialonska & Dayan 2005).

A common consequence of heavy metal toxicity is usually the direct effect on the photosynthetic apparatus and membrane permeability status or through the induced deficiency of an essential nutrients. The first type of impact part is the most studied in lichens. According to Garty et al. (1985, 1993 and 2001), degradation of chlorophyll in the symbiotic photobiont is one of the most obvious signs that damage has occurred in lichens. A direct relation between the chlorophyll content of the lichen species and the sensitivity to air pollution was demonstrated by Beltman et al. 1980. A decrease in the chlorophyll *a/b* ratio of with increasing content of Cu in lichen was showed by Chettri et al. (1998). Hauck & Paul (2005) showed that excess Mn reduced chlorophyll concentrations, chlorophyll fluorescence and degraded the chloroplast in *H. physodes* photobionts. Exposure to As mainly inhibited the quantum yield for primary photochemistry, density of reaction centers and photosynthesis performance index, and increased the dissipated energy (Wang et al. 2012).

Pollutants upset the physiological balance between the fungus and the alga or cyanobacterium, and degradation or destruction of the lichens results as pointed Brown & Beckett (1984). Di Toppi et al. (2005) suggest that among the two bionts, the algal partner appears was more susceptible to Cd stress because of the presence of delicate and sensitive components such as the chloroplast and photosynthetic pigments, whereas, Hyung-Shim et al. (2004) observed increased total chlorophyll content and the absence of any changes in the algal cell proportions of weakly polluted samples. This

indicates that the photobionts possessed higher chlorophyll contents per unit volume of the photobiont at somewhat polluted sites and that lichens have altered their storage allocation in different cellular compartments. This may be a result of symbiotic readjustment(s) between the photobiont and the mycobiont. Further studies revealed that the sensibility of lichens to pollution relates to the sensibility of the mycobionts and photobionts in the symbiosis in the particular lichen species as well (Gaio-Oliveira et al. 2005; Bačkor 2009, 2010)

The relationship between morphology and metal toxicity is still poorly understood. Nevertheless, metals can be located in different parts of lichen thalli. Thus, the content of elements of limited metabolic significance (Al, Cd, Pb) were higher in the central thallus parts, trapped in the medulla, while elements essential for metabolism (Co, Cu, Mo, Zn) were concentrated in peripheral thallus regions that are most active metabolically (Loppi et al. 1997). Additionally, Baranowska-Bosiacka et al. (2001) found that Pb and Cd remained mainly on the surface of the lichen thalli and in the cell walls, while Zn and Cu penetrated to the protoplast.

Some lichen species can react on induced pollution state by changing morphological parameters and color of thalli. Thus, Pearson & Skye (1965) mentioned that *P. sulcata*, shown morphological and photosynthetic abnormalities in polluted areas, changes happen in thallus lobes that frequently become pink. Similarly, Sigal & Taylor (1979) reported that is particular species become pigmented and turns reddish violet in lobe margins when were fumigated with peroxy-chemicals. Goyal & Seaward (1982) found by using experiments that the terricolous cyanolichens *Peltigera canina* and *Peltigera rufescens* responded to induced metal pollution (Cu, Fe, Mn, Ni, Pb and Zn) by reducing thallus size, rhizine length and darkening of their color. On the other hand, thalli from metalpolluted sites showed denser rhizinae, more profusely branched, network-forming veins, hypertrophic medulla and increased bleaching of thalli. Thallus bleaching, as a visible sign of pollution damage, was demonstrated in *Ramalina duriaei* by Garty et al. (1993) due to exposition of lichens at different pollution sites. Decreases in growth in lichen photobionts after Cu

exposure have been reported by Bačkor & Váczi (2002). Beside, Mikhailova (2007) found that Cu pollution caused growth abnormalities, distortion of apothecia, and colour changes in thalli of *Tuckermanopsis sepincola* and decreased production of soredia in *Hypogymnia physodes.* Whereas, visible morphological changes do not always support the measure of judge about the physiological status of photosynthetic apparatus. As an instance, water plant *Elodea canadensis* from a polluted watercourse looked quite healthy, but showed any net photosynthesis. Along with breakdown of photosynthesis the heavy metal chlorophyll being formed, some of which are much more stable towards irradiance than natural Mg-chlorophyll. Consequently, plants remain green even when they are dead (Küpper et al. 1996).

Tomaševic et al. (2005), by using a scanning electron microscope with an energy dispersive X-ray spectroscopy, examined the size, distribution, morphology and chemical composition of particles on the surfaces of leaf discs of deciduous trees. The majority of observed particles belonged to a class of fine particles (D<2 μm) and chemical their composition indicated that trace metals (Pb, Zn, Ni, V, Cd, As, Cu) are found on the leaf deposits. Approximately, 10-15% the leaf surface was covered with metal deposited particles with density from 5 000 to 20 000 mm^{-2}. At the same time Tasic et al. (2007) observed that different categories of atmospheric depositions form on the leaf surfaces in urban area: particles of natural sources (soil dust, pollen, bacteria, fungal spores) and metal-rich particles from anthropogenic sources, which mostly emitted due to combustion processes from cars. They identified individual particles as well as agglomerates which contained aluminosilicates (Al, Si, Fe, Ca), oxides of Al, Zn, Cu, Ni, Pb, Ti and particles with minor constituents of Fe, Mg, Ca, Ba, Pb, Zn, Ni, Cu.

The majority of presented results above results show how air-related pollution alters the viability of lichens. Because the density of road traffic is increasing, more research should be focused on the effects of pollutants on roadside ecosystems, in addition to conducting regular biomonitoring of pollutant, specially by lichens.

The study aims to investigate the impact of traffic-related pollutants on lichen viability parameters and visible damage at selected monitoring sites along the highway E6 in Akershus fylke, Norway.

Specific questions:

– how do viability parameters of lichens respond air pollution emissions?

– what is the most sensitive parameter of lichen viability?

– which pollution components key influence lichen physiology?

– which factors influence the lichen viability parameters in the field?

– which species are most sensitive to traffic-related pollution exposure?

– how important are visible signs of damage (discoloration and changing color of tissues, availability of deposits) in the process of estimating lichen viability due to air pollution?

3.2. Methods

3.2.1. Study area

The field survey location and lichens in transects installation were for the Paper II the same as for previous study and performed at Støkken, in Ås commune, Akershus fylke, south-eastern part of Norway (59°64'N and 10°74'E; 100-150 m.a.s.l).

3.2.2. Study species

For this study were used four epiphytic species of lichens as *Lobaria pulmonaria*, *Parmelia sulcata*, *Ramalina farinacea* and *Usnea filipendula* which are in use for biomonitoring studies. The details of their ecological, morphological and physiological characteristics were described in Paper I.

3.2.3. Laboratory analysis

After the transplantation period along the highway, nets with air dry lichen thalli were carefully collected from the field stands and temporary stored at room temperature in darkness until they were removed from the nets and put into paper envelopes.

The control samples had been kept air dry in the freezer since the transplantation period started. Freezing is the recommended way for long-term storage of lichen thalli for later physiological experiments (Honegger 2003).

In order to understand how environmental factors influence internal responses of the lichens in the experiment, growth (dry mass); size (area); chlorophyll fluorescence and pigment content; elemental analysis and visible damage were quantified.

Dry mass. The dry mass (DM) of all thalli was obtained by weighing air dried samples (±0.1 mg) before and after the field experiment. Four additional thalli of each lichen species were used as a controls. The dry matter growth (DM growth) was calculated in percentage by using the formula: DM growth = 100 x ($M_{end} - M_{start}$)/ M_{start}, where M_{start} - initial thallus dry mass (g), M_{end} – thallus dry mass after the harvest (g).

Thallus area. Prior to field transplantation, each thallus was placed on wet filter paper and sprayed with deionized water to revive full photosynthetic activity at low light (10 μmol photons m^{-2} s^{-1}) for 24 hours. The surface area of lichens was measured by using a leaf area meter LI-3100 Area Meter (LI-COR Inc., Lincoln, NE, USA).

Specific thallus mass (STM). Based on mass and area measurements, specific thallus mass change was computed as $STM_{start} = DM_{start}/Area_{start}$ (g cm^{-2}), (%).

Chlorophyll fluorescence (Fv/Fm). Lichens thalli after the field experiment were placed on plastic nets in the close contact with paper hydrated with deionized water on the bottom of plastic containers (26 x 56 x 6 cm). The top of containers was covered with cling film to get a high RH. This was done to hydrate the lichens with a minimum risk that mineral elements might leach from the thalli as they can do if they are directly sprayed with water. The thalli were left for 24 hours under 10 μmol photons $m^{-2}s^{-1}$ before the chlorophyll fluorescence measurement. Such conditions brought lichens to an active photosynthetic status. Maximal photosystem II activity (PSII) was measured after 15 min dark adaptation with a portable, modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany).

Chlorophyll determination. Chlorophyll content was quantified in ten thalli as the start values (Fv/Fm_{start}) and all transplanted thalli after the harvest (Fv/Fm_{end}) for each lichen species. The samples were powdered by grinding in a mill with Teflon balls (Precellys 24-Dual tissue homogenizer, Bertin Technologies, France) to produce

subsamples. Grinded material (4-10 mg) was placed into Eppendorf tubes and extracted in 1,5 ml $MgCO_3$ -saturated dimethyl sulfoxide (DMSO). The Eppendorf tubes were placed under low light in a water bath (60 ^{0}C) for 40 min for chlorophyll extraction (Palmqvist & Sundberg 2002). After an incubation period, the Eppendorf tubes were centrifuged by Hettich Universal 16 centrifuge (Germany) at 12 900 rpm for 2 min. The supernatant was transferred into 1x1 cm disposable cuvette and the absorbance was measured with Shimadzu UV-2101PC spectrophotometer at 649, 665 and 750 nm. All absorbance values were corrected against the absorbance at 750 nm. Chlorophyll *a, b* (Chl *a,* Chl *b*) concentrations were calculated according to equations (Wellburn 1994) and quantified as chlorophyll contents per thallus area (μg Chl·cm^{-2}) using individual thallus mass:

Chl *a* (microgramms/ml) $= (12.19*(A_{665} - A_{750}) - 3.45 * (A_{649} - A_{750})) * V_{total} / V_{sample}$

Chl *b* (microgramms/ml) $= (21.99 * (A_{649} - A_{750}) - 5.32 * (A_{665} - A_{750})) * V_{total} / V_{sample}$

Visible damage. All lichen thalli were inspected and photographed by Pentax *ist DL Lens: Sigma 70/2.8 DG Macro. Photos of each specimen were taken while hydrated at the start and the end of experiment in order document visible changes.
The visible color change as damage or bleaching (chlorophyll degradation) and black areas (unknown damage) were quantified for each sample of *L. pulmonaria* as the number of pixels that varied in color and presented as a percent of total thallus area. For this purpose was used the GNU Image Manipulation Program (GIMP).

3.2.4 Statistic

For the statistical analysis data distribution were examined with the application of the normality test. The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics. Graphics were performed by using Veusz plotting and graphing package. All statistical analyses were performed using the statistical package R (version 2.15.1., R Development Core Team 2012).

A Pearson correlation tests were applied to obtain correlation coefficients between physiological parameters inside the lichen species as well as correlation coefficients

for the elemental content and physiological parameters of the lichen species.
Three way ANOVA and two-way ANOVA were performed to check if the any significant differences between the changes of physiological parameters in lichens and performance of the factors “Distance”, “Side”, “Species” and their interactions.
The single linear regression between the lichen parameters and the visible level of damage was performed to find significant relations. Single linear regression was used for the comparison of visible damage in *L. pulmonaria* specimens at different field locations and the distribution was illustrated as Box-plots.

3.3. Results

On the base of physiological parameters (Tab. 21-28**;** Appx. II), were calculated Pearson correlation coefficients between tested parameters of the lichen species after period exposure (Table 29).

Table 29. – Pearson correlation coefficients between the main physiological parameters among the lichen species (df=118; n= 120)

Variable	Fv/Fm_{end}	$Chl\ (a+b)_{end}$	$Chl\ a/b_{end}$
	L. pulmonaria		
Fv/Fm_{end}	*		
$Chl\ (a+b)_{end}$	**0.44**	*	
$Chl\ a/b_{end}$	**0.73**	**0.59**	*
DM growth	**0.6**	**0.5**	**0.62**
	P. sulcata		
Fv/Fm_{end}	*		
$Chl\ (a+b)_{end}$	n.s.	*	
$Chl\ a/b_{end}$	n.s.	n.s.	*
DM growth	n.s.	n.s.	n.s.
	R. farinacea		
Fv/Fm_{end}	*		
$Chl\ (a+b)_{end}$	n.s.	*	
$Chl\ a/b_{end}$	n.s.	**0.33**	*
DM growth	n.s.	0.21	n.s.
	U. filipendula		
Fv/Fm_{end}	*		
$Chl\ (a+b)_{end}$	n.s.	*	
$Chl\ a/b_{end}$	n.s.	**0.3**	*
DM growth	n.s.	0.27	**0.3**

Note. Corr. coefficients significance is marked in bold numbers <0.001, normal <0.01 and n.s. >0.05

According to the results, the parameter Fv/Fm $_{end}$ positively correlated with most studied physiological parameters (Chl (a+b)$_{end}$, Chl *a/b* $_{end}$ and DM growth), but just in a case of *L. pulmonaria*. Thus, correlation coefficients of values were accordingly: 0.44, 0.73 and 0.60 with a significance level from P<0.001 to P=0.000. In turn, DM growth positive increases the two explanatory variables (Chl (a+b)$_{end}$ and Chl *a/b* $_{end}$) of *R. farinacea* and *U. filipendula* (r= 0.21 to 0.30, P<0.01 to P<0.001) as well as positive correlations coefficients accounted between Chl (a+b)$_{end}$ and Chl *a/b* $_{end}$ (r= 0.30 to 0.33, P<0.001). Studied physiological parameters of *P. sulcata* showed any evidence of association.

The results of Table 30 showed factor "Species" had highest influence (P=0.000) on all studied physiological parameters of lichens. The factor "Distance" found as highly significant (P=0.000) for photosynthetic status of lichens (Fv/Fm $_{end}$, Chl *a/b* $_{end,}$ Chl (*a*+*b*)$_{end}$)), in turn, factor "Side" showed highly significant influence (up to P= 0.000) on the parameter responsible for growth of lichen (DM growth). The DM growth was higher influenced (P=0.000) by the interaction of "Species x Side", while Fv/Fm $_{end,}$ and Chl *a/b* $_{end}$ by "Species x Distance". There were found a weak influence (P<0.05) of "Species x Distance x Side" and "Species x Distance" interactions on DM growth only.

On the basis of previous results possible to confirm that lichens possessed a high influence of species specific features that changed due to field conditions, therefore it need further investigation. According to the data in Table 31, factor "Distance" had a high significant influence (P=0.000) on the photosynthetic activity of *L. pulmonaria* and Chl *a/b* $_{end}$ of *U. filipendula.* Whereas, factor "Side" highly affected (P=0.000) DM growth of *P. sulcata* and photosynthetic activity of *R. farinacea* (P<0.05 to P<0.001). The interaction of factors was significant (P<0.01) for *L. pulmonaria* and *P. sulcata* in a case of Chl *a/b* $_{en\,d}$ or for *P. sulcata*.in a case of DM growth. No significant interactions found between the parameters and external factors for *U. filipendula* and *R. farinacea.*

Table 30. – The developmental design, physiological parameters of lichen transplants along with statistic results presented as three-way ANOVA

	Lichen species (Sp)					Distance from the highway (D)						Side (S)	Sp*S	Sp*D	S*D	Sp*S*D	
Variable	***Parmelia sulcata***	***Lobaria pulmonaria***	***Ramalina farinacea***	***Usnea filipendula***	***P* (Sp)**	**10 m**	**15 m**	**30 m**	**50 m**	**100 m**	***P* (D)**	***P***	***P***	***P***	***P***	***P***	r^2_{adj}
STM $_{start}$	**34.3±0.2**	13.4±0.2	21.9±0.4	23.8±0.5	0.000	**22.0±1.3**	21.8±1.2	21.9±1.2	21.5±1.1	21.9±1.2	ns	ns	ns	ns	ns	ns	84.5
DM growth	**11.6±1.2**	4.24±0.94	-1.02±0.32	2.61±0.34	0.000	1.70±1.76	**5.22±1.54**	4.90±1.01	5.07±1.36	3.87±1.05	0.006	0.000	0.000	0.035	0.035	0.042	70.8
Chl (a+b) $_{end}$	1.16±0.04	**1.32±0.07**	0.76±0.04	0.65±0.03	0.000	0.77±0.07	1.00±0.08	1.00±0.08	1.03±0.07	**1.05±0.08**	0.000	ns	ns	ns	ns	ns	62.1
Chl a/b $_{end}$	3.80±0.03	2.91±0.04	3.69±0.02	**3.82±0.04**	0.000	3.38±0.11	3.56±0.08	3.61±0.08	3.60±0.08	**3.63±0.08**	0.000	ns	ns	0.000	0.000	ns	87.3
Fv/Fm $_{end}$	**645±5**	458±20	599±6	598±6	0.000	531±31	579±18	589±13	579±14	**597±11**	0.000	ns	ns	0.000	ns	ns	72.7

Note. Values of analyzed parameters in each of the four studied lichen species (n=30; all distances combined), and at each of five distances from the highway (n= 24; all species combined). The highest value in each group is marked in bold. P-values are given according to a 3-way ANOVA with species (Sp), distance from road (D))

Table 31. – Results of two way ANOVA due to performance of the filed factors by using the physiological parameters among the lichens species

Variable	***L. pulmonaria***			***P. sulcata***			***R. fastigiata***			***U. filipendula***		
	Side	**Distance**	**Side x Distance**	**Side**	**Distance**	**Side x Distance**	**Side**	**Distance**	**Side x Distance**	**Side**	**Distance**	**Side x Distance**
STM $_{start}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
DM growth	<0.05	0.000	n.s.	0.000	n.s.	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Chl (a+b) $_{end}$	n.s.	<0.001	<0.05	n.s.	n.s.	n.s.	<0.05	<0.05	n.s.	n.s.	n.s.	n.s.
Chl a/b $_{end}$	n.s.	0.000	<0.01	n.s.	<0.05	<0.01	<0.01	n.s.	n.s.	n.s.	<0.001	n.s.
Fv/Fm $_{end}$	n.s.	0.000	n.s.	n.s.	<0.01	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	n.s.

The correlations between the mean values of parameters and the content of accumulated elements are presented in the Table 32.

