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A STUDY ON THE DIEL ACTIVITY OF MYRIAPODS (DIPLOPODA, CHILOPODA) IN NATURAL AND ANTHROPOGENICALLY INFLUENCED HABITATS

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ABSTRACT: *The present paper provides the results of a study on the diel activity of millipedes and centipedes from two different in terms of anthropogenic impact sites located in northeastern Bulgaria. The presumed differences in the diel activity of myriapods were tested using various statistical methods: the criterion of Kolmogorov, $\chi 2$ criterion of uniformity, Pearson's normalized correlation coefficients, and the multivariate regression analysis. . The results show that the diel surface activity of the established species depends on the degree of illumination (number of hours in a day), the air and the soil temperature. However, the activity was not affected by relative humidity, active seasons, and the degree of anthropogenic impact. The diurnal activity conducted in urban and natural habitats confirmed the nocturnal nature of the established species, which are most active in the time interval from 10:00 pm to 4:00 am. This activity is likely to have endogenous nature and it is controlled by internal physiological factors, but its duration may vary according to the changes in the environmental conditions.*

KEYWORDS: Circadian Rhythms, Activity, Urban, Rural, Bulgaria

INTRODUCTION

The diel and motor activity of myriapods have been relatively poorly studied. It is especially so regarding the activity of myriapods under field conditions as most of the previous studies were conducted in laboratories, Banerjee (1967). Most myriapods show distinct surface activity at night, although there are also species active in the early morning hours, during the day, and in the evening. Donadale et al. (1972) found that 90% of the studied millipedes have been caught between 10 pm and 4 am. In a similar survey of three common European species, *Cylindroiulus punctatus* (Leach, 1815), *Tachypodoiulus niger* (Leach, 1814), and *Polydesmus angustus* Latzel, 1884, Banerjee (1967) recorded two activity peaks of millipedes – from midnight to one hour before sunrise and from one hour after sunset to midnight. The same author establishes a correlation (with a coefficient higher than 0.5) between the air temperature and the activity of all studied millipedes. The crepuscular millipede *Anoplodesmus splendidus* (Verhoeff, 1936) (Polydesmida: Paradoxosomatidae) has a bimodal circadian rhythm with activity peaks at dawn and dusk [8, 17]. The peaks of activity in this species are very distinct in laboratory conditions under a regime of 12 hours of light, where the morning activity is greater. In continuous light or darkness the activity peak is completely non existant. Another study found that the respiratory activity of *Gymnostreptus olivaceus* Schubart, 1944 (Spirostreptida) in normal light mode is higher at night than during the day. In a reversed light regime the species tends to support more intense nocturnal respiratory activity, where such behavior is observed under conditions of constant light and constant darkness, Boccardo et al. (1995). Experimental data show a consistent endogeic rhythm in *G. olivaceus*, where light does not participate in the clock synchronization of its diel activity. Light is probably insufficient to block the start of the biological clock in conditions of total darkness or constant light, Boccardo et al. (1995). Internal biological rhythm occurs not only in the surface-dwelling species but also in troglobites living in constant darkness [21, 25]. Toye (1966) studied the activity of the millipedes *Spirostreptus assiniensis* Attems, 1914 (Spirostreptida), *Oxydesmus* sp. (Polydesmida: Oxydesmidae), and *Habrodesmus falx* Cook, 1896 (Polydesmida: Paradoxosomatidae) in Nigeria and reported that *S. assiniensis* reacted photonegatively both in natural light and in continuous artificial light, while *Oxydesmus* sp. showed photopositive response under both light conditions. In artificial light, *H. falx* reacted photopositively and showed indifferent reactions to natural light.

Cloudsley-Thompson (1951) indicates the reduction in temperature at night as a reason for the increase in the terrestrial physical activity of millipedes. In general, representatives of the class Diplopoda showed decreased activity during the hours of daylight compared to centipedes Donadale et al. (1972), although the latter can rarely be found in daylight. The North American species *Strigamia chionophila* Wood, 1862, which has been observed by Johnson (1952) on dry foliage during the day, marks an exception. The coastal species *Strigamia maritima* (Leach, 1817) has been observed feeding on the surface of rocks at night [9, 28], but Lewis (1961) has found that it also feeds during the day. It is very likely that this species is active round-the-clock, a peculiarity that may as well be true for other mostly underground species, Lewis (1981).

Some centipedes easily adapt their circadian rhythms to the changes in the environmental conditions. The North American species *Scolopendra heros* Girard, 1853 prefers to remain underground in warm days and comes to the surface when it is cloudy and rainy, Campbell (1932), while *Scolopendra morsitans* L., 1758 sometimes appears on the surface after a heavy rain in the Northern Nigerian savanna, Lewis (1981). Studies show that in Tunisia *S. canidens* Newport, 1844 (sub *Scolopendra clavipes* CL Koch, 1847, see Akkari et al. (2008)), similar to the African species *Rhysida immarginata togoensis* Kraepelin, 1903 (from Ghana), exhibits an endogenous rhythm with nocturnal activity when kept for several days in darkness at constant temperature and humidity [13, 14]. An unknown species of the genus *Scolopendra* from New Mexico exhibits a distinct diurnal rhythm when illuminated from 6 am to 9 pm in humid air and temperature of 27˚C. The rhythm remains steady in constant darkness, but it almost disappears in constant light, Cloudsley-Thompson et al. (1970). Mead (1968) found that in constant darkness and constant relative humidity the European species *Scolopendra cingulata* Latreille, 1829 maintains a circadian rythm for 80 days, after that variations in activity occur marked by periods much shorter or longer than 24 hours. Experiments were conducted in a humid substrate and relative air humidity from 60 to 80%. The activity stopped when the humidity rose to 100%. The period of activity lasted for about one hour every 24 hours, sometimes individuals went through a second phase of activity, three or four hours after the first, which was probably dictated by hunger. Kept in a terrarium *S. cingulata* was active on an average of 1-2 hours one in eight nights, Klingel (1960). Koilraj et al. (1999) found that the periods of activity depend on the length of daylight. In *Cyngalobolus* sp. the period of activity during 24 hours of darkness is shorter during 12 hours of darkness and 12 hours of light, rather than during 24 hours of illumination. *Lithobius forficatus* L., 1758 exhibits distinct round-the-clock rhythmic activity, when observed in laboratory conditions. Thirty individuals living together at a constant temperature, continuous exposure to light, and an intensity of 230 lux registered four times higher active rate at night than during the day. See Amouriq (1967).

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Tuf et al. (2006) investigated the diel activity of myriapods in two different sites in the Czech Republic – riparian forest and adjacent deforested area. They found that almost all investigated terrestrial myriapods show activity at night and at twilight. In spring, some species, e.g., *Lithobius mutabilis* L. Koch, 1862, *Glomeris connexa* CL Koch, 1847, *Haplogona oculodistincta* (Verhoeff, 1893), *Unciger foetidus* (CL. Koch, 1838), and *Leptoiulus proximus* (Nemec, 1896), showed low activity during the day, while *Polyzonium germanicum* Brandt, 1837 appeared to be the only diplopod active throughout the day without a distinct peak period. According to Tuf et al. (2006), the circadian rhythm in millipedes is controlled by internal physiological factors, but their activity is largely determined by environmental conditions and mainly by the temperature regime.

The present paper shows the results from a study on the diel activity of millipedes and centipedes in two sites which are different in terms of anthropogenic impact and which are located in the town of Shumen and its surroundings, in northeastern Bulgaria. The presumed differences in the diel activity of myriapods in anthropogenically influenced and undisturbed environments were tested using various statistical methods.

MATERIAL AND METHODS

The study of the diel surface activity of Myriapoda was conducted in spring and fall, i.e. during the months with highest seasonal activity. The study was conducted at two sites, different in terms of anthropogenic impact, which were located in the town of Shumen (northeastern Bulgaria) and its suburbs. The first sampling plot (Urban, hereafter U) is located in the park of Shumen University (N 43°16′750″; E 26°56′870″, 226 m alt.). It is located within the city borders and is characterized by strong human presence. The vegetation has an of anthropogenic origin, is dominated by *Pinus nigra* Arnold, *Tilia tomentosa* Moench, *Robinia pseudoacacia* L. , *Aesculus hippocastanum L.* , *Cercis siliquastrum* L. , *Hedera helix* L. , *Clematis vitalba* L., etc. See Assyov et al. (2006)The soils are predominantly chernozems with sandy clay structure with pH 7.37 measured at the collecting slot. The second sampling plot (Rural, hereafter R) is located in the Bukaka Nature Reserve (N 43°15′592″; E 26°53′310″, 350 m alt.) which is part of the Shumen Plateau Nature Park.

