

Microfauna of Lichen (*Xanthoria parietina*) in Lithuania: Diversity Patterns in Polluted and Non-Polluted Sites

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Abstract

Assemblages of lichen are served as the habitat and food resource for various microinvertebrates. The lichens' dwelling microfauna is of special interest regarding the maintenance of sustainable balance in terrestrial ecosystems.

Little is known about microfauna consisting of protists and micrometazoa. The present study focused on the composition, diversity and distribution of microfauna in lichen *Xanthoria parietina* in Lithuania. Lichens were investigated beside highways and in unpolluted sites. The microfauna of *X. parietina* were represented by 24 taxa. Two species of tardigrades, two species of testate amoebae and one species of gymnoamoebae that are new in Lithuania were founded. The study results revealed that dominating protozoa in lichen *X. parietina* are testate amoebae (genus *Arcella*) and ciliates (*Colpoda*, *Paramecium*). *Philodina* sp. (Rotifera), *Aphelenchoides* sp. (Nematoda) and *Ramazzottius oberhaeseri* (Tardigrada) were found to be dominant micrometazoa in *X. parietina*.

The dominant protozoa (*Colpoda cuculus*, *Paramecium* sp. *Arcella* sp.) and micrometazoa (rotifers *Philodina*) didn't reveal the significant difference between polluted and unpolluted sites. Meanwhile the tardigrades were more abundant in unpolluted sites. Further studies on microfauna diversity using increased number of samples, sampling sites and estimating more environmental parameters can result in more distinct conclusions.

Key words: microfauna, lichen, pollution, protozoa, Tardigrada, rotifera

Introduction

Lichens are pioneers on bare rock, old buildings, cleared soil, dead wood, animal bones, and living bark. From there they slowly begin the process of creating a foundation for habitation for other organisms.

Assemblages of lichens serve as habitat and food resource for various microinvertebrates: tardigrades (Rebecchi et al. 2006), rotifers (Sohlenius et al. 2004), nematodes (Sohlenius et al. 2004), arthropods (Søchting and Gjelstrup 1985), and protozoa (Beyens et al. 1986, Nguyen Viet et al. 2004).

The lichens' dwelling microfauna is of special interest regarding the maintenance of sustainable balance in ecosystems (Vicente 2010): microorganisms may play an important role in ecosystems' food chains (Moore et al. 1993), litter decomposition and nitrogen dynamics on the floor (Gießelmann et al. 2010).

Many microinvertebrates are ecologically specialized and can only survive in particular conditions; consequently, the effects of pollution on lichens (Jovan 2008) can cause a reduction in the species composition and specimen number of the microfauna (Uetz et al. 1980, Vargha et al. 2002).

Due to the lack of roots, mineral nutrition of lichens depends mainly on the atmospheric input. This,

together with their ability to accumulate more mineral elements than they actually need, makes lichens one of the best bio indicators of air pollution (Stolte et al. 1993). Lichen has also been found to act as accumulators of elements, such as trace metals and radioactive elements (Stolte et al. 1993, Bargagli et al. 2002).

A variety of heavy metals derived from gasoline additives and road deciding salts are to the roadside environment. The most widely documented is lead, aluminum, iron, copper, manganese, zinc and nickel (Garcia-Miragaya et al. 1981). Heavy metals accumulate in the tissues of plants (Beslaneev and Kuchmazokova 1991) and invertebrates (Read et al. 1998). The alteration of chemical environment by roads results in a number of consequences for living organisms (Trombulak and Frissell, 2000). Roads have diverse and systemic effects on many aspects of terrestrial and aquatic ecosystems (Trombulak and Frissell 2000).

The lichen *Xanthoria parietina* ((L.)Th.Fr.) is a common lichen of urban areas and one of the most widely utilized lichen species in European biomonitoring programs (Silberstein et al. 1996). Although tolerance of *X. parietina* for pollutants has been known for a long time (Hawksworth and Rose 1970), only few studies (Gerson and Seaward 1977, Uetz et al. 1980, Meininger and Uetz 1985, Stubbs 1989, Roberts and

Zimmer 1990) have focused on microorganisms living in these lichens. The aims of this study are: (1) to investigate composition of the microfauna in *X. parietina* and (2) to examine how microfauna (abundance, species richness, diversity, taxonomic composition) differs between polluted and unpolluted sites. Because of the lack of data on microfauna groups in Lithuania such as heterotrophic protists rhizopodes, ciliates and micrometazoa including nematodes, rotifers, tardigrades, the study focused on estimation of above mentioned groups.

Materials and methods

Study area

Lithuania is located in the eastern part of Europe on the coast of the Baltic Sea (56° 00' N and 24° 00' E). The wettest months are July and August (92-95 mm rainfall) and the driest month is May (30 mm). The average temperature is 17 °C in July (the mean maximum temperatures 35 °C) and – 5 °C in winter (the mean minimum temperatures -25 -30 °C). The climate is temperate.

Lichens sampling sites were chosen in several localities of Lithuania: Prienai, Birštonas and highways A1 and A16 (Fig. 1; Table 1).

Lichen sampling and extraction of microfauna

Samples of lichens (approximately 5 g of lichens at each study site) were collected in 2008 during the period from September to November from *Populus tremula* (150 cm in height from the ground; age of trees was about 30 years). In addition, highway lichens were collected from the part of the tree trunks which were facing at the road side.

