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MITE (ACARI) OCCURRENCE IN SELECTED SUBSTRATES USED FOR A RESTORATION OF DEGRADED SOILS

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Abstract

The number and groups of mites *(Acari)* and species composition of oribatid mites (Oribatida) were analysed in three different substrates used for the restoration of degraded soils: (1) pine forest litter, (2) apple orchard litter, and (3) pine chips. The study was conducted in the years 2011-2012, on microplots of the area of 1m², established in a belt of trees of a nursery in Białe Błota (Bydgoszcz Forest District). Average biannual mite density per 50 cm³ of the investigated substrates ranged from 14.6 to 54.43 individuals. The highest numbers of mites were found in shredded forest litter and the lowest in pine chips. The most abundant mites in the studied material were oribatid mites, accounting for 57.3 % of these arthropods. The highest number of oribatid mites was found in the forest litter (28), and the lowest (20) in pine chips. The number of species in both types of litter was similar in the first and second year of the study, but it rose three times in the pine chips substrate over the study period. Oribatid species in the litter substrates were dominated by the eurytopic *Tectocepheus velatus*, and the most abundant species in the pine chips substrate was *Oribatula tibialis*.

The experiment indicated a possibility of practical use of the shredded litter in the reintroduction of soil mesofauna and soil regeneration. This may facilitate the soil inoculation process, e.g., by using seeders specially adapted for this purpose. Additionally, a quick colonization of wood chips by acarofauna may suggest the possibility of using them as an excellent substrate for soil regeneration.

Keywords: forest litter, orchard litter, wood chips, reintroduction, soil regeneration, oribatid mites.

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INTRODUCTION

Soils on degraded, but also on extensively used areas, are usually characterized by low biological diversity (Klimek and Kowalska 2013; Klimek and Rolbiecki 2011). These soils, even after enriching with organic fertilizers or after phytomelioration, feature a low number of soil fauna species, especially Oribatida (Klimek *et al.* 2009; 2013). These small arthropods are known to play many important functions in the soil ecosystems, they improve pedogenic processes and spreading of bacteria and fungi, and they indirectly affect the formation of endo – and ectomycorrhizas (Klironomos and Kendrick 1996; Behan-Pelletier 1999; Remén *et al.* 2010; Schneider *et al.* 2005). They are also good bioindicators of soil biological activity (Axelsson *et al.* 1973; Behan-Pelletier 1999; 2003; Gulvik 2007).

However, this fraction of the edaphon has limited possibilities for migration and colonisation of new sites. Literature reports confirm that the colonisation of initial soils, featuring harsh conditions for small arthropod survival, is a slow process that may last for many months or even years (Lehmitz *et al.* 2011; Wanner and Dunger 2002; Klimek *et al.* 2013). Therefore, effective methods for accelerating the processes of soil regeneration are continuously searched for. Haimi (2000) claims that soil fauna is very important during reclamation of degraded soils in order to restore biological activity and improve bioremediation processes. These activities may be supported by an intentional introduction of the fauna by means of soil inoculum.

When inoculating the initial soils with the soil fauna, it is necessary to consider the type of substrate and media enriching the soils with organic matter. They should provide optimal conditions for the development of specific populations in the initial biotope. The authors of this paper have already performed a successful soil fauna introduction using fresh pine forest litter (raw humus). The soil collected from a nursery and used for edaphon inoculation was enriched with sewage sludge compost containing peat, sawdust and bark (Klimek *et al.* 2008; 2012). In laboratory conditions a substrate composed of sewage sludge compost, containing straw and wood ash, was used (Klimek *et al.* 2011).

The aim of this study was to determine the number and composition of the mite groups and species composition of oribatid mites in three different substrates intended for the regeneration of degraded soils: (1) pine forest litter, (2) apple orchard litter, and (3) pine chips.

MATERIAL AND METHODS

The study was conducted in the years 2011-2012, on the microplots established in a nursery in Białe Błota (Bydgoszcz Forest District). The experiment was carried out in a 20 m wide belt of trees (53°06'13.2"N, 17°55'46.6"E), in order to mitigate the influence of weather conditions, such as excessive sunlight, temperature fluctuations or too intensive precipitation. The tree stand was composed of the following species: Scots pine and European ash, in the undergrowth were oak and maple. Soil belonged to the poor (mainly brown rusty soil), the main type of forest here is fresh mixed coniferous.

