



MITE (*Acari*) COLONIZATION OF PINE CHIPS ALONE AND PINE CHIPS SUPPLEMENTED WITH FOREST LITTER, PEAT AND LIGNITE IN REVITALIZATION OF DEGRADED FOREST SOILS

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Abstract

The aim of this study was to compare colonization of pine chips without supplements and pine chips supplemented with forest litter, peat and lignite by mites (*Acari*), and particularly oribatid mites (*Oribatida*) in a two-year cycle. The study was conducted in the years 2013-2014, on microplots established in a belt of trees in a nursery in Białe Błota within Bydgoszcz Forest District. The experiment was established on four microplots (1 x 1 m). It included the following variants: C – pine chips alone, Lf – pine chips inoculated with fresh forest litter, Lf+Pe – pine chips enriched with deacidified high peat (20%) (pH 5.5-6.5) and inoculated with the litter, Lf+Li – pine chips enriched with granulated lignite (20%) inoculated with the litter.

In the first year of the study, mite abundance in the chips inoculated with forest litter was significantly higher than that on microplot C. In the second year of the experiment, the abundance of these microarthropods decreased probably due to unfavorable weather conditions. The most common group of mites were usually oribatid mites that accounted for 19.7 to 80.4% of all mites. An analysis of seasonal dynamics of *Oribatida* abundance over the entire study cycle revealed a clear increase in their numbers in non-supplemented pine chips only on the last sampling

date. *Oribatida* abundance in Lf variant was similar at the beginning and end of the study. Contrary to that, their number decreased in the chips supplemented with peat, and particularly those enriched with lignite. In total, 36 species of oribatid mites were identified on all microplots. The greatest boost in species diversity after the introduction of forest litter was observed in Lf chips and the smallest in Lf + Li variant. *Oribatula tibialis* was the most common oribatid mite in the investigated substrates. Low numbers of *Oppiella nova* and *Tectocepheus velatus*, the species having trophic associations with fungi, may indicate poor colonization of the chips, particularly those enriched with lignite, by saprotrophic organisms.

Keywords: soil regeneration; mulching; microarthropods; bioindication; *Oribatida*.

INTRODUCTION

Degraded soils that were once covered by forests are usually devoid of forest litter. In afforested degraded post-industrial or post-agricultural areas forest tree seedlings are planted into mineral soil lacking typical forest edaphon. Formation of forest soil takes many years. Unfortunately, proper growth of forest trees in these difficult conditions is disturbed due to lack of necessary soil microorganisms, particularly mycorrhizal fungi. Such stands often die due to infestation with pathogenic fungi.

To restore natural forest soil structure as soon as possible, it is recommended to mulch the soil with wood chips at the beginning of succession right after planting the trees. Another useful solution is reintroduction of forest edaphon into the mulched soil.

Microarthropods, and especially oribatid mites (*Oribatida*), play crucial role in soil forming processes in forest ecosystems. However, these all animals have limited possibilities of spreading and colonization of new sites (Beckmann 1988, Lehmitz *et al.* 2011, Wanner and Dunger 2002).

Haimi (2000) claimed that soil fauna was highly important for restoring biological activity during the reclamation of degraded soils and that this process might be reinforced by introducing soil inoculum. Moreover, the author proposed two ways of using microarthropods in soil restoration. The first was their direct (feeding) and indirect (stimulation of microorganisms) influence on soil metabolism. The second assumed using the microarthropods as bioindicators of biological condition of the soil. Research literature has proved that oribatid mites improve propagation of bacteria and fungi, and they indirectly affect the formation of mycorrhizas (Klironomos and Kendrick 1996, Behan-Pelletier 1999, Remén *et al.* 2010, Schneider *et al.* 2005).

This study was a continuation of the research on soil revitalization with the use of various substrates and forest litter. Our previous studies demonstrated that effective soil inoculation with edaphon required 1 cm layer of litter introduced onto a proper substrate (Klimek 2010, Klimek and Chachaj 2015, Klimek and Rolbiecki 2014, Klimek *et al.* 2008, 2011, 2012, 2013a,b). Wood chips may be a suitable substrate for soil revitalization (Klimek and Chachaj 2015, Klimek *et al.* 2014a,b). As the organic matter in the chips is not as well disintegrated as in natural forest soil, attempts are made at enriching the substrate with different structural components.

