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Data on Some Parasitic and Semi-parasitic Plant Species from Serpentines of Kosovo

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Abstract. From the richness of the vascular flora of Kosovo, the flowering plants predominate. Within this group of plants, most of them are typical autotrophic plants. In contrast, a very small group of plants consists of parasitic and semi-parasitic life. Semi-parasitic plants are able to perform the process of photosynthesis throughout their life cycle and mainly take up water and mineral salts dissolved by the host plant. In our study, we focus on the presence of certain confirmed parasitic and semi-parasitic plant species in the serpentines of Kosovo, with additional evidence of their occurrence in other parts of the country as well. Here, as a result of intensive two-year field work, we provide details on the presence of the following four species, two of them semi-parasitic: *Arceuthobium oxycedri* (DC.) M. Bieb. and *Loranthus europaeus* Jacq., and two parasitic: *Lathraea squamaria* L. and *Orobanche alba* Willd. For each species, the host plant(s), status as parasitic or semi-parasitic, and data on distribution in the region studied are given, along with a broad discussion of distribution at the state level.

Key words: natural ecosystems, serpentine flora, parasitic plants, Kosovo.

Introduction

Kosovo is located in the central part of the Balkan Peninsula. Its geographical location is defined as the country of Western Balkans, SE Europe (Pllana, 2015). Based on the geological data of Kosovo (Korolija et al., 1976), all the serpentine areas of the country (e.g. the regions of Brezovica, Gjakova, Golesh, Koznicë, Gubavc, Strofc, Rahovec, etc.) belong to the Jurassic ophiolitic complexes, which makes them characterized by their diversity and specific flora. Within the territory of Kosovo, based on its geomorphology, there is a considerable area of serpentine substrates (Pavičevič et al., 1974). Serpentine substrates of Kosovo represent dry and rather slightly warmed soils with additional alkaline reaction. They

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are particularly poor in potassium and sodium content, and deficient in nitrates, phosphates, chlorides and sulfates. These soils are also poor in terms of water capacity, although there are some exceptions. In terms of altitude and horizontal extent, serpentine substrates in Kosovo are present within mountain massifs, ranging in altitude from about 300 - 2000 m. Based on various studies conducted by other authors (Słomka et al., 2016) in these substrates have been shown to be habitats with high floristic diversity and with increased presence of endemic plant taxa (Rexhepi, 1979; Millaku et al., 2008; Millaku, 2013; Berisha et al., 2014; Krasniqi et al., 2015; 2019; Prodanović et al., 2020) and very diverse and interesting vegetation composition (Rexhepi, 1994; Krasniqi & Millaku, 2007; Millaku et al., 2011; 2017; Shuka et al., 2012; 2020; Stevanovic et al., 2003; Shuka, 2008).

On the territory of the Republic of Kosovo there are significant areas covered with serpentine substrate. They are located in the northern part of Kosovo (Kopaonik, the banks of the river Ibër), Malësia e Gjakovës (sector-Qafë e Morinë-Qafë e Prush), Badovc (near the capital Prishtina), Gurana (near Hani i Elezit), Golesh Mt., Sharr Mts. (Peak of Pashallarëve, or Peak of Ostrovica), Mushtisht district, some small fragments in Albanian Alps of Kosovo ("Bjeshkët e Nemuna" National Park), Koznik Mountains (Rahovec and Mirusha sector), Koznica, some fragments in Drenica Mt., (Llapushnik-Carralevë sector) as well as some other smaller fragments (Fig. 1).

Kosovo is characterized by a typical continental climate, with an annual average temperature of 10ºC and an average annual rainfall of 784.7 mm (Çavolli, 1997; Pllana, 2015). In terms of flora and vegetation, the serpentine substrates of Kosovo are covered with forests, shrubs, pastures and dry rocky grasslands with a rather rich floristic diversity. Deciduous forests and shrub formations are dominated by oaks and above 800 m by beech forests. The typical grasslands are dry due to the serpentine substrate characteristics. The pastures and rocky grasslands are rich in plant species, some of which are characteristic of serpentine substrates.

