

# The Impact of Air Pollution on the Occurrence of Bioindicator *Rhytisma Acerinum* L. and Its Potential Use in the Production of Biomass

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**Abstract:** The aim of our project was to find the association between the intensity of bioindication demonstrations of *Rhytisma acerinum* L. (size and number of spots) and selected meteorological factors (precipitation, SO<sub>x</sub>), compare the content of selected biogenic elements in the leaves and soil of affected seedlings of *Acer platanoides* L. and *Acer pseudoplatanus* L. In 2014 and 2015 we recorded 100 % infection rate in both species of maple in the mountains of Bukovské mountains and Slánske mountains. The major manifestation of the bioindication of fungi was recorded in the highest areas (protected areas). We observed differences in infection of the monitored species of maples: *Acer pseudoplatanus* L. had a greater number of smaller spots compared to *Acer platanoides* L. In 2014 with more precipitation the number of spots was higher (Bukovské mountains by 27,62 % more and Slánské mountains by 30,11 %), the size of spots was also larger than in 2015 (Bukovské mountains by 8,34 % and Slánské mountains by 52,31 %). In the experimental stage we found out that the more intense pH of the watering solution, the higher number and size of spots. The largest number and size of spots were recorded on the seedlings watered with the solutions of pH from 6,00 – 7,60 and on seedlings growing in the forest. The content of nitrogen increased with a decreasing pH, higher contents of carbon, hydrogen and oxygen were recorded in the maples remarkably infected with the fungus, the presence of sulfur was recorded only in the leaves of *Acer platanoides* L. (more sensitive to SO<sub>x</sub>). The highest content of calcium, magnesium, potassium and phosphorus was recorded in the soil under the maple trees intensely affected by *Rhytisma acerinum* L. After evaluating the calorific value and gross combustion heat we did not record any significant differences in the production of maple biomass. We propose to use the monitoring of occurrence of *Rhytisma acerinum* L. bioindicator as a suitable method for detection of air pollution caused by sulphur pollutants. The results are as well significant for the finding about faster defoliation of maple leaves which causes more rapid humification of soil, which in the replanting process of trees results in a less serious land degradation.

**Key words:** *Rhytisma acerinum* L., *Acer pseudoplatanus* L., *Acer platanoides* L., bioindicator, SO<sub>x</sub>, air pollution, acid rain

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## 1. Introduction

*Rhytisma acerinum* L. is a representative of the fungus division *Ascomycota*, subdivision *Pezizomycotina* and class *Leotiomycetes*. On the surface of the leaves of sycamore maple (*Acer pseudoplatanus* L.) and Norway maple (*Acer platanoides* L.) it creates black, so called 'tar' spots. This fungus is often found on the maple tree leaves in the UK, Ireland, Northern Central Europe and North America.

Bioindicators are organisms of which presence or excessive growth (positive indicator) or, on the other hand, absence or its decreasing number (negative indicators), its condition or behavior reflect the state of the environment and its changes including the presence and concentration. Environmental monitoring uses as one of control elements also evaluation of biomarkers. Each element in the ecosystem has its own importance to inform about the quality of the environment. Commonly used fungus with bioindication capabilities are many species of lichens. The aim of the project was to determine any association between the presence of the black maple fungus and the quality of the environment and propose possible options to use the monitoring of the fungus as a potential bioindication method for detection of the presence of SO<sub>x</sub> pollutants.

Acid "rain" is a broad term referring to a mixture of wet and dry deposition (deposited material) from the atmosphere containing higher than normal amounts of nitric and sulfuric acids. The precursors, or chemical forerunners of acid rain formation result from both natural sources, such as volcanoes and decaying vegetation, and man-made sources, primarily emissions of sulfur dioxide (SO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) resulting from fossil fuel combustion. [14]

We were as well interested in the economic exploitation of the results from monitoring the experimental infection of maple seedlings with a view to a potential improvement of soil quality and of properties of plants used for biomass production.

### **We set the following aims:**

1. Compare the incidence of *Rhytisma acerinum* L. in two selected areas of Eastern Slovakia: Bukovské and Slanské mountains in 2014 and 2015
2. Find the link between the intensity of symptoms of *Rhytisma acerinum* L. and selected meteorological factors (temperature, humidity, precipitation).
3. Record the changes of SO<sub>x</sub> on the occurrence of *Rhytisma acerinum* L., size and frequency of spots on the leaves of *Acer platanoides* L. and *Acer pseudoplatanus* L.
4. Compare the content of biogenic elements: carbon, nitrogen, hydrogen, oxygen and sulfur of *Acer platanoides* L. and *Acer pseudoplatanus* L. in the leaves infected by *Rhytisma acerinum* L.

5. Compare the content of biogenic elements: phosphorus, potassium, magnesium and calcium in the soil of affected seedlings of *Acer platanoides* L. and *Acer pseudoplatanus* L. infected by *Rhytisma acerinum* L. as compared with the control sample.
6. Compare the calorific value of the biomass from infested and uninfected *Rhytisma acerinum* L. maples with respect to a potential improvement of the soil quality and properties of plants used for biomass production.

## 2. Methodology

### Field Research

In 2014 and 2015 we conducted the research of *Rhytisma acerinum* L. with a permission from the Administration Office of the National Park Poloniny in the flysch mountains of Bukovské mountains, the National Nature Reserve Riaba skala with the hill called Ďurkovec. Poloniny is the easternmost Slovak National Park. The area, together with the adjacent protected areas of Poland and Ukraine is a part of the network of biosphere reserves named International Biosphere Reserve Eastern Carpathians known for its Carpathian beech primaeval forests. This area was compared with Slanské mountains with the permission from the Forest Administration Zámutov (cultivated areas), of which a nature reserve Zámutovské skaly is a part. Volcanic mountains Slanské mountains is a part of the Carpathian volcanic arc. From each sites we selected 10 trees, 35 to 50 m tall and collected 30 freshly fallen leaves of a similar size from every tree. In 2014 and 2015 we observed the average number of clearly demarcated spots and the average size of spots per 1 infected leaf on the trees *Acer platanoides* L. growing in altitudes above the sea level (Poloniny 791 - 1,077 m, Slanské mountains 443 - 791 m) and *Acer pseudoplatanus* L. (Poloniny 611 - 1,189 m, Slanské mountains 614 - 795 m).

$$x = \frac{a + b}{2}$$

x = average spot size per 1 infected leaf

a = minimum size spots

b = maximum size spots

## Experiment part

### Preparation of H<sub>2</sub>SO<sub>4</sub> solutions

In the school laboratory we prepared a stock solution of sulfuric acid with a pH 2, 00, which we subsequently diluted to get the solutions to be used (Table 1).

<b>pH of added solution</b>	<b>2, 00</b>	<b>2, 70</b>	<b>3, 00</b>	<b>3, 70</b>	<b>4, 00</b>
<b>g H<sub>2</sub>SO<sub>4</sub> / 0,5 l</b>	0,2552083333	0,0509207570	0,0255208333	0,0050920757	0,0025520833
<b>pH of added solution</b>	<b>4, 70</b>	<b>5, 00</b>	<b>5, 70</b>	<b>6, 00</b>	<b>6, 70</b>
<b>g H<sub>2</sub>SO<sub>4</sub> / 0,5 l</b>	0,0005092076	0,0002552083	0,0000509208	0,0000255208	0,0000050921

Table.1: The dilution of solutions

### Sampling maple seedlings

From June 2015 to September 2015 we planted 11 seedlings of *Acer pseudoplatanus* L. and 18 seedlings of *Acer platanoides* L., which were provided from the Forest Administration Zámotov, into the vessels of 12 cm diameter and height of 11 cm. The seedlings of the species studied came from the surroundings of one tree (area of Slanské mountains - Dubník 610 m above sea level). We weighed 350g of soil substrate (forest soil) for each vessel and planted the seedlings and put a layer of 50 g of infected leaf parts *Rhytisma acerinum* L. on the soil substrate in each vessel. Ten seedlings of *Acer platanoides* L. and ten seedlings of *Acer pseudoplatanus* L. were watered with H<sub>2</sub>SO<sub>4</sub> solution right into the soil substrate (Table 2), the control samples were watered with water from a local well and seven seedlings of *Acer platanoides* L. were affected by spraying of H<sub>2</sub>SO<sub>4</sub> solution on the leaves (Table 3). Maples were watered twice a week with three ml of sulfuric acid and once a week we watched an average size of spots and recalculated it per 1 infected leaf and average number of spots. Seedlings grown in domestic conditions under the roof in the shade (no atmospheric precipitation) were compared with 4 seedling of *Acer pseudoplatanus* L. and 4 seedlings of *Acer platanoides* L. growing in a natural environment under the same tree from which we took young seedlings to be grown at home (the control sample No. 2).

Kind of maple / number of sample	<i>Acer platanoides</i> L. - pH of added solution	<i>Acer pseudoplatanus</i> L.- pH of added solution
1.	2,00	2,00
2.	2,70	2,70
3.	3,00	3,00
4.	3,70	3,70
5.	4,00	4,00
6.	4,70	4,70
7.	5,00	5,00
8.	5,70	5,70
9.	6,00	6,00
10.	6,70	6,70
11.- control sample water from the well	7,60	7,60

Table 2: Single H<sub>2</sub>SO<sub>4</sub> solution concentrations applied into the soil substrate of *Acer platanoides* L. and *Acer pseudoplatanus* L.