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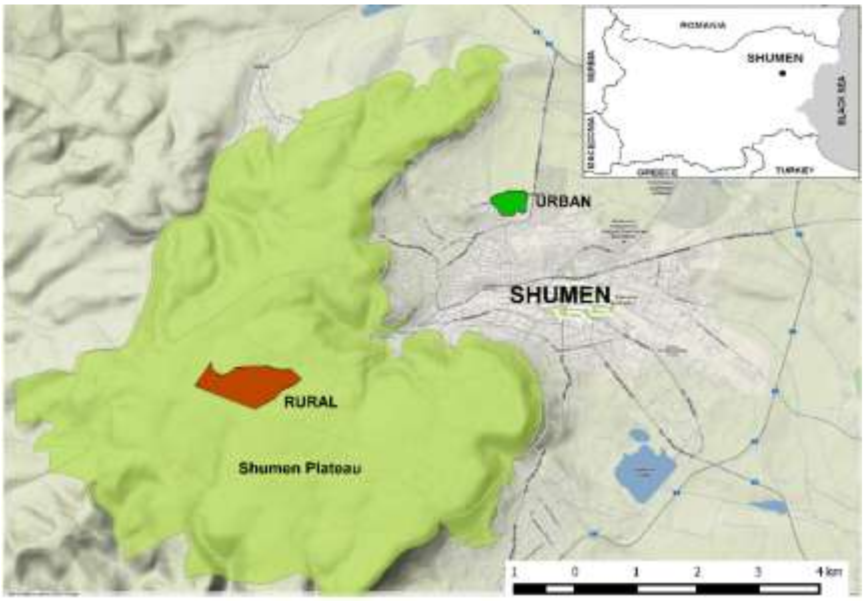

Figure 1. Map of the research area and the location of the two sites.

The area is well preserved environmentally and has only a partial human influence (scattered erosion). Luvisols, with pH 4.18, are the main soils in the sampling site. The dominant tree species is *Fagus sylvatica* L. ssp. *moesiaca* (K. Malý) Hjelmquist in places mixed with single specimens of *Carpinus betulus* L. , *Acer campestre* L., *Fraxinus exelsior* L., Assyov et al. (2006). The beech trees have a natural origin, with high canopy density, and are 80 to 100 years old.

Twenty pitfall traps, each at a distance of 10 meters from the other, were placed in a straight line in each sampling plot. The traps were checked by two different teams and emptied every two hours throughout the twenty-four-hour day for 10 days in the period 26 May – 5 June and 2 –12 October 2008. Simultaneously with the checking, the temperature and soil moisture were measured in every site with the aid of a digital thermometer and soil hygrometer (TFA, Germany). The atmospheric pressure, air temperature, and air humidity were measured with a barometer, thermometer, and hygrometer (TFA, Germany). The soil pH was determined with a digital pH meter, type HI 83141 (HANNA instruments, Romania). The geographical coordinates were reported in the central parts of the plots with a GPS-receiver (GARMIN-OREGON 300). According to the data obtained from the meteorological station of Shumen the average annual temperature in the city and adjacent areas in 2008 was 8 – 12.5C° and the mean annual precipitation was 420 mm/ m^2.

An analysis of variance determined the statistical significance of the dependence of the activity of individuals on different factors such as: the average temperature of the air and the soil, the average humidity of the air and the soil, the location of the site, the degree of illumination (time of day) and the season. In some cases the results are verified and confirmed by the criterion of Kolmogorov and χ^2 criterion of uniformity. Pearson's normalized correlation coefficients have been calculated. Multivariate regression analysis has been used to determine the form of the dependence of the activity of individuals on the statistically significant factors. A multiple linear regression was used to depict the dependence of the daily activity on the air and soil temperature. A thorougly discription of used statistical methods could be found e. g. in Afifi and Ayzen (1982).

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RESULTS

In the period 26 May – 5 June 152 specimens belonging to 10 species were trapped. Of them, 138 specimens (5 species) belong to class Diplopoda and 14 specimens to class Chilopoda (5 species). A total of 74 specimens, of which 73 millipedes (57 specimens of *Leptoiulus trilineatus* and 16 specimens of *Megaphyllum transsylvanicum*) and only one centipede, *Lithobius nigripalpis*, were found at site U (Table 1). At site R were caught altogether 78 specimens, of which 65 millipedes and 13 centipedes. They are distributed as follows: 1) Diplopoda: *L. trilineatus* (36 specimens), *M. bosniense* (18 specimens), *Cylindroiulus boleti* (5 specimens), *Pachyiulus hungaricus* (4 specimens), and *M. transsylvanicum* (2 specimens); 2) Chilopoda: *L. mutabilis*, *L. forficatus* and *Clinopodes flavidus*, each represented by 3 specimens, and *Cryptops anomalans* by 4 specimens (Table 1).

In the second trapping period 2 – 12 October, 117 specimens from 9 species were caught in total, of which 114 belong to Diplopoda (7 species) and only 3 to Chilopoda (2 species). Seventy-one specimens of Diplopoda from 4 species (*L. trilineatus* – 39 specimens, *M. transsylvanicum* – 29, *Megaphyllum unilineatum* – 2, and *C. boleti* – 1) and 2 specimens of Chilopoda (one of each *L. nigripalpis* and *C. flavidus*) were caught in U (Table 2). Forty-tree specimens of Diplopoda from five species (*L. trilineatus* – 27, *M. bosniense* – 12, *C. boleti* – 2, one of each *P. hungaricus* and *Polydesmus complanatus*, and one of Chilopoda (*L. nigripalpis*), were trapped at site R (Table 2).

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Table 1. Temporal distribution of species in the Urban and Rural site in the period 26 May – 5 June 2008.

	№	Species	12:00	14:00	16:00	18:00	20:00	22:00	0:00	2:00	4:00	6:00	8:00	10:00
Urban	1.	Leptoiulus trilineatus (C.L. Koch, 1847)	2 ♀	1 ♀				2 ♀	11 ♀, 1♂, 2♂ subad.	16♀, 1j.	12 ♀, 2♂ subad.	2 ♀, 1♂ subad.	4 ♀	
	2.	Megaphyllum transsylvanicum (Verhoeff, 1897)			1 ♂			1 ♀, 2 ♂	2 ♂	3 ♀, 6 ♂	1 ♀			
		Total Diplopoda	2	1	1	0	0	5	16	26	15	3	4	0
	1.	Lithobius nigripalpis L. Koch,1867											1 ♀	
		Total Chilopoda	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
Rural	1.	Leptoiulus trilineatus (C.L.Koch, 1847)	1 ♂				1♀, 1♂,	3 ♀, 2 j., 1subad	4 ♀, 2 j., 1subad.	2 ♀, 3 ♂, 2 subad, 1j.	3 ♀, 2 ♂	1 ♀, 3 ♂	1 ♀, 2 ♂	
	2.	Megaphyllum transsylvanicum (Verhoeff, 1897)									1 ♀		1 ♀	
	3.	Megaphyllum bosniense (Verhoeff, 1897)	1 ♂		1♀	1♀	1 ♀	2 ♂	3 ♀, 1 ♂	2 ♀, 2 ♂	1 ♂	1 ♀	1 ♀, 1 ♂	
	4.	Cylindroiulus boleti (C.L. Koch, 1847)								1 ♀, 1 ♂	2♀, 1subad.			
	5.	Pachyiulus hungaricus (Karsch, 1881)					1 ♀	1 ♀		1 ♂		1 j.		
		Total Diplopoda	**2**	**0**	**1**	**1**	**4**	**9**	**11**	**15**	**10**	**6**	**6**	**0**
	1.	Lithobius mutabilis L. Koch, 1862	1 ♀					1 ♂	1 ♀					
	2.	Lithobius forficatus (Linnaeus, 1758)							1 ♀, 1 ♂	1 ♀				
	3.	Clinopodes flavidus C.L. Koch, 1847			1 subad.			1 ♀	1 ♀					
	4.	Cryptops anomalans Newport, 1844				1			2	1				
		Total Chilopoda	**1**	**0**	**1**	**1**	**0**	**2**	**6**	**2**	**0**	**0**	**0**	**0**