The aim of this study was to compare the colonization of pine chips without supplements and pine chips supplemented with forest litter, peat and lignite by mites (*Acari*), and particularly oribatid mites (*Oribatida*) in a two-year cycle. This study may show whether the applied components may positively affect biological activity of the investigated substrates proposed for the regeneration of forest soils.

MATERIAL AND METHODS

The study was conducted in the years 2013-2014, within a belt of trees in a nursery in Białe Błota belonging to Bydgoszcz Forest District (53.103667°N 17.929611°E). The belt of trees was 20 m wide and harbored the following species: Scots pine (*Pinus sylvestris* L.), oak (*Quercus* L.) and European ash (*Fraxinus excelsior* L.), and the underbush layer was composed of European ash, silver birch (*Betula pendula* Roth) and oak. The forest stand helped to mitigate the influence of atmospheric factors, such as sun exposure, temperature fluctuations, or too intense precipitation. The soil type was albic brunic arenosol (Bydgoszcz Forest Inspectorate data).

The experiment was established on 16th April 2013 on four microplots (1 x 1 m). It included the following variants: C – pine chips alone, Lf – pine chips inoculated with fresh forest litter, Lf+Pe – pine chips enriched with deacidified high peat (20%) (pH 5.5-6.5) and inoculated with the litter, Lf+Li – pine chips enriched with granulated lignite (20%) (granule size 1-10 mm) inoculated with the litter.

Each microplot was surrounded with 18 cm high wooden frame. Forest litter was removed and the frame was placed directly on the mineral soil. To protect the microplots against rodents, plastic net and non-woven fabric were mounted to the bottom of the frame. The microplots were covered with 150 dm³ of supplemented or non-supplemented pine chips. The chips were obtained with TIMBERJACK BRUKS 805.2 wood chipper from a post-logging site located in a fresh coniferous forest. Then, the substrate in variants Lf, Lf+Pe and Lf+Li was supplemented with 10 dm³ of fresh raw humus (obtained on the same day

from a Scots pine forest) that was mixed with the chips. The material was collected from the organic horizon of the soil and it was manually sieved through 10 x 10 mm screens.

The samples for acarologic analyses were collected four times in two consecutive years in the spring and autumn on the following days: 8th May 2013, 23rd October 2013, 12th May 2014 and 28th October 2014. Each time 10 samples per microplot were collected. A total of 40 samples of 50 cm³ each were harvested from every variant. Mite extraction was carried out over seven days using Tullgren funnels. Then, the mites were preserved in 70% ethanol. All the mites were classified into orders and oribatid mites into species or genera, with regard to juvenile stages. In total 2037 mites, including 1056 oribatid mites, were identified.

Average density (N) of the mites was provided for 50 cm³ of the substrate, and the species dominance index (D) was given in percentage. Species diversity was determined based on the number of species (S) and mean number of species per sample (s). Prior to statistical analysis, the numerical data were subjected to a logarithmic transformation – $\ln(x+1)$ (Berthet and Gerard 1965). The statistical analysis was performed using Statistica 12 software: a compliance of the measurable parameters with the normal distribution was assessed using Kolmogorov-Smirnov test. As the normal distribution was not confirmed, a non-parametric analysis of variance (Kruskal-Wallis H test) was performed. For statistically significant differences ($p < 0.05$) a post-hoc analysis for each pair was carried out (Mann-Whitney U test) to identify significantly different means.

RESULTS AND DISCUSSION

Meteorological conditions. Mean annual temperature in the growing season 2013 was similar to that of a multi-year period (Table 1). In 2014 the temperature was by 1°C higher. July was an exceptionally warm month and other months with temperature clearly higher than that of the multi-year period were April and September. Total precipitation in the growing season 2013 was by over 13 mm higher than total precipitation in the multi-year period. This changed dramatically in 2014, when precipitation was by over 64 mm lower than the year before. Particularly dry months, as compared with average values for multi-year period, were July, August, and September.