Table 32.– Pearson correlation coefficients between of elemental content and physiological parameters in lichens species (df= 28, n= 30)

	L. pulmonaria		*P. sulcata*		*R. farinacea*		*U. filipendula*	
Element	Fv/Fm_{end}	DM growth	Fv/Fm_{end}	DM growth	Fv/Fm_{end}	DM growth	Fv/Fm_{end}	DM growth
Ca	**-0.75**	**-0.77**	-0.32	n.s.	n.s.	n.s.	n.s.	n.s.
Mg	**-0.76**	**-0.67**	-0.46	n.s.	-0.37	n.s.	n.s.	n.s.
Na	**-0.68**	**-0.74**	-0.35	-0.44	-0.49	n.s.	n.s.	n.s.
K	**0.82**	**0.85**	n.s.	n.s.	n.s.	n.s.	n.s.	0.47
P	0.57	0.55	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fe	**-0.81**	**-0.67**	-0.44	n.s.	-0.32	n.s.	n.s.	-0.36
Al	**-0.79**	**-0.65**	-0.41	n.s.	-0.48	n.s.	n.s.	n.s.
Mn	-0.32	-0.38	-0.47	n.s.	n.s.	n.s.	n.s.	0.39
Zn	**-0.75**	**-0.64**	-0.34	n.s.	-0.31	n.s.	n.s.	n.s.
Cu	**-0.78**	**-0.65**	-0.35	n.s	n.s.	n.s	n.s.	-0.38
Ba	**-0.72**	**-0.64**	n.s.	n.s	-0.47	n.s	n.s.	n.s
V	**-0.8**	**-0.69**	-0.42	n.s	n.s.	n.s	n.s.	-0.36
Pb	-0.34	n.s.	n.s.	n.s	n.s.	n.s	n.s.	n.s
Cr	**-0.78**	**-0.6**	-0.43	n.s	-0.37	n.s	n.s.	-0.39
Ni	**-0.8**	**-0.74**	-0.44	n.s	-0.33	n.s	n.s.	n.s
Sn	**-0.7**	**-0.61**	n.s.	n.s	n.s.	n.s	n.s.	-0.38
Co	**-0.82**	**-0.71**	-0.41	n.s	n.s.	n.s	n.s.	-0.37
Sb	**-0.76**	**-0.69**	**0.57**	n.s	n.s.	n.s	n.s.	-0.4
Mo	**-0.63**	-0.53	n.s.	n.s	-0.32	n.s	n.s.	-0.36
As	-0.47	-0.49	-0.34	n.s	n.s.	n.s	n.s.	-0.42
Cd	0.41	0..51	0.39	0.4	n.s.	n.s	-0.33	n.s

Note. Corr. coefficients significance is marked in bold numbers <0.001, normal <0.01 and n.s. >0.05

The most significant (P<0.001 to P=0.000) and high correlation values were found in *L. pulmonaria* compare to other lichen species. Thus, the Fv/Fm_{end} of *L. pulmonaria* showed a very strong negative correlation with Co, Fe, V, Ni, Al, Cu, Zn and Cr (r= -0.75 to -0.81) along with Na (r= -0.68) and Ca (r= -0.75), whereas with Sb (r= -0.57) for *P. sulcata.* There were found a moderate significant (P<0.01) correlations between Fv/Fm_{end} and Na, Al, Ba (r= -0.47 to -0.49) for *R. farinacea* and Cd (r= -0.33) for *U. filipendula.* The highest strong negative correlations (P<0.001to P=0.000) were determined between DM growth and Ca, Na, Ni, Co (r= -0.71 to -0.77) for *L. pulmonaria.* The DM growth showed significant (P <0.01) moderate negative

correlations with Na and Cd for *P. sulcata* (r= -0.39 to -0.44), while more of correlations were found between DM growth and As, Sb, Fe, Cu, V, Cr, Sn, Co, Mo (r= -0.36 to -0.42) for *U. filipendula.* Potassium (K) and P were the only elements that showed positive relationship with physiological parameters of lichens. Highly significant (P=0.000) correlations were found between K and studied parameters (r= 0.69 to 0.85) and less significant (P<0.01) with P (r= 0.5 to 0.57) for *L. pulmonaria,* whereas between K and DM growth (r= 0.47) for *U. filipendula.*

The photographs of lichen specimens were examined for any visible changes that happen with thalli after the roadside field exposure, some of them showed at Figure 13.

Figure 13. – The visible color change that detected among the lichen thalli at 10 m from the road as mark of a damage or degradation process: a) *Lobaria pulmonaria;* b) *Parmelia sulcata;* c) *Ramalina farinacea* and d) *Usnea filipendula*

As an object for a closer study specimens of *L. pulmonaria* have been chosen. The level of visible degradation caused by field exposure was calculated (Tab. 33; Appx. II) and computed for this species (Fig. 14).

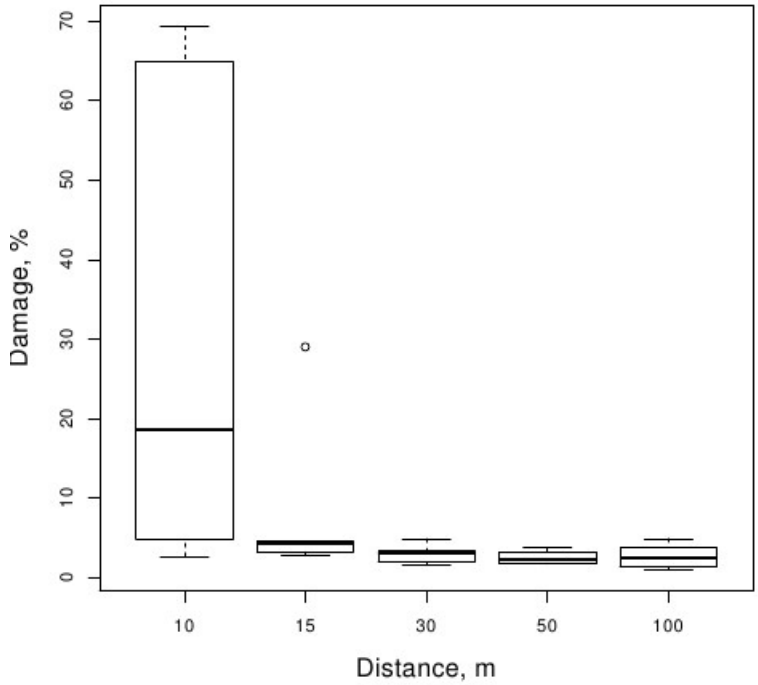

Figure 14. – The comparison of *L. pulmonaria* specimens visible damage at different field locations, averaged per stand (F= 4.13; df=25; P<0.01)

The figure shows pronounced variation, especially for thalli located at 10 m from the road. The median value of damage showed a significant (P<0.01) decrease from 19% to 2% with the distance. The box plot data collected within the stands located at 10 m showed a wide range of variation (the IQR) – from 5 to 65%, while box plots of further away locations (15, 30, 50 and 100 m) – only from 2 to 5 %.

The single linear regression between the lichen viability parameters and the visible damage after the transplantation period was performed (Tab. 34).

Table 34. – Regression summary for the assessed visible damage in relationship to the parameters of *L. pulmonaria* (df=118; n= 120)

Source of variation	df	Coef.	SE Coef.	T	F	P-value	r^2 adj
Fv/Fm_{end}	1	-95.01	8.71	-10.91	119.1	0.000	0.502
$Chl\ (a+b)_{end}$	1	-17.88	2.57	-6.96	48.4	0.000	0.291
Chl a/b end	1	-51.52	3.59	-14.35	205.9	0.000	0.636
DM growth	1	-1.36	0.23	-5.86	34.36	0.000	0.225
Error	118						
Total	122						

*degree of freedom (df), F-statistic (F), adjusted r^2 (r^2 adj), standard error coeff. (S.E. Coef.), t-value (T)

The results showed that physiological parameters of *L. pulmonaria* (Fv/Fm_{end}, $Chl\ (a+b)_{end}$, Chl a/b $_{end}$ and DM growth) were significantly (P=0.000) influenced by the visible level of damage in the species. The coefficients of determination (r^2_{adj}) for these parameters varied from 0.225 to 0.636, respectively.

Measured functional parameters in *L. pulmonaria* strongly responded to airborne pollutants. Therefore, we calculated changes in the potential quantum yield of PSII by prior to (Fv/Fm_{start}) and after field exposure (Fv/Fm_{end}). The results were visually presented as the percent of photoinhibition due to distance from the road (Fig. 15).

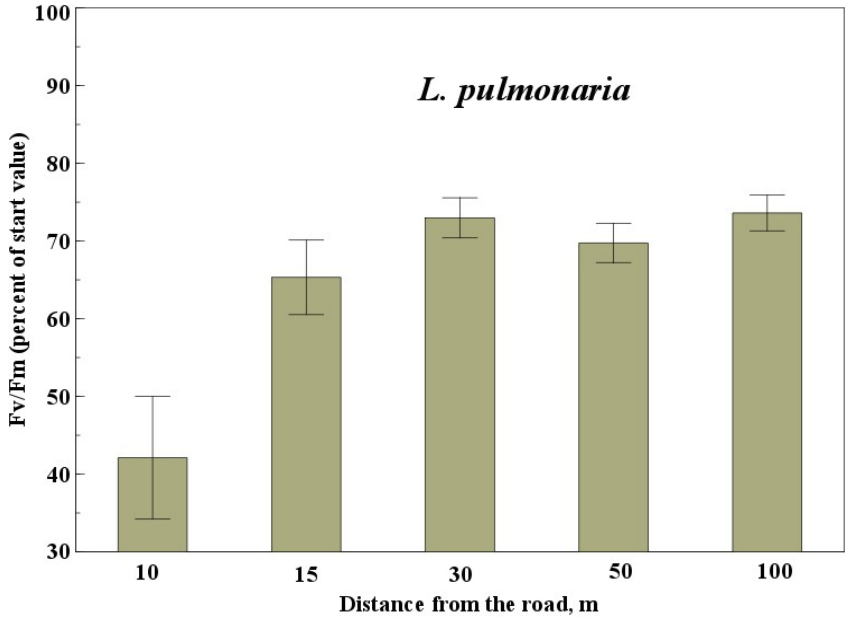

Figure 15. – Photoinhibition in *L. pulmonaria* measured as percent of Fv/Fm of start values with increasing distance from the road (Fv/Fm_{start} = 0.708 ±0.01, n=10)

The strongest reduction in Fv/Fm $_{end}$ to approximately 40% occurred in the samples collected at 10 m from the road, although a clear reduction was still evident at 15 m (65%). The examined parameter at distances 30, 50 and 100 m showed an additional increase and contributed 70-74.0% of the start value.

3.4. Discussion

The observed effects from the vehicular traffic pollution may lead to stress on species or ecosystems in the vicinity of highways and main roads. Heavy metal toxicity is one of the major abiotic stresses leading to hazardous effects in photosynthetic organisms in such location. Lichens have long been regarded reliable bioindicators of air pollution, as studies typically have indicated negative impacts in polluted areas. For instance, exposure to toxic levels of heavy metals can trigger a range of physiological and metabolic alterations. As mentioned by Wolterbeek (2002), air pollution impact on lichens behavior as biomonitors is viewed as resulting in changes in dose-response relationships.

Weight and area gain are physiological parameters of lichen in relation to atmospheric pollution stress (Lawrey & Hale 1979). Our study reveals a several species-specific significant relationships between the physiological parameters after lichen transplantation period. Thus, the parameter DM growth found positively associated with the parameters of photosynthetic activity of lichens with a variation across species. That result agrees with Dahlman & Palmqvist (2003), who reported that 80% of the variation in weight gain in lichens was explained by a linear regression model including light received during wet active periods, chlorophyll *a* concentration. They emphasized that new thallus area is produced predominantly from recently assimilated resources rather than recycling within the thallus. We found strong positive relationship between DM growth and Fv/Fm $_{end}$ for *L. pulmonaria,* consistent with the view that weight growth being primary relates to net carbohydrate gain.

Our finding indicate a statistical linkage between the chemical elemental content and lichen viability. The most significant correlations between the elemental content and physiological parameters that affected Fv/Fm of lichens than DM growth. On the importance of chlorophyll content as a measure the degree of plant stress by air pollution indicated by Peñuelas & Filella (1998). We concluded that observed significant reduction of photosynthetic activity as well as changes in growth and weight among the lichens mostly related to the concentrations in their tissue heavy metals: Co, Ni, Cu, Fe, Cr, Zn, Mg, V, As, Al and micronutrients like Na and Ca. Potassium (K) correlated significantly positive with measured physiological parameters. It is evidently, that heavy metals provoke the electrolyte leakage due to degrading cell membranes, where K is major element (Garty et al. 1998; 2000). Thereby, reduced concentrations of this macronutrient in studied lichens represent damage and can be considered to be a viability measure.

Interspecies differences in sensitivity are discussed in many studies (Garty 1995; Van Dobben et al. 2001; Gonzalez et al. 2003; Bačkor & Loppi 2009). Our investigation showed the lichen species sensitivity to airborne pollution can be assessed due to changes in physiological responses and provided the information about the specific pattern response of each species to atmospheric pollution. According to our results, several elements (Sb, Cd and Mn) had significant positive correlations with some of physiological parameters of lichen species (*P. sulcata*, *L. pulmonaria* and *U. filipendula*). It indicate on high tolerance of lichens species to heavy metals and their species specific features in the process of accumulation. We found that Pb was negatively for *L. pulmonaria.* According to Branquinho et al. (1996), Lead (Pb) uptake, particularly in lichens with cyanobacteria, caused a decrease in PS II photochemical reactions, and they were more sensitive to this element than cell membrane damage.

Lichens after the field survey displayed significant differences in the responses of physiological parameters between species and external factors. Factor “Distance” influenced viability parameters of *L. pulmonaria* and Chl a/b_{end} of *U. filipendula*,

whereas factor “Side” dependent were the photosynthetic activity of *R. farinacea* and DM growth of *P. sulcata.* Higher levels of Fv/Fm $_{end}$ and DM growth were found in the lichen species transplanted on the west side of the road, compare with Chl $(a+b)_{end}$ and Chl a/b $_{end}$ – on the east. These differences may result from higher levels of photoinhibition with an evening sun than with a morning sun. Evidently, that the concentration of pigments in lichen photobionts can change with abiotic factors such as light and moisture. In our experiment, stands with specimens of lichens located on the west side of the road received light in the morning when hydrated by humid air, whereas stands on the east received light in afternoon when lichens are often dry and susceptible to photoinhibition. Therefore, the stress by air pollution axcess may lead to an additive effect of high irradiance and air born pollutants.

The chlorophyll a/b ratio can be used as an indicator of the measurement of plant cells physiological activity. Thus, a decrease in the Chl a/b ratio may be interpreted as an enlargement of the antenna system of PS II (Lichtenthaler & Burkart 1999). Chettri et al. (1998) found that heavy metal cations caused a 10-15% decrease of Chl *a/b* in the lichens. Additionally, Vantova et al. (2013) showed that the Chl *a/b* ratio decreased in *Evernia prunastri* more rapidly compare to change of the total Chl (a+b) during the short term experiments. We suggest that in lichen specimens located on the west side some amount of Chl *a* was transformed into Chl *b* that caused the change of Chl *a/b* ratio.

The potential quantum yield of photosystem II (PSII), which characterize the photosynthetic activity of lichens, was significantly affected by air emissions from the road. Thus, in accordance with our results in *L. pulmonaria* was observed a significant photoinhibition of the photosystem, in an average from 42 to 74% of the start value. The photosystem of the species did not recovered even with 100 m distance from the road indicating on a severe damage. Our observations also detected that visible signs of degradation in *L. pulmonaria* were related to the physiological status of the species and to the amount in their tissue accumulated pollutants, whereas mostly were caused by the factor “Distance”, especially at 10 m location.

According to Estrabou et al. (2004) the deposition of particulate matter probably decrease the photosynthesis and inhibit normal thalli development in lichens. We observed dark areas and spots among the transplanted specimens of lichens that also indicated on the visible effects of air pollution (Fig.16).

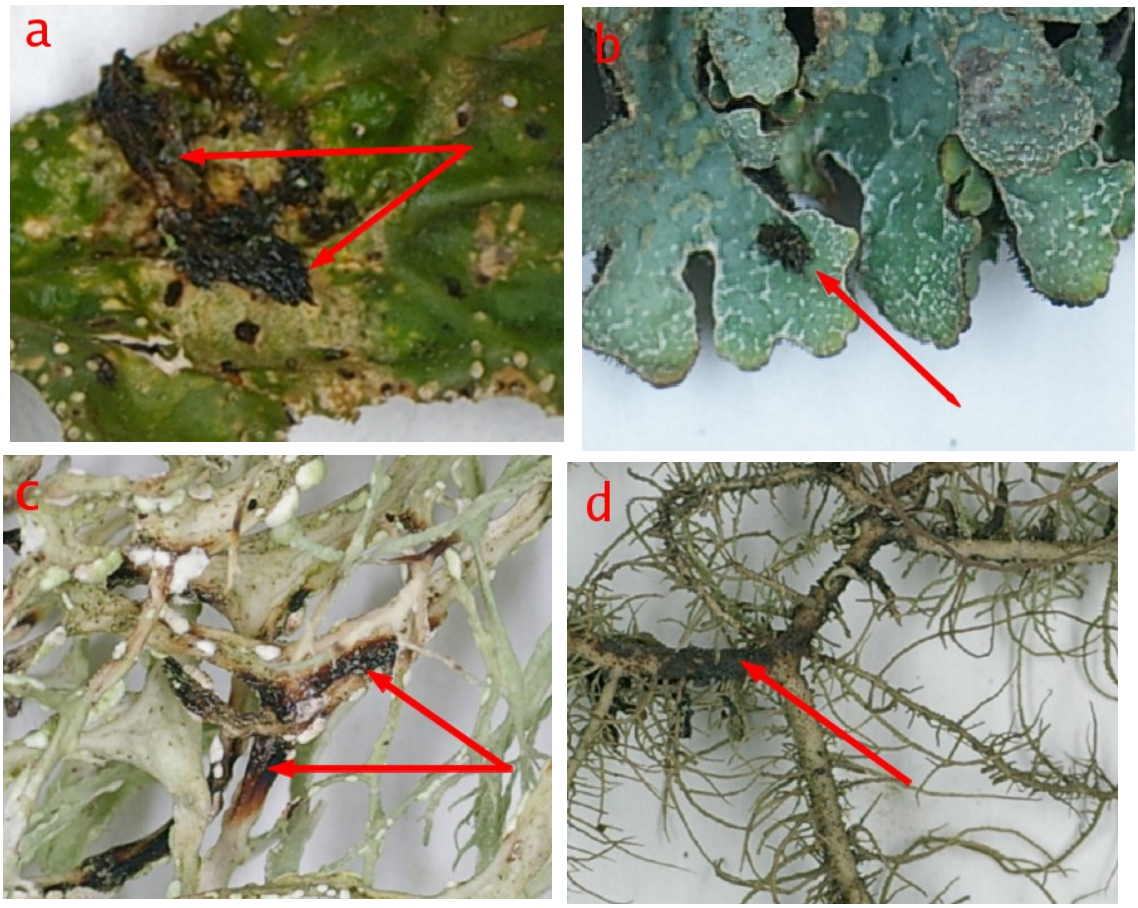

Figure 16. – Unknown black spots (deposits) on the lichen thalli observed at 10-30 m across the transplants due to exposure near the road: a) *Lobaria pulmonaria;* b) *Parmelia sulcata*; c) *Ramalina farinacea* and d) *Usnea filipendula*

In conclusion, during the field survey was assessed the influence of local air pollution on physiological status of lichens, measured as changes in physiological and morphological attributes which are responsible for lichens viability. Was observed significant reductions of the chlorophyll content in lichens which were strongly related

to the elemental accumulation patterns in lichens and heavy metals with Sodium (Na) had a key influence. Interspecies differences in sensitivity among the lichens were detected and the results pointed on *L. pulmonaria* as the best object for the physiological biomonitoring and the visible damage detection at short distances from the air emissions sources.