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Table 2. Temporal distribution of species in the Urban and Rural site in the period 2 – 12 October 2008

	№	**Species**	**12:00**	**14:00**	**16:00**	**18:00**	**20:00**	**22:00**	**0:00**	**2:00**	**4:00**	**6:00**	**8:00**	**10:00**
Urban	1.	Leptoiulus trilineatus (C.L. Koch, 1847)	1 ♂, 1j.				4♀, 1♂	4♀ 2♂	2♀, 4♂	5♀, 1♂	4♀, 3♂	1♀, 1♂	2♀, 2♂	1♂
	2.	Megaphyllum transsylvanicum (Verhoeff, 1897)			1j.			3♀, 4j.	3♂, 3j.	4♂, 3 j.	1♀, 3♂, 1j., 1 ♂ subad		1♀, 1j.	
	3.	Megaphyllum unilineatum (C.L.Koch, 1838)						2♂						
	4.	Cylindroiulus boleti (C.L. Koch, 1847)									1♂			
		Total Diplopoda	2	0	1	0	5	15	12	13	14	2	6	1
	1.	Lithobius nigripalpis L. Koch,1867						1♂						
	2.	Clinopodes flavidus C.L. Koch, 1847					1♀							
		Total Chilopoda	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Rural	1.	Polydesmus complanatus (Linneaus, 1761)										1♂		
	2.	Leptoiulus trilineatus (C.L. Koch, 1847)					1♀, 1j.	2♀, 1j.	4♀, 1♂, 1j.	4♀, 2j.	1♀	3♂, 1j.	3♀	1♀, 1j.
	3.	Megaphyllum bosniense (Verhoeff, 1897)					1♂, 1j.	2♀, 1♂		1♀, 1♂	2♂	1♀, 1♂	1♂	
	4.	Cylindroiulus boleti (C.L. Koch, 1847)								1♂			1♀	
	5.	Pachyiulus hungaricus (Karsch, 1881)								1♂				
		Total Diplopoda	0	0	0	0	4	6	6	10	3	7	5	2
	1.	Lithobius nigripalpis L. Koch, 1867									1♀			
		Total Chilopoda	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**

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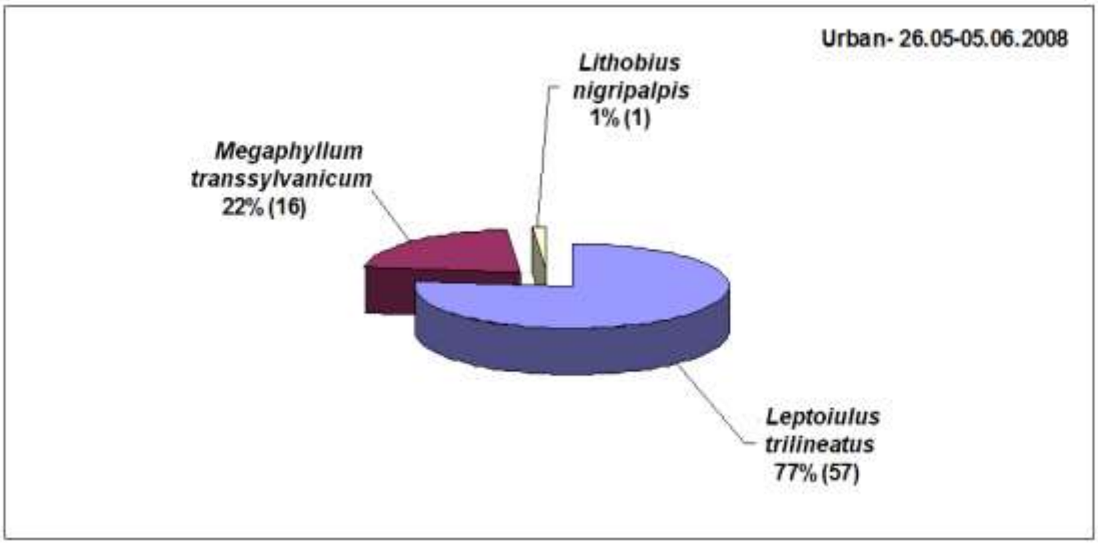
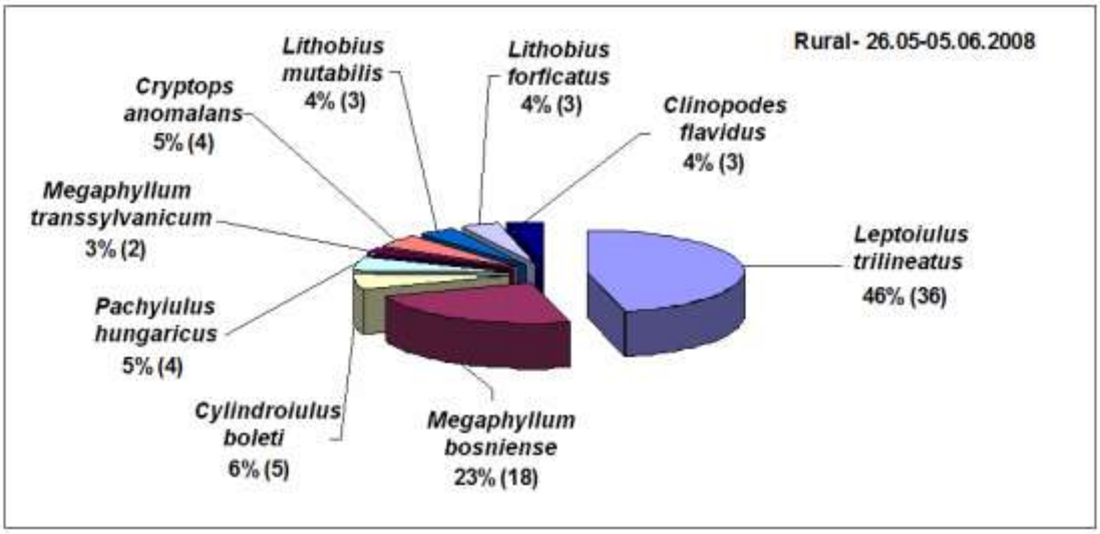
The most abundant species at both sites (R and U) in the spring and fall was *Leptoiulus trilineatus*. It is followed by *Megaphyllum transsylvanicum* in R and *Megaphyllum bosniense* in U. The total number of catches in the spring was 152 individuals, of which 138 millipedes and 14 centipedes, while in fall – 117, of which 114 millipedes and 3 centipedes respectively. The main part of the catch consisted of millipedes. Since the centipedes found constituted a very small number, they were not included in the analysis. The number of millipedes collected during the two seasons in the university park was about the same – 73 individuals in the spring and 65 in the fall. At the reserve only 43 millipedes were caught in the fall, which is significantly fewer than those collected in the spring – 65 (see Table 1 & 2).