The substrate material – forest and orchard litter and pine branches (thinning residues) were collected after 11^{th} April 2011. The forest litter and branches were collected near the nursery in a mature Scots pine forest (*Leucobryo-Pinetum* Mat.) in Białe Błota Forest District. The litter from an apple orchard was collected in Topolinek (53°19'26.1"N, 18°18'11.3"E), near Bydgoszcz. The next day the collected material was fragmented using a garden shredder VIKING GE 250 and distributed in a 10 cm layer on the exposed mineral soil in the designated places within the belt of trees. The experiment involved a total of 6 microplots of 1m^2 each, two per every variant: (1) pine forest litter, (2) orchard litter, and (3) wood chips. The microplots were isolated from the stand soil by means of 20 cm high Cellfast garden edge inserted at the depth of 5 cm and secured with garden pegs.

To maintain optimum moisture content, the microplots were hydrated by micro sprinklers, as per the guidelines and schedule for the irrigation of nurseries, and mean soil moisture was kept at the level of 5.1-9.9%.

The samples (50 cm³) for the acarologic analyses were collected in the spring, summer and autumn of each study year on the following days: 24th May 2011, 20th July 2011, 27th October 2011, 19th May 2012, 10th July 2012, and 16th October 2012. Ten samples were harvested from each variant (5 from each microplot). A total of 60 samples of 50 cm³ each were collected from every variant. Mite extraction was carried out over 7 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. A total of 5686 mites was determined, including 3720 oribatid mites.

Average density (N) of these mites was provided per 50 cm³ of the substrate, and the species dominance index (D) was given in percentage. Species diversity was determined based on the mean number of species per sample (s). According to Berthet and Gerard (1965) prior to statistical analysis the numerical data were subjected to a logarithmic transformation – ln (x+1). The statistical analysis was performed using Statistica 6.0: 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) 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 (Lomnicki 2000).

RESULTS

Mite groups occurrence

Average biannual (2011-2012) mite density in 50 cm³ of the investigated substrates ranged from 14.6 to 54.43 individuals. The highest numbers of mites were found in the shredded forest litter and the lowest in the pine chips (Table 1). The differences in numbers between forest litter and variants (2) and (3) were significant (Mann-Whitney U test, U=969.0, p=0.000013, U=649.5, p=0.000000, respectively). In the subsequent years, a density of the arthropods in both forest and orchard litter substrates remained at a similar level. In contrast, a 3-fold increase in the mite density in the wood chips substrate was observed and the differences between 2011 and 2012 were significant (Mann-Whitney U test, U=123.5, p=0.000001).

Table 1. Mite densities (in 50 cm ³ of substrate) on micropoles in years 2011-2012:
(1) pine forest litter, (2) orchard litter and (3) wood chips

		Experiment variant			Kruskal-V	Wallis test
Taxon	Year	(1) Pine forest litter	(2) Orchard litter	(3) Wood chips	Н	р
	2011	0.13	1.70	0.20		
Acaridida	2012	0	0.23*	0.23		
	mean	0.07 ^A	0.97 ^B	0.22 ^A	20.616	0.000
	2011	5.43	1.90	1.07		
Actinedida	2012	8.03	3.57*	5.10*		
	mean	6.73 ^A	2.73 ^B	3.08 ^B	23.025	0.000
	2011	5.13	14.13	5.27		
Mesostigmata	2012	4.27	3.53*	4.43		
	mean	4.70	8.83	4.85	0.934	0.627
	2011	53.27	7.13	0.43		
Oribatida	2012	32.17	18.90*	12.10*		
	mean	42.72 ^A	13.02 ^B	6.27 ^B	62.912	0.000
	2011	0.30	0.23	0.03		
Tarsonemida	2012	0.13	0.13	0.33*		
	mean	0.22	0.18	0.18	0.050	0.976
	2011	64.27	25.10	7.00		
Acari total	2012	44.60	26.37	22.20*		
	mean	54.43 ^A	25.73 ^B	14.60 ^B	39.536	0.000