Lichen samples from both “unpolluted” (Prienai, Birštonas) and “polluted” (beside roads) sites were collected.

Samples of lichens in Prienai were taken from the pinewood “*Prienų šilas*” (labeled “D”) and botanical reserve “*Degsnės forest of larches*” (labelled “E”). Car traffic is completely absent in these sites. The soil of the pinewood and botanical reserve was fine sandy loam.

Sampling site in Birštonas (labelled “C”) was located in a relatively protected place, close to the Nemunas river where car traffic is limited. The soil in this area was turf.

The highway A1 Vilnius – Kaunas – Klaipėda was distinguished by very intensive traffic (more than 17000 cars per day (Special researches, measure and analysis in transport roads, 2002). Grigalavičienė and Rutkovienė (2006) investigated moss and topsoil collected along the highway Vilnius – Kaunas and analyzed pollution by motor transport emissions. According to their results, the high concentrations of Pb, Cu, Cd were established in the soils and moss near the highway (a distance 5 – 10 m from the road). The soil

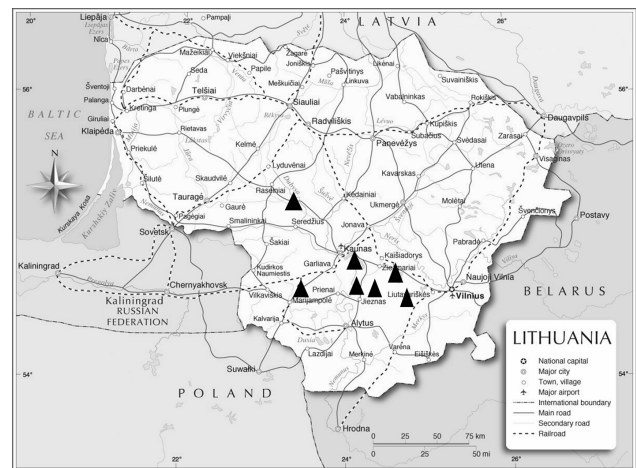


Figure 1. Location of lichens sampling sites in Lithuania

Sample number	Marking of sampling sites	Date	Sites of lichen sampling	Location latitude/ longitude	Comments	pH of lichen
1	A	21 September 2008	Section Vilnius – Kaunas of highway Vilnius – Kaunas – Klaipėda: 80 th kilometre	54°52'N; 24°27'E	The highway A1 connects the capital city of Vilnius and the Klaipėda Seaport. Length of highway 311, 40 km. Constructed in 1970.	6.45
2		21 September 2008	Section Kaunas – Klaipėda of highway Vilnius – Kaunas – Klaipėda: 120 th kilometre	54°58'N; 23°49'E		6.33
3	B	08 November 2008	Section Vilnius – Prienai of highway Vilnius – Prienai – Marijampolė: 93 th kilometre	54°36'N; 24°10'E	The highway connects the capital city Vilnius and Marijampolė, one of biggest city of Lithuania. Length of highway 137, 51 km. Constructed in 1987.	6.20
4		02 November 2008	Section Prienai – Marijampolė of highway Vilnius – Prienai – Marijampolė: 112 th kilometre	54°36'N; 23°59'E		6.21
5	C	21 September 2008	Birštonas city periphery, coast of the river Nemunas	54°37'N; 24°2'E	The centre of the regional park of the Great Nemunas river Bends	6.20
6	D	02 November 2008	Pinewood, Prienai district	54°38'N; 23°57'E	The banks of the Nemunas river	6.30
7		02 November 2008	Degsnės forest of larches, Prienai district	54°32'N; 23°53'E		Degsnė arboretum of larch trees growing in Prienai Forestry enterprise, Balbieriškis Forest. Degsnė is the most productive forest in Lithuania; age about 140.

Table 1. The dates, sites and location of lichens (*Xanthoria parietina*) sampling and measured sample pH

along roads on the sampling sites was turf with sand. Lichens were sampled at the distance of about 5 metres from the highway from two points: 1) from the section Vilnius – Kaunas (at 80 km lat long); 2) from the section Kaunas – Klaipėda (at 120 km lat long). The collected microfauna from two points of highway A1 was rather similar, therefore the obtained results were integrated together and highway A1 with two points of investigations was labelled as “A”.

The highway A16 Vilnius – Prienai – Marijampolė. The soil type along the road was turf and sandy loam. Lichens were sampled from two points: 1) from the section Vilnius – Prienai (at 93 km lat long). 2) from the section Prienai – Marijampolė (at 112 km lat long). Due to similar microfauna, the obtained results from two points of highway A16 were integrated like the case of highway A1, and labelled “B”.

Lichens from trees were harvested together with the tree bark. 2 g of lichens together with bark was used to measure sample pH using the Mettler Toledo SevenMulti pH conductivity meter; 0.5 ml of 0.1 M KCl water solution was adjust on the bark (approximately for one minute) before measurement. Lichens sampling data are presented in Table 1. Before analysis, lichen samples were stored at temperature of 4°C.

