

VARIATIONS OF PHOSPHORUS, SULPHUR AND NITROGEN CONTENT IN LICHENS IN THE FORMER MANUFACTURING AREAS

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Abstract. The aim of this research work was to evaluate the pollution level of phosphorus, sulphur and nitrogen in the former manufacturing areas (former drainage pipe factory and landfill site) and compare it with the forest area levels using lichens as bioindicators. Additionally, the correlation between the content of these elements in lichens and soil was examined. For this study, three sampling sites in Latvia were chosen: the former Kuprava drainage pipe factory, the former landfill site and Sita forest the distance between them of 10-25 km. The research results show that in the former drainage pipe factory area and landfill site, the levels of phosphorus, sulphur and nitrogen content were elevated in comparison with Sita forest. In the case of phosphorus and nitrogen contents, a positive correlation was observed between soil and lichen samples.

Keywords: phosphorus, nitrogen, sulphur, lichen, soil.

Received: 11 September 2020/ Revised final: 26 October 2020/ Accepted: 16 November 2020

Introduction

A drainage pipe factory was built in 1971 near Kuprava (Latvia), but in the mid-1980s bricks and other building materials were also manufactured. After the restoration of independence of Latvia in 1991, the factory was renamed to “Kuprava State Building Material Company”, which was closed in the spring of 1992. It was important to verify the impact of the former Kuprava drainage pipe factory, which functioned during the period from 1971-1992 and used the Kuprava deposits for the production of drainage pipes, ceramic pebbles and bricks. In those times, the surrounding area was covered with clay dust and mazut, which was intensively used as a fuel. Nowadays, the surrounding area is abandoned and there are small landfill sites. Leakages have been observed near the mazut depots, resulting in small puddles. It is important to note that mazut leakages are located near the river “Pērdeja” and in 26 years the mazut residues from the Kuprava drainage pipe factory have not been eliminated. Thus, Kuprava manufacturing area constitutes a possible pollution source that requires monitoring. Moreover, the landfill site in Kubuli was closed in 2012 and buried with dirt. During the operation of the landfill site, various types of wastes were intensively discarded such as foodstuffs, accumulators, washing machines, tires, etc., thus constituting a possible pollution source

that also requires observation. Currently, a new forest grows in this area, but there were also observed some wastes. A possibility of monitoring the pollution levels on a territory is by using lichens.

Lichens (*Lichenes*) belong to the group of symbiotic organisms which consist of three components: fungi, algae or cyanobacteria and in some cases yeasts. Until 2015, approximately 768 lichen species were discovered on the territory of Latvia and over 60 of them are included in the list of protected species [1-3]. According to their appearance, lichens are divided into three main groups: crustose, foliose (leafy) and fruticose (bushy) lichens [4]. As lichens are sensitive to the changes in environmental conditions such as humidity, temperature and even air pollution, they are widely used in environmental pollution studies [5]. There are two possible ways of using lichens as bioindicators of the pollution in a selected area. First, by mapping the diversity of lichen species in the designated area [6,7]. The lower diversity of lichens implies a higher level of pollution. In fact, some sensitive lichen species do not grow in highly polluted areas [8]. The second possible way is by sampling individual lichen species to analyse pollutants that accumulate on the thallus surface [9]. Heavy metals, non-metals and even radioactive chemical elements can be detected in lichens. Moreover, they are sensitive to air

pollution, that may negatively affect the population of lichen species [10-13]. Additionally, due to their wide geographical range, lichens allow analysing the long-range gradient air pollution [14-16].

Nowadays, several standardized methods of analysis were developed, widely used to monitor the air pollution level by using lichens; these include inductively coupled plasma mass spectrometry, atomic absorption spectrophotometry, X-ray fluorescence spectrometry, neutron activation analysis and the Kjeldahl method for determination of total nitrogen content. The most often determined chemical elements in lichens are S, N, P, Zn, F, Pb, Ni, Cu, Fe, Mg, Mn, Cr, Cd, Ca and several rare earth elements [17]. For example, in the entire territory of the USA and Scandinavian countries, mosses and lichens are successfully used to monitor metallic elements, and sulphur and nitrogen content in the forests and National parks [18].