Abundance of mite community. In 2013 the lowest number of mites was detected on the control microplot – 12.95 individuals per 50 cm³ of the substrate (Table 2). In the chips inoculated with forest litter the number of these microarthropods was considerably higher and the differences between the control and the other microplots were significant. In 2014, the abundance of the mites decreased significantly, with the greatest drop of 4.5 times in the chips supplemented with lignite. That year the differences between the variants were not

significant. Such a considerable reduction in numbers in 2014 might be caused by unfavorable weather conditions, and particularly low rainfall in the summer months (Table 1). Literature data demonstrated that microarthropods might be negatively affected by summer droughts (Lindberg *et al.* 2002, Lindberg and Bengtsson 2005, 2006).

Table 1. Air temperature and precipitation in 2013-2014 (data for the Research Center Mochelek near Bydgoszcz, processed by the Department of Melioration and Agrometeorology of University of Science and Technology in Bydgoszcz)

Parameter	Year	Months						
		April	May	June	July	August	September	April-October
Air temperature (°C)	2013	7.0	14.2	17.4	18.9	18.1	10.7	14.4
	2014	9.9	13.3	16.0	21.5	17.2	14.4	15.4
	Long-term value	8.0	13.0	16.3	18.5	17.8	13.1	14.5
Precipitation (mm)	2013	13.6	91.7	49.3	79	56.6	64.1	354.3
	2014	40.7	65.7	44.9	55.4	57.3	25.9	289.9
	Long-term value	29.0	61.2	48.8	87.7	68.6	45.6	340.9

Source: own research data

Oribatid mites were usually the most common group of mites and they accounted for 19.7 to 80.4% of all mites. Only in the first year they were dominated by *Mesostigmata* on the control plot. In 2014, their share in the mite community clearly increased as compared with the first year. In 2013, the number of oribatid mites on the microplots covered with chips inoculated with edaphon was 7.60-12.70 individuals per 50 cm³ and it was significantly higher than in the control chips. In the second year, mite abundance in the variants Lf, Lf+Pe and Lf+Li was significantly reduced and was comparable to that of C variant. Despite fairly low abundance of oribatid mites, they still dominated in the mite communities on these microplots and *D* was 77.7-80.4%. Such a high share of oribatid mites is typical of Scots pine forests with well developed organic horizon (O) (Klimek 2000).

An analysis of seasonal dynamics of *Oribatida* abundance over the entire study cycle revealed a clear increase in their numbers on the control microplot only at the last sampling date when *N* was 7.3 individuals per 50 cm³ (Figure 1). Therefore, colonization of non-inoculated chips was slower. As mentioned before, chip colonization might have also been affected by weather conditions. In another experiment, in which non-inoculated chips were irrigated, peak number of oribatid mites was observed already at the beginning of the second year and it

was markedly higher than that in this experiment – 18.3 individuals per 50 cm³ (Klimek and Chachaj 2015). In Lf variant, minimum abundance was noticed in May 2014 (1.3 individuals per 50 cm³), and maximum in October of the same year (13.7 individuals per 50 cm³). The pattern was different in the chips supplemented with peat and lignite. The mite abundance dropped considerably as soon as in October 2013, then it was still lower in May 2014, and only a slight increase was observed in the autumn 2014. It was therefore concluded that the investigated structural components, and particularly lignite, might inhibit the growth of oribatid mites.