In the laboratory, approximately 1 g of dry lichen was rehydrated in Petri dishes (100 mm x 15 mm) by adding distilled water. Then 24 hours later lichens were squeezed and filtrate was concentrated with a centrifuge at 1500 rpm for 4-6 minutes. The supernatant was removed and the debris was sorted and analyzed by compound microscope equipped with a digital camera. Specimens of microfauna were identified and counted at 100x, 400x and 600x magnifications. 140 microscopic subsamples (60 µl) of the collected lichen samples were analyzed. A microfauna was identified to the lowest possible taxonomic level. The following literature was used for species/genus identification: Protozoa - Patterson (1998), Mažeikaitė (2003), Charman et al. (2000); rotifers – Ricci and Melone (2000); tardigrades – Guidetti and Bertolani (2005), Pilato et al. (2010), nematodes – Smart and Nguyen (1990).

The relative abundance of species (p_i) was calculated by:

$$p_i = n_i / N \times 100\%,$$

where n_i is a number of individuals of particular microfauna species in a sample, N is a number of all individuals of microfauna species in a sample. Species that had $p_i > 5\%$ were considered as a dominant. All data were subjected to statistical analysis of variance (one-Way ANOVA). In order to analyze microfauna diversity, species richness (S), Shannon-Wiener's (H) diversity indices were calculated for each site. Differences at levels of $p < 0.05$ were considered significant.

Non – parametric statistics (Mann-Whitney test) was used to compare the means of diversity, number of taxa between polluted and unpolluted sites. Statistical analysis was performed using Microsoft Excel (2003) package and software SPSS 17.0.

Results

The analysis of the samples of lichen *X. parietina* revealed microfauna of 24 taxa. Fifteen taxa of microfauna had relative abundance $< 0.5\%$. A detailed list of all observed taxa, together with their relative abundance (%) in samples of lichen is given in Table 2.

Protozoa: A total of 7 testate amoebae taxa, belonging to three genera were identified. The testate amoebae community was dominated by *Arcella discoides* and *Arcella* sp., which were found at most sample sites, but presented the less relative abundance. Other species *A. arenaria* (?), *Assulina muscorum*, *A. seminulum*, *Nebela flabellum* and *Nebela* sp. were rarely present and were the least abundant (Table 2). The highest abundance of testate amoebae was observed from site A (N=21), whereas the lowest was in samples from C (N=5). The total abundance of testate amoebae was similar between polluted (N=33) and unpolluted (N=33) sites (Mann – Whitney $p > 0.05$). The species richness of testate taxa varied between 2 and 3 was rather similar at all sampling sites (Table 2).

The diversity of testacea was highest (H=1.05) at unpolluted site “C”.

A total 6 taxa of ciliates were found in the samples. *Colpoda cucullus* and *Paramecium* sp. were the dominant and were distributed in most samples of lichens ($p > 0.05$) (Fig. 2). The highest abundance of

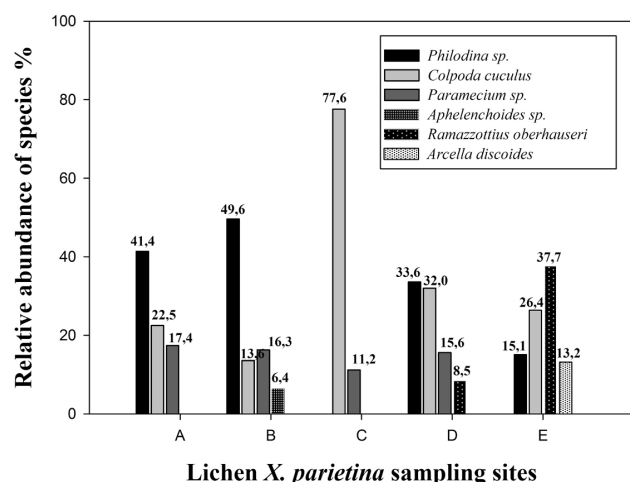


Figure 2. Dominance of microfauna in lichen *X. parietina* in sampling areas (A – Highway Vilnius-Kaunas-Klaipėda; B – Highway Vilnius-Prienai-Marijampolė; C – Birštonas; D – Pinewood, Prienai; E – Degsnės forest of larches, Prienai)

Table 2. The composition of microfauna in lichen *X. parietina* samples from five localities in Lithuania (results obtained from separate sections of highways were integrated together) (*N* – number of individuals; *p_i* – relative abundance of species; A – Highway Vilnius-Kaunas-Klaipėda; B – Highway Vilnius-Prienai-Marijampolė; C – Birštonas; D – Pinewood, Prienai; E – Degsnės forest of larches, Prienai)