^{A, B} – the same letter means the in significant difference – a *post hoc* the Mann-Whitney U test at p < 0.05) * – significant difference between 2011 and 2012– a *post hoc* the Mann-Whitney U test at p < 0.05) Source: Own study The most abundant mites in the studied material were oribatid mites, accounting for 57.3% of these arthropods. Significantly less frequent groups included Mesostigmata (25.4%) and Actinedida (14.7%), and occasionally Acaridida and Tarsonemida.

Oribatid mites were the most abundant in the forest litter substrate (42.72 individuals per 50 cm³). Their numbers decreased along the study period, but the differences were not statistically significant. In the other substrates, the mite density was low in the first year, but it increased a few times in the second year. An average density of Mesostigmata was similar in all variants and it was higher in 2011 than in 2012. Actinedida, similarly to Oribatida, were the most numerous in the first variant (6.73 individuals per 50 cm³), and more than half less abundant in the other variants. A density of these mites rose in the course of the study on all the microplots, but this growth was significant only in variants (2) and (3) (Mann-Whitney U test, U=256.0, p=0.004129, U=149.5, p=0.000000, respectively).

Oribatid occurrence

The highest number of oribatid mites was identified in the forest litter (28), and the lowest (20) in the pine chips (Table 2). The number of species in both types of litter was similar in the first and second year of the study, and it rose three times in the pine chips substrate over the study period. The statistical analysis performed for the average number of species in the sample (*s*) indicated that this parameter differed significantly in all variants of the experiment. In variants (2) and (3), a significant improvement in oribatid species diversity was observed in the subsequent years of the study (Mann-Whitney *U* test, U=302.5, p=0.029206, U=21.5, p=0.000000, respectively).

The analysed material contained more mature than juvenile forms, with larvae and nymphs accounting for 40-45% of all the oribatid mites, depending on the variant (Table 2). In the case of litter substrates, an increasing share of juvenile forms was observed in the years 2011-2012. Different situation was noticed in the wood chips substrate, where high proportion (77 %) of juvenile forms and low total numbers of Oribatida (0.43 individual per 50 cm³ – Table 1) were reported.

Oribatida populations isolated from the substrates made of forest and orchard litter were dominated by the eurytopic *Tectocepheus velatus* (Michael), accounting for 49 % and 51 % of all Oribatida species (Figure 1). Other abundant species included *Oribatula tibialis* (Nicolet) and *Chamobates schuetzi* (Oudemans 1902) of the forest litter and *Eupelops occultus* (C.L. Koch) and *Punctoribates punctum* (C.L. Koch) of the orchard litter. Additionally, both populations included fairly numerous *Suctobelba* mites. A dominant species of wood chips

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substrate was *Oribatula tibialis* (D=41.8%), with *Tectocepheus velatus* as the second most common mite (D=18.6%).

Table 2. Number of species (S), average number of species (s) and% of juveniles onmicro-plots in years 2011-2012: (1) pine forest litter, (2) orchard litter and(3) wood chips

		E	Kruskal-V	Wallis test		
Index	Year	(1) Forest litter	(2) Orchard litter	(3) Wood chips	Н	р
	2011	24	20	6		
S	2012	22	22	18		
	mean	28	25	20	-	-
	2011	5.13	2.60	0.30		
S	2012	4.67	4.10*	3.63*		
	mean	4.90 ^A	3.35 ^B	1.97 ^c	41.115	0.000
	2011	33.35	41.59	76.92		
% juv	2012	50.78	43.03	43.80		
	mean	39.91	42.64	44.95	-	-

^{A, B} – the same letter means the in significant difference – a *post hoc* the Mann-Whitney U test at p < 0.05) * – significant difference between 2011 and 2012– a *post hoc* the Mann-Whitney U test at p < 0.05) Source: Own study

In total, 40 species of oribatid mites were reported in the experiment (Table 3). Fourteen of them were found in all the experimental variants. Ten species were found only in the forest litter substrate, 8 inhabited exclusively the orchard litter, and 3 were found in the wood chips substrate alone.