A



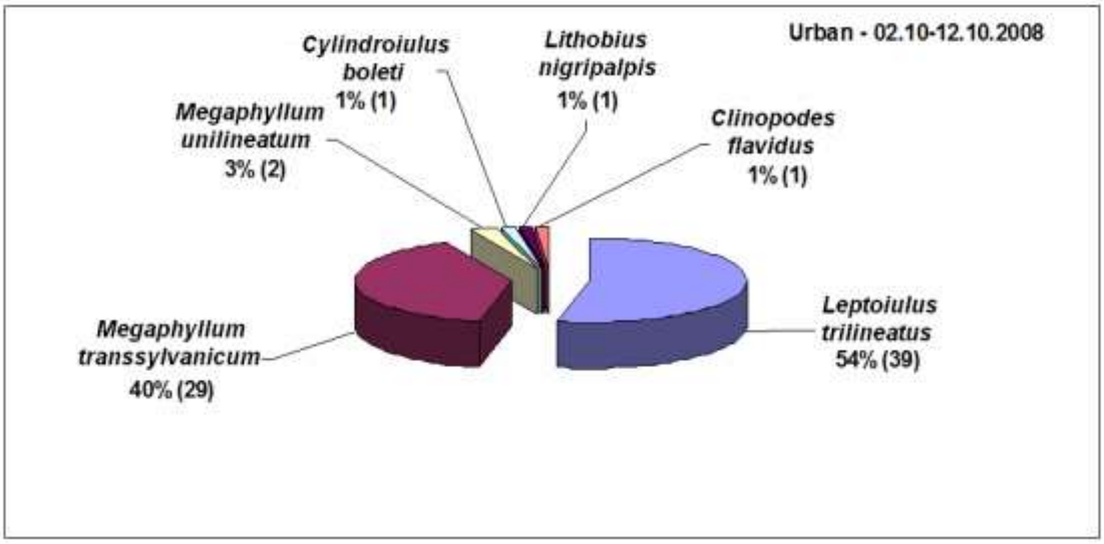
B

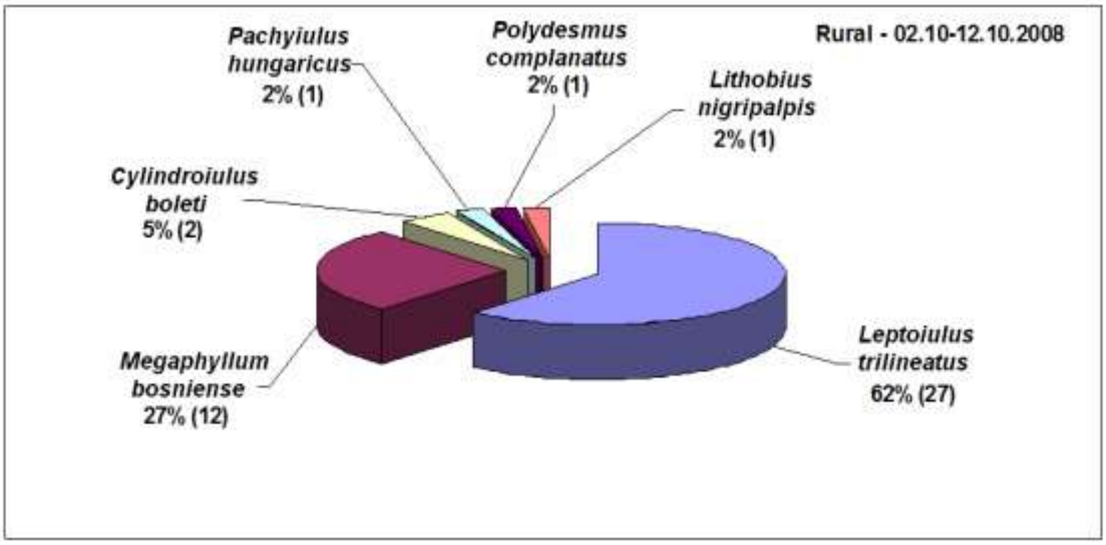
Figure 2. Number and proportion of species collected in the study at the two sites in the period 26 May – June 5, 2008 (Explanations: A: in Urban, B: in Rural; % – relative share of species of the total catch; in brackets – number of individuals of this species).

In the natural habitat (R) the species' composition was richer, especially in the spring when the catches included 6 millipede species and 5 centipede species. At the urban site (U) during the same season only 2 species millipede and one centipede were caught. In both seasons the millipedes *L. trilineatus* (May-June – 57 individuals; October – 39) and *M. transsylvanicum* (May-June – 16; October – 29 individuals) prevailed in the urban area. At the reserve the dominant species were also two: *L. trilineatus* (May-June – 36; October – 27) and *M. bosniense* (May-June – 18; October – 12 individuals) (Fig. 2 and 3).

The surface diel activity of collected myriapods both, in spring and fall, is highest in the time interval from 22:00 to 04:00 (Fig. 4) – at which are collected in total 133 individuals (see Table 1 and Table 2).

A





B

Figure 3. Number and proportion of the species detected during the study at the two sites in the period 2 – 12.10.2008 (Explanations: A: in Urban, B: in Rural; % – relative share of species of the total catch; in brackets – number of individuals of this species).

The measured values of the air and soil temperature in both sites during season show much greater stability and similar diel dynamics. The same is true for the indicators of soil and air humidity, except for the fall period when the humidity in the suburban site showed some increase in values during the twilight and the dark hours of the day – form 6 pm to 8 am. According to the Meteorological Station in Shumen, in the period May – June, sunrise occurred

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at the time interval from 05:41 am to 05:36 am, and sunset – in the range from 20:54 pm to 20:47 pm, and in October from 07:10 am to 7:22 am and from 6:53 pm to 6:36 pm respectively.

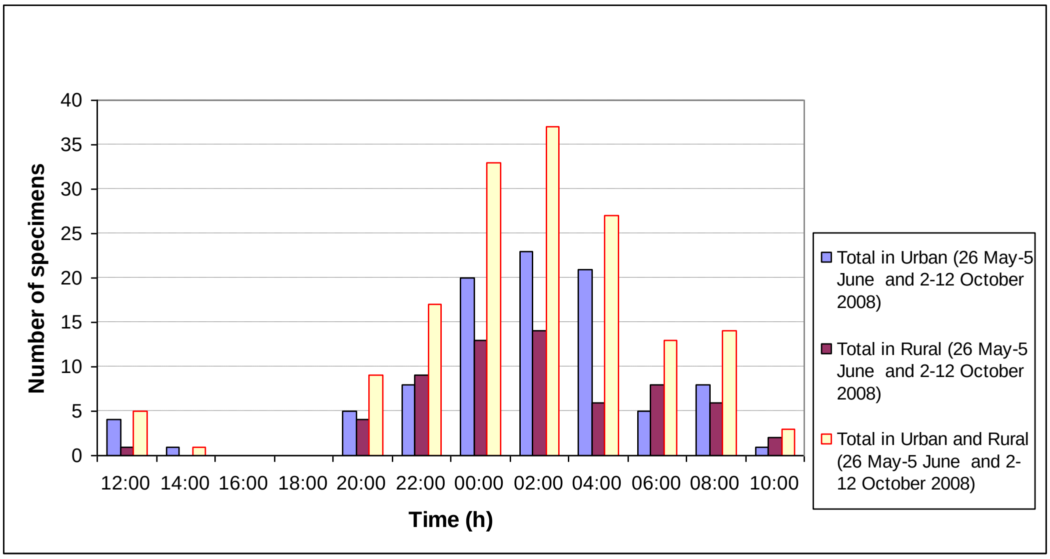

Figure 4. Common surface diel activity of myriapods (Diplopoda) at both study sites.

The dependence of the activity of the identified species on the various types of environmental factors was examined using variance analysis, the results of which are summarized in Table 3.

Table 3. Analysis of the variation (ANOVA) of the millipedes activity against the tested factors. (Explanations: SS – the sum of squires of the differences of observed values and the corresponding averages; df – degrees of freedom; MS – the variances (the sum of squires divided by the degrees of freedom); F – fraction of the variance between groups and the variance within groups; P-value – the tail probability that corresponds to F; F crit – the 0.95 quantile of the F – distribution with degrees of freedom (df1, df2);.

Factor	**ANOVA**						
	Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Time of day	Between Groups	255.3014	11	23,2092	17.5736	6.35E-25	1.8315
	Within Groups	295.8342	224	1,32069			
	Total	551.1356	235				
Season	Between Groups	5.1907	1	5,1907	2.228	0.1369	3.8815
	Within Groups	545.161	234	2,3297			
	Total	550.3517	235				
Soil Humidity	Between Groups	20.1135	6	3,35225	1.447774	0.197357	2.138314
	Within Groups	530.2382	229	2,315451			
	Total	550.3517	235				
Air Humidity	Between Groups	21.47907	8	2,684884	1.152392	0.329353	1.979345
	Within Groups	528.8726	227	2,329835			
	Total	550.3517	235				

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Air Tempe-rature	Between Groups	73.8060	3	24,6020	11.9772	0.0000255	2.6435
	Within Groups	476.5457	232	2,0541			
	Total	550.3517	235				
Soil Tempe-rature	Between Groups	9.8396	3	3,2799	3.2468	0.0218	2.6238
	Within Groups	477.8249	473	1,0102			
	Total	487.6646	476				

The factor "hour" (*P-value* = 6.35 E-25) has the greatest and statistically the most significant impact on the daily activity of the individuals. To analyze the shape of the millipedes' activity dependence on the hour, a polynomial regression has been used (Fig. 5).