Table 2. Abundance of mites (individuals per 50 cm³), number of *Oribatida* species (*S*), and average number of *Oribatida* species (*s*) in the experimental variants

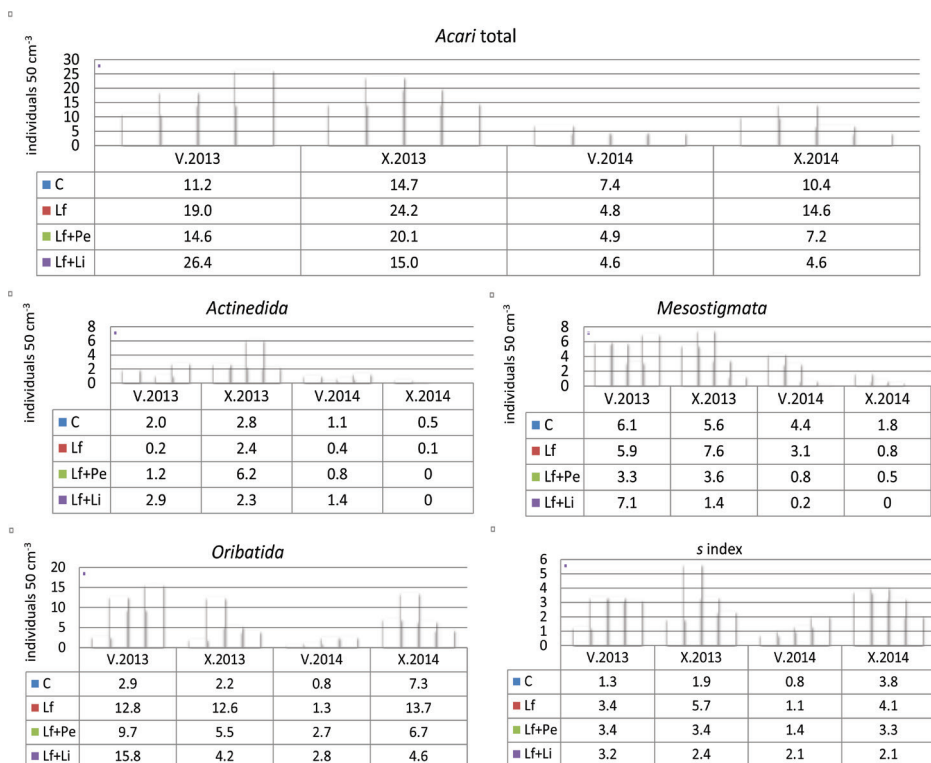
Index – Taxon	Year	Variant				Kruskal-Wallis test	
		C	Lf	Lf+Pe	Lf+Li	<i>H</i>	<i>p</i>
<i>N – Acaridida</i>	2013	1.90 ^A	0.65 ^A	2.55 ^A	3.75 ^A	26.60	0.000
	2014	0.50 ^{A*}	0	0.20 ^{A*}	0.10 ^{A*}		
<i>N – Actinedida</i>	2013	2.40 ^A	1.30 ^A	3.70 ^A	2.60 ^A	43.10	0.000
	2014	0.80 ^{A*}	0.25 ^{A*}	0.40 ^{A*}	0.70 ^{A*}		
<i>N – Mesostigmata</i>	2013	5.85 ^A	6.75 ^A	3.45 ^A	4.25 ^A	62.62	0.000
	2014	3.10 ^{A*}	1.95 ^{AB*}	0.65 ^{BC*}	0.10 ^{C*}		
<i>N – Oribatida</i>	2013	2.55 ^A	12.70 ^B	7.60 ^B	10.00 ^B	31.72	0.000
	2014	4.05 ^A	7.50 ^{A*}	4.70 ^{A*}	3.70 ^{A*}		
<i>N – Tarsonemida</i>	2013	0.25	0.20	0.05	0.10	8.47	0.292
	2014	0.45	0	0.10	0		
<i>N – Acari total</i>	2013	12.95 ^A	21.60 ^B	17.35 ^{AB}	20.70 ^{AB}	50.61	0.000
	2014	8.90 ^{A*}	9.70 ^{A*}	6.05 ^{A*}	4.60 ^{A*}		
<i>S – Oribatida</i>	2013	9	24	21	19	-	-
	2014	13	14	14	12		
<i>s – Oribatida</i>	2013	1.45 ^A	4.55 ^B	3.40 ^{BC}	2.80 ^C	28.65	0.000
	2014	1.05 ^A	2.60 ^{A*}	2.35 ^{A*}	2.10 ^A		

Explanations: A, B, C – the same letter denotes insignificant difference – Mann-Whitney U test at $p < 0.05$.
 * – significant difference between 2011 and 2012 – Mann-Whitney U test at $p < 0.05$.