Overall discussion

Mobilization of trace metals along with the deicing road salt application has attracted much attention. The observed effects from the vehicular traffic pollution may lead to multiple stress on species or ecosystems in the vicinity of highways and main roads (Antgold 1997; Farmer 1999; Forman et al. 2003). Therefore, the present study focuses on accumulation and effects of traffic related pollutants assessed by using transplanted lichens as biomonitoring organisms of atmospheric depositions along the highway E6 in Akershus fylke, southeastern Norway.

The experimental setup was designed to facilitate comparisons on lichens performance in the laboratory conditions with responses from extensive field studies. The aim was to integrate knowledge on physiology and species-specific response of lichens, their elemental composition and concentration of airborne pollution, and their stress response and tolerance.

In the laboratory experiment (Paper I) we simulated the variables salinity, heavy metals (Cu, Zn) and high light, which are important factors during winter in the roadside environment and studied their effects on lichens separately or in combination. We hypothesized that such design will help to understand the physiological mechanisms of lichens, their tolerance limits and to determine if they are within or outside of their normal function range.

Road de-icing salt is the major contaminant in run-off from a modern road surfaces specially in Northern countries. According to Lundmark (2003), concentrations of

Chloride (Cl^-) and Sodium (Na^+) in highway runoff during winter time were 35 000 and 22 500 mg L^{-1}, which is 1.8 and 2.1 times higher than in the synthetic sea water we modeled in the laboratory conditions, and match 0.4 M NaCl. Our results showed high tolerance of lichens to the lowest concentration of NaCl (0.01M), sensitive to medium (0.2 M) and susceptible at high (0.6 M) concentration.

Copper and Zinc are commonly present in highway stormwater runoff. Vollertsen et al. (2009) estimated that average concentrations of these metals in storm water runoff near Skullerud junction of E6 highway with 42 000 average daily traffic load in Oslo area were 86 and 272 mg m^{-3}, respectively. These amounts are 2.6 and 8.5 times more than the highest concentration applied in the laboratory experiment (500 μM). Our experiment showed that lichens species were damaged by metal applications starting from 100 μM.

The results in Paper II are in agreement with the literature and confirm that traffic and road related emissions must be considered as a source of pollution for the roadside environment biota. Thus, our field survey showed the highest amount of Na (36 mg g^{-1}) in *U. filipendula* at 10 m from the road with a mean of 19 mg g^{-1} Na in averaged sample and 0.32 mg g^{-1} as a background value. Subsequently, these data showed that lichens species were efficient accumulators of Na. Similarly, studies of Fostad & Pedersen (2000), Kayama et al. (2003) suggested a relationship between Sodium (Na) accumulation in leaves, soil and injury to roadside plants. According to Bryson & Barker (2002), Na accumulation occurred on the pine needles on the tree side that faced the road, ranging from 3.36 g kg^{-1} DM at 3 m to 1.51 g kg^{-1} at 6 m, compared to an average of 2.13 g kg^{-1} Na in the samples of damaged needles and 28 g kg^{-1} Na in healthy.

Regarding to Pahlson (1989), Cu and Zn are normally present from 5-15 μg g^{-1} and up to 100 μg g^{-1} DM in most plants, while their critical concentrations affecting growth are 15-20 μg g^{-1} and 200-300 μg g^{-1} DM, respectively. Tarhanen et al. (1998) found that damaging concentration of Cu for algal biont inside the lichen thalli was >50 μg g^{-1},

while Chettri at al. (1998) reported a critical concentration of Zn >140 μg g^{-1} that agrees with our investigations. The highest amounts of Zn and Cu we detected in *U. filipendula* (40 μg g^{-1} and 245 μg g^{-1} DM) at 10 m from the road, while the background values were 3 and 74 μg g^{-1}, respectively.

The lichen bionts responded in various ways to desiccation. The mycobionts of *L. pulmonaria* and *P. sulcata* were more adversely affected by NaCl applications than photobionts (Paper I). Similarly, the field experiment (Paper II, Part II) showed that accumulated Na negatively correlated with Fv/Fm (r= -0.68 and -0.35) and with DM growth (r= -0.74 and -0.44) in *L. pulmonaria* and *P. sulcata,* respectively. Copper (Cu) and Zn applications (Paper I) showed stronger impact on photobionts of *L. pulmonaria* than on the mycobiont and was an opposite response of *P. sulcata*. Results of the field survey (Paper II, Part II) showed that Fv/Fm of transplanted *L. pulmonaria* was altered by Cu and Zn more than DM growth, r= -0.78 and -0.75 against -0.66 and -0.63, while in a case of *P. sulcata* was impacted only photobiont *(*r= -0.35 and -0.34).

Paper I supported also the hypothesis that damaging effects of NaCl and heavy metals (Copper, Zinc) differed between of algal and fungal partners as well as between the lichen species. Thus, the photobiont of *P. sulcata (Trebouxia)* showed high tolerance to NaCl applications (up to 0.6 M), while photobionts of *L. pulmonaria (Dictyochloropsis* and *Nostoc)* were much sensitive (from 0.01 M). Furthermore, photobionts of *L. pulmonaria* showed higher sensitivity to Cu than the *P. sulcata* photobiont.

The field survey (Paper II, Part II) showed that accumulated Na, Cu and Zn by lichens negatively influenced the viability of transplanted lichens that support investigations of the laboratory study (Paper I). According to the results, Na was negatively related to Fv/Fm of *L. pulmonaria* (r= -0.68) and *P. sulcata* (r= -0.35). This element also affected DM growth of *L. pulmonaria (*r= -0.74) and *P. sulcata* (r= -0.44), closely related to mycobiont viability. Field experiment showed higher sensitivity of *L. pulmonaria* viability to accumulated Cu and Zn (r= -0.78 to -0.64) than *P. sulcata*

does (r= -0.35 to -0.34) that support the results of the laboratory study.
According to Garthy et al. (2000, 2001), metal toxicity in lichens is evidenced by adverse effects on cell membrane integrity assessed as Potassium (K) loss. The results of Paper II, Part I highlights the role of Na, Cu and Zn for the lichen membrane permeability. Thus, correlation coefficients between K and these pollutants for *L. pulmonaria* varied from -0.82 to -0.86 and for *P. sulcata* from -0.51 to -0.68. Also in for these species were found positive relationships between Na and Cu, Na and Zn (r= 0.55 to 0.66) and highly positive between Cu and Zn (r= 0.94 to 0.99) suggesting a common source.

Morphological characteristics difference can play important roles in the elemental accumulation in lichens. We can assume that growth form may assist in element accumulation and in particulate matter entrapment, as indicated Garty et al. (2001). However, our findings suggest that species with a rough, fruticose thallus (*U. filipendula*) and a flat, foliose (*P. sulcata*) accumulated similar amounts of elements (20.9 and 21.2 mg g^{-1} DM). These results may be influenced by the fact that we compare lichen species that are quite tolerant to airborne pollutants species (*P. sulcata*) and sensitive (*U. filipendula*). Presumably, some of the elemental depositions, including metal-rich particulate matter, might have been trapped on the lichen surface (not toxic fraction), while other parts of trapped elements were located within inter- and extracellular spaces and can be soluble, cross the membranes and eventually alter the cell viability of lichens (toxic fraction). Thus, Paper II, Part II showed that *U. filipendula* characterized by severe accumulation of airborne pollutants. However, only accumulation of Fe, Sb, Cu, Cr, Ni, Co, Mo, V and As reduced the viability parameter DM growth (r= -0.36 to -0.42), while accumulation of Na, Zn, Ca, Al, Ni, Pb, Ba and Sn did not significantly correlate with damage and may therefore be considered deposited at the thallus surface or as not toxic. In *P. sulcata*, exceeded levels of Na, V, Al, Fe, Mn, Cu, Zn, Ni and As altered viability parameters responsible for photosynthetic capacity and growth (r= -0.32 to -0.47), and without any damage from accumulated elements as Ba, Pb, Sn and Mo. These finding agrees with several publications. In order to tolerate high trace elements concentrations

exceeding their physiological requirements, lichens can sequester elements extracellularly as oxalate crystals or chelatine complexes (Nieboer et al. 1978). Hale (1983) reviewed, that intercellular spaces of the lichen thallus can accumulate and retain heavy metals in particulate form or bound to cation exchange sites. Finally, Baranowska-Bosiacka et al. (2001) found that Pb and Cd remained mainly on the surface of the lichen thalli and in the cell walls, while Zn and Cu penetrated to the protoplast.

During our laboratory studies (Paper I) we studied the influence of multiple stress on lichen viability. For example, high light increased the desiccation stress among lichen species. Thus, viability parameters of lichens (Fv/Fm and electrical conductivity) become significantly altered by the lowest concentration of NaCl (0.01 M) compare to the only salt treatment. Furthermore, application of salt along with Cu or Zn increased the negative effect of salt (especially in presence of Cu) by lowering thresholds. The stress of the lichen species from the mixed salt-metal applications started to grow from their lowest concentrations (0.01M and 10 μM). There was found that the mixture of metals (Cu or Zn) with salt did not show noteworthy difference from the application of metal by itself on the lichen viability, especially in the presence of Zn. Presumably, it was metal ion chloro- complexes formation (Babich & Stotzky 1978) that reduced the adverse effects of the agents. The field study (Paper II, Part I), showed higher positive association of Na to Zn (r= 0.46 to 0.78) than to Cu (r= 0.36 to 0.62) in accumulated content among lichens that support our laboratory results and hypotheses.

Biomonitoring of pollutants by lichens gives an information about pollutants and their effects on roadside ecosystems and therefore can be a useful tool. We propose to establish a biomonitoring with lichens possessing differing in their sensitivity to quantitative and qualitative changes in airborne roadside pollutants. Our findings suggested that the common *P. sulcata* and *U. filipendula* in the region can be used to monitor elemental pollution, while the rare *L. pulmonaria* can be a good monitor of visible damage of traffic origin.

Our results evidenced that heavy metals and Sodium strongly accumulated in lichens transplanted in a vicinity of the road, therefore roadside vegetation represent a potential pool of these pollutants for the environment. The materials of Salt SMART review (2010) recommended to avoid the planting of species that are sensitive to salt spray closer than about 10-15 m from a roads edge where the speed limit reaches 80-100 km/h. Local conditions should be considered in the planning of green areas, and when upgrading green areas in the plantation near the road to the experience and thorough knowledge of place is necessary. In order to reduce environmental impact on roadside ecosystems and improve their management, we propose to implement the following steps: use regular scientific-based biomonitoring; control the amount of applied deicing salt; reduce metals inputs from vehicles by modification of their lower parts; lower speed of vehicles on a highways during winter period; installation of storm water treatment systems along roads, restriction of 30 m zone for arable lands.

Our studies showed that the chemical composition changed in transplanted lichens along with the morphological and physiological responses and largely reflected the environmental status of air quality in the vicinity of a road. General conclusions:

- laboratory experiments and field surveys showed similar response of lichens to external stresses; the species-specific sensitivity to external stresses depended on biont type and stress factor level;
- according to the laboratory study salinity stress increased the adverse effects of excess irradiation and heavy metals (Cu, Zn);
- field survey evidenced that airborne pollutants from the road are responsible for the physiological disruptions and observed visible changes, where heavy metals and Sodium had a key influence;
- chemical content of lichens along with damage significantly declined with increasing distance from the road in species-specific pattern;
- beyond a distance of 30 m from the road, the concentration of major and trace elements in the lichens significantly starts to decrease; lichen sensitivity to accumulation and their tolerance to certain airborne pollutants is useful information to biomonitoring studies.

Outlook

Results of literature review demonstrated a strong relationship between metal mobilization and deicing in roadside conditions. Thus, salinity influenced solubility, speciation and uptake of metals (Goetz & Bauske 1993; Granato et al. 1995). According to Reinosdotter & Viklander (2007), one of the major mobilisation mechanisms of heavy metals due to deicing salt applications was chloride complex formation.

In laboratory studies we have found direct evidence for increased lichens sensitivity by combining road salt (NaCl) and heavy metals (Copper, Zinc). Along with that, we detected species-specific differences in tolerance to stresses at the biont level. The field experiment demonstrated high distance from the road dependency of accumulation of the most heavy metals and Sodium (Na) in the transplanted lichen specimens, indicating their traffic and road maintenance origin. The results also showed that these accumulated pollutants are responsible for the lichen viability parameters disruption.

We did not determined chlorocomplexes formation with heavy metals and their influence on lichen bionts under simulated roadside conditions in the laboratory. Therefore, further studies should focus more on specific physico-chemical abiotic factors: temperature, pH, redox potential, O_2, CO_2, resource availability (nutrients) and inhibitors of the recipient environment, which may profoundly affect the toxicity of the pollutant. One is the interaction of UV-B radiation and roadside pollutants. Regarding Garty et al. (2004, 2007), ultraviolet light exposure alters the chemical structure of lichens and enhance the toxicity of airborne pollutants. In addition, there is incomplete information about the impacts of deicers on lichens such as type of deicer, its concentration, duration of exposure, lichen species sensitivity and their interactions.

Because morphological, anatomical and physiological differences and play a role in the process of accumulation of airborne elements (Garty 2001; Cercasov et al. 2002; Stamenkovic et al. 2013), such factors should be considered before selecting lichen species for biomonitoring. Our results showed interspecies differences in sensitivity and accumulation of certain elements among lichens that should concidered in biomonitoring practice in the vicinity of roads. For instance, we found interspecific variability among lichens in elemental accumulation process. According to the results, transplanted samples of *L. pulmonaria* accumulated highly exceeded concentrations of Mo and Ni, *P. sulcata* – V, Co, Pb, As, Mn, *R. farinacea* – Ca, Ni, while *U. filipendula* – preferentially tendency to accumulate Na, Zn, Cu, Al, Fe, Ba, Sn and Sb. Further investigations should focus on monitoring local lichen species along with elaborating the scale of them which are may provide early warning signs of road born pollutants.

There are currently no studies on how deicing chemicals used during winter road maintenance affect the arable land in Norway, it is therefore difficult to estimate its effect on soil quality and crop yield over time (Salt SMART, 2010). The knowledge of the level of traffic originated pollutants, especially heavy metals, can be a great importance for land used as farm land or plants fodder by animals (Jozic et al. 2009). Our field experiment suggests that in order to prevent, regulate and reduce the airborne pollutants dissemination, 30 m safe distance (buffer) should be selected between highway and roadside ecosystem including farming territories. Future guidelines or recommendations for farmland use and crop rotation with strong scientific background in a case of possible roadside pollution impact should be eventually elaborated.

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Appendix I

Table 1. – Mean ± SD of the conductivity parameter for the three lichen species treated by salt and irradience (n=5)

No.	Treatment		*L. pulmonaria*			*P. sulcata*			*X. aureola*		
	NaCl, M	D/L	X	±	SD	X	±	SD	X	±	SD
1	0	D	3.19	±	1.43	3.83	±	3.95	3.75	±	1.82
2	0	L	2.93	±	1.87	2.89	±	1.09	3.35	±	0.98
3	0.01	D	11.84	±	1.88	6.85	±	1.18	3.73	±	1.29
4	0.01	L	13.21	±	3.08	6.73	±	1.75	3.49	±	0.60
5	0.2	D	30.17	±	3.96	25.78	±	7.90	7.91	±	2.02
6	0.2	L	35.47	±	3.35	31.61	±	6.47	8.10	±	1.13
7	0.6	D	38.54	±	5.71	22.71	±	4.08	10.66	±	0.74
8	0.6	L	42.91	±	10.26	26.42	±	2.06	10.36	±	2.44

Table. 2. – Mean ± SD of the conductivity parameter for the three lichen species treated by heavy metals (n=5)

No.	Treatment		*L. pulmonaria*			*P. sulcata*			*X. aureola*		
	Metal, µM		X	±	SD	X	±	SD	X	±	SD
1	Cu0	0	0.87	±	0.24	2.69	±	0.82	2.71	±	0.88
2	Zn0	0	1.00	±	0.13	1.78	±	0.56	2.29	±	0.64
3	Cu	10	1.08	±	0.45	2.34	±	0.61	8.43	±	10.82
4	Zn	10	1.28	±	0.57	2.68	±	0.98	5.37	±	4.04
5	Cu	100	35.61	±	13.94	22.72	±	5.65	26.05	±	12.24
6	Zn	100	6.54	±	3.17	20.85	±	7.38	4.59	±	2.49
7	Cu	500	54.76	±	9.81	40.61	±	16.88	59.98	±	5.79
8	Zn	500	5.44	±	3.31	44.55	±	10.35	8.17	±	1.06

Table 3. – Mean ± SD of the chlorophyll fluorescence parameter for the three lichen species treated by salt and irradience (n=5)