Kind of maple / number of sample	<i>Acer platanoides</i> L.- pH of added solution
1.	2,00
2.	2,70
3.	3,00
4.	4,00
5.	5,00
6.	6,00
7.	6,70

Table 3: Single H<sub>2</sub>SO<sub>4</sub> solution concentrations applied onto the leaves of *Acer platanoides* L. trees



Figure 1: Seedlings of *Acer platanoides* L. (author of photo: Miriam Feretová)



Figure 2: Seedlings of *Acer pseudoplatanus* L. (author of photo: Miriam Feretová)

### CHNSO analysis

The device works on the principles of the Dumas chromatography by combustion of the sample in a stream of oxygen. In the mortar we mashed individual samples of *Acer platanoides* L. and *Acer pseudoplatanus* L. leaves, which were watered and sprayed on the leaves with solutions of (pH 2,00 – 4,00 – 6,70 – 7,60) and control samples of both types of maple from Slanské and Bukovské vrchy. The device was calibrated on the number of standards required. The analysis was evaluated using the Eager Xperience program.



Figure 3: FLASH 2000 – CHNSO Analyzer (author of photo: Miriam Feretová)

### Determining of combustion heat

We poured into an agitating tank 2 liters of water (18 – 25 °C) and tablets from 4 samples from the Slanské mountains and 4 samples from the Bukovské mountains were prepared on a press (Figure 6). Then we dosed the required amount of the sample (1 g of a dried sample) into the press and by rotating we pressed out a tablet into a bomb calorimeter. We filled the bomb calorimeter with oxygen to the pressure of 30 Pa, poured 2 ls of water into the interior chamber. The made-ground was poured in a cylinder and pressed. An empty quartz crucible was weighed, the tab below the cylinder was opened and the tablet was pressed out into the pre- weighed crucible and then weighed again. When calibrating, we used 2 tablets of benzoic acid.

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Number of sample and sampling place	Kind of maple
1. Slanské mountains	<i>Acer pseudoplatanus</i> L. with <i>Rhytisma acerinum</i> L.
2. Slanské mountains	<i>Acer pseudoplatanus</i> L. with <i>Rhytisma acerinum</i> L.
3. Slanské mountains	<i>Acer pseudoplatanus</i> L. without <i>Rhytisma acerinum</i> L.
4. Slanské mountains	<i>Acer pseudoplatanus</i> L. without <i>Rhytisma acerinum</i> L.
5. Bukovské mountains	<i>Acer platanoides</i> without L. <i>Rhytisma acerinum</i> L.
6. Bukovské mountains	<i>Acer platanoides</i> without L. <i>Rhytisma acerinum</i> L.
7. Bukovské mountains	<i>Acer platanoides</i> L. with <i>Rhytisma acerinum</i> L.
8. Bukovské mountains	<i>Acer platanoides</i> L. with <i>Rhytisma acerinum</i> L.

Table 6: Samples and parts of the leaves and parts of the trees infected and uninfected by *Rhytisma acerinum* L. from the Bukovské and Slanské mountains



Figure 5: Calorimeter C 200



Figure 6: Tablets press IKA

(author of photo: Miriam Feretová) (author of photo: Miriam Feretová)



### **Calculation of combustion heat to caloric value $Q_v$**

Combustion heat is the amount of heat released by complete combustion of fuel in a calorimetric pressure tank in a medium with compressed oxygen at temperature 24 - 25 °C, to its weight unit.

The formula for the caloric value was calculated from the relation:  $Q_v = Q_s - 24,42 \cdot (W + 8,94 H_h)$ ,  $Q_v$  is caloric value and  $Q_s$  - combustion heat in  $J \cdot g^{-1}$ . The coefficient equivalent to 1 % of the water in the sample ( $W$  - the water content in the sample) at 24 °C is 24,42 and the coefficient for calculating the amount of hydrogen in the water ( $H_h$  - content of hydrogen in the sample) is 8,94.

### **PKMgCa analysis**

The samples were arranged to fine earth (soil fractions with the diameter of grains smaller than 2 mm), the remains of the roots were removed and kept dry in the air at 40°C. We weighed 10g of the sample into a polyethylene bottle, added 100ml of the extraction solution according to Mehlich 3 and extracted the sample on a rotation beater for 10 minutes. After the extraction we filtered the suspension through filter paper.

### **Determining of phosphorus in the extract by Mehlich 3 spectrophotometric method**

We pipetted 1ml of the soil extract and colouring molybden-antimonite agent in the ration of 1:24 into a test tube. The coloured sample was measured on spectrophotometer Cary 50. The content of phosphorus was determined from the calibrating curve.

### **Determining of kalium in the extract by Mehlich 3 method of atomic emission spectrophotometry (FAES)**

In the soil extract the emmision of radiation with the wave length 766,5 nm was measured. The content of kalium was determined from the calibrating curve with atomic absorption spectrophotometer GBC 904AA.

### **Determining of magnesium and calcium in the extract by Mehlich 3 method of atomic absorption spectrophotometry (FAAS)**

We pipetted 0,5ml of the soil extract into test tubes, 3 and 5mls of the solution of lanthan with the concentration of 1mg/ml. After mixing the sample we measured the absorption of radiation with atomic absorption spectrophotometer (AAS). Atomic absorption spectrophotometer Varian AA240FS was used.

### Meteorological data and determining of sulphur in the air

The data about the amount of rainfall, temperature and air humidity, SO<sub>2</sub> (Osadné and Kamenica nad Cirochou (the Bukovské mountains) and Čaklov (the Slanské mountains) in 2014 and 2015 were provided by Slovak Hydrometeorological Institute.

## 3. Results

### Observations

In the Slanské (Table 4) and Bukovské mountains (Table 5) in September 100 % infectedness of *Acer platanoides* L. and *Acer pseudoplatanus* L. was observed. The highest occurrence of *Rhytisma acerinum* L. was in the highest localities (NPR Ďurkovec – the Bukovské mountains, PR Zámotovské skaly – the Slanské mountains). *Acer pseudoplatanus* L. was more infected than *Acer platanoides* L., it had significantly infected areas of leaves blades, in 2014 by 85,53 %, in 2015 by 90,64 % (a higher number of smaller spots). The higher number of spots on *Acer pseudoplatanus* L. was observed in the Slanské mountains (in 2014 by 21,93 % and in 2015 by 25,68 %, on *Acer platanoides* L. in the Bukovské mountains (in 2014 by 91,51 % and in 2015 by 88,36 %. On *Acer platanoides* L. the number of spots was comparable on trees in a different altitude (Table 4 and 5). Faster defoliation was observed in *Acer pseudoplatanus* L., in *Acer platanoides* L. leaves did not start to fall off. On tree No. 9 in the Bukovské mountains the presence of rare lichen *Lobaria pulmonaria* L. (Figure 7), a critically endangered, protected and sensitive to pollution species, was discovered but in the Bukovské mountains it has not been observed in the last 40 years, in 2015 it almost dried up. On *Acer campestre* L. no pathogen was observed in the localities.

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Number of tree and kind of maple	Altitude	Average number of spots in 1 infected leaf in 2015 (30 leaves / 1 tree)	Average number of spots in 1 infected leaf in 2014 (30 leaves / 1 tree)	Average size of spots in 1 infected leaf in cm in 2014	Average size of spots in 1 infected leaf in cm in 2015
1. <i>Acer platanoides</i> L.	443 m. n. m.	1, 23 ± 0, 37	1, 45 ± 0, 60	2, 10 ± 0, 25	0, 53 ± 0, 05
2. <i>Acer platanoides</i> L.	458 m. n. m.	1, 33 ± 0, 47	1, 50 ± 0, 67	2, 00 ± 0, 23	0, 57 ± 0, 05
3. <i>Acer platanoides</i> L.	518 m. n. m.	1, 60 ± 0, 53	1, 70 ± 0, 62	1, 88 ± 0, 19	0, 77 ± 0, 12
4. <i>Acer pseudoplatanus</i> L.	614 m. n. m.	10, 33 ± 3, 30	17, 62 ± 4, 08	0, 75 ± 0, 08	0, 43 ± 0, 08
5. <i>Acer platanoides</i> L.	615 m. n. m.	1, 33 ± 0, 47	1, 40 ± 0, 32	1, 30 ± 0, 16	0, 83 ± 0, 12
6. <i>Acer pseudoplatanus</i> L.	643 m. n. m.	14, 67 ± 2, 50	20, 72 ± 4, 36	0, 74 ± 0, 09	0, 58 ± 0, 09
7. <i>Acer pseudoplatanus</i> L.	667 m. n. m.	44, 67 ± 6, 18	61, 61 ± 6, 74	0, 86 ± 0, 09	0, 44 ± 0, 05
8. <i>Acer pseudoplatanus</i> L.	716 m. n. m.	68, 33 ± 6, 24	99, 42 ± 8, 95	1, 00 ± 0, 11	0, 60 ± 0, 12
9. <i>Acer platanoides</i> L.	791 m. n. m.	1, 25 ± 0, 47	2, 02 ± 0, 65	1, 50 ± 0, 15	0, 84 ± 0, 11
10. <i>Acer pseudoplatanus</i> L.	795 m. n. m.	77, 00 ± 11, 44	109, 73 ± 13, 42	0, 83 ± 0, 07	0, 58 ± 0, 07

Table 4: Slanské mountains: *Acer pseudoplatanus* L. maple and *Acer platanoides* L. in 2014 a 2015- average number and size of spots

**The Impact of Air Pollution on the Occurrence of Bioindicator *Rhytisma Acerinum* L. and Its Potential Use in the Production of Biomass**