Several lichen sampling protocols have been developed. For example, it is recommended in the monitoring of the air pollution that the lichens be collected on the trees, at a distance of 1-2 m from the ground and those on the ground. Moreover, to describe the air pollution level of a specific area, it is necessary to choose several sampling plots with a square grid between 0.2 and 4 km². Sampling plots are divided into two types: the main plot and subplots. The main plot is an area that is considered a potential source of pollution. Subplots are areas located at a certain distance from the main plot. The comparison of chemical element analysis of lichens between main plots and subplots are used to analyse air pollution level [19].

In the framework of this research three areas were selected: former Kuprava drainage pipe factory and former landfill site in Kubuli region as areas with potential levels of pollution and Sita forest with unpaved roads and low

vehicle traffic was considered as the reference area with the lowest level of pollution.

The aim of this research work was to evaluate the pollution level by determining the contents of phosphorus, sulphur and nitrogen in the former manufactured areas (former Kuprava drainage pipe factory and landfill site) and to compare with the Sita forest area levels (used as a reference area) with the lowest level of pollution using lichens as bioindicators.

Experimental

Reagents

Phosphate standard solution (Merck, $\gamma_{\text{PO}_4} = 999 \pm 2$ mg/L), ammonium molybdate tetrahydrate (Sigma-Aldrich, ACS reagent, 99.98%), ascorbic acid (Enola, analytical grade), potassium sulphate (Sigma-Aldrich, ACS reagent, $\geq 99.0\%$), barium chloride dihydrate (Acros Organics, ACS reagent, $\geq 99.0\%$), potassium nitrate (Enola, analytical grade), hydrochloric acid (Sigma-Aldrich, ACS reagent, 37%) were used in this study.

Description of sample collection

The research was performed in 2017 at the University of Latvia, Faculty of Chemistry, Department of Analytical chemistry laboratories Riga, Latvia. Lichen and soil samples were collected in September of 2017. Three lichen species were chosen for this study: *Cladonia rangiferina* (fruticose), *Xanthoria parietina* (foliose) and *Peltigera collina* (foliose) (Figure 1).

Samples were collected in three different growth areas – on the ground, trees and boulder stones. A knife was used to separate the lichens from the substrate. In each sampling site, about 6 to 14 lichen samples were collected. Soil samples were collected at the depth level ~5 cm with a shovel. Lichen and soil samples were collected in plastic bags and stored in air atmosphere, in the laboratory at room temperature (25°C).

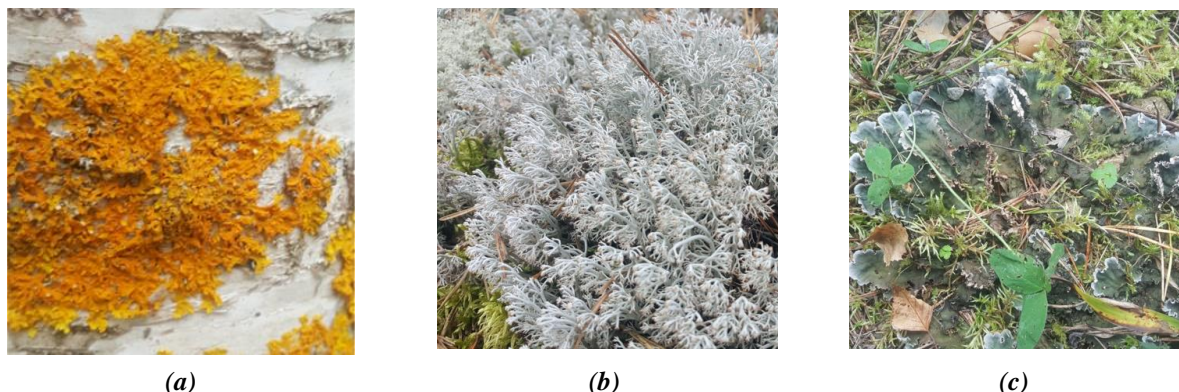


Figure 1. Collected lichen species: *Xanthoria parietina* (a), *Cladonia rangiferina* (b) and *Peltigera collina* (c).