			Fv/Fm1									Fv/Fm2									Fv/Fm3					
	Treatment		*L. pulmonaria*			*P. sulcata*			*X. aureola*			*L. pulmonaria*			*P. sulcata*			*X. aureola*			*L. pulmonaria*			*P. sulcata*			*X. aureola*		
No.	NaCl, M	D/L	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0	D	0.674	±	0.02	0.787	±	0.02	0.750	±	0.01	0.671	±	0.01	0.767	±	0.01	0.748	±	0.02	0.709	±	0.01	0.744	±	0.03	0.735	±	0.02
2	0	L	0.684	±	0.02	0.780	±	0.00	0.767	±	0.01	0.686	±	0.02	0.755	±	0.01	0.759	±	0.01	0.609	±	0.03	0.676	±	0.02	0.724	±	0.01
3	0.01	D	0.679	±	0.01	0.784	±	0.01	0.751	±	0.01	0.678	±	0.01	0.762	±	0.01	0.751	±	0.01	0.720	±	0.01	0.759	±	0.02	0.735	±	0.01
4	0.01	L	0.682	±	0.01	0.781	±	0.01	0.764	±	0.00	0.683	±	0.01	0.757	±	0.01	0.753	±	0.01	0.537	±	0.03	0.656	±	0.03	0.711	±	0.01
5	0.2	D	0.680	±	0.01	0.789	±	0.01	0.763	±	0.01	0.668	±	0.01	0.762	±	0.01	0.754	±	0.00	0.694	±	0.02	0.758	±	0.01	0.728	±	0.03
6	0.2	L	0.673	±	0.01	0.784	±	0.01	0.745	±	0.01	0.669	±	0.01	0.763	±	0.01	0.753	±	0.01	0.520	±	0.04	0.605	±	0.05	0.679	±	0.02
7	0.6	D	0.677	±	0.01	0.779	±	0.01	0.756	±	0.01	0.656	±	0.01	0.764	±	0.02	0.743	±	0.01	0.701	±	0.02	0.766	±	0.02	0.729	±	0.00
8	0.6	L	0.686	±	0.01	0.779	±	0.01	0.757	±	0.01	0.659	±	0.01	0.757	±	0.01	0.740	±	0.01	0.425	±	0.04	0.633	±	0.04	0.676	±	0.02

Table 4. – Mean ± SD of the chlorophyll fluorescence parameter for the three lichen species treated by heavy metals (n=5)

			Fv/Fm1									Fv/Fm2								
	Treatment		*L. pulmonaria*			*P. sulcata*			*X. aureola*			*L. pulmonaria*			*P. sulcata*			*X. aureola*		
No.	Metal	µM	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	Cu0	0	0.676	±	0.01	0.741	±	0.02	0.757	±	0.01	0.677	±	0.01	0.757	±	0.01	0.765	±	0.01
2	Zn0	0	0.675	±	0.01	0.751	±	0.01	0.757	±	0.01	0.672	±	0.02	0.764	±	0.00	0.763	±	0.00
3	Cu	10	0.685	±	0.02	0.741	±	0.01	0.760	±	0.01	0.688	±	0.01	0.758	±	0.01	0.757	±	0.02
4	Zn	10	0.686	±	0.01	0.738	±	0.02	0.758	±	0.01	0.685	±	0.01	0.750	±	0.01	0.743	±	0.02
5	Cu	100	0.674	±	0.01	0.751	±	0.01	0.757	±	0.02	0.603	±	0.07	0.757	±	0.01	0.759	±	0.01
6	Zn	100	0.681	±	0.01	0.748	±	0.02	0.760	±	0.02	0.678	±	0.00	0.757	±	0.01	0.751	±	0.01
7	Cu	500	0.670	±	0.02	0.750	±	0.01	0.761	±	0.01	0.229	±	0.04	0.724	±	0.04	0.738	±	0.02
8	Zn	500	0.680	±	0.02	0.737	±	0.01	0.765	±	0.01	0.668	±	0.03	0.738	±	0.01	0.755	±	0.01

Table. 11. – Mean ± SD of the main studied variables for *L. pulmonaria* treated by salt and heavy metals (n=5)

No.	Treatment			Fv/Fm1			Fv/Fm2			Conductivity, %		
	NaCl, M	Metal,	µM	X	±	SD	X	±	SD	X	±	SD
1	0	Cu0	0	0.662	±	0.01	0.670	±	0.01	0.94	±	0.49
2	0.01	Cu	10	0.679	±	0.01	0.671	±	0.01	10.61	±	1.31
3	0.01	Cu	100	0.669	±	0.01	0.656	±	0.01	30.32	±	5.34
4	0.01	Cu	500	0.671	±	0.01	0.392	±	0.06	52.75	±	12.03
5	0.2	Cu	10	0.670	±	0.01	0.662	±	0.01	18.41	±	2.49
6	0.2	Cu	100	0.668	±	0.01	0.650	±	0.01	39.45	±	9.58
7	0.2	Cu	500	0.670	±	0.01	0.590	±	0.06	56.08	±	3.49
8	0.6	Cu	10	0.672	±	0.01	0.656	±	0.01	35.95	±	5.64
9	0.6	Cu	100	0.673	±	0.01	0.632	±	0.03	55.16	±	7.13
10	0.6	Cu	500	0.680	±	0.01	0.534	±	0.06	77.12	±	2.63
11	0	Zn0	0	0.674	±	0.01	0.677	±	0.01	0.72	±	0.22
12	0.01	Zn	10	0.675	±	0.02	0.664	±	0.01	11.04	±	2.65
13	0.01	Zn	100	0.664	±	0.01	0.656	±	0.02	12.45	±	2.64
14	0.01	Zn	500	0.663	±	0.02	0.649	±	0.02	12.52	±	1.49
15	0.2	Zn	10	0.665	±	0.02	0.660	±	0.02	4.07	±	1.71
16	0.2	Zn	100	0.672	±	0.01	0.656	±	0.02	28.75	±	2.09
17	0.2	Zn	500	0.680	±	0.01	0.666	±	0.01	24.34	±	3.58
18	0.6	Zn	10	0.679	±	0.01	0.659	±	0.00	38.71	±	4.47
19	0.6	Zn	100	0.677	±	0.00	0.659	±	0.00	35.14	±	2.76
20	0.6	Zn	500	0.678	±	0.01	0.661	±	0.01	35.10	±	2.62

Table 12. – Mean ± SD of the main studied variables for *P. sulcata* treated by salt and heavy metals (n=5)

No.	Treatment			Fv/Fm1			Fv/Fm2			Conductivity, %		
	NaCl, M	Metal,	µM	X	±	SD	X	±	SD	X	±	SD
1	0	Cu0	0	0.757	±	0.01	0.764	±	0.01	4.46	±	0.96
2	0.01	Cu	10	0.759	±	0.00	0.772	±	0.00	10.11	±	3.54
3	0.01	Cu	100	0.758	±	0.01	0.765	±	0.01	20.88	±	4.51
4	0.01	Cu	500	0.762	±	0.01	0.754	±	0.02	36.60	±	13.02
5	0.2	Cu	10	0.764	±	0.01	0.760	±	0.01	22.80	±	2.52
6	0.2	Cu	100	0.763	±	0.01	0.761	±	0.01	44.06	±	8.58
7	0.2	Cu	500	0.756	±	0.01	0.770	±	0.01	46.70	±	6.09
8	0.6	Cu	10	0.759	±	0.01	0.760	±	0.01	32.30	±	7.79
9	0.6	Cu	100	0.758	±	0.01	0.760	±	0.01	47.03	±	10.53
10	0.6	Cu	500	0.753	±	0.01	0.754	±	0.01	64.29	±	6.15
11	0	Zn0	0	0.753	±	0.01	0.765	±	0.01	3.19	±	1.57
12	0.01	Zn	10	0.761	±	0.01	0.771	±	0.01	9.62	±	3.12
13	0.01	Zn	100	0.755	±	0.01	0.752	±	0.01	8.36	±	2.94
14	0.01	Zn	500	0.761	±	0.01	0.769	±	0.01	47.48	±	18.12
15	0.2	Zn	10	0.754	±	0.01	0.766	±	0.01	12.02	±	8.55
16	0.2	Zn	100	0.758	±	0.01	0.756	±	0.01	37.85	±	3.53
17	0.2	Zn	500	0.765	±	0.01	0.726	±	0.03	36.23	±	3.46
18	0.6	Zn	10	0.759	±	0.01	0.753	±	0.01	10.04	±	2.80
19	0.6	Zn	100	0.756	±	0.01	0.733	±	0.02	30.64	±	4.14
20	0.6	Zn	500	0.758	±	0.01	0.743	±	0.01	34.22	±	3.30

Table 13. – Mean ± SD of the main studied variables for *X. aureola* treated by salt and heavy metals (n=5)

No.	Treatment			Fv/Fm1			Fv/Fm2			Conductivity, %		
	NaCl, M	Metal,	μM	X	±	SD	X	±	SD	X	±	SD
1	0	Cu0	0	0.742	±	0.02	0.756	±	0.02	1.55	±	0.32
2	0.01	Cu	10	0.737	±	0.02	0.752	±	0.01	5.00	±	0.69
3	0.01	Cu	100	0.740	±	0.02	0.767	±	0.00	7.99	±	1.09
4	0.01	Cu	500	0.746	±	0.02	0.759	±	0.02	29.18	±	5.31
5	0.2	Cu	10	0.744	±	0.01	0.758	±	0.01	9.39	±	1.16
6	0.2	Cu	100	0.736	±	0.01	0.747	±	0.01	15.44	±	5.57
7	0.2	Cu	500	0.751	±	0.01	0.746	±	0.01	28.19	±	2.10
8	0.6	Cu	10	0.749	±	0.01	0.762	±	0.01	13.63	±	2.61
9	0.6	Cu	100	0.757	±	0.02	0.757	±	0.01	25.90	±	16.31
10	0.6	Cu	500	0.744	±	0.02	0.747	±	0.01	39.55	±	3.79
11	0	Zn0	0	0.747	±	0.01	0.743	±	0.01	1.59	±	0.27
12	0.01	Zn	10	0.751	±	0.01	0.751	±	0.01	5.20	±	1.62
13	0.01	Zn	100	0.764	±	0.00	0.758	±	0.01	5.90	±	2.33
14	0.01	Zn	500	0.751	±	0.01	0.737	±	0.02	19.70	±	8.06
15	0.2	Zn	10	0.763	±	0.00	0.762	±	0.00	4.03	±	0.87
16	0.2	Zn	100	0.762	±	0.01	0.761	±	0.00	6.69	±	1.08
17	0.2	Zn	500	0.757	±	0.00	0.740	±	0.00	8.81	±	4.07
18	0.6	Zn	10	0.765	±	0.01	0.737	±	0.02	4.78	±	0.83
19	0.6	Zn	100	0.739	±	0.02	0.723	±	0.02	7.00	±	1.91
20	0.6	Zn	500	0.754	±	0.01	0.749	±	0.01	7.58	±	2.28

Appendix II

Table 2. – The volume of traffic in both directions of E6 selected sections during 24 hours period averaged over the year

Location	Distance, m	Year	Class	Total
Korsegården	10	2006	20	28539
Korsegården	10	2006	21	24299
Korsegården	10	2006	26	4240
Korsegården	10	2008	20	31466
Korsegården	10	2008	21	26865
Korsegården	10	2008	26	4601
Korsegården_1	4432	2010	20	35000
Korsegården_1	4432	2010	21	30100
Korsegården_1	4432	2010	26	4900
Korsegården_2	2900	2010	20	34500
Korsegården_2	2900	2010	21	29670
Korsegården_2	2900	2010	26	4830
Smihagen tunnel	3413	2006	20	29555
Smihagen tunnel	3413	2006	21	26432
Smihagen tunnel	3413	2006	26	3123
Smihagen tunnel	3413	2007	20	31282
Smihagen tunnel	3413	2007	21	27751
Smihagen tunnel	3413	2007	26	3531
Smihagen tunnel	3413	2008	20	32149
Smihagen tunnel	3413	2008	21	28371
Smihagen tunnel	3413	2008	26	3778
Smihagen tunnel	3413	2009	20	32573
Smihagen tunnel	3413	2009	21	28937
Smihagen tunnel	3413	2009	26	3636
Smihagen tunnel	3413	2010	20	35259
Smihagen tunnel	3413	2010	21	31150
Smihagen tunnel	3413	2010	26	4109
Smihagen tunnel	3413	2011	20	36657
Smihagen tunnel	3413	2011	21	32298
Smihagen tunnel	3413	2011	26	4359

Note. 20 – sum of all vehicles, 21 – sum of light vehicles and 26 – sum of heavy vehicles

Tables 3-6. – The element concentrations (µg g $^{-1}$ DM) and background element values as control in transplanted lichen species (element content analysed by ICP-OES), n=4

a) *Lobaria pulmonaria*

Stand	Side	Distance	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
1	E	10	1611	2946	5.0	26	2694	1418	1200	70	14823	3.8	131
2	E	15	841	1647	3.1	15	1430	3790	827	107	7956	2.1	106
3	E	30	654	2229	2.4	11	1029	4378	931	71	4833	1.6	86
4	E	50	583	2080	2.4	12	921	5166	895	84	3206	1.8	70
5	E	100	480	1809	2.0	12	665	5461	828	68	2054	1.6	70
6	E	10	1353	2664	3.9	24	2307	1482	1041	73	14235	3.0	118
7	E	15	1389	2353	4.3	21	2345	1919	1148	97	8803	3.4	120
8	E	30	787	1884	2.7	22	1362	4206	962	127	5323	2.4	108
9	E	50	502	1973	1.6	10	711	5064	787	118	2555	1.5	75
10	E	100	390	2460	1.3	10	540	5094	803	81	1720	1.4	67
11	E	10	1403	2299	4.2	26	2307	2293	1103	81	11300	2.4	121
12	E	15	1055	2018	3.5	19	1791	4036	1084	73	7274	2.4	110
13	E	30	656	2094	2.4	16	1135	4796	882	63	5525	1.5	92
14	E	50	577	1430	2.0	14	871	5251	862	69	3896	1.4	80
15	E	100	380	1909	1.3	9	516	5677	787	70	1901	0.9	80
16	W	10	1354	2496	4.5	29	2439	2822	1187	72	6045	3.1	126
17	W	15	953	1900	3.2	23	1585	3472	842	65	4262	1.8	104
18	W	30	913	1567	3.0	18	1388	4962	815	59	2344	1.4	101
19	W	50	770	1790	2.7	14	949	6137	843	108	1700	1.4	103
20	W	100	519	1200	1.8	13	610	6629	709	52	1066	0.9	84
21	W	10	2778	3561	9.3	50	5060	973	1739	108	6570	5.3	161
22	W	15	1456	2079	4.2	20	2071	5224	1028	70	3301	2.5	106
23	W	30	967	1769	2.6	15	1209	5549	821	71	2187	1.7	95
24	W	50	707	1550	2.4	15	1069	6062	870	74	1778	1.9	97
25	W	100	433	1395	1.6	12	539	6493	706	58	1026	1.3	78
26	W	10	2307	3476	7.1	44	4141	960	1531	109	7694	4.4	157
27	W	15	1090	2152	4.0	23	1861	4746	1048	102	4683	2.3	123
28	W	30	820	1395	2.9	15	1335	5560	796	55	2541	1.7	94
29	W	50	494	1067	1.7	11	676	5680	588	53	1381	1.1	75
30	W	100	493	1705	1.5	11	508	6452	875	93	1170	0.9	79
Control	EW	0	250	3601	0.9	8	287	11440	1257	236	305	0.4	86

b) *Parmelia sulcata*

Stand	Side	Distance	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
1	E	10	3237	3614	8.3	25	4636	3169	1743	86	17134	4.9	125
2	E	15	2233	5140	6.2	15	3180	4795	1433	86	9066	4.1	90
3	E	30	1408	4220	3.8	11	1775	4611	1206	63	5300	2.5	68
4	E	50	1262	2248	3.5	13	1587	5145	1070	62	3605	2.3	70
5	E	100	1369	2198	4.1	12	1736	5059	1159	73	2388	2.7	76
6	E	10	2593	3892	6.6	20	3589	3573	1426	79	15228	4.3	104
7	E	15	2346	3447	6.8	17	3240	4387	1435	84	8550	4.1	104
8	E	30	1859	4002	5.0	13	2458	5010	1409	63	6442	3.3	68
9	E	50	1398	2134	4.4	11	1794	5365	1170	110	3109	2.8	70
10	E	100	1912	2344	5.7	11	2641	4710	1330	101	2292	3.5	63
11	E	10	3317	3603	9.8	23	5029	3643	1831	99	13273	5.4	116
12	E	15	3239	5182	9.0	18	4761	4959	1817	96	9045	5.2	90
13	E	30	1750	3264	4.7	15	2295	4670	1291	64	7252	3.0	75
14	E	50	1352	1907	3.7	12	1655	5000	967	65	3611	2.2	70
15	E	100	1433	2577	3.8	11	1781	5143	1245	70	2788	2.5	61
16	W	10	3273	5167	9.2	29	4526	3324	1909	113	7179	5.5	148
17	W	15	1940	2271	5.5	16	2664	4400	1118	75	3937	3.3	85
18	W	30	1748	2271	5.0	16	2420	4553	1160	87	2549	3.0	82
19	W	50	1140	4604	3.0	11	1330	4927	1095	63	1423	2.1	67
20	W	100	1061	2337	2.8	9	1182	4749	944	44	930	2.1	54
21	W	10	4349	4692	12.4	38	7006	2920	2455	134	6210	7.1	190
22	W	15	2537	3790	7.7	17	3909	4648	1524	99	3244	4.2	99
23	W	30	1505	2793	3.9	13	1978	4087	1039	75	2095	2.6	77
24	W	50	1507	2075	4.4	12	2003	4814	1123	98	1712	2.9	70
25	W	100	1332	2377	3.7	10	1687	4966	1121	127	925	2.9	64
26	W	10	4522	6125	12.6	35	7278	3891	2456	140	6892	7.1	162
27	W	15	2511	3371	5.6	17	2883	5203	1260	105	4790	3.6	95
28	W	30	1517	3336	4.8	12	2008	5020	1109	76	3220	3.1	71
29	W	50	1469	2482	4.2	11	2027	4890	1072	74	1496	2.9	67
30	W	100	1051	1790	3.1	7	1447	3269	830	55	649	2.2	40
Control	EW	0	1145	3993	3.3	10	1524	6039	1136	39	276	2.3	54