Source: own research data

Mesostigmata order were much less abundant than oribatid mites and their density was 0.10-6.75 individuals per 50 cm³ (Table 1). In the first year of the study their abundance was invariable. In the second year, it was significantly reduced in all variants, especially in the chips supplemented with peat and lignite. They were not detected at all in Lf+Li variant on the last sampling date (Figure

1). On this date, the chips mixed with peat and lignite were also devoid of *Actinedida* that had been previously relatively abundant there. The abundance of mites of this order, similarly as of most *Acari*, significantly decreased in 2014 as compared with 2013 (Table 1). A similar pattern of abundance was observed for *Acaridida* order. The mites of *Tarsonemida* order were clearly less abundant.



Source: own research data

Figure 1. The dynamics of abundance of the mite groups and *s* index of Oribatida in the investigated experimental variants

Species richness of oribatid mites. In total, 36 species of oribatid mites were identified on all microplots (Table 2). In 2013, the control plot harbored nine species of oribatid mites. Next year their number grew up to 13 (Table 1). The chips inoculated with forest litter were much more abundant in *Oribatida* and their *S* was 19-24 species. However, the number considerably decreased in the second year (12-14 species). An analysis of mean number of species *s* revealed significant differences in this parameter between the control microplot and the microplots enriched with the forest litter. The greatest (4.55) increase in

s after inoculation with the forest litter was observed in the chips without any structural components, and the lowest in those supplemented with lignite (2.80). However, high species diversity of 2013 was not maintained in 2014 when it dropped and was similar in all experimental variants. The analysis of seasonal dynamics of *s* showed that under weather conditions prevailing in 2014 that were unfavorable for most mites, *Oribatida* communities previously present in Lf and Lf+Pe variants were renewed in October. This pattern was not observed only in the chips supplemented with lignite. In a study conducted in the years 2011-2012 within the same area species diversity of oribatid mites in irrigated pine chips without any supplements was high and stable in May, July and October of 2012 with *s* between 3.50 and 4.0 species (Klimek and Chachaj 2015). In this study, this parameter reached such a high value on microplot C only in October 2014.

Analysis of occurrence of selected *Oribatida* species. The most common oribatid mite on all microplots was *Oribatula tibialis* (Table 3). This species is considered eurytopic (Weigmann 1991, Weigmann and Kratz 1981), and prefers forest soils (Rajski 1968). It was the most abundant in the chips supplemented with lignite (4.35 individuals per 50 cm³). In this variant *O. tibialis* was particularly dominating with *D* as high as 63.5%. However, dynamics of this population in subsequent seasons showed a marked downward trend (Figure 2). It therefore seems that *O. tibialis* was probably very common in the forest litter used for inoculation and did not find suitable growing conditions in the chips. Similar changes in abundance were observed in *Chamobates schuetzi*, a species typical of Scots pine forests (Usher 1975).

The second most abundant *Oribatida* on all microplots was *Oppiella nova* with *D* of 13.5-17.6%. Mean highest abundance of this species was found in Lf variant (1.63 individuals per 50 cm³), but the differences between variants were not significant (Table 2). In the beginning of the study cycle, *O. nova* was rare or not present at all (Figure 2). Its high abundance (2.3-6.3 individuals per 50 cm³) was observed only in October 2014. Literature reports have confirmed *O. nova* to be a parthenogenetic species with a short life cycle of 20 days and rapid growth of its populations (Siepel 1994, Skubala and Gulvik 2005).