Taxa	A		B		C		D		E	
	N	<i>p_i</i>	N	<i>p_i</i>	N	<i>p_i</i>	N	<i>p_i</i>	N	<i>p_i</i>
Sarcodaria: Rhizopoda										
Thecolobosea										
<i>Arcella discoidea</i> Ehrenberg, 1843	17	2.5	10	2.8					14	13.2
<i>Arcella arenaria</i> (?) Greef, 1866							10	2.4		
<i>Arcella</i> sp.			2	0.5	2	0.6	2	0.5		
<i>Assulina muscorum</i> Greef, 1888	2	0.29								
<i>Assulina seminulum</i> Ehrenberg, 1871	2	0.29								
<i>Nebela</i> sp.					2	0.6			2	1.9
<i>Nebela flabellulum</i> Leidy, 1874					1	0.3				
Gymnoamoebidae										
<i>Amoeba</i> sp.			5	1.3						
<i>Mayorella</i> sp.			2	0.5						
<i>Thecamoeba striata</i> Penard, 1902			3	0.8						
Ciliata										
<i>Colpoda cucullus</i> Müller, 1773	157	22.5	51	13.6	264	77.6	135	32.0	28	26.4
<i>Frontonia</i> sp.	5	0.7								
<i>Oxytrichum</i> sp.	7	1.0					1	0.23		
<i>Paramecium</i> sp.	121	17.4	61	16.3	38	11.2	66	15.6		
<i>Epistylis</i> sp.					7	2.1	2	0.5		
<i>Euplotes</i> sp.					3	0.9				
Tardigrada: Eutardigrada										
<i>Macrobiotus hufelandi</i> C.A.S. Schultze, 1834.	3	0.4	9	2.4	4	1.2	6	1.42	4	3.8
<i>Ramazzottius oberhaeuseri</i> Doyère, 1840	24	3.5	16	4.3	5	1.5	36	8.5	40	37.7
<i>Milnesium tardigradum</i> Doyère, 1840			1	0.3						
Nemathelminthes										
<i>Aphelenchoides</i> sp.	28	4.0	24	6.4	4	1.2	19	4.5	2	1.9
<i>Tylenchus</i> sp.	2	0.3								
Rotifera: Bdelloidea										
<i>Habrotrocha</i> sp.	15	2.2	5	1.3	1	0.3	4	0.94		
<i>Philodina</i> sp.	287	41.4	186	49.6	9	2.6	142	33.6	16	15.1
<i>Rotaria rotatoria</i> Pallas, 1766	23	3.3								
Total Abundance	693	100	375	100	340	100	423	100	106	100
Shannon-Weaver diversity index (H)		1.69		1.64		0.92		1.65		1.54
Species richness (S)		14.0		13.0		12.0		12.0		7.00

Colpoda cucullus was observed from the site “C” (N=264; 77.6%) whereas the lowest was in samples from “E” (N=28; 26.4). *Paramecium* sp. dominated in “A” (17.4%) and “B” (16.3%). The total abundance of ciliates was highest in “C” (N=312) and lowest in “E” (N=28). The comparison of abundance of ciliates between polluted (“A”, “B”) and unpolluted (“C”, “D”, “E”) sites showed that more ciliates (N=544) were recorded from unpolluted sites.

The species richness of ciliates varied between 1 and 4 taxa. The Shannon-Wiener diversity index was highest in lichen samples from highway (“A”, H=0.86). Only *C. cucullus* was found in samples from site “E” (Table 2).

A total 16 taxa of protozoa were recorded. General species richness of protozoa ranged from 3 to 7 taxa (Table 2). According to the Mann-Whitney test there was no significant difference ($p > 0.05$) between species richness of polluted (“A”; “B”) and unpolluted (“C”; “D”; “E”) sites. The minimal number of protozoan taxa (3) was observed in samples from site “E”. The lichens collected from the highways characterized by highest diversity (indices range from 1.08 to 1.25) of protozoa comparing with lowest diversity from

clean sites (indices range from 0.62 to 0.9), but according to Mann-Whitney test ($p > 0.05$) these differences were not significant. Abundances of protozoan between polluted and clean sites did not differ significantly (one-way ANOVA, $F=0.32$, $p > 0.05$).

Micrometazoa: The eight genera of micrometazoa (tardigrades, rotifers, nematodes) were found (Table 2). The dominate groups were rotifers, tardigrades and nematodes.

A total 3 taxa of rotifers belonging to order *Bdelloidea* were identified in analyzed samples. *Philodina* sp. was the dominant taxon between rotifers in all sample sites. The highest species richness (3), abundance (N=325) and diversity (H=0.44) of rotifers were recorded from highway “A” (Table 2). The lowest abundance (N=10) of rotifers were observed in unpolluted site (“C”). Site “E” did not show diversity whereas dominated only *Philodina* sp.

A total 3 species of tardigrades were identified in samples (Table 2). *Ramazzottius oberhaeuseri* with high relative abundance was found in all samples (Fig. 2). *Macrobiotus hufelandi* was detected in all samples as well; however, was less numerous and non-domi-

nant. The highest diversity of tardigrades ($H=0.99$) was recorded in lichen from site "B" (highway). Species richness of tardigrades was similar ($S=2.0$) between most investigated sites except the highway ("B") where four species of tardigrades were founded. Tardigrades were less abundant ($N=52$) at polluted sites ("A", "B") than at unpolluted ($N=95$).

The diversity of micrometazoa varied from 0.84 ("B") to 0.94 ("A") at polluted sites, meanwhile diversity indices were highest at clean sites and varied from 0.92 ("E") to 1.44 (site "C") (Table 2). Despite these results Mann – Whitney analysis revealed no significant ($p > 0.05$) differences in the mean Shannon-Wiener diversity between sampling sites. Species richness of micrometazoa varied from 4 taxa ("E") to 7 ("A") and was highest in polluted sites ("A" – $S=7.0$; "B" – $S=6.0$) (Table 2).