c) *Ramalina farinacea*

Stand	Side	Distance	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
1	E	10	1777	12332	4.4	20	2272	1346	937	54	13310	2.7	95
2	E	15	1033	2068	2.4	12	1131	1546	585	47	8384	1.3	70
3	E	30	869	15483	2.5	11	940	1063	567	33	4586	1.3	64
4	E	50	752	7122	2.2	10	806	1345	599	38	4735	1.2	63
5	E	100	526	3555	1.8	10	571	1670	476	25	2210	1.0	54
6	E	10	1562	7156	3.8	20	1910	1208	813	48	12863	2.3	92
7	E	15	1367	17385	3.6	15	1648	1245	750	45	7179	2.3	82
8	E	30	1097	9661	3.3	12	1291	1524	627	40	5303	1.7	64
9	E	50	757	3871	2.1	10	834	1341	546	27	3305	1.2	52
10	E	100	623	5484	2.0	9	677	1633	623	49	2629	1.0	64
11	E	10	1290	6655	3.4	20	1600	1286	704	41	10277	1.8	88
12	E	15	1583	8262	4.3	21	2062	1489	882	50	7867	2.2	89
13	E	30	560	3368	2.1	10	898	1185	479	34	3367	1.0	48
14	E	50	485	8289	1.8	9	628	1467	541	35	3092	1.1	50
15	E	100	889	5994	3.0	17	1504	916	584	38	3617	1.8	67
16	W	10	911	5274	3.0	15	1382	1180	621	39	3527	1.7	65
17	W	15	694	5400	2.5	10	1056	1404	626	40	2048	1.3	54
18	W	30	484	7586	1.8	8	655	1162	409	24	1259	1.0	39
19	W	50	1005	17756	2.9	15	1246	1433	678	58	6373	1.5	75
20	W	100	364	5831	1.4	7	451	1632	466	27	1233	0.8	47
21	W	10	1504	7787	4.8	25	2623	1217	961	56	4637	2.7	97
22	W	15	818	2139	2.6	14	1298	1329	559	35	2556	1.3	58
23	W	30	568	5001	1.9	11	826	1373	442	35	1708	1.1	46
24	W	50	535	2933	1.9	11	733	1585	505	26	1501	1.2	50
25	W	100	414	4918	1.5	9	518	1349	419	37	1279	0.8	41
26	W	10	1415	12028	4.5	25	2413	990	953	57	4312	2.6	101
27	W	15	1036	6012	3.4	18	1655	1206	687	45	3504	1.9	73
28	W	30	609	3131	1.9	11	828	1511	494	30	2755	1.2	56
29	W	50	448	4310	1.6	8	565	1509	420	30	1584	1.0	43
30	W	100	340	2465	1.2	9	424	1527	425	28	1067	0.8	47
Control	EW	0	206	3439	1.0	5	190	1909	508	22	431	0.3	28

d) *Usnea filipendula*

Stand	Side	Distance	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
1	E	10	1400	3623	4.1	34	1807	901	1248	144	35987	2.4	195
2	E	15	1170	2941	3.0	21	1412	1515	1176	348	25246	2.1	160
3	E	30	836	2357	2.4	19	1009	1376	1171	222	17336	1.7	105
4	E	50	775	2424	2.3	19	911	1574	1174	305	17188	1.5	122
5	E	100	499	1878	1.9	16	592	1938	1080	205	9720	1.0	88
6	E	10	1675	3897	4.7	40	2220	975	1329	109	34628	2.8	233
7	E	15	1066	2626	2.7	18	1269	1561	1089	171	25844	1.7	138
8	E	30	845	2066	2.3	18	952	1391	938	179	19501	1.4	98
9	E	50	678	2649	1.8	16	759	2393	1225	343	14556	1.5	112
10	E	100	477	2201	1.6	16	544	1638	1057	281	3369	1.3	75
11	E	10	1627	3786	4.4	33	2008	1509	1356	193	15247	2.8	188
12	E	15	1302	3606	3.9	33	1634	1263	1294	130	12638	2.5	182
13	E	30	1067	3279	3.0	28	1346	1497	1093	277	8594	2.0	142
14	E	50	890	2909	2.5	24	1055	1499	1334	276	6848	1.9	126
15	E	100	486	2278	1.6	15	563	1818	1138	305	4528	1.3	103
16	W	10	1402	4643	4.0	32	1926	1455	1273	137	9285	2.6	178
17	W	15	1183	4716	3.2	33	1484	1472	1567	285	7075	2.3	176
18	W	30	961	3444	2.8	21	1149	1623	1459	326	4786	1.9	118
19	W	50	538	2203	1.8	15	636	2135	1011	271	2527	1.5	89
20	W	100	1587	4953	4.6	37	2224	1722	1677	182	7834	3.0	194
21	W	10	1824	5643	5.5	39	2755	1586	1880	205	8104	3.4	210
22	W	15	1415	4283	4.2	29	1770	1560	1595	206	7682	2.8	188
23	W	30	985	3076	2.8	20	1209	1664	1224	206	4498	2.0	110
24	W	50	674	2889	2.1	13	797	2133	1279	304	2701	1.3	89
25	W	100	481	3121	1.7	12	556	2359	1516	432	2151	1.6	101
26	W	10	2140	6200	6.7	35	3335	1344	1939	169	11308	3.6	245
27	W	15	1258	4120	3.4	24	1596	1841	1420	300	7290	2.2	179
28	W	30	856	3218	2.4	16	1042	1606	1182	305	5127	1.4	114
29	W	50	552	2471	1.8	12	650	2229	1185	277	3414	1.4	91
30	W	100	328	2192	1.3	10	417	1941	1231	289	2192	1.2	77
Control	EW	0	87	2270	0.4	3	78	5456	1516	705	320	0.6	74

Tables 7-10. – The element concentrations (µg g^{-1} DM) and background element values as control in transplanted lichen species (element content analysed by ICP-MS), n=4

a) *Lobaria pulmonaria*

Stand	Side	Distance	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
1	E	10	1300	6.0	1.4	0.38	0.86	0.09	1.6	0.89	21	2.4
2	E	15	1400	3.0	0.69	0.25	0.63	0.13	1.1	0.60	14	1.9
3	E	30	1800	2.1	0.49	0.28	0.54	0.11	0.73	0.47	14	1.7
4	E	50	1800	1.9	0.4	0.28	0.59	0.10	0.85	0.43	15	2.4
5	E	100	1800	1.5	0.32	0.26	0.53	0.13	0.64	0.39	15	2.7
6	E	10	1300	5.1	1.3	0.31	0.76	0.09	1.5	1.30	18	2.2
7	E	15	1700	5.3	1.1	0.28	0.74	0.15	1.4	0.71	18	2.5
8	E	30	2100	3.3	0.72	0.27	0.73	0.17	1.6	0.67	20	2.7
9	E	50	1800	1.6	0.34	0.3	0.56	0.12	0.63	0.32	14	3.1
10	E	100	1800	1.5	0.27	0.34	0.51	0.19	0.58	0.26	14	2.3
11	E	10	1700	5.2	1.2	0.38	0.81	0.09	1.6	1.10	18	2.1
12	E	15	1700	4.2	0.93	0.30	0.71	0.14	1.3	0.73	17	1.9
13	E	30	1600	2.2	0.56	0.24	0.63	0.15	1.1	0.59	15	1.7
14	E	50	2500	1.9	0.43	0.25	0.7	0.16	0.76	0.49	12	1.7
15	E	100	1900	1.3	0.23	0.25	0.52	0.17	0.54	0.30	13	1.6
16	W	10	2300	5.6	1.3	0.39	0.9	0.12	1.8	1.00	18	2.7
17	W	15	1700	2.9	0.75	0.32	0.71	0.11	1.6	0.72	16	2.4
18	W	30	1600	3.2	0.66	0.25	0.62	0.20	1.4	0.63	16	2.2
19	W	50	2000	1.9	0.42	0.31	0.62	0.21	0.84	0.44	19	2.5
20	W	100	2400	1.5	0.27	0.22	0.56	0.18	0.87	0.34	14	2.8
21	W	10	1400	10	2.4	0.45	1.2	0.11	2.4	1.30	26	3.8
22	W	15	2100	4.7	0.87	0.38	0.95	0.12	1.2	0.74	19	2.7
23	W	30	2100	2.8	0.55	0.32	0.83	0.15	0.94	0.51	17	2.5
24	W	50	2100	2.1	0.44	0.27	0.65	0.15	0.89	0.47	14	2.6
25	W	100	2400	1.3	0.25	0.25	0.56	0.20	0.75	0.37	13	2.2
26	W	10	1700	9.2	2.1	0.39	1.2	0.11	2.2	1.20	26	3.3
27	W	15	2600	4.2	0.96	0.29	0.79	0.14	1.6	0.84	19	3.0
28	W	30	2200	3.0	0.60	0.27	0.82	0.14	0.98	0.56	13	2.2
29	W	50	2100	1.6	0.30	0.28	0.62	0.18	0.75	0.35	12	1.9
30	W	100	2500	1.2	0.23	0.32	0.62	0.15	0.52	0.26	14	2.4
Control	EW	0	2600	1.0	0.12	0.23	0.37	0.21	0.29	0.13	32	2.7

b) *Parmelia sulcata*

Stand	Side	Distance	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
1	E	10	1400	12	2.1	0.56	0.57	0.13	1.6	0.13	25	5.3
2	E	15	1900	8.1	1.3	0.52	0.48	0.19	1.2	0.33	17	4.9
3	E	30	2100	4.7	0.7	0.40	0.32	0.23	0.72	0.36	14	3.6
4	E	50	1800	4.1	0.7	0.43	0.34	0.20	0.78	0.43	15	4.4
5	E	100	1700	5.1	0.69	0.57	0.37	0.22	0.83	0.40	17	7.8
6	E	10	1800	9.1	1.6	0.54	0.55	0.16	1.4	0.17	19	5.4
7	E	15	1800	7.9	1.4	0.52	0.52	0.18	1.2	0.35	21	5.2
8	E	30	1800	6.6	1.0	0.52	0.43	0.20	0.82	0.23	15	5.1
9	E	50	1900	4.5	0.7	0.48	0.35	0.19	0.72	0.31	14	4.7
10	E	100	1600	6.6	0.93	0.51	0.4	0.19	0.73	0.28	17	5.7
11	E	10	1500	12	2.2	0.89	0.57	0.15	1.6	0.04	23	7.6
12	E	15	1500	11	1.7	0.61	0.46	0.16	0.8	0.04	21	7.1
13	E	30	1500	6.0	0.92	0.46	0.42	0.20	1.0	0.40	18	4.4
14	E	50	1800	4.3	0.65	0.46	0.34	0.19	0.84	0.53	16	4.6
15	E	100	1900	4.8	0.68	0.43	0.31	0.21	0.63	0.26	14	4.2
16	W	10	1400	13	2.3	0.67	0.67	0.23	0.81	0.20	27	7.7
17	W	15	1400	6.9	1.1	0.65	0.55	0.20	1.1	0.26	20	5.7
18	W	30	1700	6.9	1.0	0.52	0.63	0.19	1.1	0.47	20	5
19	W	50	1900	3.6	0.56	0.38	0.29	0.24	0.62	0.30	15	4.2
20	W	100	2000	3.6	0.48	0.42	0.25	0.19	0.54	0.25	14	4.1
21	W	10	1700	17	3.3	0.87	0.64	0.17	0.98	0.04	39	8.4
22	W	15	1500	9.1	1.5	0.63	0.57	0.17	1.2	0.24	22	7.4
23	W	30	1500	5.0	0.79	0.46	0.35	0.17	0.85	0.33	18	4.5
24	W	50	1500	5.4	0.74	0.46	0.34	0.19	0.76	0.31	17	6.4
25	W	100	1700	4.7	0.6	0.49	0.29	0.21	0.6	0.26	18	5.5
26	W	10	1600	17	3.0	0.78	0.57	0.21	1.0	0.08	30	7.6
27	W	15	1600	6.9	1.1	0.53	0.43	0.25	1.5	0.37	25	5
28	W	30	1500	5.1	0.76	0.45	0.35	0.22	0.77	0.40	17	4.6
29	W	50	1500	5.3	0.72	0.48	0.34	0.21	0.66	0.37	16	5.1
30	W	100	850	3.7	0.55	0.33	0.17	0.16	0.26	0.10	12	4.3
Control	EW	0	1800	4.3	0.55	0.43	0.26	0.20	0.44	0.14	11	4.5

c) *Ramalina farinacea*

Stand	Side	Distance	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
1	E	10	440	5.2	1.1	0.43	0.45	0.29	1.6	0.37	15	3.4
2	E	15	410	2.2	0.55	0.41	0.39	0.09	0.93	0.72	9.9	1.7
3	E	30	350	1.9	0.47	0.33	0.32	0.40	0.88	0.66	10	3.2
4	E	50	400	1.6	0.37	0.30	0.29	0.26	1.1	0.64	9	3.3
5	E	100	520	1.3	0.26	0.33	0.24	0.18	0.75	1.10	6.8	2.5
6	E	10	430	4.2	1.0	0.41	0.54	0.21	1.6	0.81	15	2.9
7	E	15	410	3.7	0.78	0.48	0.51	0.42	1.6	0.86	14	4.1
8	E	30	470	2.8	0.54	0.51	0.43	0.40	1.1	1.00	11	5.6
9	E	50	370	1.6	0.37	0.36	0.3	0.17	0.82	0.53	8.2	2.5
10	E	100	510	1.4	0.31	0.33	0.28	0.20	0.74	0.52	8.6	3.3
11	E	10	460	3.4	0.81	0.35	0.48	0.20	1.4	1.10	14	2.4
12	E	15	520	4.6	0.92	0.47	0.59	0.26	1.6	0.94	15	4.3
13	E	30	450	1.7	0.38	0.36	0.3	0.16	0.89	1.20	6.9	2.0
14	E	50	490	1.5	0.29	0.35	0.25	0.35	0.93	1.50	7.3	3.3
15	E	100	400	3.5	0.73	0.36	0.38	0.13	1.3	0.89	8.4	2.3
16	W	10	460	3.2	0.65	0.45	0.4	0.16	1.3	0.84	9.7	3.0
17	W	15	520	2.3	0.51	0.39	0.32	0.24	1.1	0.64	9.8	3.3
18	W	30	420	1.5	0.29	0.29	0.22	0.24	0.75	0.48	7.3	3.0
19	W	50	510	2.8	0.62	0.33	0.46	0.58	1.4	1.30	14	5.1
20	W	100	590	1.2	0.20	0.31	0.19	0.21	0.47	0.37	6.5	2.8
21	W	10	520	6.4	1.3	0.63	0.64	0.21	1.8	1.60	12	4.3
22	W	15	430	3.1	0.57	0.53	0.38	0.11	1.2	0.73	8.1	2.2
23	W	30	400	1.7	0.39	0.35	0.26	0.21	0.9	0.53	7.8	3
24	W	50	540	1.7	0.31	0.41	0.26	0.13	0.87	0.50	7.6	2.8
25	W	100	450	1.2	0.22	0.31	0.23	0.22	0.62	0.41	6.9	2.9
26	W	10	440	5.6	1.2	0.47	0.59	0.38	1.9	1.30	12	3.9
27	W	15	440	3.6	0.76	0.49	0.44	0.19	1.4	0.95	9.9	3.3
28	W	30	480	1.7	0.42	0.27	0.28	0.12	0.82	0.58	7.8	2.7
29	W	50	640	1.3	0.27	0.29	0.23	0.20	0.68	0.42	6.8	2.5
30	W	100	400	1.0	0.19	0.29	0.18	0.15	0.6	0.35	5.5	2.4
Control	EW	0	450	0.70	0.12	0.36	0.1	0.18	0.42	0.11	2.7	1.7

d) *Usnea filipendula*

Stand	Side	Distance	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
1	E	10	430	4.0	1.6	0.42	0.98	0.10	2.3	1.30	26	3.7
2	E	15	420	3.2	1.1	0.37	0.62	0.15	1.6	0.96	23	3.4
3	E	30	410	2.2	0.75	0.36	0.5	0.13	1.6	0.91	17	3.4
4	E	50	420	1.8	0.62	0.39	0.45	0.19	1.3	0.92	18	3.5
5	E	100	520	1.4	0.39	0.35	0.36	0.14	1.1	1.90	15	3.8
6	E	10	520	5	1.8	0.46	0.74	0.10	2.7	1.30	29	4.1
7	E	15	690	2.5	0.95	0.35	0.52	0.13	1.4	0.90	21	3.3
8	E	30	400	1.9	0.65	0.35	0.46	0.13	1.4	0.89	18	3.8
9	E	50	700	1.6	0.57	0.33	0.4	0.16	1.1	0.76	21	3.1
10	E	100	410	1.3	0.32	0.29	0.32	0.17	1.4	0.54	11	3.3
11	E	10	480	4.2	1.4	0.39	0.8	0.10	2.1	1.30	28	3.5
12	E	15	480	3.7	1.4	0.43	0.81	0.11	2.0	1.40	25	4.2
13	E	30	440	2.4	0.81	0.33	0.60	0.17	1.9	1.30	23	3.9
14	E	50	490	2.0	0.69	0.34	0.50	0.17	1.6	1.30	20	3.7
15	E	100	420	1.2	0.36	0.31	0.32	0.23	1.0	0.67	13	3.3
16	W	10	600	4.2	1.4	0.44	0.64	0.15	1.7	1.00	26	4.2
17	W	15	510	3.1	1.1	0.39	0.56	0.17	2.0	0.92	26	4.6
18	W	30	470	2.3	0.73	0.44	0.46	0.24	1.4	0.81	20	4.6
19	W	50	970	1.4	0.4	0.36	0.35	0.26	1.0	0.70	13	4.0
20	W	100	740	4.5	1.6	0.47	0.65	0.15	1.9	1.20	28	4.7
21	W	10	630	5.9	1.8	0.54	0.73	0.17	2.4	1.10	32	5.5
22	W	15	530	3.7	1.3	0.48	0.61	0.16	1.7	0.89	27	5.3
23	W	30	460	2.4	0.77	0.44	0.47	0.19	1.4	0.81	21	5.5
24	W	50	530	1.6	0.49	0.35	0.33	0.17	1.0	0.64	20	4.1
25	W	100	610	1.3	0.36	0.40	0.28	0.25	1.1	0.50	20	5.1
26	W	10	660	7.3	2.4	0.60	0.69	0.19	1.7	0.38	35	5.3
27	W	15	610	3.5	1.2	0.44	0.55	0.15	1.6	0.80	27	4.6
28	W	30	470	1.9	0.66	0.36	0.37	0.24	1.1	0.74	21	4.1
29	W	50	700	1.3	0.44	0.36	0.31	0.21	1.2	0.55	14	3.9
30	W	100	450	0.92	0.29	0.37	0.24	0.20	1.1	0.46	15	4.1
Control	EW	420	0	0.34	0.072	0.12	0.062	0.25	0.14	0.06	9.7	1.3

Table 11. – Coefficient of Variance (CV) for the major elements in transplanted lichens

Species	P	K	Ca	Mg	Na	Fe	Al	Zn	Mn
L. pulmonaria	19.0	39.1	29	25	78.2	69.2	58.7	24.1	25.6
P. sulcata	14.8	15.2	35.4	29.6	81.1	55	45.5	37.6	27.6
R. farinacea	14.0	14.2	60.8	26.6	75	51.9	47	28.2	25.7
U. filipendula	24.1	21.6	33	18.4	81.7	53.4	45	34.6	31.1

Table 12. – Coefficient of Variance (CV) for the trace elements in transplanted lichens

L. pulmonaria	55.2	50.6	50.1	66.2	71.8	18.3	24.8	24.6	42.7	47.9	21.7	20.
P. sulcata	45.6	46.1	38.4	50.6	61.4	24.7	30.1	14.2	34.8	47.4	29.5	24.
R. farinacea	38.1	38.7	38.7	55.0	55.1	22.3	35.5	46.8	35.0	43.6	29.9	27.
U. filipendula	43.2	18.3	34.4	55.4	57.2	17.1	34.9	26.3	28.8	36.2	27.2	16.