<b>Number of tree and kind of maple</b>	<b>Altitude</b>	<b>Average number of spots in 1 infected leaf in 2015 (30 leaves / 1 tree)</b>	<b>Average number of spots in 1 infected leaf in 2014 (30 leaves / 1 tree)</b>	<b>Average size of spots in 1 infected leaf in cm in 2015</b>	<b>Average size of spots in 1 infected leaf in cm in 2014</b>
1. <i>Acer pseudoplatanus</i> L.	611 m. n. m.	36, 38 ± 8, 57	47, 67 ± 13, 42	0, 45 ± 0, 06	0, 60 ± 0, 08
2. <i>Acer pseudoplatanus</i> L.	638 m. n. m.	29, 46 ± 6, 42	48, 00 ± 5, 72	0, 50 ± 0, 05	0, 73 ± 0, 05
3. <i>Acer pseudoplatanus</i> L.	746 m. n. m.	19, 67 ± 4, 11	33, 37 ± 6, 65	0, 63 ± 0, 08	0, 57 ± 0, 09
4. <i>Acer platanoides</i> L.	791 m. n. m.	12, 2 ± 3, 05	18, 00 ± 2, 83	0,98 ± 0, 10	0, 97 ± 0, 12
5. <i>Acer platanoides</i> L.	871 m. n. m.	13, 17 ± 0, 62	19, 33 ± 4, 78	1, 12 ± 0, 13	1, 20 ± 0, 14
6. <i>Acer platanoides</i> L.	1077 m. n. m.	13, 33 ± 2, 11	19, 67 ± 4, 64	1, 20 ± 0, 10	1, 33 ± 0, 17
7. <i>Acer pseudoplatanus</i> L.	1077 m. n. m.	31, 00 ± 7, 96	42, 00 ± 4, 90	0,57 ± 0, 12	0, 60 ± 0, 08
8. <i>Acer pseudoplatanus</i> L.	1154 m. n. m.	40, 58 ± 9, 14	42, 33 ± 9, 46	0, 40 ± 0, 07	0, 40 ± 0, 00
9. <i>Acer pseudoplatanus</i> L.	1192 m. n. m.	56, 00 ± 11, 43	44, 33 ± 9, 10	0,33 ± 0, 13	0, 37 ± 0, 05
10. <i>Acer pseudoplatanus</i> L.	1189 m. n. m.	52, 35 ± 8, 81	64, 00 ± 16, 87	0, 37 ± 0, 07	0, 37 ± 0, 05

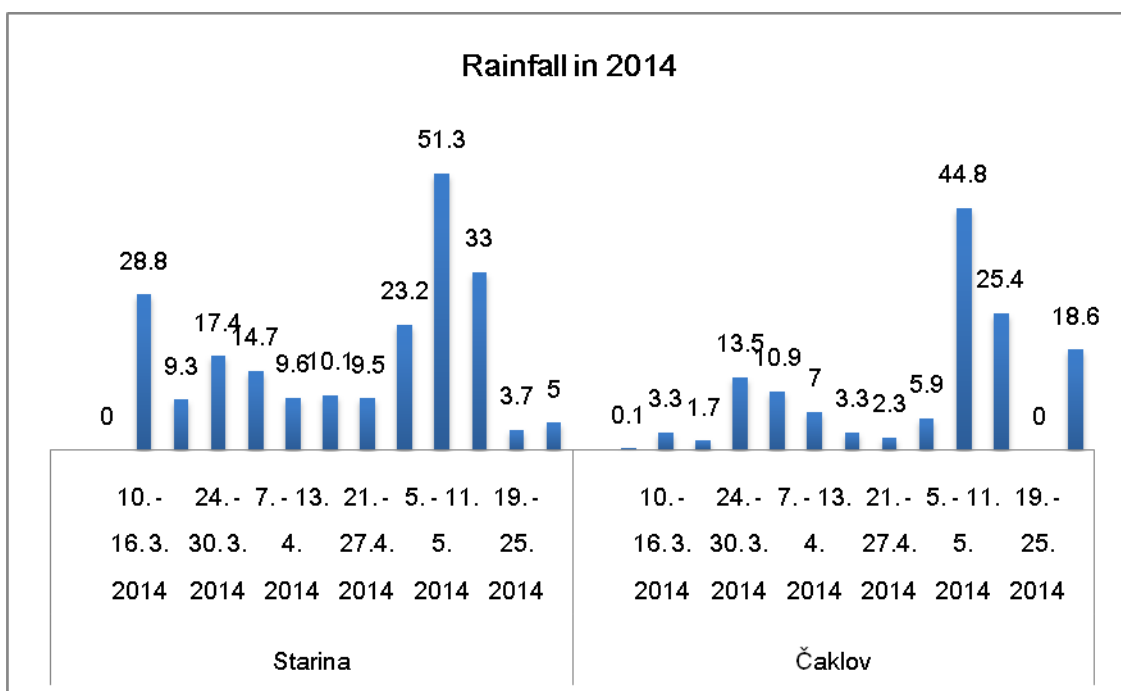
Table 5: Bukovské mountains: *Acer pseudoplatanus* L. maple and *Acer platanoides* L. in 2014 a 2015- average number and size of spots



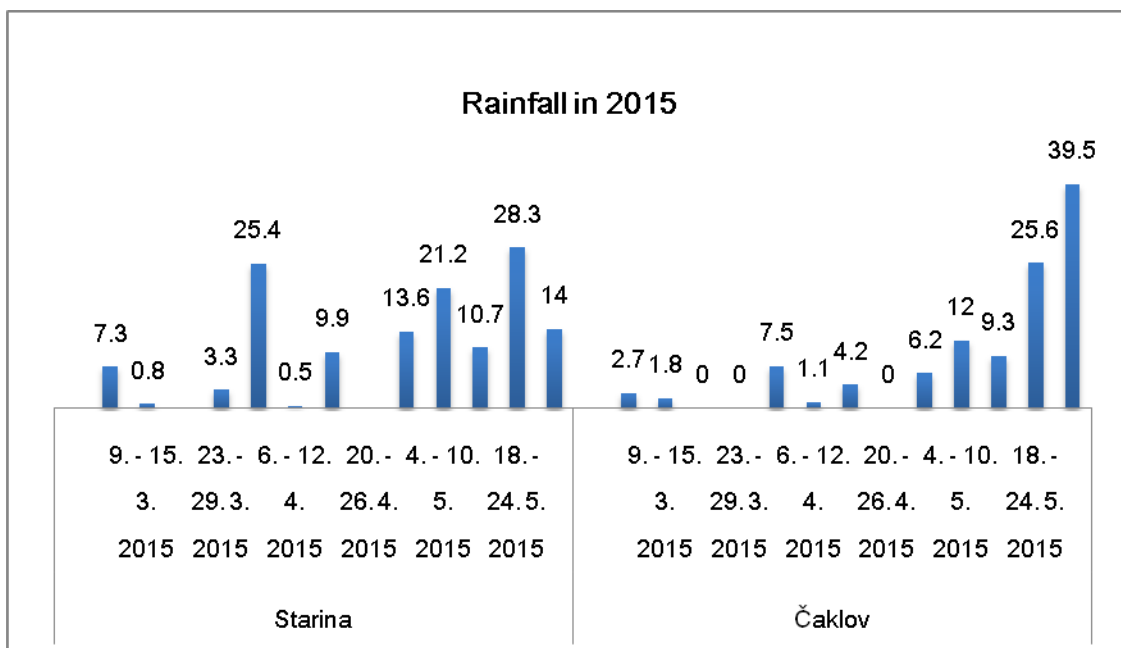
Figure 7: Rare lichen *Lobaria pulmonaria* found in NP Poloniny in 2014

### Meteorological characteristics

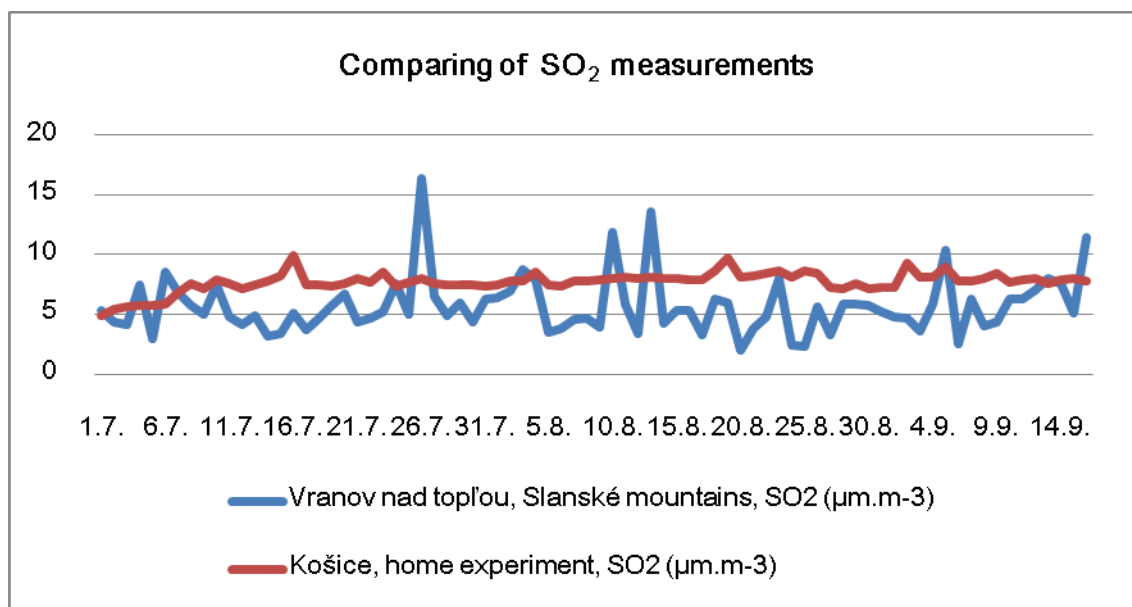
During August and September 2014 in the Bukovské mountains there was more rainfall by 52,31 % than in 2015. The average humidity of the air was by 17,50 % higher than in 2015 and the average daily temperature in 2015 was by 20,48 % lower. In 2014 the number of spots was higher by 27,62 % and the size of spots was bigger by 8,34 % than in 2015 in NP Poloniny. In August and September 2014 in the Slanské mountains there was more rainfall by 46 % than in 2015. The humidity of the air was by 16,91 % higher than in 2015 and the daily temperature in 2015 was by 25 % lower (Graph 1, Graph 2). In 2014 the number of spots was higher by 30,11 % and also the size of spots was bigger even by 52,31 % than in 2015. We discovered that the higher humidity was, the higher number of spots and the bigger size of spots were at both kinds of maples, in rainier year 2014 the number of spots was higher (in the Bukovské mountains by 27,62 %, in the Slanské mountains by 30,11 %, the size of spots was also bigger than in 2015 (in the Bukovské mountains by 8,34 % and in the Slanské mountains by 52,31 % ).