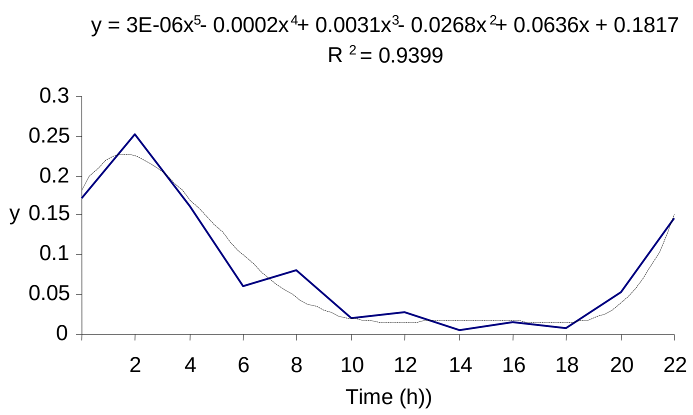

Figure 5. Empirical (solid line) and valued (with a dotted line) the relative frequencies of the individuals caught by hours.

The coefficient $R^2 = 0.9399$ indicates that 93.99% of the variation in relative frequencies is due to the regression. The remaining 6.01% are due to factors not included in the model. The highest activity rate of the individuals at night is between 10:00 pm and 4:00 am. Pearson's normed correlation coefficient. See Afifi and Aysen (1982). Is in this case 0.72. It shows again the big dependence of daily activity on the hours.

Table 4.mIntermediate and final results of the test used to verify or deny the hypotheses about the importance of the activity of individuals on the location of the site (Explanations: Mean – mean number of individuals caught per two hours; Known Variance – the variance of the number of individuals caught per two hours; Observations – the number of the observed couples of hours;

Hypothesized Mean Difference – description of the tested hypothesis; z – the Z-test statistic; P-value one-tail – the area under the standard normal curve after z; z Critical one-tail – the 0.95 quantile of the standard normal distribution; P-value two-tail – the area under the standard normal curve after z and before -z; z Critical two-tail – the 0.975 quantile of the standard normal distribution).

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z-Test: Two Sample for Means		
	The Park of Shumen University	***The Bukaka Nature Reserve***
Mean	0.6125	0.5021
Known Variance	0.9992	1.0236
Observations	240	237
Hypothesized Mean Difference	0	
z	**1.1986**	
P-value one-tail	0.1153	
z Critical one-tail	1.6449	
P-value two-tail	0.2307	
z Critical two-tail	**1.96**	

When compared in the two sites which differ in their degree of anthropogenic impact, no significant differences were found. In the University park Pearson's normed correlation coefficient was 0.67 and in the Bukaka Reserve (R) – 0.55, confirming that the terrestrial round-the-clock activity of the Myriapoda is statistically significantly dependent on the degree of illumination of the ground during certain hours of the day and also that it does not depend on the site location. The latter assertion is supported by the current review of hypotheses of equality between the means of two general sets normally distributed with unknown variances. In the quantile value of the error of the empirical data – z = 1.1986, lower than that of z Critical two-tail = 1.96, the species at the site has no statistically significant influence on the activity of the identified species (Table 4).

In both areas the hourly distribution of the activity of the individuals was identical, seen from χ^2-the homogeneity criteria, which in this case is equal to 14.1751 and is less than the constant $C_\alpha = 18.3$ (C_α is equal to 1-α quintile of χ^2 (k-1)). Similarly, using the criterion of Kolmogorov, the maximum difference between the empirical distribution functions is 0.4251. The theoretical description is 1.37 (with confidence level $P < 0.05$), which again confirms that the terrestrial distribution of the daily activity of individuals by hours is identical in both sites (Fig. 6 and 7).

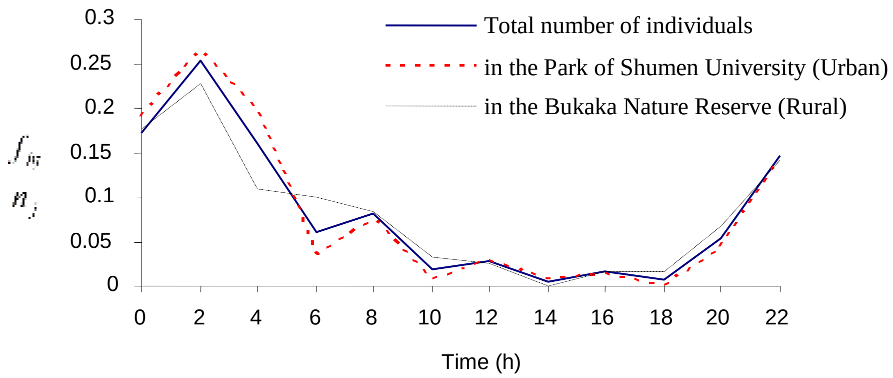

Figure 6. Empirical densities of distribution of individuals caught in both sites by the hour (Explanations: *j* = 1 for all observation, *j* = 2 for Urban, *j* = 3 for Rural; f_{hj} – the number of observations in the h-th group of *j*, n_j – the total number of individuals in *j*).

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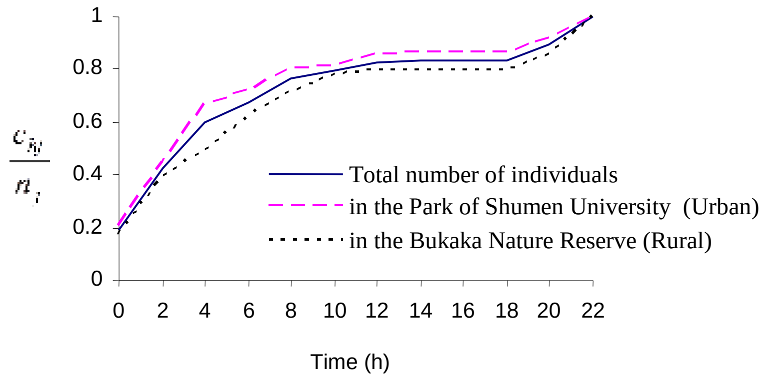

Figure 7. Empirical distribution function of the individuals caught in both sites by the hour (Explanations: j = 1 for all observation, j = 2 for Urban, j = 3 for Rural. c_{hj} – the number of observations in the groups 1, 2, …, h of j, n_j – the total number of individuals in j).

To a large extent the character of the surface diel activity of Myriapoda is due to the three species occurring most frequently in both work sites – *L. trilineatus*, *M. transsylvanicum*, and *M. bosniense*. To determine the dependence of the activity of the most ubiquitous species – *L. trilineatus* (R^2 = 0.9489) on the different hours in a day, the criterion χ^2 and Kolmogorov criterion of identity of two samples have been used. Their values (χ^2 = 12.26 and 0.17 for the criterion of Kolmogorov) show similar dynamics in the activity of *L. trilineatus* by hours at both sites (Fig. 8 and 9).