Table 13. – The comparison of transplanted lichen species with the background value due to the element content accumulation capacity (the major element content)

Species	Times higher than background value								
	P	K	Ca	Mg	Na	Fe	Al	Zn	Mn
L. pulmonaria	4.6	0.4	0.6	0.8	15.6	5.3	3.8	1.2	0.3
P. sulcata	3.9	0.7	0.8	1.2	18.9	1.9	1.8	1.6	2.2
R. farinacea	1.1	0.7	2.0	1.2	10.2	6.2	4.3	2.3	1.8
U. filipendula	1.3	0.3	1.5	0.9	35.1	16.9	11.9	1.9	0.3

Table 14. – The comparison of transplanted lichen species with the background value due to the element content accumulation capacity (the trace element content)

Species	Times higher than background value											
	Cr	Cu	Ni	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
L. pulmonaria	3.4	2.3	5.1	9.9	10.4	2.5	11.5	0.60	8.3	10.5	1.7	1.8
P. sulcata	1.7	1.6	1.6	21.6	16.6	4.5	6.9	0.80	6.6	4.6	2	4.2
R. farinacea	2.8	2.9	4.7	7.7	7.8	3.2	5.8	0.90	7.9	13.2	1	2.4
U. filipendula	6.7	9.2	3.6	8.2	13.1	3.3	8.4	0.70	11.1	15.5	2.2	3.2

Table 15. – Pearson's correlation coefficients between elemental concentration within the lichen species (n=30; df=28)

	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
Al	1	**0.81**	**0.99**	**0.96**	**0.99**	**-0.83**	**0.94**	n.s	**0.60**	**0.95**	**0.94**	-0.42	**0.99**	**0.98**	**0.79**	**0.93**	-0.53	**0.90**	**0.89**	**0.90**	**0.58**
Ca		1	**0.80**	**0.79**	**0.83**	**-0.84**	**0.90**	0.43	**0.63**	**0.86**	**0.74**	-0.55	**0.83**	**0.85**	**0.78**	**0.69**	**-0.59**	**0.71**	**0.74**	**0.82**	0.49
Cr			1	**0.97**	**0.99**	**-0.82**	**0.94**	n.s	0.57	**0.95**	**0.93**	-0.42	**0.98**	**0.98**	**0.75**	**0.91**	-0.52	**0.91**	**0.88**	**0.89**	**0.59**
Cu				1	**0.97**	**-0.82**	**0.92**	n.s	0.55	**0.92**	**0.94**	n.s	**0.96**	**0.97**	**0.74**	**0.91**	-0.47	**0.95**	**0.90**	**0.90**	**0.61**
Fe					1	**-0.87**	**0.95**	n.s	**0.62**	**0.96**	**0.94**	-0.45	**0.99**	**0.99**	**0.76**	**0.92**	-0.54	**0.92**	**0.90**	**0.89**	0.57
K						1	**-0.82**	n.s	**-0.86**	**-0.89**	**-0.82**	**0.65**	**-0.87**	**-0.90**	**-0.64**	**-0.69**	**0.66**	**-0.83**	**-0.88**	**-0.74**	n.s
Mg							1	0.44	**0.58**	**0.94**	**0.89**	-0.38	**0.95**	**0.95**	**0.77**	**0.85**	-0.55	**0.84**	**0.83**	**0.89**	**0.58**
Mn								1	n.s	0.41	0.38	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.52	0.51
Na									1	**0.68**	**0.66**	**-0.63**	**0.65**	**0.68**	0.42	0.45	**-0.66**	**0.62**	**0.77**	0.52	n.s
Ni										1	**0.90**	-0.48	**0.96**	**0.96**	**0.74**	**0.86**	**-0.57**	**0.87**	**0.86**	**0.88**	**0.58**
Zn											1	-0.36	**0.95**	**0.95**	**0.67**	**0.87**	-0.44	**0.94**	**0.91**	**0.89**	0.51
P												1	-0.42	-0.46	n.s	n.s	0.45	-0.37	-0.44	-0.36	n.s
V													1	**0.99**	**0.76**	**0.92**	-0.53	**0.92**	**0.91**	**0.90**	0.56
Co														1	**0.75**	**0.90**	-0.56	**0.93**	**0.93**	**0.89**	0.54
As															1	**0.77**	-0.52	**0.64**	**0.66**	**0.74**	0.54
Mo																1	-0.48	**0.84**	**0.82**	**0.84**	**0.58**
Cd																	1	-0.46	**-0.61**	-0.39	n.s
Sn																		1	**0.92**	**0.86**	0.52
Sb																			1	**0.79**	0.39
Ba																				1	**0.68**
Pb																					1

L. pulmonaria

	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
Al	1	**0.73**	**0.99**	**0.95**	**0.99**	**-0.61**	**0.97**	**0.70**	**0.64**	**0.99**	**0.93**	n.s	**0.99**	**0.98**	**0.87**	**0.80**	n.s	**0.58**	**-0.67**	**0.90**	**0.73**
Ca		1	**0.71**	**0.67**	**0.72**	n.s	**0.77**	0.41	0.51	**0.73**	**0.67**	n.s	**0.72**	**0.70**	0.50	0.53	n.s	n.s	-0.49	0.56	0.40
Cr			1	**0.94**	**0.99**	**-0.60**	**0.97**	**0.72**	**0.59**	**0.99**	**0.93**	n.s	**0.99**	**0.98**	**0.89**	**0.81**	n.s	0.52	**-0.68**	**0.89**	**0.78**
Cu				1	**0.95**	**-0.68**	**0.94**	**0.67**	0.57	**0.95**	**0.99**	n.s	**0.97**	**0.98**	**0.85**	**0.82**	n.s	0.52	**-0.58**	**0.94**	**0.71**
Fe					1	**-0.63**	**0.97**	**0.70**	**0.60**	**0.99**	**0.93**	n.s	**0.99**	**0.99**	**0.88**	**0.78**	n.s	0.52	**-0.69**	**0.89**	**0.75**
K						1	-0.56	n.s	-0.51	**-0.61**	**-0.65**	0.47	**-0.66**	**-0.70**	-0.50	-0.51	0.53	n.s	**0.65**	**-0.60**	-0.40
Mg							1	**0.71**	0.57	**0.97**	**0.92**	n.s	**0.97**	**0.97**	**0.84**	**0.74**	n.s	0.44	**-0.67**	**0.87**	**0.74**
Mn								1	n.s	**0.74**	**0.69**	n.s	**0.70**	**0.68**	**0.68**	0.54	n.s	n.s	-0.42	**0.73**	**0.69**
Na									1	**0.60**	0.55	n.s	**0.61**	**0.61**	0.47	**0.61**	-0.51	**0.76**	-0.45	0.42	n.s
Ni										1	**0.93**	n.s	**0.99**	**0.98**	**0.88**	**0.79**	n.s	0.51	**-0.69**	**0.89**	**0.77**
Zn											1	n.s	**0.95**	**0.97**	**0.84**	**0.82**	n.s	0.54	-0.52	**0.95**	**0.72**
P												1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
V													1	**0.99**	**0.88**	**0.82**	n.s	0.52	**-0.68**	**0.91**	**0.76**
Co														1	**0.87**	**0.81**	n.s	0.52	**-0.67**	**0.92**	**0.74**
As															1	**0.79**	n.s	0.56	**-0.58**	**0.83**	**0.85**
Mo																1	n.s	**0.72**	n.s	**0.78**	**0.64**
Cd																	1	n.s	0.50	n.s	n.s
Sn																		1	n.s	0.52	n.s
Sb																			1	-0.49	-0.54
Ba																				1	**0.73**
Pb																					1

P. sulcata

Note. Corr. coefficients significance is marked in bold numbers <0.001, normal <0.01 and n.s. >0.05

	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
Al	1	0.51	**0.96**	**0.89**	**0.94**	-0.37	**0.94**	**0.79**	**0.85**	**0.94**	**0.95**	n.s	**0.91**	**0.94**	**0.67**	**0.93**	n.s	**0.91**	0.39	**0.93**	0.44
Ca		1	0.50	0.37	0.42	n.s	0.51	0.53	0.42	0.51	0.51	n.s	0.42	0.43	n.s	0.48	**0.92**	0.51	n.s	**0.65**	**0.70**
Cr			1	**0.95**	**0.99**	-0.47	**0.95**	**0.80**	**0.69**	**0.98**	**0.94**	n.s	**0.97**	**0.98**	**0.77**	**0.96**	n.s	**0.96**	0.51	**0.86**	0.51
Cu				1	**0.97**	-0.50	**0.91**	**0.76**	**0.62**	**0.94**	**0.93**	n.s	**0.97**	**0.97**	**0.70**	**0.93**	n.s	**0.94**	0.52	**0.78**	n.s
Fe					1	-0.50	**0.94**	**0.79**	**0.66**	**0.98**	**0.93**	n.s	**0.99**	**0.99**	**0.77**	**0.95**	n.s	**0.96**	0.49	**0.82**	0.42
K						1	n.s	n.s	n.s	-0.48	n.s	0.55	-0.49	-0.52	n.s	-0.43	n.s	-0.53	n.s	n.s	n.s
Mg							1	**0.86**	**0.70**	**0.94**	**0.96**	n.s	**0.93**	**0.94**	**0.69**	**0.92**	n.s	**0.92**	0.47	**0.86**	0.52
Mn								1	**0.62**	**0.77**	**0.85**	n.s	**0.78**	**0.80**	0.55	**0.83**	0.44	**0.80**	0.48	**0.79**	0.54
Na									1	**0.68**	**0.78**	n.s	**0.61**	**0.68**	n.s	**0.68**	n.s	**0.65**	n.s	**0.87**	n.s
Ni										1	**0.93**	n.s	**0.97**	**0.98**	**0.73**	**0.93**	n.s	**0.95**	0.46	**0.83**	0.47
Zn											1	n.s	**0.91**	**0.94**	**0.61**	**0.93**	n.s	**0.91**	0.48	**0.89**	0.42
P												1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
V													1	**0.99**	**0.78**	**0.94**	n.s	**0.95**	0.50	**0.78**	0.43
Co														1	**0.72**	**0.95**	n.s	**0.95**	0.49	**0.82**	0.39
As															1	**0.76**	n.s	**0.72**	0.48	0.50	0.42
Mo																1	n.s	**0.95**	**0.59**	**0.87**	0.51
Cd																	1	n.s	0.36	0.49	**0.79**
Sn																		1	0.57	**0.84**	0.48
Sb																			1	n.s	0.39
Ba																				1	0.54
Pb																					1

R. farinacea

	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
Al	1	**0.88**	**0.98**	**0.92**	**0.99**	**-0.63**	**0.68**	**-0.61**	0.41	**0.96**	**0.96**	n.s	**0.98**	**0.98**	**0.81**	**0.86**	-0.48	**0.81**	n.s	**0.95**	0.44
Ca		1	**0.90**	**0.82**	**0.91**	-0.36	**0.89**	n.s	n.s	**0.92**	**0.87**	n.s	**0.90**	**0.87**	**0.86**	**0.64**	n.s	**0.64**	n.s	**0.91**	**0.67**
Cr			1	**0.91**	**0.99**	**-0.59**	**0.74**	**-0.62**	n.s	**0.97**	**0.94**	n.s	**0.99**	**0.98**	**0.87**	**0.83**	-0.42	**0.78**	n.s	**0.93**	0.51
Cu				1	**0.90**	**-0.70**	**0.60**	**-0.65**	0.39	**0.92**	**0.93**	n.s	**0.89**	**0.91**	**0.69**	**0.89**	-0.54	**0.92**	0.48	**0.86**	0.37
Fe					1	**-0.58**	**0.74**	**-0.60**	n.s	**0.97**	**0.95**	n.s	**0.99**	**0.98**	**0.85**	**0.81**	-0.41	**0.77**	n.s	**0.94**	0.49
K						1	n.s	**0.68**	**-0.65**	-0.53	**-0.62**	0.47	**-0.58**	**-0.64**	n.s	**-0.77**	**0.60**	**-0.76**	-0.43	-0.48	n.s
Mg							1	n.s	n.s	**0.78**	**0.68**	n.s	**0.73**	**0.69**	**0.84**	0.40	n.s	0.44	n.s	**0.76**	**0.74**
Mn								1	-0.49	-0.54	-0.56	n.s	**-0.60**	**-0.64**	-0.43	**-0.67**	**0.67**	**-0.62**	-0.49	-0.45	n.s
Na									1	n.s	0.46	n.s	0.36	0.45	n.s	**0.63**	**-0.70**	0.55	0.41	n.s	n.s
Ni										1	**0.94**	n.s	**0.96**	**0.95**	**0.85**	**0.81**	-0.37	**0.80**	n.s	**0.92**	0.55
Zn											1	n.s	**0.95**	**0.97**	**0.78**	**0.87**	-0.49	**0.84**	n.s	**0.94**	0.40
P												1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
V													1	**0.99**	**0.86**	**0.81**	-0.43	**0.77**	n.s	**0.92**	0.48
Co														1	**0.83**	**0.87**	-0.50	**0.81**	n.s	**0.93**	0.43
As															1	0.56	n.s	0.53	n.s	**0.81**	**0.78**
Mo																1	**-0.69**	**0.89**	0.53	**0.79**	n.s
Cd																	1	**-0.65**	**-0.62**	-0.42	n.s
Sn																		1	0.51	**0.74**	n.s
Sb																			1	n.s	n.s
Ba																				1	0.54
Pb																					1

U. filipendula

Note. Corr. coefficients significance is marked in bold numbers <0.001, normal <0.01 and n.s. >0.05

Table 16. – Pearson's correlation coefficients of element concentration in the total atmospheric deposition, combination of all studied species (n=120; df=118)

	Al	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Zn	P	V	Co	As	Mo	Cd	Sn	Sb	Ba	Pb
Al	1	n.s	**0.99**	**0.57**	**0.98**	n.s	**0.78**	n.s	0.29	**0.97**	**0.46**	0.21	**0.98**	**0.91**	**0.84**	**0.36**	n.s	**0.30**	-0.21	**0.67**	**0.72**
Ca		1	n.s	n.s	n.s	**-0.46**	n.s	-0.21	n.s	n.s	n.s	**-0.45**	n.s	n.s	0.20	n.s	**0.77**	0.21	0.24	n.s	0.18
Cr			1	**0.60**	**0.99**	n.s	**0.78**	n.s	0.25	**0.98**	**0.47**	0.24	**0.99**	**0.92**	**0.82**	**0.41**	n.s	**0.32**	-0.19	**0.66**	**0.70**
Cu				1	**0.62**	**-0.34**	**0.67**	0.28	**0.53**	**0.60**	**0.90**	n.s	**0.54**	**0.83**	**0.38**	**0.75**	-0.27	**0.85**	**0.45**	**0.80**	0.27
Fe					1	n.s	**0.77**	n.s	0.25	**0.98**	**0.47**	0.24	**0.99**	**0.93**	**0.80**	**0.43**	n.s	**0.33**	n.s	**0.65**	**0.65**
K						1	n.s	-0.23	**-0.37**	n.s	-0.25	**0.89**	n.s	n.s	n.s	n.s	n.s	**-0.52**	**-0.63**	n.s	n.s
Mg							1	**0.48**	**0.34**	**0.83**	**0.71**	n.s	**0.76**	**0.83**	**0.67**	**0.40**	n.s	**0.40**	n.s	**0.89**	**0.71**
Mn								1	0.27	n.s	**0.47**	-0.29	n.s	n.s	n.s	n.s	n.s	**0.31**	0.20	**0.47**	0.22
Na									1	0.25	**0.62**	-0.27	0.21	**0.46**	n.s	**0.40**	-0.25	**0.63**	**0.38**	**0.48**	n.s
Ni										1	**0.49**	0.29	**0.98**	**0.91**	**0.79**	**0.42**	n.s	**0.31**	-0.21	**0.70**	**0.70**
Zn											1	n.s	**0.40**	**0.74**	**0.31**	**0.67**	-0.29	**0.77**	**0.36**	**0.89**	0.28
P												1	0.28	n.s	**n.s**	**0.35**	-0.23	**-0.31**	**-0.49**	n.s	n.s
V													1	**0.90**	**0.82**	**0.37**	n.s	0.24	-0.25	**0.62**	**0.69**
Co														1	**0.74**	**0.57**	n.s	**0.56**	n.s	**0.81**	**0.59**
As															1	n.s	n.s	0.20	-0.20	**0.52**	**0.84**
Mo																1	**-0.38**	**0.64**	**0.33**	**0.58**	n.s
Cd																	1	n.s	n.s	-0.23	0.26
Sn																		1	**0.65**	**0.58**	n.s
Sb																			1	n.s	-0.26
Ba																				1	**0.55**
Pb																					1

Note. Corr. coefficients significance is marked in bold numbers <0.001, normal <0.01 and n.s. >0.05

Table 18. – Results of two way ANOVA with distance from the road and side of the road as factors that determine the element content for each studied lichen species

	L. pulmonaria			*P. sulcata*			*R. farinacea*			*U. filipendula*		
Element	**Side**	**Distance**	**S x D**	**Side**	**Distance**	**S x D**	**Side**	**Distance**	**S x D**	**Side**	**Distance**	**S x D**
Ca	n.s.	0.000	0.04	n.s.	0.000	<0.01	n.s.	n.s.	n.s.	<0.001	0.000	n.s.
Mg	n.s.	0.000	0.02	n.s.	0.000	<0.001	n.s.	n.s.	n.s.	<0.001	0.01	n.s.
Na	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	<0.001	0.000	0.01	n.s.
K	<0.01	0.000	n.s.	n.s.	0.000	n.s.	n.s.	n.s.	n.s.	<0.01	<0.001	n.s.
P	<0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.
Fe	n.s.	0.000	n.s.	n.s.	0.000	0.03	n.s.	0.000	n.s.	n.s.	0.000	n.s.
Al	0.03	0.000	n.s.	n.s.	0.000	0.02	<0.01	0.000	n.s.	n.s.	0.000	n.s.
Mn	n.s.	n.s.	n.s.	n.s.	0.03	n.s.	n.s.	0.01	n.s.	n.s.	0.02	n.s.
Zn	0.01	0.000	n.s.	0.03	0.000	<0.001	0.01	0.000	n.s.	n.s.	0.000	n.s.
Cu	<0.01	0.000	0.02	0.03	0.000	0.000	n.s.	<0.001	n.s.	n.s.	<0.001	n.s.
Ba	n.s.	<0.001	n.s.	0.01	0.000	0.04	0.01	<0.001	n.s.	0.04	0.000	n.s.
V	n.s.	0.000	n.s.	n.s.	0.000	<0.001	n.s.	0.000	n.s.	n.s.	0.000	n.s.
Pb	n.s.	0.010	n.s.	0.34	<0.01	n.s.	n.s.	n.s.	n.s.	0.000	n.s.	n.s.
Cr	0.04	0.000	n.s.	n.s.	0.000	0.02	n.s.	0.000	n.s.	n.s.	0.000	n.s.
Ni	n.s.	0.000	n.s.	0.34	0.000	<0.01	n.s.	0.000	n.s.	n.s.	0.000	n.s.
Sn	0.03	0.000	n.s.	0.02	0.000	0.01	n.s.	0.000	n.s.	n.s.	0.000	n.s.
Co	n.s.	0.000	0.04	n.s.	0.000	0.02	n.s.	0.000	n.s.	n.s.	0.000	0.04
Sb	n.s.	0.000	n.s.	n.s.	<0.001	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.
Mo	<0.01	0.000	n.s.	n.s.	0.000	n.s.	n.s.	0.000	n.s.	n.s.	0.000	n.s.
As	n.s.	<0.001	n.s.	n.s.	0.000	0.02	n.s.	<0.001	n.s.	<0.001	<0.001	n.s.
Cd	0.04	<0.001	n.s.	n.s.	n.s.	0.04	n.s.	n.s.	n.s.	<0.001	<0.01	n.s.