Graph 1: Rainfall totals during spring months (March – May) in Čaklov – Slanské mountains and Starina – Bukovské mountains in 2014 [15]



Graph 2: Rainfall totals during spring months (March – May) in Čaklov – Slanské mountains and Starina – Bukovské mountains in 2015 [15]

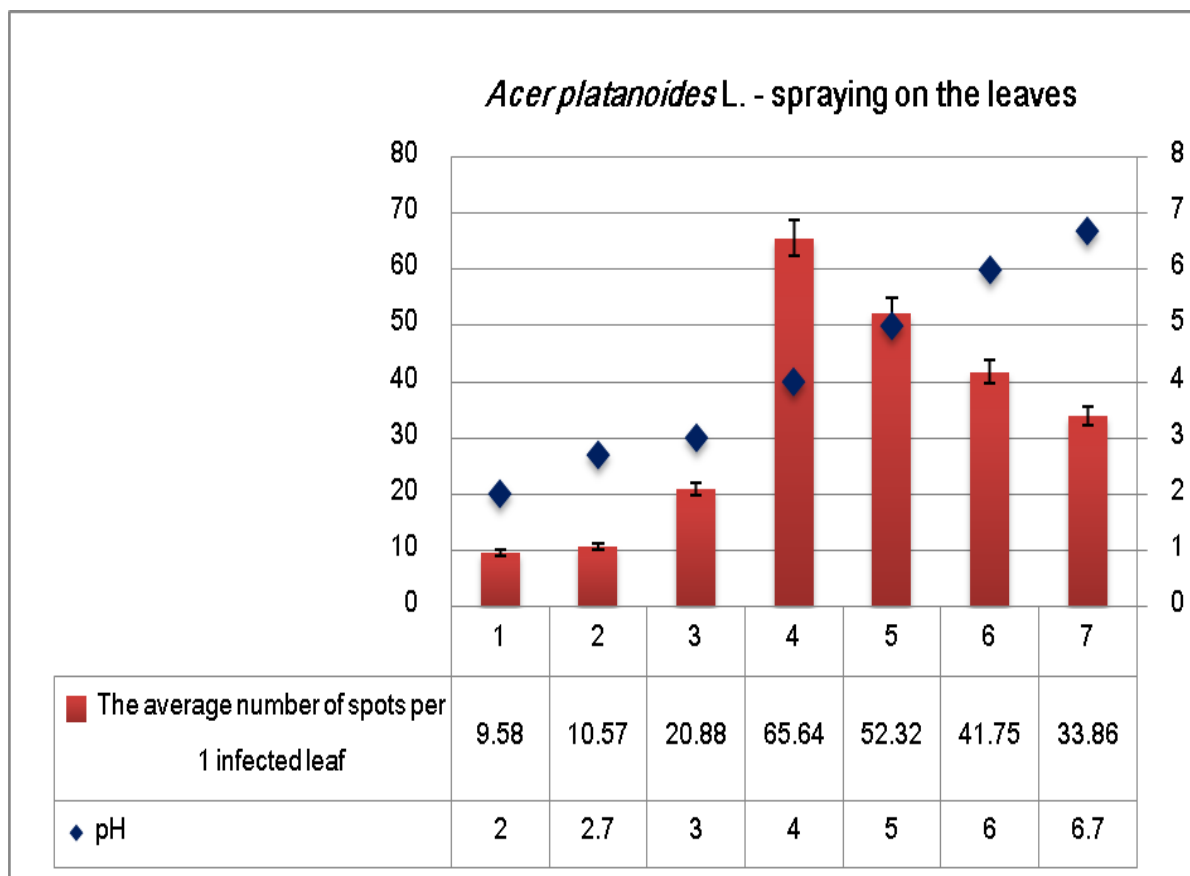


Graf. 3: SO<sub>2</sub> levels in summer 2015 in Slanských mountains and during home experiment [14]

In the last two years the maximum daily SO<sub>2</sub> level in the air in the studied area has been 17 µm.m<sup>-3</sup>. According to Bevan and Greenhalgh, the SO<sub>2</sub> tolerance of *Rhytisma acerinum* L. is 90 µm.m<sup>-3</sup> [1].

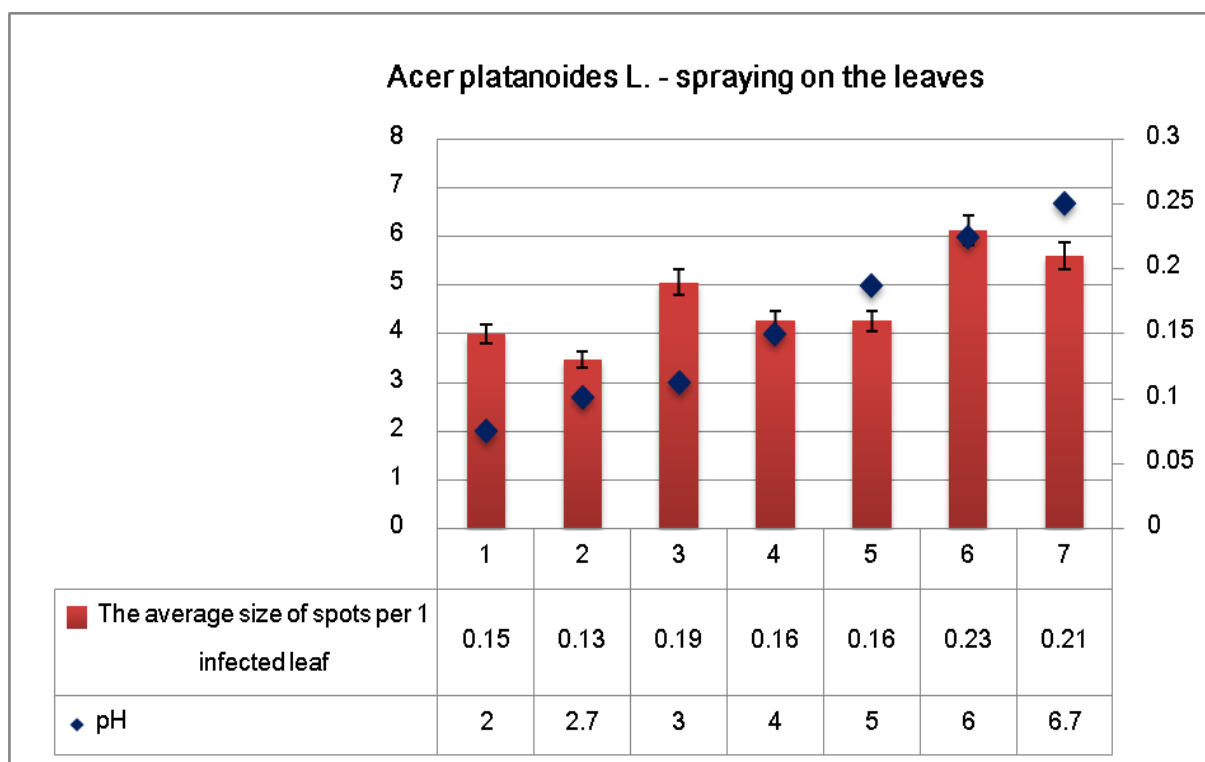
### Experimental part

With the increasing pH of the solutions for watering the number of spots and the size of spots on *Acer platanoides* L. and *Acer pseudoplatanus* L. increased, too. The highest number of spots and the size of spots were at the seedlings of *Acer platanoides* L. and *Acer pseudoplatanus* L. (Graph 8 and 9), watered with the water from a well with pH 7,60, or more precisely, the seedlings growing in natural conditions below Dubník (the Slanské mountains - young individuals). The lowest number and the smallest size of spots were at the seedlings of both kinds of maples with pH 2,00. We recorded the differences between the infectedness of the seedlings of *Acer platanoides* L. sprayed with sulphur acid solutions on leaves (Graph 4 and 5) and into a soil substrate (Graph 6 and 7). A significantly lower number of smaller spots was observed at seedlings from the soil substrate watered by sulphur acid solutions, the number of spots was lower by 84,98 % and the size of spots by 63,89 %. The faster defoliation of maples was discovered in *Acer pseudoplatanus* L., approximately 2 weeks sooner than at the seedlings watered by the solutions with lower pH. The most infected leaves having pH 5,00 – 6,70 were falling fastest. In *Acer platanoides* L. no defoliation of leaves was observed.