Although the influence of the anthropogenic factor is evident, there are also a considerable number of endemic plant taxa in these areas and those that are also protected by law and evaluated accordingly, in accordance with IUCN rules and criteria (Millaku, 2013). Within the richness of the vascular flora of these substrates, there are also some parasitic and semi-parasitic species, confirmed both during field surveys and from literature sources, which are the subject of study in this paper. In particular, we studied the parasitic species during field observations in 2018-2020. Here we will present the results of two semi-parasitic plant species from the family *Loranthaceae* [*Arceuthobium oxycedri* (DC.) M. Bieb. and *Loranthus europaeus* Jacq.] and those of two parasitic plant species from the family *Orobanchaceae* [*Lathraea squamaria* L. and *Orobanche alba* Willd].

Material and Methods

The floristic material was collected during research conducted mainly in the period 2018-2020. In order to expand the knowledge about parasitic and semi-parasitic plant species in serpentine substrates of Kosovo, research expeditions were carried out in other areas with serpentine substrates in Kosovo. For this purpose, about 56 research expeditions were conducted during these two years. All studied plant taxa were photographed, data on habitat types, floristic composition and plant communities, threats and human impacts were recorded. Samples were dried and herbarised according to known standards (Bridson & Forman, 1998) and their habitat and GPS data collected. For the parasitic and semi-parasitic plant species, their presence was confirmed in some other serpentine localities of Kosovo.

For taxonomic identification of plant species, we relied on the Flora Europaea volumes (Tutin et al., 1964; 1972), and in addition, local and regional floras and taxonomic keys were also consulted for certain taxa (Pajazitaj, 2017; Qosja et al., 1996; Paparisto et al., 1988; Demiri, 1983; Josifovic et al., 1970 - 1977). The taxa nomenclature was updated accordingly, based on the Euro-Med Plant Database (Euro+Med, 2006-2021).

Results and Discussion

As part of our study, mainly in the period 2018-2020, we conducted research expeditions in the serpentines of Malësia e Gjakovës, specifically in the sector Qafë e Morinës-Qafë e Prushit (on the territory of Kosovo). This research has led to the identification and documentation of plant species of the vascular flora, including parasitic and semi-parasitic species. The parasitic and semi-parasitic plant species enrich the floristic diversity of the space studied in this case, but also the flora and vegetation of Kosovo in general. From parasitic and semi-parasitic plants we present in this paper the results for these species: *Arceuthobium oxycedri* (DC.) M. Bieb., *Loranthus europaeus* Jacq., *Lathraea squamaria* L. and *Orobanche alba* Willd. The presence of these plant species has been confirmed in some additional serpentine localities of Kosovo (Table 1).

Fam. Loranthaceae Juss.

Arceuthobium oxycedri (DC.) M. Bieb.

Small shrub up to 20 cm, green to yellow. Articulated, often dichotomous stem. Semiparasitic plant, usually on *Prickly Juniper* (*Juniperus oxycedrus*). I-VII. Figure 2 – 1. (Pajazitaj, 2017; Tutin et al., 1964). From the syntaxonomic point of view, the species was recorded on plant communities belonging to the Association: *Astero-Juniperetum oxycedri* Rexhepi 1990, respectivelly within the Alliance: *Pruno tenellae-Syringion* Jov 1979, Order: *Quercetalia pubescentis* Br. Bl. 1932 and Class: *Querco-Fagetea* Br. Bl. et Vlieger 1937. (Rexhepi, 1994).

Table 1. Some parasitic and semi-parasitic plant species in the serpentine terrains of the Republic of Kosovo.

Nr.	Family / Plant species	Habitat	Host plants	Parasitic / Semi-parasitic	Locality
	Loranthaceae				
1.	*Arceuthobium oxycedri* (DC.) M. Bieb.	Rocky habitats, nearby the bushes	*Juniperus oxycedrus* L.	Semi-parasitic	Malësia e Gjakovës, Rajoni i Mirushës, Mali Drenicë, Zatriq-Koznik, Mushtisht
2.	*Loranthus europaeus* Jacq.	Oak forests	*Quercus petraea* (Matt.) Liebl.	Semi-parasitic	Malësia e Gjakovës
	Orobanchaceae				
3.	*Lathraea squamaria* Jacq.	Beech forest	*Fagus sylvatica* L.	Parasitic	Malësia e Gjakovës Zatriq-Koznik, Rajoni i Mirushës, Mali Drenicë
4.	*Orobanche alba* Wild.	Grasslands and rocky places	*Lamiaceae (Thymus sp.)*	Parasitic	Malësia e Gjakovës, Guriq, Golesh