Source: Own study

Figure 1. The structure of domination in Oribatida investigated experimental variants

	Exper	Kruskal-Wallis test			
Species	(1) Pine forest litter	(2) Orchard litter	(3) Wood chips	Н	р
Adoristes ovatus (Koch, 1839)	0.05	0	0.02	3.560	0.167
Autogneta longilamellata (Michael, 1885)	0	0.02	0	2.000	0.368
Brachychthonius sp.	0.47	0.12	0	7.784	0.020
Camisia spinifer (C.L. Koch, 1835)	0.03	0	0	4.022	0.134

Table 3. Density of Oribatida (in 50 cm³ of substrate) on micropoles in years 2011-2012: (1) pine forest litter, (2) orchard litter and (3) wood chips

	Experiment variant			Kruskal-Wallis test	
Species	(1) Pine forest litter	(2) Orchard litter	(3) Wood chips	H	р
Carabodes forsslundi Sellnick, 1953	0.03	0	0	4.022	0.134
Carabodes labyrinthicus (Michael, 1879)	0.07	0	0	8.136	0.017
Carabodes subarcticus Trägardh, 1902	0.07	0	0	8.136	0.017
Chamobates schuetzi (Oudemans, 1902)	6.22 ^A	0.28 ^B	0.85 ^B	73.916	0.000
Cultroribula bicultrata (Berlese, 1905)	0	0	0.02	2.000	0.368
Damaeus sp.	0.02 ^A	0.33 ^B	0.25 ^B	11.263	0.004
Eremaeus oblongus (C.L. Koch, 1836)	1.13 ^A	0.03 ^B	0	53.056	0.000
Eupelops occultus (C.L. Koch, 1835)	0.02 ^A	1.12 ^B	0.05 ^A	49.379	0.000
Eupelops torulosus (C.L. Koch, 1839)	0.08	0.10	0.12	0.154	0.926
Galumna lanceata (Oudemans, 1900)	0.17	0.18	0.12	1.659	0.436
Gymnodamaeus bicostatus (C.L. Koch, 1835)	0.12	0.43	0.37	2.098	0.350
Liacarus coracinus (C.L. Koch, 1841)	0	0.03	0	4.022	0.134
Liebstadia similis (Michael, 1888)	0	0.10	0	10.227	0.006
Liochthonius sp.	0.62	0.15	0.02	5.891	0.053
Metabelba pulverulenta (C.L. Koch, 1839)	0.18	0.25	0.12	1.727	0.422
Micreremus brevipes (Michael, 1888)	0	0.02	0.03	2.023	0.364
Microzetorchestes emeryi (Coggi, 1898)	0.07	0.05	0.02	1.192	0.551
Nanhermannia nanus (Nicolet, 1855)	0.10	0	0	6.067	0.048
Lauroppia neerlandica (Oudemans, 1900)	0.02	0	0	2.000	0.368
Oppiella nova (Oudemans, 1902)	0.72 ^A	0.05 ^B	0.07 ^B	14.274	0.001
Oribatula tibialis (Nicolet, 1855)	9.48 ^A	0.60 ^B	2.62 ^B	56.565	0.000
Pergalumna nervosa (Berlese, 1914)	0.02	0	0	2.000	0.368
Phthiracarus longulus (C.L. Koch, 1841)	0	0	0.02	2.000	0.368
Phthiracarus sp.	0	0.02	0	2.000	0.368
Protoribates variabilis (Rajski, 1958)	0	0.02	0	2.000	0.368
Punctoribates punctum (C.L. Koch, 1839)	0	1.03	0	49.598	0.000
Quadroppia quadricarinata (Michael, 1885)	0.02	0	0.02	1.006	0.605
Rhysotritia duplicata (Grandjean, 1953)	0.03	0	0	4.022	0.134
Scheloribates laevigatus (C.L. Koch, 1836)	0	0	0.02	2.000	0.368
Scheloribates latipes (C.L. Koch, 1844)	0	0.03	0	4.022	0.134
Suctobelba sp.	1.88 ^A	1.35 ^B	0.38 ^B	13.975	0.001
Tectocepheus velatus (Michael, 1880)	20.80 ^A	6.63 ^B	1.17 ^c	32.109	0.000