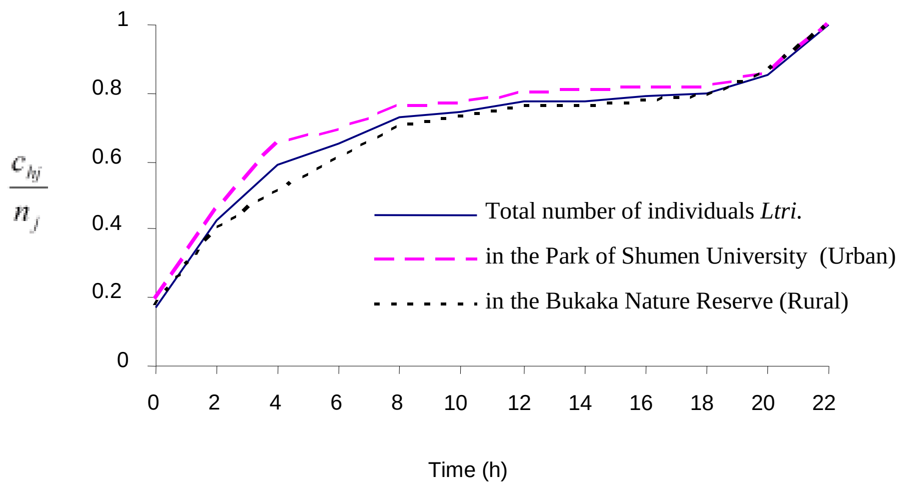

Figure 8. Empirical distribution function of the caught individuals *Leptoiulus trilineatus* (*Ltri*) by the hour at both sites (Explanations: j = 1 for all observation, j = 2 for Urban, j = 3 for Rural; c_{hj} – the number of observations in the groups 1, 2, …, h of j, n_j – the total number of individuals in j).

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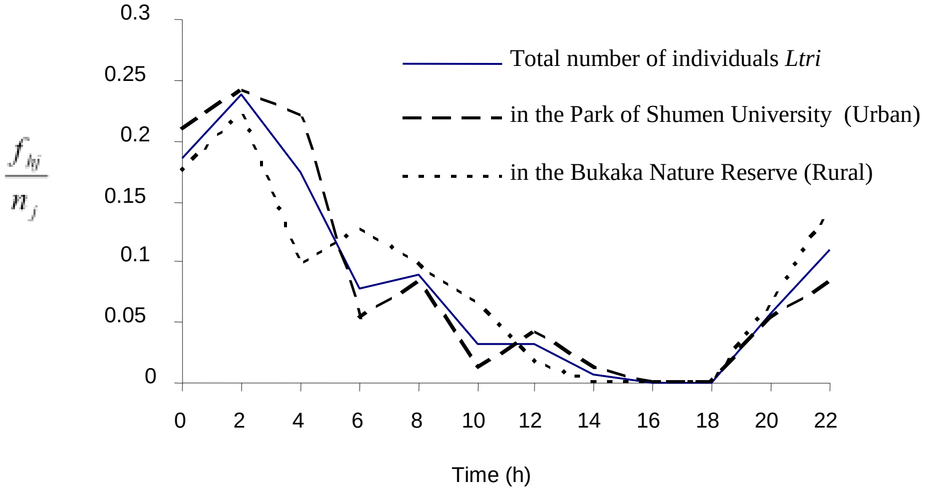

Figure 9. Empirical density functions of distribution of the caught individuals *Leptoiulus trilineatus* (*Ltri*) by the hour at both sites (Explanations: *j* = 1 for all observation, *j* = 2 for Urban, *j* = 3 for Rural; f_{hj} – the number of observations in the *h*-th group of *j*, n_j – the total number of individuals in *j*).

Likewise the round-the-clock activity of *M. transsylvanicum* and *M. bosniense* have been analyzed. The value χ^2 is 11.60 and the maximum difference between the empirical functions of distribution is 0.24. There are some differences in the nature of the ground activity of *M. transsylvanicum* and *M. bosniense*. For example, the activity period of *M. transsylvanicum* is short and very clear in the time interval from 8:00 pm to 6:00 am, while that of *M. bosniense* is longer and includes the interval from 6:00 pm to 8:00 am, which indicates that the species exhibits some activity during the daylight hours (Figs. 10 and 11). This could be explained by the higher storey of the forest reserve, compared to that in the urban areas, which determines the lower level of illumination of the ground and creates twilight in the early hours of the day.

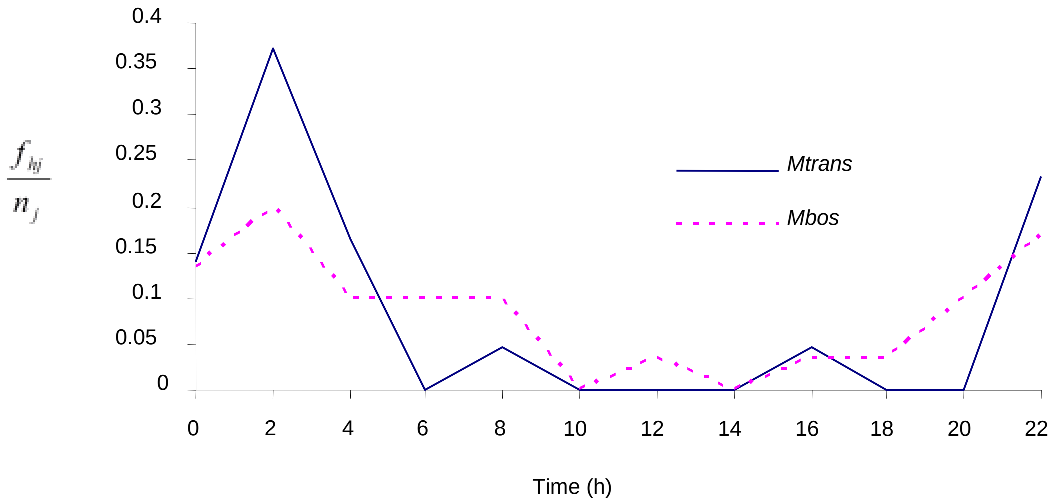

Figure 10. Empirical densities of distribution of the captured individuals *Megaphyllum transsylvanicum* (*Mtrans*) and *Megaphyllum bosniense* (*Mbos*) by the hour at both sites

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(Explanations: *j* = 1 for *Mtrans*, *j* = 2 for *Mbos*; f_{hj} – the number of observations in the *h*-th group of *j*, n_j – the total number of individuals in *j*).

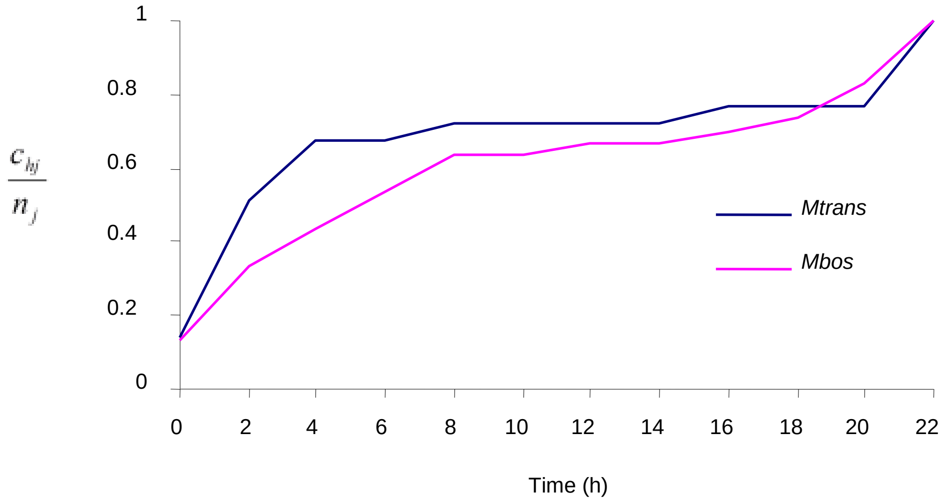

Figure 11. Empirical distribution function of the caught individuals *Megaphyllum transsylvanicum* (*Mtrans*) and *Megaphyllum bosniense* (*Mbos*) the hour at both sites (Explanations: *j* = 1 for *Mtrans*, *j* = 2 for *Mbos*; c_{hj} – the number of observations in the groups 1, 2, …, *h* of *j*, n_j – the total number of individuals in *j*).

The air and soil temperature (*P-value* = 0.0000255 and *P-value* = 0.0218) (Table 5) have statistically significant impact on the activity of the species. This has also been confirmed by Pearson's normalized correlation coefficients with values 0.4567 for air temperature and 0.31 for soil temperature. The dependence of the round-the-clock activity *Y* (measured in percentage of individuals caught during a certain hour) on the air temperature *X*1 and the soil temperature *X*2 has been modelled using multiple linear regression. See Afifi and Aysen (1982).