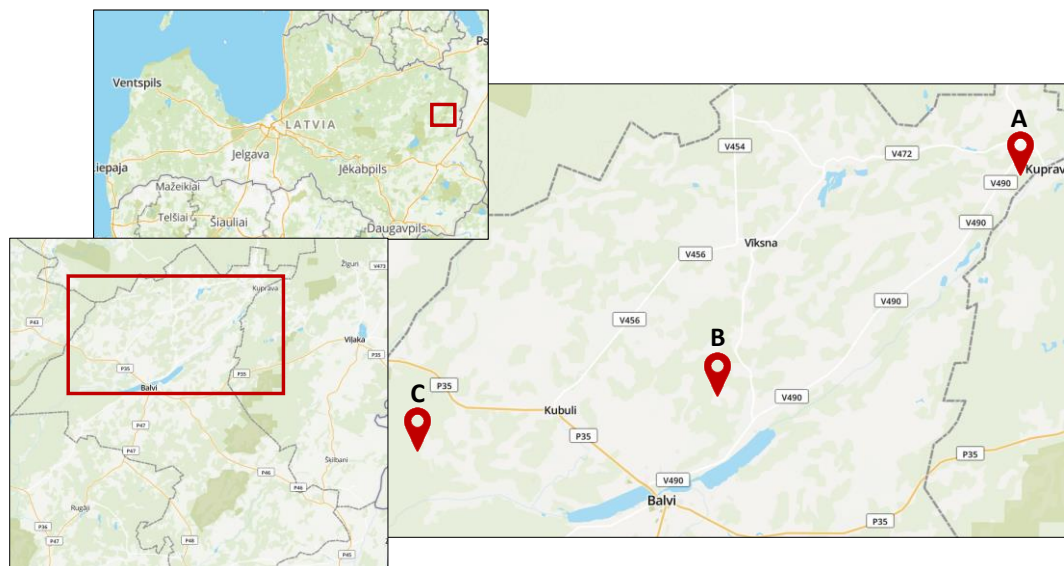


Figure 2. Sampling sites: A – former Kuprava drainage pipe factory, B – former landfill site in Kubuli and C – Sita forest.

Description of sampling sites

Three sampling sites were selected for the research purposes in Latvia: former Kuprava drainage pipe factory ($57^{\circ}14'00.0''\text{N}$ $27^{\circ}29'06.0''\text{E}$), former landfill site ($57^{\circ}09'30.5''\text{N}$ $27^{\circ}18'01.7''\text{E}$) and Sita forest dominated by pine species (*Pinus sylvestris* L.), and also birch (*Betula*) and aspen (*Populus tremula*) trees were observable ($57^{\circ}08'46.2''\text{N}$ $27^{\circ}07'06.2''\text{E}$) with the distance between sites of 10-25 km (Figure 2). During sample collection it was observed that lichen diversity in the former factory and landfill sites was lower than in the forest area.

Lichen and soil samples preparation

Lichens were separated and cleaned from soil, moss and leaves, afterwards dried at 105°C for 2 h in the oven (Memmert UNB 100).

Lichens samples were mineralized using the dry digestion method. Samples were ashed in the muffle (Nabertherm L 9/14) at 450°C for 4 h, dissolved in 2 mL of 2 M HCl and brought to a volume of 25 mL with deionized water. The undissolved particles were removed from the samples solution by filtration.

Soil samples were air-dried, sieved ($d_m = 2$ mm) and subjected to extraction procedure with 0.2 M HCl or 1% potassium alum solution depending on the analysis method.