	Exper	Kruskal-Wallis test			
Species	(1) Pine forest litter	(2) Orchard litter	(3) Wood chips	Н	р
Steganacarus carinatus (C.L. Koch, 1841)	0.05	0	0	4.022	0.134
Trhypochthonius tectorum (Berlese, 1896)	0.22	0	0	14.481	0.001
Trichoribates trimaculatus C.L. Koch, 1835	0.05	0.03	0.02	1.029	0.598
Xenillus tegeocranus (Hermann, 1804)	0	0.03	0	4.022	0.134

 A_B – the same letter means the in significant difference – a *post hoc* the Mann-Whitney U test at p < 0.05) Source: Own study

DISCUSSION

Soil environment degradation processes may occur very quickly, but pedogenesis and soil regeneration are extremely slow (Kwiatkowska 2007). Degradation of agricultural and horticultural soils usually results from too intensive use, over-fertilization and the use of chemical plant protection products. Some activities, e.g. lignite mining, may even lead to a transformation of deep lithosphere layers. Reclamation of these areas is based on such corrective measures as fertilization, liming, renewal of vegetation, organic stimulation of the soil and some bioremediation techniques (Haimi 2000).

The soils destroyed by human activities are characterized by a markedly reduced biological activity. Apart from phytomelioration, the practical approaches adopted to restore this activity include treatments with microorganisms, such as bacteria and fungi. However, soil animals are usually underestimated in this respect, except for earthworms that have been for some time proposed as useful in improving soil quality (Dunger 1969; Boyer and Wratten 2010). Bradshaw and Hüttl (2001) claim that the main purpose of soil regeneration should be to restore the ecosystem functionality. None soil-based terrestrial ecosystems may function correctly without the presence of mesofauna. Unfortunately, this part of edaphon, as less visible and less known, is mainly ignored (Langer *et al.* 1999; Majer *et al.* 2007). So far, no effective methods for spreading soil mesofauna, especially mites, have been developed.

As mentioned before, the early stages of biological succession, occurring on degraded areas or afforestation areas used earlier as farming lands, lack the typical stages of the mite fauna (Klimek and Kowalska 2013; Klimek and Rolbiecki 2011), and phytomelioration or organic fertilization alone do not improve the situation. Therefore, it seems that the only solution to this problem may be a reintroduction of mesofauna. This is why the authors of this study are trying to develop an effective method of inoculating the soils with edaphon. The mites, and particularly oribatid mites, analysed in this study, were treated, according to the concept of Haimi (2000), as the organisms whose high share in the edaphon increase soil metabolic activity and as bioindicators of general soil biological activity.

Mite density reported in the analysed substrates ranged from 8790 to 32770 individuals per 1 m² of soil. For comparison, a density of these arthropods in an apple orchard turf was 25070 individuals m⁻², similarly to strawberry crop muchled with wood chips (Klimek *et al.* 2014a). The soils of Scots pine forests with well-developed raw humus, feature much higher density of these arachnids, from 142480 to 260780 individuals m² (Klimek 2000).

As already mentioned, the oribatid mites were the most common mites in all variants of the study. Their share in the substrate made of pine forest raw humus was the highest and was maintained at a similar level throughout the study period (72-83%). The mite density on the microplots covered with the orchard litter increased in the same period by 2.5 times, and on the plots covered with wood chips by 28 times. A higher number of oribatid mites than other orders of mites, especially Actinedida, may indicate a progressive stabilization of soil biological system (Werner and Dindal 1990; Gulvik 2007).