General observed microfauna was most diverse ($H=1.69$) in lichen samples from highways Vilnius – Kaunas – Klaipėda ("A"). On the same site was recorded the highest species richness of microfauna ($S=14$). The lowest diversity ($H=0.92$) was observed from unpolluted site Birštonas („C"). The highest species richness of microfauna was recorded from highway ("A"). Species richness from forest of larches ("E") was only 7 taxa (Table 2).

General abundance, number of taxa and diversity of microfauna revealed no significant differences between polluted and clean sites (one – way ANOVA, $F=0.43$, $p > 0.05$; Mann-Whitney $p > 0.05$).

Discussion and conclusions

Protists in *X. parietina*. To our knowledge, this is the first study on lichen testate amoebae in Lithuania. Mažeikaitė (Mažeikaitė 2003) observed 25 species of testacea amoebae in freshwater from various localities of Lithuania. Among seven testate amoeba taxa observed in the present study, two species (*Assulina muscorum*, *Nebela flabellulum*) are new for the fauna of Lithuania (Table 2).

The number of taxa (7) observed in this single lichen species is relatively low compared to that found by other studies on moss testate amoebae. Nguyen-Viet (Nguyen-Viet et al. 2007) recorded 23 taxa of testate amoebae in one species of moss and Mitchell (Mitchell et al. 2004) – 25 species of testate amoebae in moss *Hylocomium splendens*. However, because of the habitation differences, the comparison between the species richness of testate amoebae estimated in the present study with that obtained by in others (described above) is not completely accurate. It is likely that more species of testacea could be found in the mosses, that are richer in humus and have more con-

stant moisture conditions than lichen. Low abundance of testate amoebae in *X. parietina* could be affected by lichen chemistry (Charman et al. 2000) too. The genus *Arcella* (Table 2) is common among bryophytes (Chardez and Beyens 1987). Domination of *Arcella discoidea* in *X. parietina* may be related to amoebae tolerance of dry habitats or for pollution of heavy metals (Nguyen-Viet et al. 2008). *A. arenaria* (?) new testate species for Lithuania was found in samples from unpolluted site "D". Though *Assulina muscorum* is regarded as an acidophilus species with xerophilous tendencies (Beyens et al. 1986, Lamentowicz and Mitchell 2005) only few individuals similar as *Nebela* were found in lichen samples (Table 2). Contrary to Balik (1991), Nguyen-Vieta et al. (2004), and Nguyen-Vieta et al. (2008) reports, the present study did not show a significant impact of pollution on species richness and abundance of testate amoebae.

Gymnoamoebae were not abundant in lichen. Three taxa were observed and one of them (*Thecamoeba striata*) was found in Lithuania for the first time.

Unlike as testacea, the ciliates *Colpoda* and *Paramecium* have remarkably higher relative abundance and dominated in almost all samples (Table 2) (Fig. 2). These data go in line with Roberts and Zimmer (1990) findings that *Colpoda* is one of the most frequent genus of ciliates found in lichens.

A comparison of the all protozoa observed in lichens showed some differences between polluted and unpolluted sites. In contrast to some authors (Nguyen-Viet et al. 2004; 2007; 2008) the general diversity of protozoa was higher at polluted sites (Table 2). However the obtained results were not statistically reliable (Mann-Whitney $p > 0.05$) and were determined by the some protozoan groups, which are ecological plastic and have rather easily adapted to various environments and to pollution of heavy metals.

Micrometazoa in *X. parietina*.

Rotifers in the lichen *X. parietina* were represented by 3 genera of the order *Bdelloidea*. The bdelloid rotifers are common in mosses (Ricci 1987) and lichens (Fontaneto et al. 2004). The *Philodina* considerably dominated in the samples collected close to highways ("A" – 41.4%; "B" – 49.6%) where transport emission is high. This is not surprising since *Philodina* have a capability to survive in the environment loaded with heavy metals (Rehman et al. 2008).

As reported by Siddiqi and Hawksworth (1982), lichens can be inhabited by a large number of various nematodes. *Aphelenchoides* spp. with slight differences in abundance was found in all samples (Table 2). The obtained results agree with those of Gadea (1974) who found that *Aphelenchoides* spp. are frequent in

X. parietina. Other genus *Tylenchus* was negligible in all of the samples.

Among three tardigrades taxa observed in the present study, two species (*Macrobotus hufelandi*, *Milnesium tardigradum*) are new for the fauna of Lithuania (Table 2). *X. parietina* namely dominated by tardigrade *Ramazzottius oberhaeuseri*, which is in agreement with Rebecchi et al. (2006) who found that *R. oberhaeuseri* is one of the most abundant of five tardigrade species in lichens. Some morphological and anatomical features of *Ramazzottius* spp. such as size (Jönsson et al. 2001), surviving longevity and “armor” (Guidetti et al. 2008), allow them to survive in such variable habitats as lichens. Moreover, this tardigrade has red pigmentation of the body, which can serve as protection against UV damage to DNA in a cryptobiotic state and allow tardigrades to live in more open habitats such as cryptogamic crusts (Hebert 2008). In this study *R. oberhaeuseri* was found in the samples from highways (although they were less abundant there). These results agree with Peluffo (de Peluffo, Peluffo et al. 2006) finding that *R. oberhaeuseri* and *Milnesium cf. tardigradum* were the only species of tardigrades rendered heavy traffic paved sites.