Table 3. Abundance of oribatid mites (individuals per 50 cm³) in the experimental variants

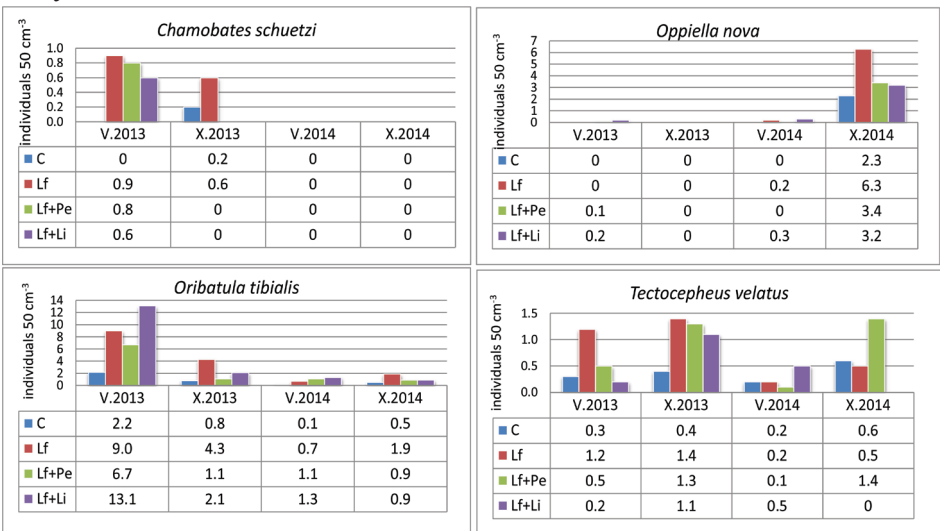
Species	Variant				Kruskal-Wallis test	
	C	Lf	Lf+Pe	Lf+Li	H	p
<i>Adoristes ovatus</i> (Koch)	0.18	0.60	0.20	0.15	5.76	0.123
<i>Autogneta longilamellata</i> (Michael)	0.03	0	0	0.05	3.71	0.294
<i>Autogneta traegardhi</i> Forsslund	0	0	0.08	0.10	8.69	0.033
<i>Carabodes forsslundi</i> Sellnick	0	0.10	0.05	0	7.57	0.055

Species	Variant				Kruskal-Wallis test	
	C	Lf	Lf+Pe	Lf+Li	H	p
<i>Carabodes labyrinthicus</i> (Michael)	0	0.05	0	0	6.03	0.109
<i>Carabodes minusculus</i> Berlese	0	0.03	0.03	0	2.01	0.569
<i>Carabodes subarcticus</i> Trägårdh	0	0.08	0.03	0.03	3.89	0.272
<i>Chamobates schuetzi</i> (Oudemans)	0.05 ^A	0.38 ^{BC}	0.20 ^{AC}	0.15 ^A	8.63	0.034
<i>Cultroribula bicultrata</i> (Berlese)	0.03	0	0	0	3.00	0.391
<i>Cymbaeremaeus cymba</i> (Nicolet)	0	0.03	0	0	3.00	0.391
<i>Damaeus</i> sp.	0.03	0	0.05	0	3.71	0.294
<i>Dissorhina ornata</i> (Oudemans)	0	0.05	0	0	6.03	0.109
<i>Eremaeus oblongus</i> C.L. Koch	0.03	0.70	0.08	0.10	5.81	0.121
<i>Eupelops occultus</i> (C.L. Koch)	0	0	0.05	0	6.03	0.109
<i>Eupelops torulosus</i> (C.L. Koch)	0.03	0.13	0.03	0	3.97	0.263
<i>Galumna lanceata</i> (Oudemans)	0	0.03	0.08	0.03	3.89	0.272
<i>Gymnodamaeus bicostatus</i> (C.L. Koch)	0.38 ^A	0.03 ^B	0.08 ^A	0	13.89	0.003
<i>Hemileius initialis</i> (Berlese)	0.05	0.03	0	0	3.71	0.294
<i>Heminothrus peltifer</i> (C.L. Koch)	0.08	0.03	0.05	0.08	2.01	0.569
<i>Lauroppia neerlandica</i> (Oudemans)	0	0.03	0	0	3.00	0.391
<i>Liacarus coracinus</i> (C.L. Koch)	0	0.05	0	0.03	3.71	0.294
<i>Liochthonius</i> sp.	0	0.05	0.05	0	3.67	0.298
<i>Metabelba pulverulenta</i> (C.L. Koch)	0.15	0.10	0.05	0.05	1.46	0.689
<i>Micreremus brevipes</i> (Michael)	0	0.03	0.03	0.03	1.01	0.798
<i>Oppiella nova</i> (Oudemans)	0.58	1.63	0.88	0.93	3.78	0.285
<i>Oribatula tibialis</i> (Nicolet)	0.90 ^A	3.98 ^B	2.45 ^B	4.35 ^B	17.36	0.000
<i>Pergalumna nervosa</i> (Berlese)	0.03	0.10	0.03	0.08	2.11	0.548
<i>Protoribates pannonicus</i> Willmann	0	0.03	0	0	3.00	0.391
<i>Punctoribates punctum</i> (C.L. Koch)	0	0	0.03	0	3.00	0.391
<i>Rhinoppia subpectinata</i> (Oudemans)	0.03	0.15	0	0	3.74	0.290
<i>Quadroppia quadricarinata</i> (Michael)	0	0	0	0.03	3.00	0.391
<i>Scheloribates latipes</i> (C.L. Koch)	0	0.10	0.08	0.10	4.17	0.243
<i>Suctobelba</i> sp.	0.38 ^A	0.75 ^B	0.68 ^B	0.05 ^A	13.07	0.004
<i>Tectocepheus velatus</i> (Michael)	0.38	0.83	0.83	0.45	6.66	0.083
<i>Trhypochthonius tectorum</i> (Berlese)	0	0	0.03	0.05	2.01	0.569
<i>Trichoribates trimaculatus</i> C.L. Koch	0	0.08	0.08	0.05	2.79	0.423
<i>Oribatida</i> total	3.28 ^A	10.10 ^B	6.15 ^B	6.85 ^B	15.81	0.001