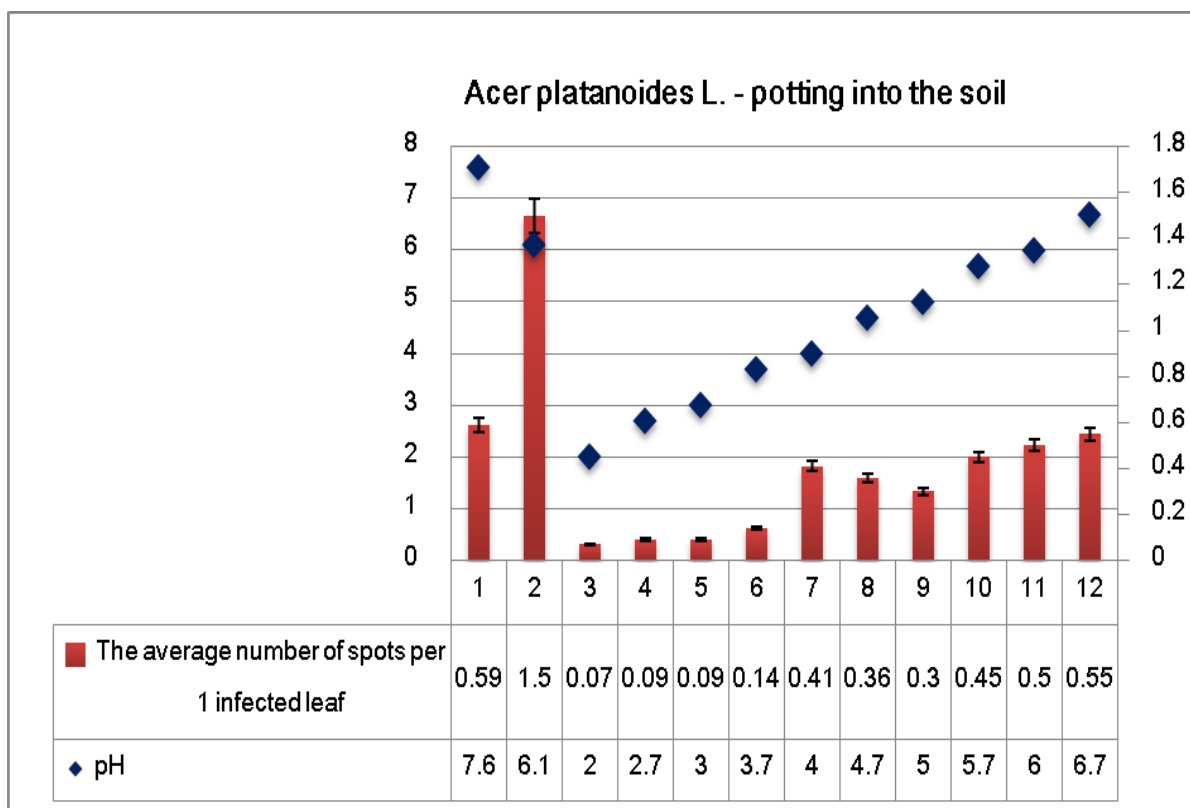


Graph 4: Average number of spots of seedlings *Acer platanoides* L. sprayed on leaves

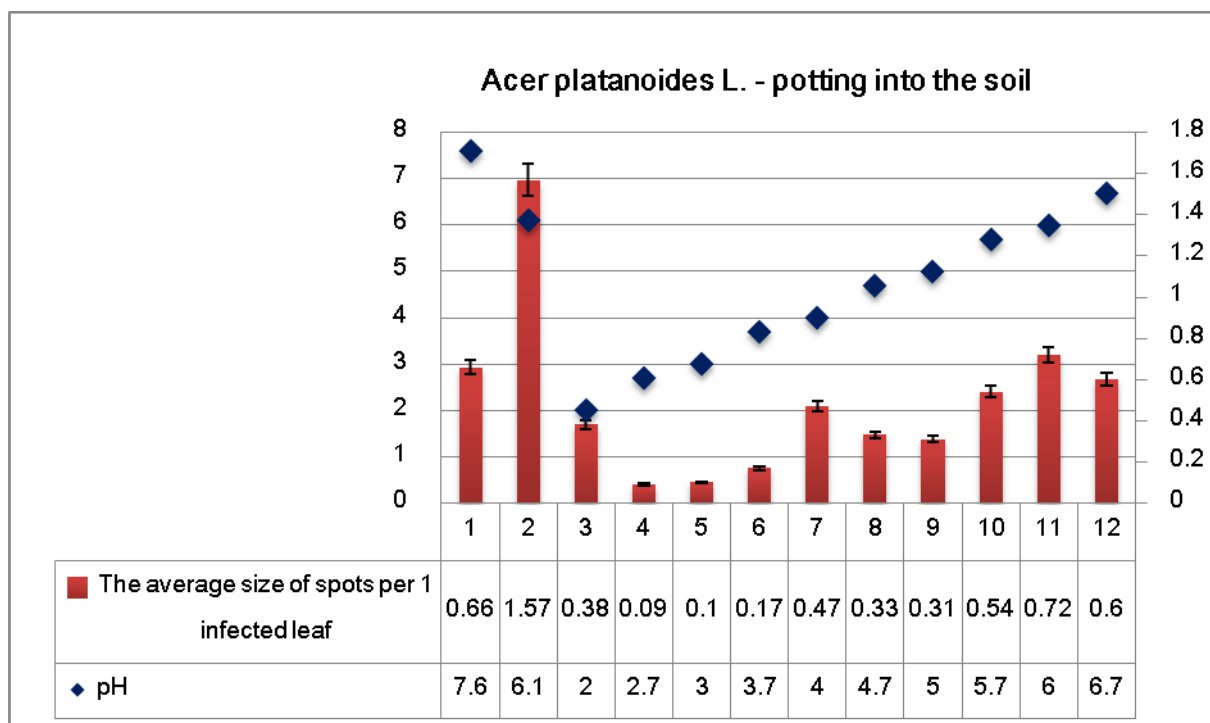




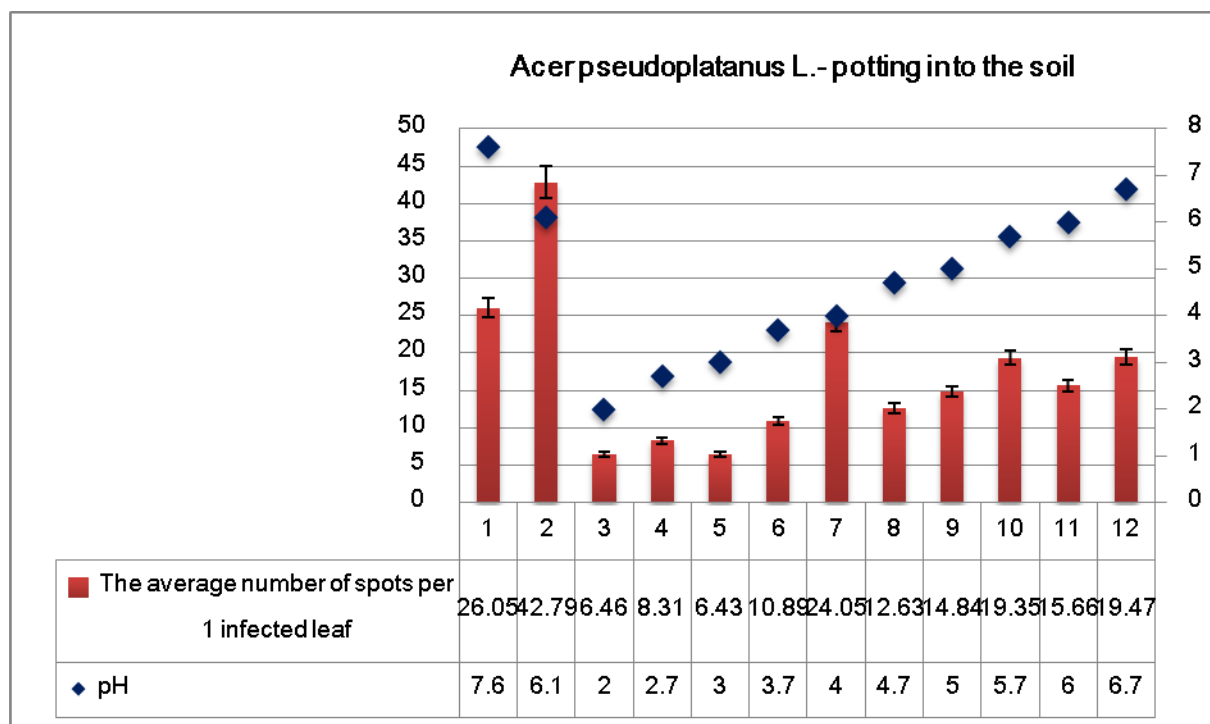
Graph 5: Average size of spots of seedlings of *Acer platanoides* L. sprayed on leaves