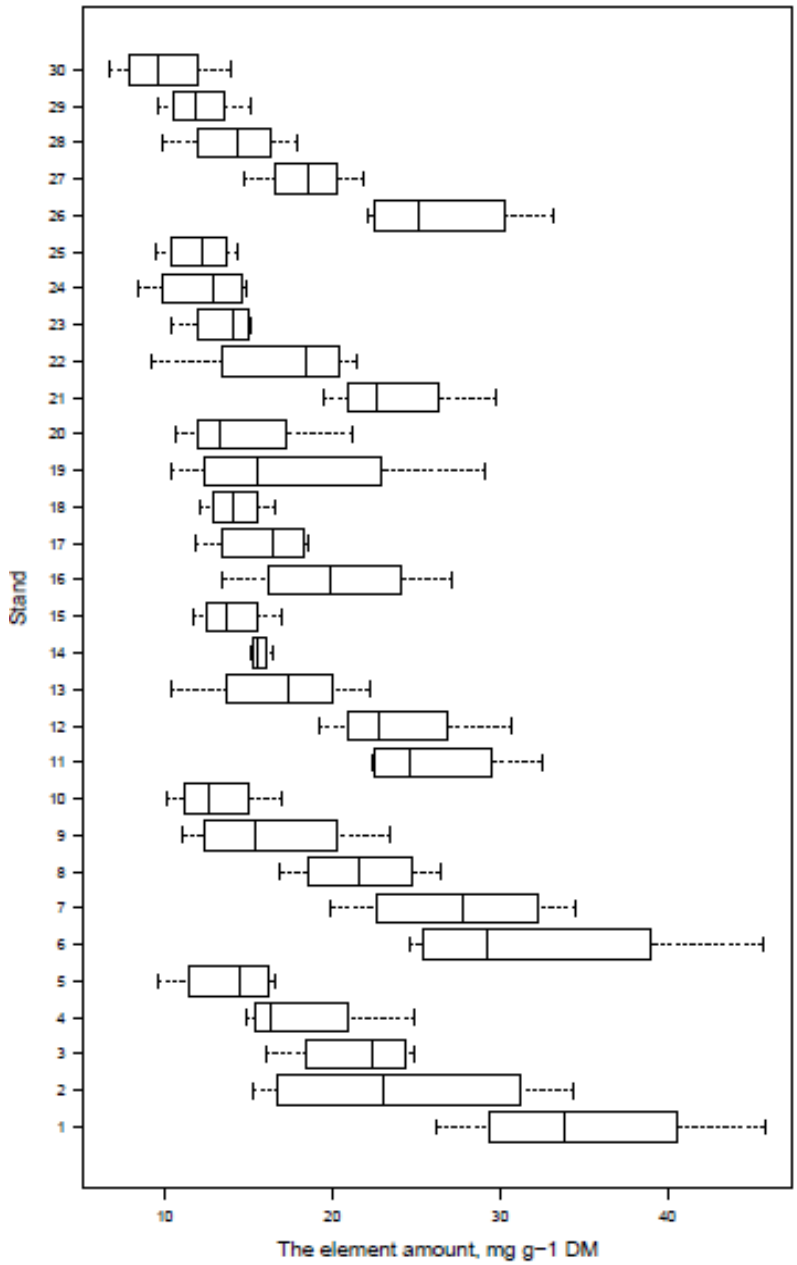

Figure 12. – Box plots of total element accumulation by lichen samples (in mg g^{-1} DM) among the stands at the field site (F= 6.62; df=90; P=0.000)

Tables 21-24. – Means ± (SD) of the physiological parameters for the samples of the lichen species analyzed by prior to ($Wght_{start}$ – weight, g; $Area_{start}$ – thallus area surface, cm^2) and after the field exposure ($Wght_{end}$) with the determination of dry matter growth (DM growth, %), (n= 4)

a) *Lobaria pulmonaria*

Stand	$Wght_{start}$			$Wght_{end}$			DM growth			$Area_{start}$		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.10	±	0.02	0.10	±	0.02	-1.60	±	1.15	8.90	±	2.73
2	0.12	±	0.01	0.12	±	0.01	4.87	±	2.80	8.76	±	1.58
3	0.21	±	0.07	0.23	±	0.08	6.51	±	3.48	15.21	±	5.44
4	0.25	±	0.06	0.26	±	0.07	4.07	±	6.12	17.50	±	2.11
5	0.20	±	0.06	0.22	±	0.07	8.02	±	3.35	15.29	±	5.05
6	0.16	±	0.01	0.14	±	0.03	-6.58	±	15.67	12.98	±	0.51
7	0.16	±	0.02	0.15	±	0.01	-3.74	±	6.54	12.46	±	1.48
8	0.19	±	0.03	0.20	±	0.04	1.51	±	6.57	13.93	±	1.87
9	0.22	±	0.08	0.23	±	0.08	6.84	±	4.78	14.47	±	6.00
10	0.22	±	0.13	0.22	±	0.12	4.17	±	5.93	14.02	±	4.72
11	0.14	±	0.03	0.14	±	0.04	0.76	±	2.97	10.12	±	1.91
12	0.20	±	0.10	0.21	±	0.11	7.37	±	7.30	14.92	±	7.29
13	0.18	±	0.01	0.19	±	0.01	7.07	±	3.84	14.70	±	2.20
14	0.23	±	0.06	0.25	±	0.06	6.82	±	1.01	16.65	±	2.80
15	0.21	±	0.06	0.22	±	0.07	3.68	±	3.43	15.63	±	1.56
16	0.21	±	0.11	0.22	±	0.12	3.34	±	5.55	14.56	±	9.00
17	0.15	±	0.03	0.16	±	0.03	3.49	±	1.65	12.91	±	3.71
18	0.17	±	0.05	0.18	±	0.05	9.31	±	3.30	13.49	±	2.15
19	0.22	±	0.05	0.24	±	0.04	7.35	±	6.85	15.40	±	2.74
20	0.18	±	0.04	0.19	±	0.05	7.56	±	4.44	12.70	±	3.01
21	0.14	±	0.03	0.14	±	0.04	-0.90	±	4.52	10.66	±	2.47
22	0.22	±	0.02	0.24	±	0.02	9.34	±	4.07	14.88	±	2.49
23	0.23	±	0.05	0.24	±	0.06	5.09	±	3.03	16.53	±	3.84
24	0.19	±	0.05	0.20	±	0.06	6.06	±	3.76	14.37	±	4.85
25	0.13	±	0.01	0.14	±	0.01	8.90	±	2.91	10.83	±	0.22
26	0.16	±	0.03	0.14	±	0.02	-7.78	±	4.80	11.97	±	2.46
27	0.14	±	0.01	0.16	±	0.02	9.22	±	9.62	11.33	±	0.53
28	0.20	±	0.03	0.22	±	0.03	9.26	±	3.60	14.95	±	2.61
29	0.18	±	0.03	0.20	±	0.03	9.82	±	3.93	13.47	±	1.87
30	0.19	±	0.03	0.20	±	0.02	7.15	±	4.61	13.48	±	2.05

b) *Parmelia sulcata*

Stand	Wght start			Wght end			DM growth			Area start		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.41	±	0.20	0.43	±	0.22	3.83	±	9.36	14.75	±	5.76
2	0.34	±	0.05	0.36	±	0.03	9.89	±	25.33	11.89	±	1.84
3	0.24	±	0.03	0.26	±	0.05	7.95	±	14.20	10.50	±	1.84
4	0.30	±	0.05	0.34	±	0.06	14.86	±	2.01	12.31	±	1.37
5	0.38	±	0.15	0.41	±	0.15	9.52	±	5.73	14.35	±	4.30
6	0.34	±	0.17	0.34	±	0.17	3.20	±	16.84	12.67	±	4.38
7	0.34	±	0.14	0.38	±	0.14	13.34	±	6.55	12.92	±	1.71
8	0.49	±	0.21	0.54	±	0.22	12.10	±	5.46	14.54	±	3.08
9	0.40	±	0.03	0.44	±	0.03	10.81	±	3.87	13.15	±	2.00
10	0.30	±	0.03	0.33	±	0.03	9.00	±	1.15	10.58	±	.69
11	0.36	±	0.11	0.34	±	0.13	-6.36	±	11.98	11.89	±	3.32
12	0.42	±	0.16	0.47	±	0.15	11.60	±	5.77	13.11	±	2.38
13	0.36	±	0.22	0.39	±	0.23	9.88	±	4.10	12.43	±	3.80
14	0.35	±	0.08	0.39	±	0.07	12.49	±	4.66	13.06	±	2.33
15	0.45	±	0.25	0.47	±	0.27	3.35	±	7.27	13.87	±	5.00
16	0.34	±	0.15	0.40	±	0.18	16.85	±	2.20	13.35	±	5.69
17	0.28	±	0.09	0.32	±	0.10	15.96	±	5.11	10.11	±	2.18
18	0.30	±	0.09	0.35	±	0.09	17.96	±	5.62	11.63	±	1.23
19	0.28	±	0.09	0.33	±	0.10	19.50	±	8.27	9.97	±	1.86
20	0.31	±	0.08	0.34	±	0.07	11.78	±	9.87	12.07	±	1.47
21	0.25	±	0.11	0.30	±	0.14	17.92	±	15.18	9.90	±	1.43
22	0.29	±	0.12	0.35	±	0.14	24.77	±	9.00	11.46	±	3.10
23	0.26	±	0.04	0.28	±	0.04	10.05	±	5.20	10.00	±	1.99
24	0.41	±	0.10	0.45	±	0.12	8.17	±	5.77	13.75	±	2.71
25	0.38	±	0.23	0.42	±	0.25	12.69	±	5.00	11.98	±	4.06
26	0.46	±	0.16	0.59	±	0.21	27.18	±	10.35	13.08	±	2.08
27	0.36	±	0.07	0.43	±	0.08	20.13	±	2.31	12.57	±	2.99
28	0.37	±	0.13	0.42	±	0.14	12.41	±	7.75	13.93	±	3.46
29	0.33	±	0.13	0.38	±	0.14	15.27	±	6.23	12.31	±	3.08
30	0.29	±	0.13	0.33	±	0.13	15.56	±	10.66	10.44	±	2.62

c) *Ramalina farinacea*

Stand	Wght start			Wght end			DM growth			Area start		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.23	±	0.05	0.23	±	0.07	-0.85	±	10.50	12.11	±	2.32
2	0.25	±	0.07	0.25	±	0.06	3.69	±	5.26	11.31	±	2.45
3	0.30	±	0.09	0.30	±	0.09	-0.31	±	3.14	12.57	±	4.17
4	0.20	±	0.06	0.17	±	0.04	-10.35	±	8.36	8.58	±	3.05
5	0.19	±	0.10	0.19	±	0.12	-2.58	±	13.81	9.65	±	4.33
6	0.26	±	0.08	0.25	±	0.08	-2.60	±	2.65	10.49	±	2.27
7	0.30	±	0.08	0.27	±	0.08	-11.76	±	7.08	11.98	±	2.52
8	0.20	±	0.11	0.19	±	0.12	-6.02	±	13.37	9.92	±	3.83
9	0.25	±	0.09	0.26	±	0.09	3.85	±	5.82	12.11	±	4.07
10	0.24	±	0.02	0.23	±	0.01	-3.67	±	7.26	11.83	±	1.52
11	0.22	±	0.08	0.21	±	0.09	-4.93	±	10.74	9.68	±	1.19
12	0.31	±	0.06	0.32	±	0.06	2.18	±	4.24	11.95	±	1.37
13	0.17	±	0.05	0.17	±	0.05	-1.24	±	.71	8.36	±	2.97
14	0.24	±	0.13	0.24	±	0.12	4.35	±	8.56	11.93	±	5.12
15	0.21	±	0.08	0.20	±	0.07	-2.35	±	5.68	9.95	±	3.02
16	0.21	±	0.08	0.20	±	0.07	-5.53	±	9.30	9.21	±	2.78
17	0.16	±	0.03	0.16	±	0.03	3.34	±	2.95	7.96	±	1.28
18	0.24	±	0.13	0.24	±	0.12	-1.22	±	5.74	10.62	±	4.78
19	0.19	±	0.04	0.19	±	0.03	-1.16	±	8.29	9.60	±	1.95
20	0.28	±	0.11	0.27	±	0.11	-4.29	±	7.06	11.85	±	3.58
21	0.23	±	0.05	0.24	±	0.06	0.50	±	6.58	10.76	±	1.68
22	0.20	±	0.04	0.20	±	0.05	-3.33	±	5.05	8.34	±	1.71
23	0.17	±	0.03	0.18	±	0.04	5.16	±	5.11	8.17	±	1.31
24	0.20	±	0.07	0.18	±	0.06	-6.82	±	14.19	9.95	±	2.53
25	0.26	±	0.12	0.26	±	0.13	-3.39	±	11.72	10.98	±	3.95
26	0.22	±	0.10	0.21	±	0.10	-3.45	±	2.31	10.14	±	3.47
27	0.17	±	0.03	0.16	±	0.04	-5.63	±	4.41	7.16	±	1.14
28	0.21	±	0.14	0.22	±	0.12	6.37	±	9.20	10.55	±	5.76
29	0.24	±	0.08	0.24	±	0.07	1.82	±	11.14	10.29	±	3.02
30	0.24	±	0.06	0.24	±	0.09	-4.80	±	17.19	11.50	±	2.98

d) *Usnea filipendula*

Stand	Wght start			Wght end			DM growth			Area start		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.20	±	0.06	0.20	±	0.05	0.32	±	4.36	7.27	±	2.44
2	0.25	±	0.09	0.25	±	0.09	3.04	±	4.26	10.58	±	4.35
3	0.24	±	0.10	0.25	±	0.10	3.06	±	2.14	9.94	±	4.26
4	0.23	±	0.10	0.23	±	0.10	1.11	±	4.70	9.28	±	2.17
5	0.19	±	0.08	0.19	±	0.08	0.42	±	3.98	7.64	±	2.88
6	0.22	±	0.05	0.22	±	0.04	-0.89	±	6.33	9.21	±	3.07
7	0.19	±	0.04	0.20	±	0.05	5.55	±	6.38	9.65	±	3.17
8	0.26	±	0.14	0.27	±	0.14	1.79	±	1.58	9.20	±	4.11
9	0.27	±	0.05	0.29	±	0.05	6.99	±	6.84	13.24	±	3.09
10	0.26	±	0.08	0.27	±	0.08	5.13	±	5.10	12.74	±	4.58
11	0.21	±	0.06	0.22	±	0.07	4.57	±	1.85	7.73	±	2.28
12	0.18	±	0.05	0.18	±	0.06	1.86	±	3.70	7.34	±	2.84
13	0.19	±	0.13	0.20	±	0.14	1.19	±	3.73	6.95	±	3.91
14	0.25	±	0.09	0.26	±	0.10	5.27	±	2.85	11.69	±	5.54
15	0.21	±	0.06	0.22	±	0.07	2.46	±	4.82	9.10	±	4.86
16	0.18	±	0.08	0.18	±	0.08	3.56	±	2.45	7.80	±	3.81
17	0.22	±	0.10	0.23	±	0.09	5.33	±	3.17	9.41	±	2.70
18	0.22	±	0.07	0.22	±	0.07	2.42	±	1.48	11.34	±	5.19
19	0.16	±	0.06	0.17	±	0.06	3.69	±	2.77	6.71	±	3.67
20	0.22	±	0.11	0.22	±	0.11	0.79	±	4.52	9.44	±	4.17
21	0.22	±	0.05	0.22	±	0.04	1.28	±	2.82	10.24	±	4.24
22	0.24	±	0.11	0.24	±	0.13	2.53	±	8.09	11.33	±	5.46
23	0.29	±	0.20	0.30	±	0.20	2.37	±	1.93	10.47	±	6.27
24	0.27	±	0.17	0.27	±	0.17	4.20	±	3.45	11.63	±	7.03
25	0.24	±	0.09	0.25	±	0.08	3.47	±	6.78	9.88	±	3.26
26	0.25	±	0.10	0.25	±	0.11	1.78	±	4.31	10.52	±	3.70
27	0.17	±	0.02	0.18	±	0.02	3.06	±	1.88	7.66	±	1.02
28	0.19	±	0.03	0.19	±	0.04	0.76	±	3.75	7.32	±	1.38
29	0.22	±	0.08	0.22	±	0.08	3.69	±	2.38	8.94	±	3.43
30	0.24	±	0.11	0.24	±	0.11	3.77	±	3.51	9.03	±	1.77