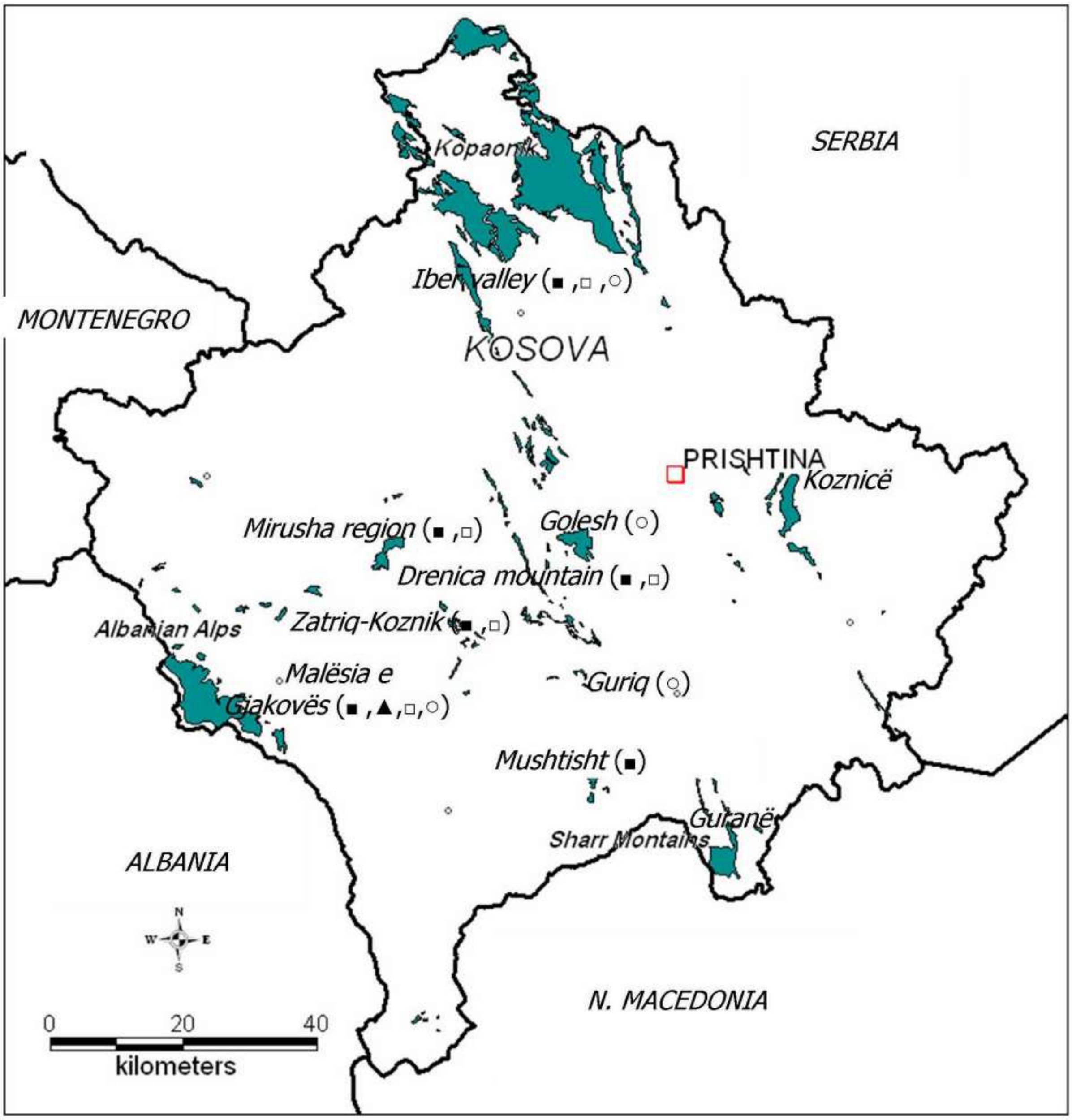

Fig. 1. Plant species localities on the map of Kosovo serpentines. Serpentine areas (colored in green). *Arceuthobium oxycedri* (DC.) M. Bieb. [■], *Loranthus europaeus* Jacq. [▲], *Lathraea squamaria* Jacq. [□] and *Orobanche alba* Wild. [○].