Observed groups of the micrometazoa showed some differences of diversity and distribution on through the entire sampling sites (polluted and unpolluted). Some micrometazoa (bdelloid rotifers, nematodes) were distributed fairly uniformly through the entire sampling sites (polluted and non-polluted) included in the study and did not show clear difference ($p > 0.05$) between samplings. Thus, since the abundance of bdelloidea rotifers, nematodes do not correlate with pollution, these organisms are not useful for bioindication. The similar conclusion was proposed by Roberts and Zimmer (1990) who had also found that abundance of bdelloidea rotifers, nematodes, oribatides mites do not correlate with pollution.

Species richness of micrometazoa was relatively low in *X. parietina* and varied from 4 to 7 taxa, but the most lichens are normally less populated. The highest species richness of micrometazoa (7) was observed in polluted sites of highways. The exception was *Ramazzottius oberhaeuseri* which was numerous on all investigated sites, but have highest relative abundance (8.8% in “D” and 37.7% in “E”) on clean sites. The obtained results agree with some authors (Vargha et al. 2002) who found these tardigrades to be more numerous and diverse in the unpolluted environment.

One important factor affecting the assemblages of microfauna is pH (Charman and Warner 1997, Vincke et al. 2007). However, since our results indicated that pH was similar in all samples (Table 1), this allows us to suggest that presumably pH was not an essential

factor determining species assemblages. Nevertheless, slightly acidic environment of lichen can affect the composition and abundance of microfauna.

In conclusion, our results revealed that dominating protozoa in lichen *X. parietina* are testate amoebae (genus *Arcella*) and ciliates (*Colpoda*, *Paramecium*). *Philodina* sp. (Rotifera), *Aphelenchoides* sp. (Nematoda) and *Ramazzottius oberhaeuseri* (Tardigrada) are the dominated micrometazoa in *X. parietina*. Some organisms such as *Colpoda*, *Paramecium*, *Philodina* are undemanding for environment and successfully specialized for some less suitable habitat as lichen or polluted areas where they became dominant and displaced other species.

The obtained results did not show a significant differences of microfauna between polluted and unpolluted sites however this does not necessary indicate that pollution has not impact on the diversity of microfauna. Due to low number of samples, the obtained results should be regarded as preliminary. In addition the absence of significant difference can be explained by the fact that identified organisms in lichen such as *Colpoda*, *Paramecium*, *Philodina* are undemanding for environment and displace other species. Moreover, the fact that only one abiotic factor – pH was analyzed in this study, certainly underestimate the real distribution and diversity of microfauna in lichen *X. parietina* from polluted and unpolluted sites.

Meanwhile, the data of tardigrades showed a tendency that tardigrades are more numerous and diverse in unpolluted environment. Further studies on microfauna diversity using increased number of samples, sampling sites and estimating more environmental parameters can result in more distinct conclusions.

References

- Bargagli, R., Monaci, F., Borghini, F., Bravi, F. and Agnorelli, C. 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on *Hypnum cupressiforme* and *Parmelia caperata* in a former mining district in Italy. *Environmental Pollution* 116 (2): 279 - 87.
- Balik V. 1991. The effect of the road traffic pollution on the communities of testate amoebae (Rhizopoda, Testacea) in Warsaw (Poland). *Acta Protozoologica*. 30: 5-11
- Beslaneev, V. D. and Kuchmazokova, F. A. 1991. The effect of motorways on the accumulation of toxic substances in walnuts. *Sadovodstvo i Vinogradarstvo* 5: 38.
- Beyens, L., Chardez, D., and de Landtsheer, R. (with collaboration of de Bock, P. and Jacques, E.). 1986. Testate amoebae populations from moss and lichen habitats in the Arctic: *Polar Biology* 5: 165 - 173.
- Chardez, D. and Beyens, L. 1987. *Arcella ovaliformis* sp. nov. a new testate amoebae from Edgeoya, a high Arctic island. *Archiv fuer Protistenkunde* 134: 297-301.