Tables 25-28. – Means (±SD) of the physiological parameters which characterize the photosynthetic activity of the lichen photobionts among the species analyzed after the field exposure, where concentration of pigments in μg mg^{-1}, (n= 4)

a) *Lobaria pulmonaria*

Stand	Chl *a* end			Chl *b* end			Chl a/*b* end			Chl (a+b) end			Fv/Fm end		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.689	±	0.13	0.276	±	0.02	2.48	±	0.26	0.965	±	0.15	0.341	±	0.15
2	0.661	±	0.22	0.242	±	0.08	2.73	±	0.05	0.903	±	0.30	0.432	±	0.08
3	1.024	±	0.40	0.365	±	0.16	2.87	±	0.25	1.389	±	0.56	0.517	±	0.06
4	0.859	±	0.57	0.296	±	0.21	2.97	±	0.13	1.155	±	0.78	0.477	±	0.07
5	0.956	±	0.31	0.311	±	0.10	3.07	±	0.08	1.267	±	0.40	0.531	±	0.12
6	0.707	±	0.37	0.265	±	0.12	2.60	±	0.25	0.972	±	0.49	0.225	±	0.21
7	0.627	±	0.36	0.218	±	0.11	2.77	±	0.35	0.845	±	0.47	0.309	±	0.23
8	1.118	±	0.59	0.368	±	0.18	2.96	±	0.25	1.486	±	0.77	0.503	±	0.04
9	1.046	±	0.39	0.346	±	0.12	3.00	±	0.13	1.392	±	0.51	0.549	±	0.04
10	1.345	±	0.63	0.447	±	0.20	2.97	±	0.12	1.792	±	0.83	0.555	±	0.05
11	0.987	±	0.30	0.348	±	0.09	2.82	±	0.11	1.334	±	0.39	0.397	±	0.20
12	1.087	±	0.80	0.365	±	0.25	2.90	±	0.17	1.452	±	1.04	0.503	±	0.11
13	1.214	±	0.32	0.396	±	0.10	3.06	±	0.10	1.611	±	0.41	0.441	±	0.08
14	0.875	±	0.27	0.283	±	0.08	3.07	±	0.08	1.158	±	0.34	0.475	±	0.09
15	1.036	±	0.25	0.339	±	0.08	3.05	±	0.12	1.376	±	0.34	0.469	±	0.07
16	1.007	±	0.42	0.346	±	0.12	2.87	±	0.17	1.353	±	0.54	0.499	±	0.08
17	1.058	±	0.05	0.356	±	0.02	2.97	±	0.02	1.414	±	0.07	0.492	±	0.04
18	1.254	±	0.26	0.402	±	0.09	3.14	±	0.12	1.656	±	0.35	0.514	±	0.03
19	1.249	±	0.20	0.411	±	0.06	3.03	±	0.05	1.660	±	0.25	0.435	±	0.15
20	1.327	±	0.49	0.454	±	0.18	2.94	±	0.13	1.780	±	0.67	0.482	±	0.03
21	0.177	±	0.05	0.090	±	0.03	1.98	±	0.15	0.266	±	0.08	0.165	±	0.14
22	0.839	±	0.33	0.270	±	0.11	3.12	±	0.05	1.108	±	0.43	0.544	±	0.04
23	0.954	±	0.36	0.318	±	0.10	2.97	±	0.20	1.272	±	0.47	0.567	±	0.04
24	1.316	±	0.25	0.435	±	0.07	3.02	±	0.09	1.751	±	0.32	0.542	±	0.07
25	1.193	±	0.33	0.388	±	0.11	3.09	±	0.04	1.581	±	0.44	0.518	±	0.05
26	0.275	±	0.34	0.107	±	0.11	2.25	±	0.42	0.382	±	0.46	0.162	±	0.12
27	1.478	±	0.50	0.510	±	0.18	2.91	±	0.10	1.988	±	0.68	0.494	±	0.03
28	1.028	±	0.19	0.338	±	0.06	3.04	±	0.08	1.366	±	0.25	0.557	±	0.05
29	1.222	±	0.21	0.398	±	0.06	3.06	±	0.12	1.620	±	0.27	0.484	±	0.11
30	0.924	±	0.19	0.306	±	0.06	3.01	±	0.04	1.230	±	0.25	0.570	±	0.04
Control	1.830	±	0.64	0.602	±	0.22	3.06	±	0.08	2.431	±	0.86	0.708	±	0.01

b) *Parmelia sulcata*

Stand	Chl *a* end			Chl *b* end			Chl a/*b* end			Chl (a+b) end			Fv/Fm end		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.641	±	0.30	0.196	±	0.11	3.37	±	0.26	0.836	±	0.41	0.607	±	0.05
2	1.126	±	0.37	0.293	±	0.09	3.83	±	0.05	1.419	±	0.46	0.652	±	0.02
3	0.824	±	0.31	0.218	±	0.07	3.75	±	0.25	1.042	±	0.38	0.662	±	0.01
4	0.797	±	0.36	0.206	±	0.10	3.90	±	0.13	1.004	±	0.45	0.669	±	0.01
5	0.747	±	0.20	0.185	±	0.05	4.03	±	0.08	0.931	±	0.25	0.674	±	0.02
6	0.964	±	0.18	0.262	±	0.05	3.68	±	0.25	1.225	±	0.23	0.619	±	0.03
7	0.865	±	0.25	0.226	±	0.06	3.82	±	0.35	1.091	±	0.31	0.674	±	0.03
8	1.153	±	0.39	0.317	±	0.10	3.63	±	0.25	1.470	±	0.48	0.649	±	0.03
9	0.739	±	0.36	0.191	±	0.08	3.83	±	0.13	0.930	±	0.45	0.603	±	0.03
10	0.784	±	0.18	0.204	±	0.05	3.86	±	0.12	0.988	±	0.24	0.658	±	0.03
11	0.788	±	0.09	0.221	±	0.05	3.64	±	0.11	1.009	±	0.13	0.610	±	0.05
12	0.982	±	0.27	0.256	±	0.08	3.85	±	0.17	1.239	±	0.34	0.617	±	0.02
13	0.706	±	0.20	0.179	±	0.05	3.96	±	0.10	0.885	±	0.25	0.683	±	0.03
14	0.954	±	0.16	0.226	±	0.04	4.23	±	0.08	1.180	±	0.21	0.675	±	0.02
15	1.214	±	0.56	0.310	±	0.15	3.95	±	0.12	1.525	±	0.70	0.613	±	0.03
16	0.680	±	0.38	0.175	±	0.09	3.89	±	0.17	0.855	±	0.47	0.649	±	0.03
17	0.996	±	0.58	0.258	±	0.14	3.81	±	0.02	1.253	±	0.72	0.676	±	0.01
18	1.040	±	0.52	0.262	±	0.13	3.92	±	0.12	1.303	±	0.65	0.664	±	0.02
19	0.758	±	0.19	0.204	±	0.06	3.77	±	0.05	0.962	±	0.25	0.646	±	0.03
20	0.683	±	0.21	0.187	±	0.06	3.65	±	0.13	0.870	±	0.27	0.653	±	0.01
21	0.640	±	0.27	0.171	±	0.06	3.69	±	0.15	0.811	±	0.33	0.634	±	0.05
22	1.244	±	0.13	0.318	±	0.03	3.92	±	0.05	1.561	±	0.16	0.642	±	0.02
23	0.836	±	0.30	0.225	±	0.08	3.69	±	0.20	1.061	±	0.38	0.623	±	0.03
24	0.982	±	0.53	0.256	±	0.13	3.81	±	0.09	1.238	±	0.66	0.612	±	0.02
25	1.217	±	0.67	0.312	±	0.17	3.83	±	0.04	1.529	±	0.84	0.632	±	0.02
26	1.139	±	0.49	0.300	±	0.14	3.84	±	0.42	1.440	±	0.64	0.600	±	0.11
27	1.153	±	0.16	0.303	±	0.04	3.81	±	0.10	1.456	±	0.20	0.684	±	0.02
28	0.917	±	0.29	0.237	±	0.08	3.88	±	0.08	1.155	±	0.37	0.639	±	0.06
29	1.109	±	0.37	0.308	±	0.09	3.57	±	0.12	1.417	±	0.46	0.667	±	0.02
30	0.894	±	0.24	0.241	±	0.07	3.74	±	0.04	1.135	±	0.31	0.677	±	0.00
Control	1.180	±	0.40	0.331	±	0.10	3.53	±	0.08	1.511	±	0.50	0.713	±	0.03

c) *Ramalina farinacea*

Stand	Chl *a* end			Chl *b* end			Chl a/*b* end			Chl (a+b) end			Fv/Fm end		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.553	±	0.20	0.146	±	0.05	3.79	±	0.11	0.698	±	0.25	0.541	±	0.06
2	0.626	±	0.35	0.163	±	0.09	3.82	±	0.25	0.789	±	0.44	0.578	±	0.02
3	0.690	±	0.22	0.183	±	0.04	3.73	±	0.52	0.873	±	0.26	0.570	±	0.04
4	0.543	±	0.26	0.151	±	0.08	3.63	±	0.21	0.695	±	0.34	0.586	±	0.03
5	0.746	±	0.37	0.199	±	0.10	3.78	±	0.10	0.945	±	0.48	0.639	±	0.04
6	0.393	±	0.25	0.105	±	0.06	3.62	±	0.27	0.498	±	0.31	0.582	±	0.03
7	0.638	±	0.29	0.169	±	0.08	3.77	±	0.06	0.807	±	0.36	0.583	±	0.04
8	0.550	±	0.10	0.146	±	0.03	3.76	±	0.13	0.697	±	0.12	0.569	±	0.03
9	0.538	±	0.17	0.147	±	0.05	3.67	±	0.11	0.685	±	0.21	0.534	±	0.03
10	0.874	±	0.44	0.228	±	0.11	3.80	±	0.12	1.102	±	0.55	0.618	±	0.02
11	0.816	±	0.19	0.225	±	0.05	3.65	±	0.21	1.041	±	0.24	0.595	±	0.07
12	0.717	±	0.38	0.195	±	0.10	3.68	±	0.07	0.912	±	0.48	0.518	±	0.05
13	0.528	±	0.19	0.141	±	0.05	3.74	±	0.18	0.668	±	0.24	0.611	±	0.04
14	0.769	±	0.20	0.202	±	0.06	3.81	±	0.08	0.971	±	0.25	0.629	±	0.02
15	0.682	±	0.21	0.184	±	0.06	3.71	±	0.21	0.866	±	0.27	0.579	±	0.03
16	0.464	±	0.12	0.127	±	0.02	3.60	±	0.46	0.592	±	0.14	0.631	±	0.04
17	0.389	±	0.13	0.108	±	0.03	3.57	±	0.16	0.498	±	0.17	0.573	±	0.08
18	0.546	±	0.25	0.151	±	0.06	3.56	±	0.25	0.697	±	0.31	0.629	±	0.02
19	0.721	±	0.40	0.204	±	0.11	3.48	±	0.19	0.925	±	0.51	0.619	±	0.02
20	0.447	±	0.12	0.128	±	0.03	3.48	±	0.26	0.575	±	0.15	0.625	±	0.06
21	0.431	±	0.13	0.121	±	0.04	3.54	±	0.14	0.553	±	0.17	0.595	±	0.07
22	0.453	±	0.18	0.131	±	0.04	3.39	±	0.36	0.584	±	0.22	0.623	±	0.03
23	0.596	±	0.28	0.159	±	0.07	3.76	±	0.07	0.754	±	0.35	0.597	±	0.05
24	0.563	±	0.40	0.147	±	0.09	3.66	±	0.37	0.710	±	0.49	0.611	±	0.06
25	0.422	±	0.17	0.116	±	0.04	3.56	±	0.45	0.538	±	0.20	0.574	±	0.04
26	0.246	±	0.12	0.068	±	0.03	3.63	±	0.19	0.314	±	0.15	0.620	±	0.01
27	0.409	±	0.24	0.110	±	0.07	3.72	±	0.07	0.519	±	0.31	0.655	±	0.03
28	0.533	±	0.10	0.140	±	0.03	3.82	±	0.10	0.673	±	0.13	0.645	±	0.04
29	0.953	±	0.10	0.261	±	0.03	3.68	±	0.45	1.214	±	0.11	0.618	±	0.05
30	0.926	±	0.21	0.252	±	0.06	3.70	±	0.18	1.177	±	0.27	0.617	±	0.03
Control	0.773	±	0.42	0.210	±	0.11	3.68	±	0.02	0.983	±	0.53	0.711	±	0.01

d) Usnea filipendula

Stand	Chl *a* end			Chl *b* end			Chl a/*b* end			Chl (a+b) end			Fv/Fm end		
	X	±	SD	X	±	SD	X	±	SD	X	±	SD	X	±	SD
1	0.354	±	0.28	0.097	±	0.07	3.63	±	0.36	0.451	±	0.36	0.626	±	0.02
2	0.543	±	0.24	0.141	±	0.06	3.79	±	0.19	0.685	±	0.30	0.578	±	0.03
3	0.570	±	0.11	0.146	±	0.03	3.91	±	0.10	0.716	±	0.13	0.601	±	0.04
4	0.422	±	0.21	0.111	±	0.05	3.76	±	0.18	0.533	±	0.27	0.501	±	0.10
5	0.491	±	0.17	0.128	±	0.05	3.86	±	0.14	0.619	±	0.22	0.629	±	0.04
6	0.418	±	0.20	0.117	±	0.05	3.48	±	0.49	0.535	±	0.24	0.592	±	0.06
7	0.669	±	0.33	0.184	±	0.10	3.68	±	0.17	0.853	±	0.43	0.576	±	0.15
8	0.345	±	0.14	0.093	±	0.03	3.66	±	0.28	0.437	±	0.17	0.579	±	0.01
9	0.655	±	0.19	0.166	±	0.05	3.98	±	0.32	0.821	±	0.24	0.596	±	0.03
10	0.743	±	0.25	0.172	±	0.06	4.35	±	0.27	0.915	±	0.31	0.594	±	0.05
11	0.323	±	0.19	0.081	±	0.05	4.00	±	0.12	0.404	±	0.23	0.613	±	0.04
12	0.446	±	0.26	0.115	±	0.06	3.80	±	0.27	0.561	±	0.33	0.645	±	0.01
13	0.480	±	0.15	0.118	±	0.03	4.06	±	0.30	0.597	±	0.18	0.617	±	0.02
14	0.626	±	0.22	0.162	±	0.05	3.85	±	0.20	0.788	±	0.27	0.588	±	0.08
15	0.517	±	0.26	0.128	±	0.06	4.05	±	0.11	0.645	±	0.32	0.594	±	0.04
16	0.637	±	0.08	0.173	±	0.02	3.67	±	0.13	0.810	±	0.10	0.605	±	0.05
17	0.620	±	0.15	0.159	±	0.04	3.88	±	0.09	0.780	±	0.19	0.581	±	0.04
18	0.745	±	0.20	0.190	±	0.05	3.92	±	0.30	0.936	±	0.25	0.493	±	0.06
19	0.494	±	0.18	0.126	±	0.04	3.89	±	0.25	0.620	±	0.22	0.547	±	0.05
20	0.646	±	0.12	0.163	±	0.03	3.98	±	0.15	0.810	±	0.15	0.593	±	0.05
21	0.314	±	0.14	0.097	±	0.04	3.24	±	0.46	0.411	±	0.18	0.622	±	0.04
22	0.336	±	0.20	0.092	±	0.05	3.63	±	0.26	0.428	±	0.25	0.611	±	0.04
23	0.580	±	0.32	0.149	±	0.08	3.86	±	0.30	0.728	±	0.40	0.593	±	0.01
24	0.598	±	0.19	0.168	±	0.06	3.67	±	0.51	0.765	±	0.25	0.616	±	0.00
25	0.446	±	0.13	0.116	±	0.03	3.83	±	0.21	0.562	±	0.16	0.619	±	0.03
26	0.552	±	0.24	0.154	±	0.06	3.51	±	0.40	0.706	±	0.30	0.624	±	0.06
27	0.689	±	0.22	0.181	±	0.06	3.81	±	0.09	0.871	±	0.28	0.652	±	0.02
28	0.318	±	0.11	0.085	±	0.03	3.68	±	0.22	0.403	±	0.14	0.621	±	0.02
29	0.490	±	0.13	0.125	±	0.02	3.91	±	0.32	0.615	±	0.15	0.608	±	0.02
30	0.415	±	0.20	0.107	±	0.05	3.90	±	0.21	0.522	±	0.26	0.620	±	0.02
Control	0.566	±	0.24	0.140	±	0.05	3.98	±	0.26	0.706	±	0.29	0.720	±	0.01

Tables 33. – Means (±SD) of the thallus area of *L. pulmonaria,* accounted as a number of pixels by using the Image Manipulation Program (GIMP), regarding to the measure of damage (%) caused by the field exposure (n= 4)

Stand	Total area			Damaged area			Damage		
	X	±	SD	X	±	SD	X	±	SD
1	526973	±	288207	128795	±	75068	24.40	±	1.91
2	621097	±	159366	27357	±	29147	4.47	±	4.15
3	944696	±	277856	32227	±	25526	3.37	±	2.08
4	1033109	±	332817	16674	±	10879	1.85	±	1.31
5	949644	±	373320	8888	±	1951	1.04	±	0.40
6	830291	±	173026	105168	±	34378	12.86	±	3.89
7	722062	±	16165	207596	±	147152	29.04	±	20.85
8	771689	±	257703	35911	±	21803	4.90	±	3.54
9	873588	±	335486	13653	±	9868	1.73	±	1.17
10	1030373	±	241757	42618	±	50451	3.85	±	4.06
11	767572	±	273153	32625	±	4917	4.86	±	2.42
12	936625	±	415424	27746	±	5656	3.30	±	1.27
13	982512	±	118987	15462	±	5038	1.56	±	0.41
14	958920	±	246112	24879	±	14408	2.62	±	1.20
15	950436	±	161123	16418	±	8928	1.76	±	0.98
16	739118	±	329821	19346	±	10068	2.68	±	1.34
17	916886	±	247947	38246	±	23564	3.94	±	1.46
18	931665	±	177990	26876	±	11252	2.84	±	0.92
19	1001347	±	149146	36431	±	23245	3.79	±	2.73
20	889194	±	195611	43346	±	11349	4.93	±	1.08
21	769339	±	264333	582718	±	428237	69.28	±	41.98
22	1007916	±	156590	27942	±	10155	2.80	±	1.09
23	1252866	±	239558	40063	±	9711	3.27	±	0.85
24	1043228	±	285956	31416	±	5177	3.19	±	0.93
25	715941	±	56853	21782	±	6994	3.02	±	0.90
26	724448	±	119213	491547	±	261617	64.92	±	32.07
27	776025	±	62494	34790	±	18310	4.63	±	2.59
28	976377	±	176770	20842	±	9878	2.06	±	0.69
29	951390	±	142705	17449	±	2439	1.84	±	0.16
30	872311	±	175444	11978	±	8186	1.34	±	0.74