Phosphorus, sulphur and nitrogen determination in lichen and soil samples

Phosphorus determination

Phosphorus content in lichens and soil samples was determined photometrically using a spectrophotometer (Spectrophotometer Jenway 6300, UK) as phosphate ions by ammonium molybdate ($\lambda = 720$ nm, $l = 5$ cm). The calibration curve was obtained by using a phosphate standard

solution (Merck $\gamma_{\text{PO}_4} = 999 \pm 2$ mg/L). During measurements, three replicates were performed for each sample. Phosphorus content (W_P) in lichens and soil samples was calculated using Eq.(1).

$$W_P = \frac{(\gamma_{\text{PO}_4} \cdot V \cdot \frac{M_P}{M_{\text{PO}_4}})}{m_{\text{sample}}} \quad (1)$$

where, W_P – concentration of phosphorus (g/kg);
 γ_{PO_4} – mass concentration of phosphate in the sample (mg/L);
 V – volume of sample (L);
 M_P , M_{PO_4} – molar mass of phosphorus and phosphate (g/mol);
 m_{sample} – sample weight (g).

Sulphur determination

Sulphur content in both samples was determined turbidimetrically on a spectrophotometer (Spectrophotometer Jenway 6300, UK) as sulphate ions using barium chloride solution ($\lambda = 450$ nm, $l = 5$ cm). The calibration curve was obtained by using the K_2SO_4 solution as the standard solution ($\gamma_{\text{SO}_4} = 200$ mg/L). During measurements, three replicates were performed for each sample. Sulphur content (W_S) in samples was calculated using Eq.(2).

$$W_S = \frac{(\gamma_{\text{SO}_4} \cdot V \cdot \frac{M_S}{M_{\text{SO}_4}})}{m_{\text{sample}}} \quad (2)$$

where, W_S – concentration of sulphur (g/kg);
 γ_{SO_4} – mass concentration of sulphate in the sample (mg/L);
 V – volume of sample (L);
 M_S , M_{SO_4} – molar mass of sulphur and sulphate (g/mol);
 m_{sample} – sample weight (g).

Nitrogen determination

Nitrogen content in both samples was determined as nitrate ions ionometrically (Adrona pH meter AD 1405, Latvia). The calibration curve was obtained by using 0.1 mol/L KNO₃ standard solution. During measurements, three replicates were performed for each sample. Nitrogen content (W_N) in samples was calculated using Eq.(3).

$$W_N = \frac{(C_{NO_3^-} \cdot V \cdot M_N)}{m_{\text{weight}}} \quad (3)$$

where, W_N – nitrogen concentration (g/kg);
 $C_{NO_3^-}$ – molar concentration of nitrate in the sample (mol/L);
 V – volume of sample (L);
 M_N – molar mass of nitrogen (g/mol);
 m_{sample} – sample weight (g).

Results and discussion

Phosphorus, sulphur and nitrogen content in lichens

The nitrogen and sulphur contents were evaluated in this study, as they are the most common chemical elements for study air pollution levels. Additionally, the content of phosphorus was analysed due to the fact that in the former factory, high-temperature resistant bricks and blocks containing high amounts of sodium pyrophosphate were produced. The determined phosphorus, sulphur and nitrogen contents in lichens from the different locations are presented in Table 1.

Firstly, it should be noted that lichens *Cladonia rangiferina* that grew on the ground in the former factory and landfill sites contained similar amounts of phosphorus, which was overall higher than the content in the lichens from the forest area. Phosphorus content in lichens *Xanthoria parietina* were similar in the samples collected from all three studied areas on the

different trees. The phosphorus content evaluation in the collected samples of lichens *Peltigera collina* from the former drainage factory indicated that the element concentration decreased in the following order: ground> tree> boulder stone.

Secondly, the sulphur content in lichens *Cladonia rangiferina* collected on the ground at the factory and landfill site was similar, however it was three times higher than in same lichens collected from the forest area. Results show that in the drainage pipe factory area there are no significant differences of sulphur content between lichen species *Peltigera collina* and *Xanthoria parietina* which grew on different trees. Sulphur contents in lichens *Xanthoria parietina* which grew on the trees were similar for all territories.

The determined nitrogen content at the former drainage pipe factory and landfill site on the trees growing lichens (*Xanthoria parietina*) was higher than in the forest area (~10 times). Irrespective of the lichen growth site, the nitrogen content in the Sita forest in all analysed lichens was similar.