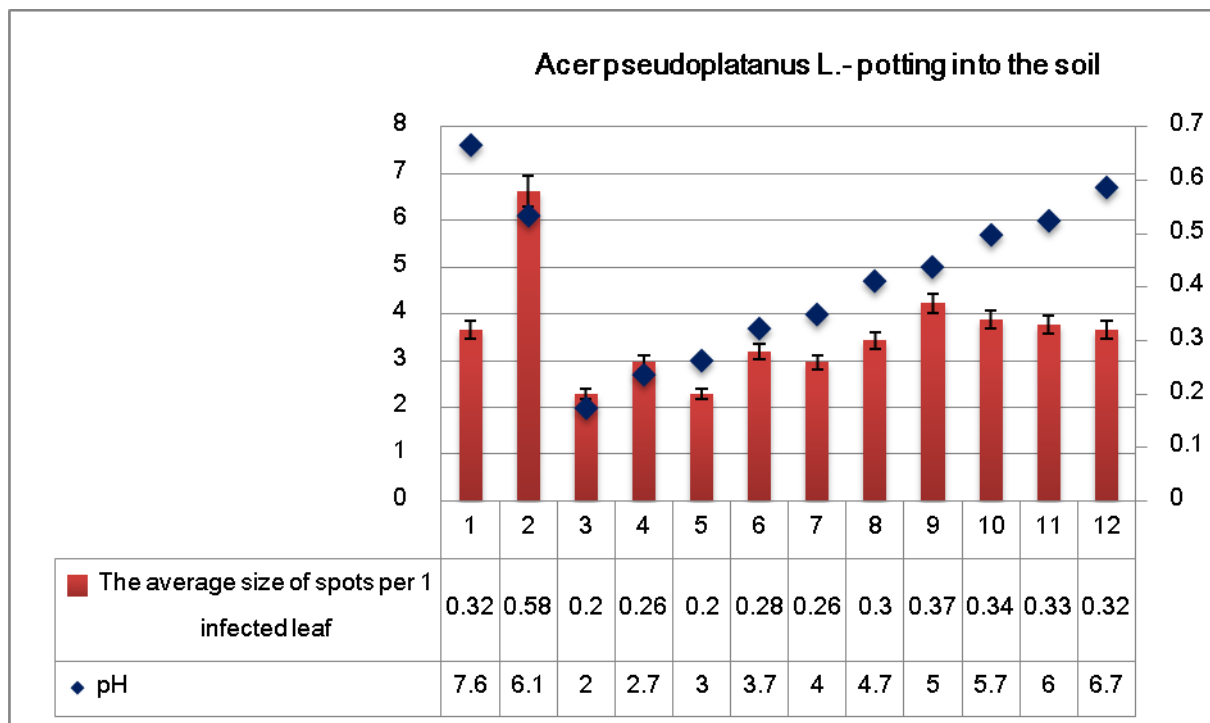
Graph 6: Average number of spots of seedlings of *Acer platanoides* L. potting into the soil



Graph 7: Average size of spots of seedlings of *Acer platanoides* L. potting into the soil



Graph 8: Average number of spots of seedlings of *Acer pseudoplatanus* L. potting into the soil



Graph 9: Average size of spots of seedlings of *Acer pseudoplatanus* L. potting into the soil

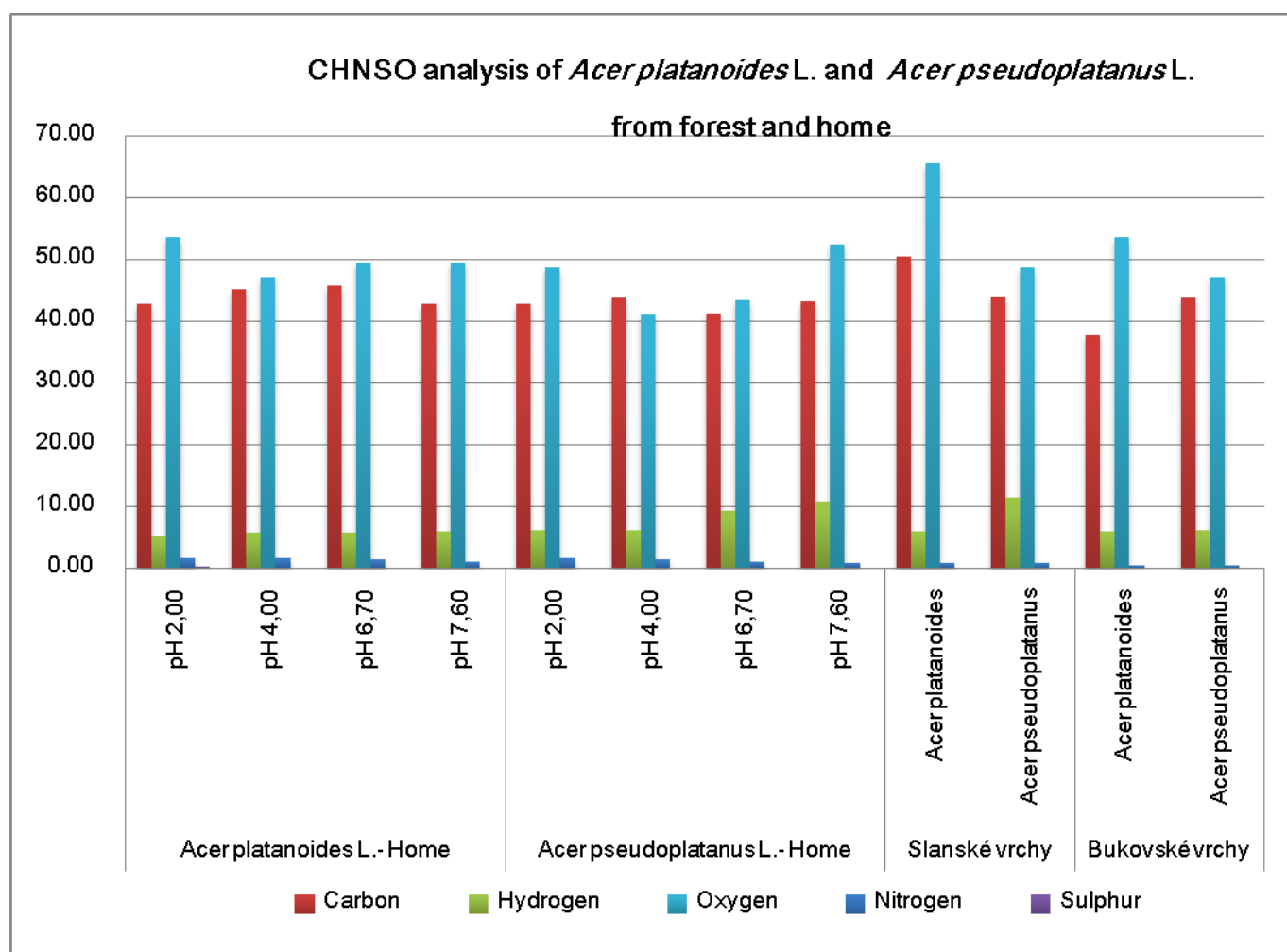
### CHNSO Analysis

**Nitrogen determination:** In comparison with the natural environment, the content of nitrogen in the affected plants was higher and was increasing with decreasing pH of the solution for watering (Graph 10). In *Acer platanoides* L. with pH 2,00 we observed by 39,50 % higher content of hydrogen than with pH 7,60, in *Acer pseudoplatanus* L. by 45,96 %. In the Slanské mountains the content of nitrogen in *Acer platanoides* L. was higher by 55,19 % than in the Bukovské mountains. In the Bukovské mountains in *Acer pseudoplatanus* L. it was lower by 48,29 % than in the Slanské mountains.

**Determination of carbon, hydrogen, oxygen:** The higher content of carbon, hydrogen and oxygen was discovered at the maples most infected by *Rhytisma acerinum* L. (Graph 10). In *Acer platanoides* L. we measured by 6,53 % higher content of carbon at pH 6,70 than at pH 2,00, and in *Acer pseudoplatanus* L. by 3,67 % higher content at pH 2,00. In the Slanské mountains the content of nitrogen in *Acer platanoides* L. was higher by 25,37 %, in *Acer pseudoplatanus* L. by 0,87 % higher than in the Bukovské mountains. The content of hydrogen in *Acer platanoides* L. was higher by 11,74 % at pH 6,70, in *Acer pseudoplatanus* L. by 33,97 %. At *Acer platanoides* L. no differences were discovered. In *Acer pseudoplatanus* L. the content of

hydrogen was higher by 46,05 % in the Slanské mountains. The content of oxygen was higher by 7,71 % in *Acer platanoides* L. with pH 2,00, in *Acer pseudoplatanus* L. by 10,86 %. In *Acer platanoides* L. the content of oxygen was higher by 18,34 % in the Slanské mountains, in *Acer pseudoplatanus* L. by 3,31 %.

**Sulphur determination:** Plants receiving sulphur in a form of  $\text{SO}_4^{2-}$ . Its presence was discovered in the leaves of *Acer platanoides* L. (Graph 10).



Graph 10: The results of the CHNS alnalysis of *Acer platanoides* L. and *Acer pseudoplatanus* L. from forest and home