When using normalized values

$$\tilde{Y}_i = \frac{Y_i - \overline{Y}_n}{S_Y},\ \tilde{X}1_i = \frac{X1_i - \overline{X}1_n}{S_{X1}} \text{ and } \tilde{X}2_i = \frac{X2_i - \overline{X}2_n}{S_{X2}},$$

the analytic presentation of the regression line is $\tilde{Y} = \beta_0 + \beta_1\tilde{X}1 + \beta_2\tilde{X}1\tilde{X}2 + \beta_3\tilde{X}2$,

where β_i (i = 0, 1, 2, 3) are unknown parameters.

Table 5 contains the estimates of the coefficients in the regression equation (Column 2), together with their standard errors (column 3) and confidence intervals (columns 6 and 7).

Table 5. Values, statistical significance and confidence intervals of the regression coefficients in the present model (Explanations: *Coefficients* – the statistical estimation of the coefficient; *Std. Error* – the standard error of the coefficient; *t Stat* – the *t*-test statistic for testing hypothesis for statistically significance; *P-value* – the area under the *t*-curve after *t* and before *-t; Lower 95%* – the lower bound of the 0.95-confidence interval of the

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corresponding coefficient; *Upper 95%* –the upper bound of the 0.95-confidence interval of the corresponding coefficient).

Initial coefficients	***Coefficients***	***Std. Error***	***t Stat***	***P-value***	***Lower 95%***	***Upper 95%***
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Intercept (β_0)	0					
Average air temperature(β_1)	-0.73048	0.080429	-9.08224	8.62E-16	-0.88948	-0.57147
Joint influence of both factors (β_2)	0.304163	0.080403	3.782997	0.000228	0.145212	0.463114
Average soil temperature (β_3)	-0.09718	0.053309	-1.82291	0.070436	-0.20257	0.008211

In our case the evaluation of the multiple regression equation has the form:

$$\tilde{Y} = -0.73048\tilde{X}1 - 0.304163\tilde{X}1\tilde{X}2 + 0.09718\tilde{X}2$$

Before testing the adequacy of the model and the statistical significance of the coefficients in the equation of the regression, the conditions under which this model is good have been examined, namely: are there any occasional residues, are they equally distributed, do they have equal variances, and are they uncorrelated. The results of those checks show that the models are adequate.

How well the new model reproduces the empirical data is illustrated by the diagrams in Fig. 12-14, reflecting the relationship between the normalized average air and soil temperature and the number of individuals caught.

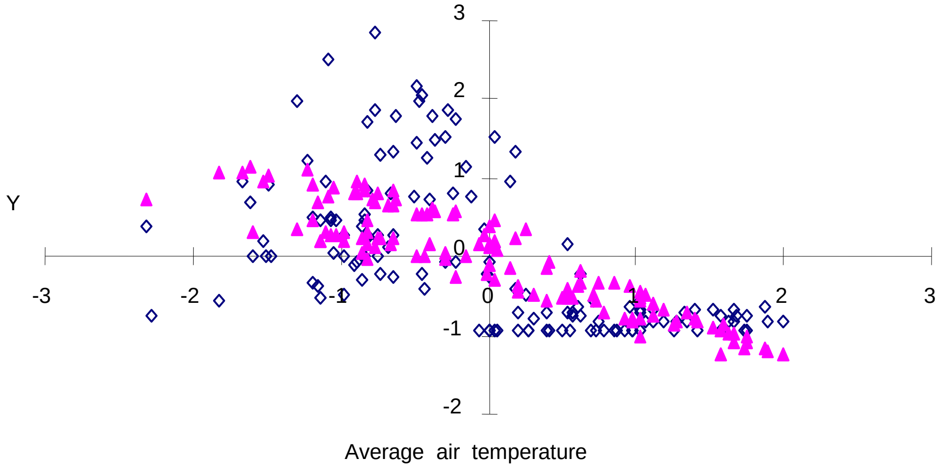

Figure 12. Diagram of the dependence of the normalized average air temperature ($\tilde{X}_1$) and the normalized number of individuals caught (Y) (Explanations: The empirical values are depicted with circles and the values resulting from the regression equation are shown with solid triangles).

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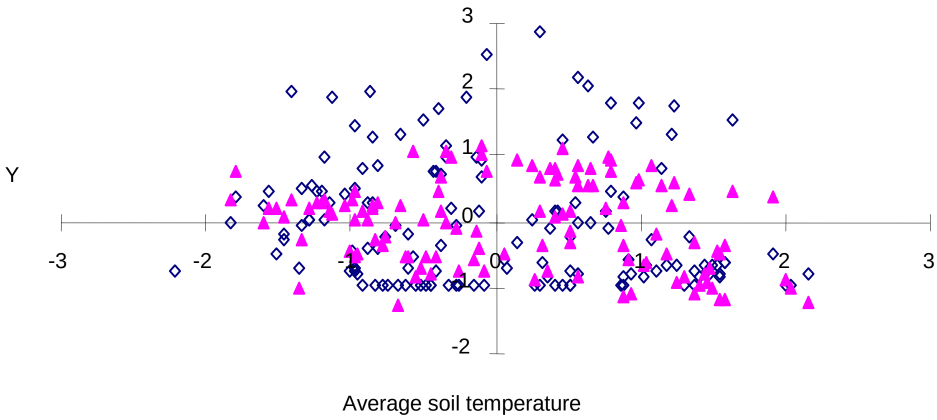

Figure 13. Diagram of the dependence of the normalized average soil temperature $(\tilde{X}_2)$ and the normalized number of individuals caught (Y) (Explanations: The empirical values are depicted with circles and the values resulting from the regression equation are shown with solid triangles).

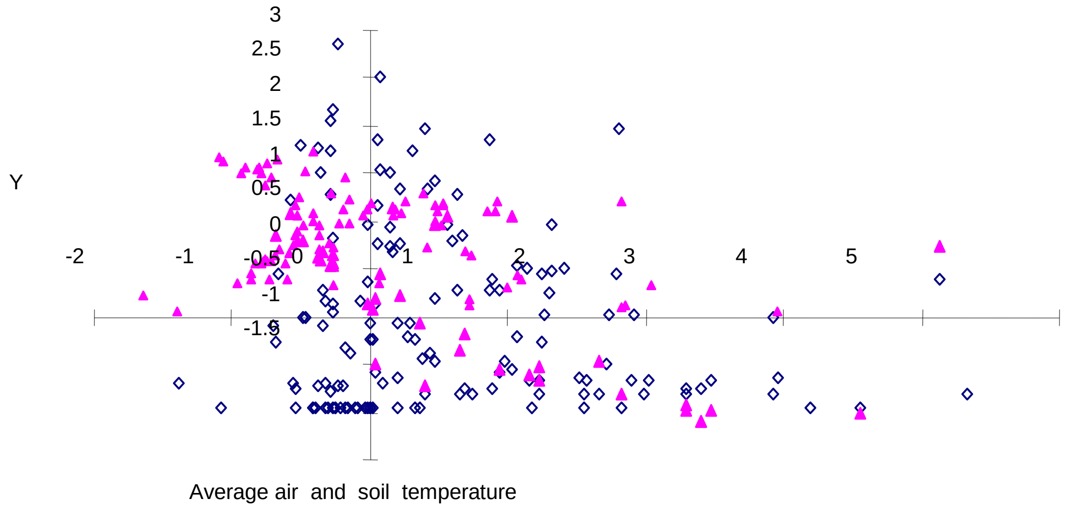

Figure 14. Diagram of the dependence of the normalized number of individuals caught (Y) on the simultaneous action of the temperatures of air and soil $(\tilde{X}_1\tilde{X}_2)$. (Explanations: The empirical values are depicted with circles and the values resulting from the regression equation are shown with solid triangles).

The empirical and the theoretical values of F indicate that the model is adequate (Table 6) and the determination coefficient R^2 is statistically significant (Table 7), meaning 39.37% of the variance of *Y* are due to the regression of the factors included in the model, while the remaining 60.63% are due to the variables that have not been included in the model. Column (5) of Table 5 has been used to check the statistical significance of the coefficients in the regression equation.