- Charman, D. J., Hendon, D. and Woodland, W. A. 2000. The Identification of Testate Amoebae (Protozoa: Rhizopoda) in Peats. *Technical Guide No. 9*, Quaternary Research Association, London, 147 pp.
- Charman, D. and Warner, B. 1997. The ecology of testate amoebae (Protozoa: Rhizopoda) in oceanic peatlands in Newfoundland, Canada. Modelling hydrological relationships for paleoenvironmental reconstruction. *Ecoscience* 4: 555 - 562.
- Fontaneto, D. and Ricci, C. 2004. Rotifera: Bdelloidea. In: Yule, C.M. & Yong, H. S. (eds.). *Freshwater invertebrates of the Malaysian Region*. Academy of Sciences Malaysia, p. 121-126.
- Gadea, E. 1974. Nematodes liquenicolas de Columbretes. *Miscellanea zoologica hungarica* 3: 3 - 8.
- Garcia-Miragava, J., Castro, S. and Paolini, J. 1981. Lead and zinc levels and chemical fractionation in road-side soils of Caracas, Venezuela. *Water, Air, & Soil Pollution* 15: 285 - 297.
- Gerson, U. and Seaward, M. R. D. 1977. Lichen-invertebrate associations. *Lichen Ecology*. New York p. 69-119.
- Giebelmann, U. Ch., Martins, K. G., Brändle, M., Schädelner, M., Marques, R., and Brandl, R. 2010. Diversity and ecosystem functioning: Litter decomposition dynamics in the Atlantic Rainforest. *Applied Soil Ecology* 46: 283 - 290.
- Grigalavičienė, I. and Rutkoviėnė, V. 2006. Heavy metals accumulation in the forest soils and mosses along highway Vilnius – Kaunas. *Miškininkystė* 2: 12 - 19.
- Guidetti, R. and Bertolani, R. 2005. Tardigrade taxonomy: an updated check list of the taxa and a list of characters used in their identification. *Zootaxa* 845: 1 - 46.
- Guidetti, R., Boschini, D., Altiero, T., Bertolani, R. and Rebecchi, L. 2008. Diapause in tardigrades: a study of factors involved in encystment. *Journal of Experimental Biology* 211: 2296 - 2302.
- Hawksworth, D. L. and Rose, F. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. *Nature* 227: 145-148.
- Hebert, P. D. N. and Leszek, A. B. 2008. "Tardigrada". In: *Encyclopedia of Earth*. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment).
- Jovan, S. 2008. Lichen bioindication of biodiversity, air quality, and climate: baseline results from monitoring in Washington, Oregon, and California. Gen. Tech. Rep. PNW-GTR-737. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, 115 pp.
- Jönsson, K. I., Borsari, S. and Rebecchi, L. 2001. Anhydrobiotic survival in populations of the tardigrades *Richtersius coronifer* and *Ramazzottius oberhaeuseri* from Italy and Sweden. *Zoologischer Anzeiger - A Journal of Comparative Zoology* 240: 419 - 423.
- Lamentowicz, M. and Mitchell, E. A. D. 2005. The ecology of testate amoebae (Protists) in *Sphagnum* in north-western Poland in relation to peatland ecology. *Microbial Ecology* 50: 48 - 63.
- Mažeikaitė, S. 2003. Lietuvos gėlo vandens telkinių planktono heterotrofiniai protistai [Heterotrophic protists of Freshwater plankton in Lithuania]. Vilnius. Botanikos instituto leidykla. 222 pp. (in Lithuanian).
- Meininger, C. A., Uetz, G. W. and Snider, J. A. 1985. Variation in epiphytic microcommunities (tardigrade — lichen — bryophyte assemblages) of the Cincinnati, Ohio area. *Urban Ecology* 9: 45 - 61.
- Mitchell, E. A., Bragazza, L. and Gerdol, R. 2005. Testate amoebae (Protista) communities in *Hylocomium splendens* (Hedw.) B.S.G. (Bryophyta): relationships with altitude, and moss elemental chemistry. *Protist* 155 (4): 423 - 36.
- Moore, J. C., De Ruiter, P. C. and Hunt, H. W. 1993. Soil invertebrate/micro-invertebrate interactions: disproportionate effects of species on food web structure and function. *Veterinary Parasitology* 48: 247 - 260.
- Nguyen-Viet, H., Gilbert, D., Bernard, N., Mitchell, E. A. D. and Badot, P. M. 2005. Relationship between atmospheric pollution characterized by NO₂ concentrations and testate amoebae density and diversity. *Acta Protozoologica* 43: 233 - 239.
- Nguyen-Viet, H., Bernard, N., Mitchell, E.A.D., Cortet, J., Badot, P. M. and Gilbert, D. 2007. Relationship between testate amoeba (Protist) communities and atmospheric heavy metals accumulated in *Barbula indica* (Bryophyta) in Vietnam. *Microbial Ecology* 53: 53 - 65.
- Nguyen-Vieta, H., Bernarda N., Mitchell, E. A. D., Badota, P. M. and Gilberta, D. 2008. Effect of lead pollution on testate amoebae communities living in *Sphagnum fallax*: An experimental study. *Ecotoxicology and Environmental Safety* 69 (1): 130 - 138.
- Patterson, D. J. 1998. *Free-Living Freshwater Protozoa: A Colour Guide*. John Wiley & Sons, New York. 224 pp.
- Peluffo, M. C. M., Peluffo J. R., Rocha A. M. and Doma, I. L. 2006. Tardigrade distribution in a medium-sized city of central Argentina. *Hydrobiologia* 558: 141-150.
- Pilato, G. and Binda, M. G. 2010. Definition of families, subfamilies, genera and subgenera of the Eutardigrada, and keys to their identification. *Zootaxa* 2404: 1 - 52.
- Read, H. J., Martin, H. and Rayner, J. M. V. 1998. Invertebrates in woodlands and polluted by heavy metals – an evaluation using canonical correspondence analysis. *Water, Air, and Soil Pollution* 106: 17 - 42.
- Rebecchi, L., Guidetti, R., Borsari, S., Altiero, T. and Bertolani, R. 2006. Dynamics of long-term anhydrobiotic survival of lichen-dwelling tardigrades. *Hydrobiologia* 558: 23 - 30.
- Rehman, A., Shakoori, F. R. and Shakoori, A. R. 2008. Heavy Metals Resistant Rotifers from a Chromium Contaminated Wastewater can Help in Environmental Cleanup. *Pakistan Journal zoology* 40(5): 309 - 316.
- Ricci, C. N. 1987. Ecology of bdelloids: How to be successful. *Hydrobiologia* 147: 117 - 127.
- Ricci, C. N. and Melone G. 2000. Key to the identification of the genera of bdelloid rotifers. *Hydrobiologia* 418: 73 - 80.
- Roberts, D. and Zimmer, D. 1990. Microfaunal communities associated with epiphytic lichens in Belfast. *The Lichenologist* 22: 163 - 171.
- Siddiqi, M. R. and Hawksworth, D. L. 1982. Nematodes associated with galls on *Cladonia glauca*, including two new species. *The Lichenologist* 14: 175 - 184.
- Silberstein, L. L., Siegel B. Z., Siegel, S. M., Mukhtar, A. and Galun, M. 1996. Comparative studies on *Xanthoria parietina*, a pollution resistant lichen and *Ramalina duriaei*, a sensitive species. *The Lichenologist* 28: 367-383.
- Smart, J. G. C. and Nguyen, K. B. 1990. An illustrated key to the orders of soil-dwelling nematodes. In *Plant Nematology, Laboratory manual*. (Zuckerman, B. M., Mai, W. F. and Krusberg, L. R.) Agricultural Experiment Station, University of Massachusetts, Amherst, Massachusetts.
- Søchting, U. and Gjelstrup, P. 1985. Lichen communities and the associated fauna on a rocky sea shore on Bornholm in the Baltic. *Holarctic Ecology* 8: 66 - 75.
- Sohlenius, B., Boström, S. and Jönsson, I. 2004. Occurrence of nematodes, tardigrades and rotifers on ice-free areas in East Antarctica. *Pedobiologia* 48: 395 - 408.