### PKMgCa Analysis of Soils

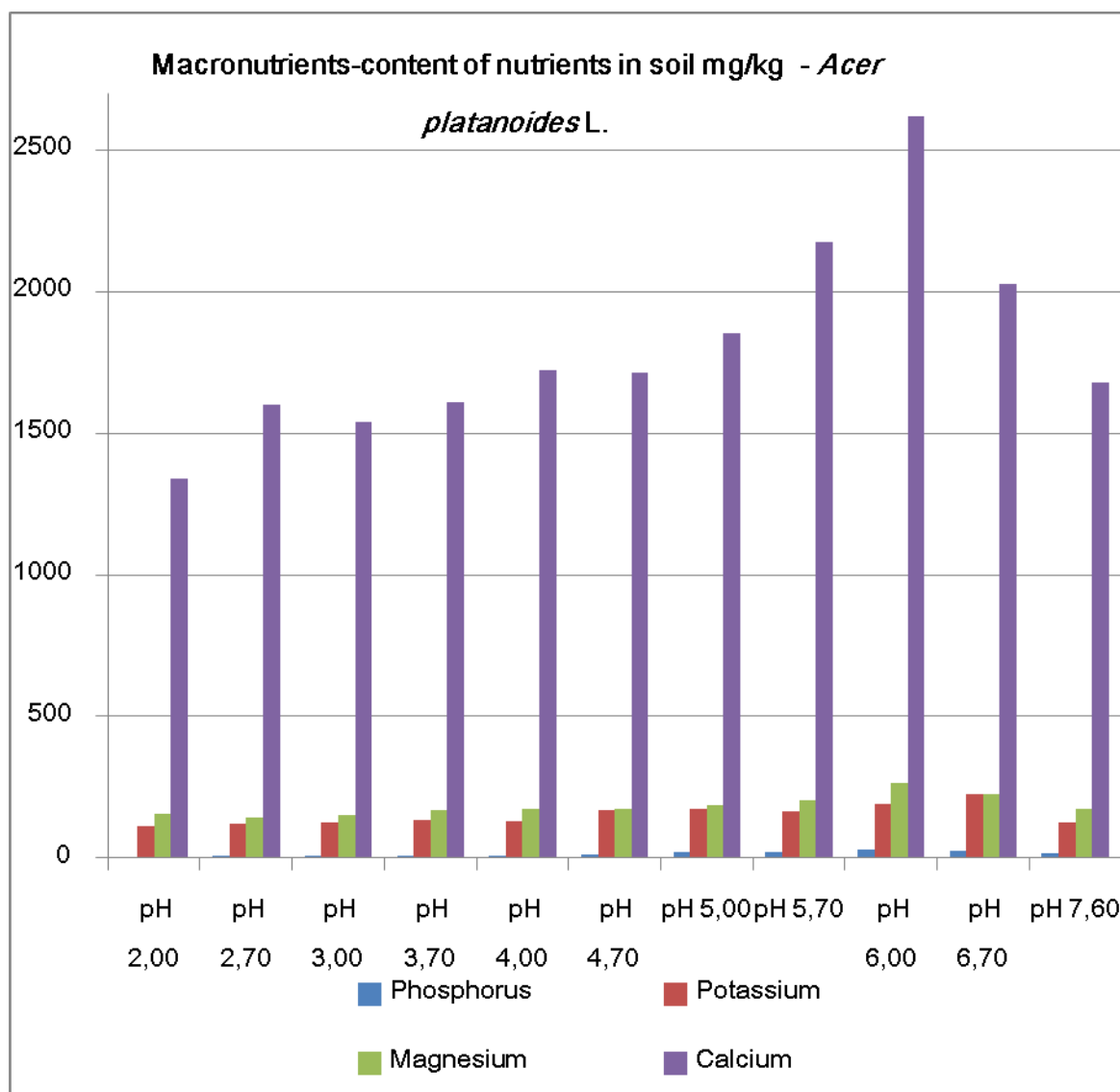
The soil samples were evaluated at the same time like the leaves.

**Phosphorus:** The highest content of phosphorus was in the soil watered with the acid solutions with pH 5,00 - 6,70. The lower content of phosphorus by 86,67 % was in the soil watered with the solutions of H<sub>2</sub>SO<sub>4</sub> with pH 2,00 in *Acer platanoides* L. (Graph 11) and by 55,56 % lower in *Acer pseudoplatanus* L. (Graph 12). The highest levels of phosphorus were measured in *Acer platanoides* L. The verification samples did not show the highest figures.

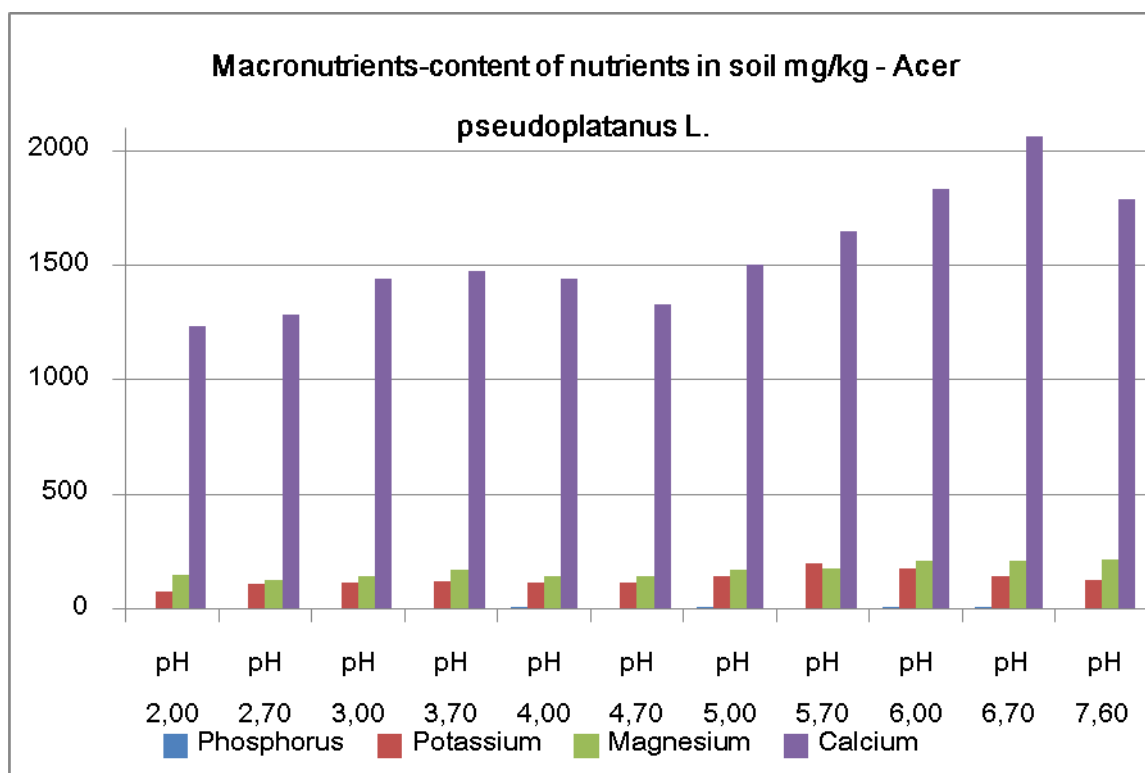
**Potassium:** The highest content of potassium was in the soil watered with the acid solutions with pH 5,00 - 6,70. By 49,33 % lower content of kalium was in the soil watered with the solutions of H<sub>2</sub>SO<sub>4</sub> with pH 2,00 in *Acer platanoides* L. (Graph 11) and by 61,69 % in *Acer pseudoplatanus* L. (Graph 12). The highest levels of kalium were discovered again in *acer platanoides* L. The verification samples did not show the highest figures.

**Magnesium:** The highest content of magnesium was in the soil watered with the acid solutions with pH 5,00 - 6,50 in *Acer platanoides* L. and with pH 5,70 - 6,00 and in the verification sample (pH 7,60) in *Acer pseudoplatanus* L. By 45,25 % lower content of magnesium was in the soil watered with the solutions of H<sub>2</sub>SO<sub>4</sub> with pH 2,00 in *Acer platanoides* L. and by 30 % in *Acer pseudoplatanus* L. The highest levels of magnesium were again measured in *Acer platanoides* (Graph 11). The verification sample showed the highest level in *Acer pseudoplatanus* L. (Graph 12), (by 2,33 % higher than at pH 6,70).

**Calcium:** The highest level of calcium was in the soil watered with the acid solutions with pH 5,00 - 6,70 in *Acer platanoides* L. and with pH 5,70 - 6,00 + the verification sample (pH 7,60) in *Acer pseudoplatanus* L. By 49,03 % lower content of calcium was in the soil watered with the solutions of H<sub>2</sub>SO<sub>4</sub> with pH 2,00 in *Acer platanoides* L. and by 40,14 % in *Acer pseudoplatanus* L. The highest levels of calcium were discovered again in *Acer platanoides* L. (Graph 11). The verification sample showed one of the highest figures in *Acer pseudoplatanus* L. (Graph 12).



Graph 11 : Results of PKMgCa analysis of soils of *Acer platanoides* L. watered with  $H_2SO_4$  solutions

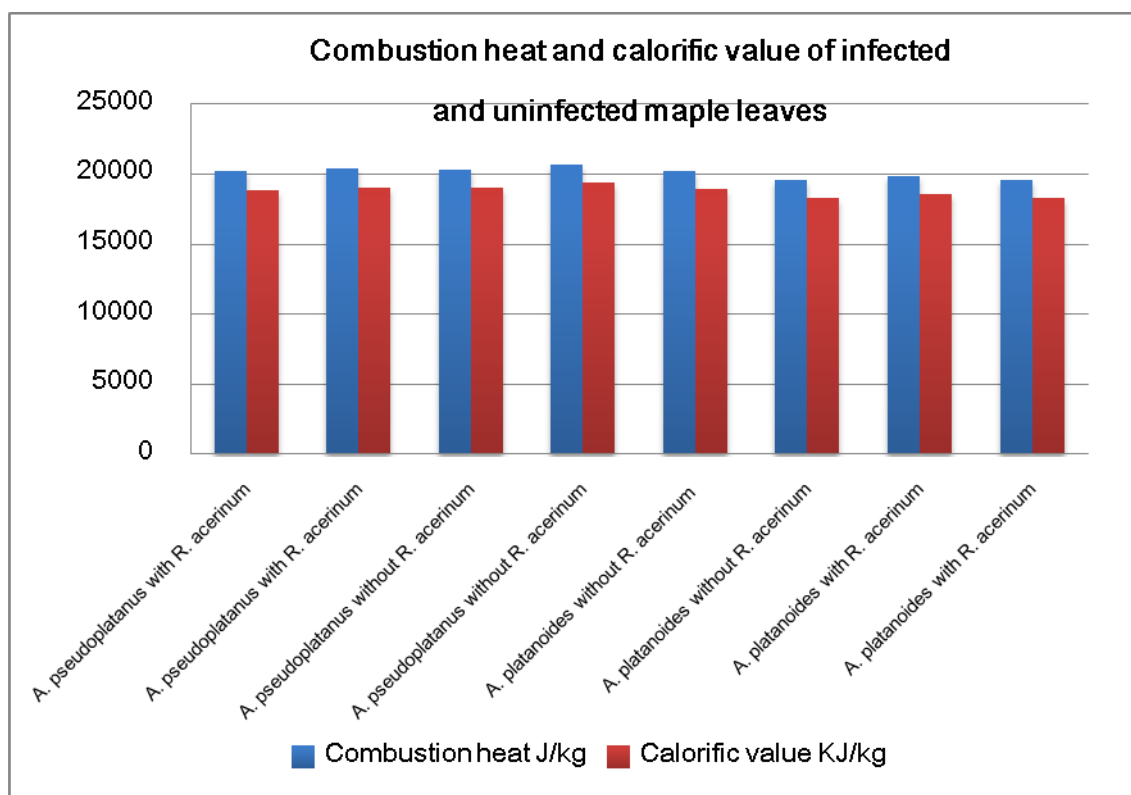


Graph 12 : Results of PKMgCa analysis of soils of *Acer pseudoplatanus* L. watered with  $H_2SO_4$  solutions