Comparison of elements contents in soil and lichens grown on the ground

In this study, phosphorus, sulphur and nitrogen content in soil were compared with elements content in lichen samples which grow on the ground (Table 2). Results show that phosphorus, sulphur and nitrogen contents were higher in lichens than in soil samples. In the case of phosphorus and nitrogen content in soil samples from different areas, a trend was attested that the content of these elements decreases in the following order: factory> landfill> forest. The sulphur content was similar in all the soil samples regardless of the territory. In the former factory and landfill site, phosphorus and nitrogen content is ~15-30 times higher than in the forest area.

Table 1

Phosphorus, sulphur and nitrogen content (g/kg) in lichens in the three chosen locations.				
Territory	Growth site, lichen species	W_P	W_S	W_N
Former Kuprava drainage factory	Ground, (<i>Peltigera collina</i>)	0.87±0.06	0.85±0.07	13.92±0.62
	Ground (<i>Cladonia rangiferina</i>)	0.91±0.22	0.32±0.03	0.93±0.21
	Tree (<i>Populus tremula</i>), (<i>Peltigera collina</i>)	4.02±0.03	0.75±0.07	3.53±0.22
	Tree (<i>Populus tremula</i>), (<i>Xanthoria parietina</i>)	1.40±0.10	1.04±0.20	12.91±0.81
Former landfill	Boulder stone, (<i>Peltigera collina</i>)	7.72±0.32	0.35±0.07	5.31±0.32
	Ground, (<i>Cladonia rangiferina</i>)	0.73±0.22	0.30±0.05	10.32±0.53
	Tree (<i>Salix caprea</i> L.), (<i>Xanthoria parietina</i>)	1.13±0.15	1.50±0.14	11.32±0.61
Sita forest	Boulder stone, (<i>Peltigera collina</i>)	1.45±0.15	0.55±0.07	1.51±0.31
	Ground, (<i>Cladonia rangiferina</i>)	0.43±0.03	0.11±0.03	1.31±0.22
	Ground, (<i>Peltigera collina</i>)	0.52±0.03	0.35±0.07	1.23±0.42
	Tree (<i>Betula</i>), (<i>Xanthoria parietina</i>)	1.22±0.40	1.12±0.22	1.42±0.33

Results are presented as mean values with standard deviation, n= 6.

Table 2

Phosphorus, sulphur and nitrogen content (g/kg) in soil and the ground grown lichens samples.

Territory	W_P		W_S		W_N	
	Soil	Lichens	Soil	Lichens	Soil	Lichens
Former Kuprava drainage factory	0.310±0.022	0.870±0.060	0.098±0.002	0.850±0.070	0.222±0.002	13.92±0.62
Former landfill	0.190±0.006	0.720±0.040	0.091±0.003	0.310±0.070	0.113±0.007	10.32±0.53
Sita forest	0.005±0.001	0.430±0.060	0.094±0.003	0.110±0.03	0.008±0.001	1.31±0.22

Results are presented as mean values with standard deviation, $n = 6$.

Table 3

Phosphorus, sulphur and nitrogen content (g/kg) in lichens collected at different distances from the drainage factory area.

Growth site	Element	Drainage pipe factory	Landfill (15 km distance)	Sita forest (25 km distance)
Ground (<i>Cladonia rangiferina</i>)	P	0.91±0.22	0.73±0.22	0.43±0.03
	S	0.32±0.03	0.30±0.05	0.11±0.07
	N	0.93±0.21	10.32±0.53	1.31±0.22
Trees (<i>Xanthoria parietina</i>)	P	1.40±0.10	1.13±0.15	1.22±0.40
	S	1.04±0.20	1.50±0.14	1.12±0.22
	N	12.91±0.81	11.32±0.61	1.42±0.33

Results are presented as mean values with standard deviation, $n = 6$.