DISCUSSION

The results clearly show that the diel surface activity of the established species is related to the degree of illumination (number of hours in a day), the air and the soil temperature (Fig. 15), and it does not depend on the soil and air humidity, the season of study (see Table 3) or the degree of urbanization of the site (see Table 4). Comparing the values of Pearson's normalized correlation coefficient shows that the dependence on the hour (0.7147) is the strongest, followed by air temperature (0.4567) and soil temperature (0.31). According to the sample, the other factors were not statistically significant for the diel activity of the species identified in the surveyed sites. The surface activity is likely to have endogenous nature and it is controlled by internal physiological factors, but its duration may vary according to the changes in the environmental conditions. Baker (1979) conducted a study on the surface activity of the introduced millipede *Ommatoiulus moreletii* in grassland and woodland habitats in Australia. He found out that the weekly fluctuations in the activity of the species are related to the changes in temperature and moisture. The same author also stated that under moist conditions, the activity depended on the temperature alone. In another study Baker (1984) ascertained that females of the same species are more active on the ground during daylight while males are much more active in the night hours. Field observations and laboratory investigations carried out by Toye (1966) on three species of African millipedes clearly show that temperature is an important environmental factor affecting the locomotor activity of the millipedes. After numerous laboratory experiments the author established the preferred temperature ranges for the three species and analysed their daily activity in exposed and hidden microhabitats.

Table 6. Results of the variance analysis used to verify the adequacy of the regression model (Explanations: *df* – degrees of freedom; *SS* – the sum of squires of the differences of observed values and the corresponding averages; *MS* – the variances (the sum of squires divided by the degrees of freedom); *F* – fraction of the variance between groups and the variance within groups; *Significance F (P-value)* – the tail probability that corresponds to F.

ANOVA					
	df	***SS***	***MS***	***F***	***Significance F***
Regression	3	56.29854	18.76618	30.51888	3.04E-15
Residual	141	86.70146	0.614904		
Total	144	143			

Table 7. Characteristics of the correlation coefficient (R) (Explanations: *Multiple R* – multiple coefficient of correlation between the observed variables; *R Square* – determination coefficient (R^2); *Adjusted R Square* – standardized determination coefficient; *Std. Error* – the standard error of the coefficient of correlation; *Observations* – the number of observations included in the regression analysis).

Regression Statistics	
Multiple R	0.6275
R Square	0.3937
Adjusted R Square	0.3780
Std. Error	0.7842
Observations	144

The study of the diurnal activity conducted in urban and natural habitats, confirmed the nocturnal activity of the established species, most pronounced in the time interval from 10:00

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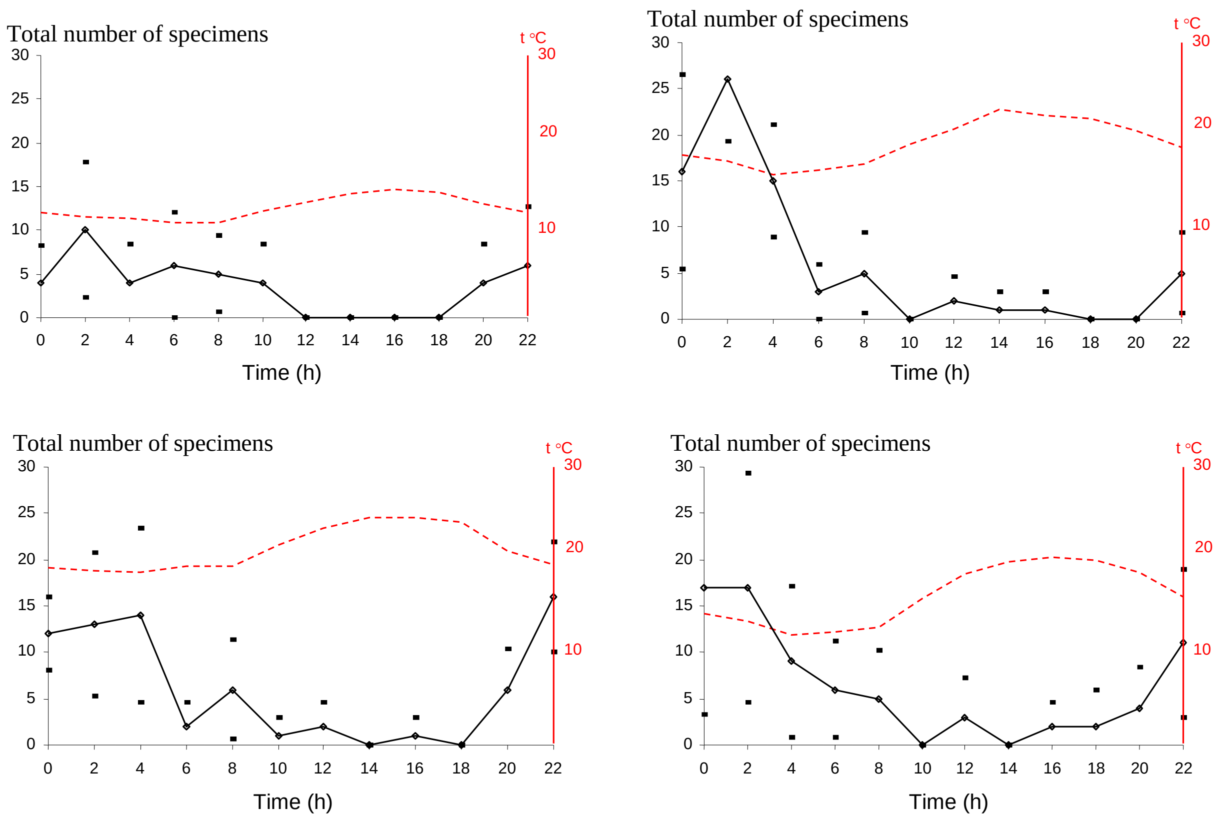
pm to 4:00 am (Fig. 4). This corresponds well with the findings on other myriapods [16, 17, 20]. The dynamics of the light regime at different times of the day statistically has the most significant impact on the diel activity of *L. trilineatus*, *M. transsylvanicum*, and *M. bosniense*. Pearson's normalized correlation coefficient in both sites is very close in value (0.67 in the park of the University of Shumen and 0.55 in reserve Bukaka), hence, the surface diel activity of millipedes depends significantly on the degree of illumination of the earth surface, but it is not affected by the location and the degree of urbanization of the site. This is confirmed by the values of the χ^2 -uniformity criterion and Kolmogorov's criterion under which the distribution of the diel activity of individuals by hour is identical at both sampling sites. The soil and air temperature (*P-value* = 0.0000255 and *P-value* = 0.0218, respectively) are also statistically significant for the diel surface activity of the identified species. Pearson's normalized correlation coefficient amounts to 0.4567 (air temperature) and 0.31 (soil temperature).

1. a) 1. b)



2. a) 2. b)

Figure 15. The dynamics of the diel activity of the individuals in the Urban – Shumen University Park (1) and the Rural – Reserve Bukaka (2) during the spring (a) and the fall (b) seasons (Explanations: The black solid line shows the activity and the dotted line – the average air temperature during the hours of observations. The points are the corresponding 0.95 confidence interval).

The empirical evidence suggests that the nature of the ground activity of *M. transsylvanicum*, which was found only in the park of the University of Shumen, and which was absent in the reserve Bukaka, differs from that of *M. bosniense*, which is absent in the urban area. *M. transsylvanicum* is characterized by shorter activity period in the time interval between 8:00

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pm and 6:00 am, while *M. bosniense*'s ground activity period is longer in the range 6:00 pm - 8:00 am. The latter also exhibits some, although weak, activity during the daylight hours.

The season selected for conducting the survey period, i. e. 26 May – 5 June and October 2 – 12, which coincide with the spring and fall peaks in the seasonal activity of the species, has no statistically significant impact on the activity of the diplopod species. This is valid for *L. trilineatus*, for which the *P-value* = 0.0753, as well as for the other two species – *M. transsylvanicum* (*P-value* = 0.0806) and *M. bosniense* (*P-value*=0.2767). The soil humidity (*P-value* = 0.1973) and air humidity (*P-value* = 0.3293) also do not have statistically significant impact on the round-the-clock activity of Diplopoda in the condition under which the study was conducted. This is probably due to the insignificant differences in the values of these indicators during the duration of the experiment.

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