### Combustion heat and calorific value of healthy leaves and leaves infected by *Rhytisma acerinum* L.

We did not measure significant differences in production of biomass by the leaves infected by *Rhytisma acerinum* L. and by the parts of branches (Graph 13). In *Acer platanoides* L. the difference between the calorific value of the infected and the uninfected leaves and the tree branches was 1,18 % - without *Rhytisma acerinum* L. and in the combustion heat was 0,94 % - without *Rhytisma acerinum* L. In *Acer pseudoplatanus* L. the difference between the calorific value of the infected and the uninfected leaves and the tree branches was 1,29 % - without *Rhytisma acerinum* L. and in the combustion heat was 1,11 % - without *Rhytisma acerinum* L.





Graph 13: Combustion heat and calorific value of infected and uninfected maple leaves

## 4. Discussion

All the mentioned authors did the research of *Rhytisma acerinum* L. in natural conditions.

### Coherence between *Rhytisma acerinum* L. and SO<sub>2</sub> in the air

I. D. Leith and D. Fowler [6] studied the relative importance of the actual concentration of SO<sub>2</sub> in the air to the occurrence of infection *Rhytisma acerinum* L. They discovered that the average concentration of SO<sub>2</sub> lower than 50 µm.m<sup>-3</sup> has a small impact. In the studied areas (the Bukovské and Slanské mountains) there has been the maximum daily level of SO<sub>2</sub> in the air 17 µm.m<sup>-3</sup> in the last two years. Despite this we did not spot *Rhytisma acerinum* L. in the nearby industrial and urban zones of cities Prešov and Košice, only in the forests of e.g. above mentioned mountains. We proved the connection between the pollution of the environment by the emissions of SO<sub>x</sub> and the presence and dynamics of bio-indicating demonstrations of *Rhytisma acerinum* L.

P. Kosiba [5] studied if the presence of *Rhytisma acerinum* L. and the variability of the size of tar spots on leaves of *Acer platanoides* L. depends on the measure of the pollution of the environment. He compared industrial areas with city agglomerations. He discovered that different pollution of the air considerably

influences the size of spots of *Rhytisma acerinum* L. on leaves of *Acer platanoides*. During our field research we compared the average number and size of spots on the leaves of *Acer platanoides* and *Acer psedoplatanus*. The highest occurrence was discovered in NP Poloniny – an isolated protected area without significant human interference. Also the difference between the presence of bioindicating demonstrations of infection *Rhytisma acerinum* L. on the maples in protected and industrial areas was discovered.

R. J. Bevan and G. N. Greenhalgh [1] studied the connection between the occurrence of black leaf-spots and the presence of *Rhytisma acerinum* L., which is connected with the annual concentration of SO<sub>2</sub> in the air, estimated on the calculation of tar spots for an area unit of leaf surface. They present that the tolerance of fungus to the presence of SO<sub>2</sub> in the air is 90 µm.m<sup>-3</sup>.

We discovered that in rainier year 2014 bioindicating demonstrations (the number and size of spots) of *Rhytisma acerinum* L. were more significant. The probable reason was lower rainfall and humidity of the air. We proved the dependence of the occurrence of *Rhytisma acerinum* L. on a change of the chosen meteorological features, as *Rhytisma acerinum* L. needs cold weather and frequent rainfall. The favourable weather in the spring (rainfall, humidity) could have had an influence on the number of spots, then new spots were appearing and the formation of the new ones could have been connected with the sufficiently infected leaves at the beginning of the year. The rainfall in the next season did not have to have a more significant impact on spots spread (only as a modifier, but the rate of such influence is difficult to quantify).

By comparison of the content of biogenic elements in the leaves we discovered that the higher content of nitrogen in the affected plants can be related to increased photosynthesis of dinitrogen dioxide in plants of maples, also to their reaction to stress. Organic nitrogen compounds are formed in big measures during proteins decomposition in ageing and by stress-harmed leaves. We measured also a higher content of carbon, hydrogen and oxygen in the maples most infected by *Rhytisma acerinum* L. An immediate reaction of an infected cell of a host (maple leaves) is coarsening of cell walls and forming a wrapping saccharides layer around the hyphas of fungus. The protoplasm of the xylem parenchyma encrusts, which leads to immobility of water and water stress. Leaves infected by fungus have a smaller area of the photosynthetic parenchyma. The reaction of plants to fungus infection is also production of superoxide and peroxide anions – toxic to pathogen (oxidant flare) – it leads to a higher level of catabolism and respiration. All these factors lead to faster defoliation of leaves. *Acer platanoides* L. was more sensitive to the presence of sulphur.

The highest content of calcium, magnesium, kalium and phosphorus was in the soil of the maples strongly infected by *Rhytisma acerinum* L. Phosphorus – tolerableness of the soil is influenced mainly by pH, acid soils tolerate phosphorus less. Kalium is present in the soil in an anorganic form, bonded with soil minerals. Soils

high in smectics are able to bond kalium and offer it to plants in a sufficient amount. Magnesium exists in soils in salts, it moves easily, and it is washed away. Calcium is very important for a good soil structure. At low pH it supports phosphorus intake ,permanent humus production and decreases soil acidity.

After the evaluation of the calorific value and combustion heat we did not discover significant differences in production of biomass of the maples.

Except from the studied factors (chosen meteorological agents and  $\text{SO}_x$ ) also other factors (other types of pollution: heavy metals, alkali dust, organic pollutants; types of soil substrate; intraspecific variability) can influence the occurrence of *Rhytisma acerinum* L. The impact of  $\text{SO}_x$  seems to be relevant, but since it does not have to be the only one, we would like to foccus on other possible factors during our future research.

## 5. Conclusion

In 2014 the infection of pathogen was more significant than in 2015 - which was according to meteorological features warmer and dryer. The leaves of both kinds of maple were infected by *Rhytisma acerinum* L. in the highest localities of the studied areas, which are protected areas without human interference and with the highest quality of the environment. All the trees of *Acer pseudoplatanus* L. and *Acer platanoides* L. were infected. We did not discover the infection at *Acer campestre* L. The highest measure of the infection was observed in the area without intense industrial activity in the Bukovské mountains. In *Acer platanoides* L. a lower number of bigger spots was discovered and a slower beginning of the infection than in *Acer pseudoplatanus* L.

Thanks to studying the connection between  $\text{SO}_x$  (acid rain and occurrence of maples defecation at the seedlings of the maples) we discovered that the lower pH of the solution for watering, the higher measure of the infectedness of the maples. On *Acer pseudoplatanus* L. from pH 2,00 - 4,00 spots grew minimally, from pH 4,70 spots grew bigger. *Acer platanoides* L. was more sensitive to  $\text{SO}_2$ , after pH 4, 00 there were few spots. We proved the relation between the amount of  $\text{SO}_x$  and occurrence of *Rhytisma acerinum* L. Significant demonstrations of the maples infection by fungus *Rhytisma acerinum* L. were present already in the summer . *Rhytisma acerinum* L. directly influences the measure of photosynthesis, because it weakens maples leaves and photosynthetic tissue. This leads to faster defoliation of leaves. The most infected trees of *Acer pseudoplatanus* L. showed 2 weeks sooner fall of their leaves than the trees of *Acer pseudoplatanus* L. watered by the solutions with low pH. The strongly infected leaves fell down first. In *Acer platanoides* L. we did not observe any leaves fall.

Based on the analyses of the mineral structure of the leaves and soils (at the end of the experiment) we pointed out at a higher content of carbon and hydrogen in the infected maple leaves, as well as the content of phosphorus, kalium, calcium and magnesium in the soil of the trees strongly infected by *Rhytisma acerinum* L. The studied fungus fastens the process of soil humification through faster decomposition. We suppose that it is possible to apply our results about infecting maple leaves by *Rhytisma acerinum* L. to an important biomass source, a willow and its pathogen *Rhytisma salicinum* L. More humified, enriched soil can contribute to faster growth during reforestation.

We did not measure any significant differences in the production of biomass from the leaves and parts of branches of the maples infected and uninfected by *Rhytisma acerinum* L. We measured minimal differences between the calorific value and combustion heat of both kinds of the maples- either healthy ones or infected by *Rhytisma acerinum* L.

*Rhytisma acerinum* L. can be supposed to be an appropriate bioindicator of the environment, indicating the presence of acid rain ( $\text{SO}_x$ ,  $\text{NO}_x$ ). Sulphur is an important part of manufactured fungicidal products, therefore, we would like to focus on experimental research of the influence of  $\text{SO}_x$ . We plan to perform a similar experiment studying the influence of  $\text{NO}_x$ .

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