An overview of the determination of phosphorus, sulphur and nitrogen contents

The obtained results on the evaluation of the phosphorus, sulphur and nitrogen contents in lichens were summarized in Table 3. A tendency was observed in nitrogen content in lichens *Xanthoria parietina* showing a decrease in the following order: factory > landfill > forest. A similar tendency was observed in the content of phosphorus in lichens *Cladonia rangiferina*, where the content decreases in the same order: factory > landfill > forest. The sulphur content in the ground grown lichens *Cladonia rangiferina* was similar in samples collected from all three studied areas, as the atmospheric emission was reduced after factory site closure 26 years ago. The comparison of the obtained results on the two lichens species in each investigated area showed that a slightly lower phosphorus content was observed in the ground grown fruticose lichens *Cladonia rangiferina* samples, compared to the foliose lichens (*Xanthoria parietina*).

This study shows that the former factory and landfill site are still acting as local pollution sources. A possible transboundary pollution could arise from heavy traffic and boiler houses from

the nearest urban areas (Balvi, Gulbene, Aluksne, etc). Also, there are some small private farms near the former factory and landfill site, but their contribution to the pollution is minimal. Thus, it could be concluded that the former factory and landfill site act as pollution sources even after their closure. In particular, the collected soil samples have shown that the former factory and landfill site had higher phosphorus and nitrogen content than in the Sita forest area.

Comparison of the contents of phosphorus and sulphur in lichens with those communicated in the literature (Table 4) leads to the conclusion that these element contents can vary in a wide range and it depends on the environment (air quality). Russian researchers analysed lichens which were collected at different distances from the metallurgical factory and results show that by increasing the distance from the pollutant source, phosphorus and sulphur content in lichens decreases [20,21]. Comparing the obtained results with the elements content in lichens from the metallurgical plant in the Murmansk oblast, it can be concluded that the content of phosphorus and sulphur are similar.

Table 4

Phosphorus and sulphur content (g/kg) in lichens, a comparison with other research results.					
Territory	Lichen specie	Growth site	W_P	W_S	Comments
Moscow districts, Russia [20]	<i>Xanthoria parietina</i>	Different trees	0.016±0.004	0.30±0.04	Manchihina*
			0.033±0.006	0.50±0.06	Zhohova-Klenova*
			0.021±0.002	0.42±0.05	Chirikova*
Former drainage pipe factory, Latvia	<i>Xanthoria parietina</i>	Tree (<i>Populus tremula</i>)	1.40±0.10	1.04±0.20	Current study
	<i>Cladonia rangiferina</i>	Ground level	0.91±0.22	0.32±0.03	
	<i>Peltigera collina</i>	Ground level	0.87±0.06	0.85±0.07	
Metallurgical factory, Murmansk region, Russia [21]	<i>Cladonia stellaris</i>	On the ground	0.38±0.08	0.22±0.06	175 km**
			0.56±0.02	0.32±0.09	48 km**
			0.79±0.05	0.89±0.04	15 km**

*Districts of Moscow.

**Distance from metallurgical factory.

Results are presented as mean values with standard deviation.

Conclusions

The variation of phosphorus, sulphur and nitrogen levels at the former Kuprava (Latvia) drainage pipe factory and landfill site was determined in samples of three lichen species: *Cladonia rangiferina* (fruticose), *Xanthoria parietina* (foliose) and *Peltigera collina* (foliose) and soil. The obtained results were compared with those obtained in samples from Sita forest, which was used as the reference area with the lowest level of pollution. This study demonstrated that elevated levels of phosphorus, sulphur and nitrogen content are observed in the former drainage pipe factory area and landfill site, in comparison with Sita forest. The results show that the content of phosphorus and sulphur in lichens *Cladonia rangiferina* that grew on the ground was higher in the former factory and landfill site. A similar tendency was observed regarding the nitrogen content in lichens *Xanthoria parietin* that grew on the trees.

The collected soil samples showed that phosphorus and nitrogen contents decreased in the following order: factory > landfill > forest. The soil samples collected on the factory and landfill sites contained much higher levels of phosphorus and nitrogen than those from the forest area, ~15-30 times which can be identified as contamination and thus soil remediation is required. Despite the fact that the factory was closed 26 years ago and the landfill site 5 years ago, phosphorus, sulphur and nitrogen pollution are still observed in these areas being detected in both lichens and soil samples.

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