The most abundant oribatid mite in the investigated substrates was Tectocepheus velatus (Michael 1880). It clearly dominated in the forest and orchard litters (Figure 1). Its density was the highest in the substrate (1) - 20.8 individuals per 50 cm³, and the differences between individual variants were significant (Mann-Whitney U test, U=1170.0, p=0.000944, U=776.0, p=0.000000, U=1428.0, p=0.030434, respectively). It is a parthenogenetic species, characterized by short reproduction cycle, high reproduction rate and high ability to colonise new environments (Gulvik 2007, Siepel 1994, Skubała and Gulvik 2005). It belongs to fungivores (Luxton 1972, Ponge 1991), feeding on ectomycorrhizal fungi (Remén et al. 2010, Schneider et al. 2005). This way, it may contribute to spreading of these fungi. This may be of practical importance, particularly at the early stages of forest succession on degraded areas, where a reintroduction of T. velatus could improve the mycorrhizal interactions. A significant increase in the abundance of this species in the course of the study was observed in the orchard litter and wood chips (Figure 2). However, a significant decline in T. velatus was noticed in the forest litter in 2012 (Mann-Whitney U test, U=192.0, p=0.000137).

Another abundant species in the examined substrates was *Oribatula tibialis* (Nicolet 1855). This species preferred microplots covered with the forest litter (Table 3). The density of this oribatid mite increased many times in all variants throughout the study. *O. tibialis* is a eurytopic species (Weigmann 1991; Weigmann and Kratz 1981), classified as a forest oribatid (Rajski 1968). Interestingly, a rise in this species abundance was also reported in a birch nursery irrigated after soil inoculation with forest raw humus (Klimek *et al.* 2013).



Source: Own study

- Figure 2. The density of selected species of moss mites in the tested variants of the experiment: (1) pine forest litter, (2) orchard litter, and (3) wood chips.
 - A, B the same letters for the species mean no differences between years 2011 a 2012, a *post hoc* the Mann-Whitney U test at p < 0.05

A slightly less numerous oribatid mite was *Chamobates schuetzi* (Oudemans 1902). This species was most common in the forest litter, similarly as the previously discussed species (Table 3). It was more abundant in all the variants in the second year of the study (Figure 2). It is the oribatid mite classified as Scots pine forest oribatid (Usher 1975).

Eupelops occultus (C.L. Koch 1835) and *Punctoribates punctum* (C.L. Koch 1839) are classified as meadow species (Rajski 1968). It is therefore not surprising that the latter was found only on the microplots covered with the orchard litter (Table 3), collected from under the apple trees surrounded by grasses. *E. occultus* was also preferably found in this variant. However, both species experienced a decline in numbers over the study period (Figure 2).

The density of oribatid mites in the nurseries mulched with raw humus usually ranged from a few to several thousand individuals per square meter of soil (Klimek *et al.* 2008, 2012, 2013). These earlier studies involved fresh and non-shredded forest litter, collected from the same place as in the present exper-

iment. A practical use of non-shredded litter, containing small pieces of wood, cones or branches may pose problems during storage or application on the soil during regeneration. Therefore, this experiment utilized the substrates made of litters shredded two times with a garden shredder. Based on the analysis of the abundance and diversity of oribatid species, and after comparing these results with previous studies, it can be concluded that this relatively "invasive" procedure of a double shredding had no apparent effect on the average abundance or the species diversity of soil mesofauna. Similar outcomes were reported in a laboratory study, when the mites were investigated in optimum thermal and moisture conditions in the non-shredded litter applied on composts made of sewage sludge (Klimek *et al.* 2011).

Interesting trends were observed regarding Oribatida species abundance and diversity in wood chips: their mean density *N* and mean number of species *s* were lower than in the other substrates, but over two years of the study these parameters rose by 28 and 12 times, respectively. It should be mentioned that no species of mites were found in fresh wood chips before establishing the experiment. The chips were probably colonised by the mites immigrating from the soils surrounding the microplots. Therefore, this substrate seems to be favourable for a development of these arthropods. This claim was confirmed by another study, in which using wood chips for mulching strawberries created very favourable conditions for the development of many oribatid species (Klimek *et al.* 2014a).

The experiment indicated a possibility of practical use of shredded litter in a reintroduction of soil mesofauna and soil regeneration. This may facilitate the soil inoculation process, e.g., by using seeders specially adapted for this purpose. Additionally, a quick colonization of wood chips by acarofauna may suggest the possibility of using them as an excellent substrate for soil regeneration.

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