- Specialieji tyrimai, matavimai ir analizė automobilių keliuose. Valstybinių kelių automobilių eismo intensyvumo tyrimai 2001 m. [Special researches, measure and analysis in transport roads]. Transporto ir kelių tyrimo institutas. Kaunas 2002, (in Lithuanian).
- Stolte, K. W., Mangis, D., Doty, R., Tonnessen, K. and Huckaby, L. S.** 1993. Lichen as bioindicators of air quality. *USDA Forest Service General Technical Report RM-224*.
- Stubbs, C. S.** 1989. Patterns of distribution and abundance of corticolous lichens and their invertebrate associates on *Quercus rubra* in Maine. *The Bryologist* Vol. 92 (4): 453-460.
- Trombulak, S. C. and Frissell, Ch. A.** 2000. Review of Ecological Effects of Roads on Terrestrial and Aquatic Communities. *Conservation Biology* 14: 18 - 30.
- Vargha, B., Otvos, E. and Tuba, Z.** 2002. Investigations on ecological effects of heavy metal pollution in Hungary by moss-dwelling water bears (Tardigrada), as bioindicators. *Annals of Agricultural and Environmental Medicine* 9: 141 - 146.
- Vicente, F.** 2010. Micro-invertebrates conservation: forgotten biodiversity. *Biodiversity and Conservation* 19: 3629 - 3634.
- Vincke, S., Vijver, B., Ledeganck, P., Nijs, I. and Beyens, L.** 2007. Testacean communities in perturbed soils: the influence of the wandering albatross. *Polar Biology* 30: 395 - 406.
- Uetz, G.W., Meininger, C. A. and Snider, J.** 1980. Effect of air pollution on microcommunities of invertebrates in lichens and mosses. Electric Power Research Institute, Palo Alto, California. 94 pp.

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МИКРОФАУНА ЛИШАЙНИКОВ (*XANTHORIA PARIETINA* L.) В ЛИТВЕ: РАЗНОВИДНОСТЬ В ЧИСТЫХ И ЗАГРЯЗНЕННЫХ МЕСТАХ

И. Шаткаускиене

Резюме

Лишайники являются пригодным местообитанием для микробезпозвоночных и протистов. Микрофауна, обитающая в лишайниках, важна для циркуляции азота, дроблении органических веществ и как источник пищи для нормального функционирования экосистемы. Цель этих исследований – определить состав и разнообразие микрофауны в лишайнике *Xanthoria parietina* и выявить изменения состава микрофауны в зависимости от местопроизрастания лишайников. Лишайники были собраны около магистральных дорог и в чистых от загрязнения местах. Были найдены и определены 24 рода микрофауны, из которых два вида тардиград, два вида раковинных амёб и один вид голых амёб впервые обнаружены в Литве. В лишайниках доминировали инфузории *Colpoda cuculus*, *Paramecium* sp., раковинные амёбы рода *Arcella*, коловоротки *Philodina* sp. и тардиграды *Ramazzottius oberhaeuseri*. Значительных различий между доминирующими видами протозоа (*Colpoda cuculus*, *Paramecium* sp., *Arcella* sp.) и коловоротками *Philodina* в чистых и загрязнённых биотопах не обнаружено, но тардиграды были обильнее в незагрязнённых местах.

Ключевые слова: микрофауна, лишайники, загрязнение, protozoa, Tardigrada, rotifer.