Explanations: see Table 1.

Source: own research data

Similar survival strategy is exercised by *Tectocepheus velatus* (Gulvik 2007) but its life cycle is slightly longer. It is a eurytopic soil species (Weigmann and Kratz 1981), present in a range of different biotopes and characterized by high reproduction rate and capability of colonizing new environments. For these reasons, its abundance was expected to be much higher than the actual average values of 0.38-0.83 individuals per 50 cm³, all the more so since it is usually a predominant oribatid mite in Scots pine forests and it is a good bioindicator of soil biological activity (Klimek 2000). It seems that low density of *Oppiella nova* and *Tectocepheus velatus* may be due to the trophic conditions prevailing in the investigated substrates. Both species are considered fungivores (Luxton 1972, Ponge 1991, Remén *et al.* 2010). Therefore, the presence of saprotrophic fungi was necessary for their populations to grow. These fungi probably failed to develop in the investigated substrates. Proper development of the microorganisms might have been hampered by unfavorable weather conditions, and especially low rainfall in the summer.



Source: own research data

Figure 2. The dynamics of abundance of selected species of oribatid mites in the investigated experimental variants

SUMMARY

In the first year of the study mite density was significantly higher in the chips inoculated with the forest litter than on the control microplot. In the second year, the numbers of the microarthropods decreased, probably due to adverse

weather conditions. The most common group of mites were usually oribatid mites that accounted for 19.7 to 80.4% of all mites. An analysis of seasonal dynamics of *Oribatida* abundance over the entire study cycle revealed a clear increase in their numbers in non-supplemented pine chips only on the last sampling date. The chips without any structural components but enriched with the forest litter contained similar number of oribatid mites at the beginning and end of the study. Contrary to that, the number of mites decreased in the chips supplemented with peat, and particularly those enriched with lignite.

In total, 36 species of oribatid mites were identified on all microplots. The greatest increase in species diversity after inoculation with the forest litter was observed in the chips without any structural components, and the lowest in those supplemented with lignite.

Oribatula tibialis was the most common oribatid mite in the investigated substrates. Low number of *Oppiella nova* and *Tectocephus velatus*, the species having trophic associations with fungi, may indicate poor colonization of the chips, particularly those enriched with lignite, by saprotrophic organisms.

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