Loranthus europaeus Jacq.

Small shrub, 5-15 (-50) cm, leaves obovate-oblong, obtuse, dull green, 1-5 cm. Stamen 4-6, embedded in the base of the petals. Semiparasitic plant on *Oak*, *Chestnut*, *Pine* etc. V-VI. Figure 2 – 2. (Pajazitaj, 2017; Tutin et al., 1964). From the syntaxonomic point of view, the species was recorded on plant communities belonging to the Association: *Erico-Quercetum petraeae serpentinicum* Rexhepi 1988, respectivelly within the Alliance: *Orno-Ostryon* Tomazic 1940, Order: *Erico-Pinetalia* Oberd. 1949 emend. Ht. 1959 and Class: *Erico-Pinetea* Ht. 1959 (Rexhepi, 1994).

Fig. 2. Habitus of four surveyed plant species. 1. *Arceuthobium oxycedri* (DC.) M. Bieb., 2. *Loranthus europaeus* Jacq., 3. *Lathraea squamaria* Jacq. and 4. *Orobanche alba* Wild.

Fam. Orobanchaceae Vent.

Lathraea squamaria L.

The common toothwort (*L. squamaria*) have been wrongly included in the *Scrophulariaceae* family by various authors (Tutin et al., 1972; Weber, 1976). Perennial plant, 10-25 cm, with fleshy steam, reddish to pink color. Flowers in dense unilateral clusters, with red upper lip, the lower lip is white. In moist forests, as parasites on beech and oak trunks. IV-V. Fig. 2 – 3. (Pajazitaj, 2017; Tutin et al., 1972). From the syntaxonomic point of view, the species was recorded on plant communities belonging to the Association: *Fagetum moesiacae montanum* Blec. et Lakusic 1970, respectivelly within the Alliance: *Fagion moesiacae* Blec. et Lakusic 1970, Order: *Fagetalia sylvaticae* Pawl. 1928 and Class: *Querco-Fagetea* Br. Bl. et Vlieger 1937. (Rexhepi, 1994).

Orobanche alba Wild.

The upper edge of the corona is full, lower lip glandular ciliate. The edges of the stamens at the base with dense hairs, 35-70 cm. The species parasites in the roots of *Lamiaceae* species. VI-VII. Figure 2 – 4. (Pajazitaj, 2017; Tutin et al., 1972). From the syntaxonomic point of view, the species was recorded on plant communities belonging to the Association: *Polygalo-Genistetum hassertianae* Blec. et al. (1969), respectivelly within the Alliance: *Centaureo-Bromion fibrosi* Blec. et al. 1969, Order: *Halacsyetalia sendtneri* H. Ritter-Studnicka 1970 and Class: *Festuco vaginatae* Soo'1968 emend. Vicherek 1972.

Conclusions

In the research that we conducted in the vascular flora of Malësia e Gjakovës (territories between Qafë Morinë to Qafë e

Prushit), as well as in some other serpentine substrates on the territory of the Republic of Kosovo, we identified parasitic and semi-parasitic plant species among many plant species. From 2018 until now, we have identified and documented 4 plant species from this group of plants, grouped in two families of vascular plants. For these plant species, their occurrence was confirmed in some other serpentine localities in Kosovo, such as in Mirusha Region, Drenica Mountain, Zatriq-Koznik, Guriq, Mushtisht and Golesh. From the family *Loranthaceae*, we identified the semi-parasitic species *A. oxycedrus* occurring on Prickly Juniper (*J. oxycedrus*) and *L. europaeus* occurring on Sessile Oak (*Q. petraea*). From the family *Orobanchaceae*, we identified the parasitic species *L. squamaria*, which parasitizes on beech trunks (*F. sylvatica*) and *Orobanche alba*, which parasitizes mainly on plant roots of species belonging to the family *Lamiaceae*. For each plant species, we have also provided its phytosociological data and vegetation classification. The presence of these plant species not only enriches the floristic diversity of the studied area, but also contributes to the general knowledge about the flora of Kosovo.

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Copulatory Behavior of the Egyptian Vulture (Neophron percnopterus) in the Eastern Rhodopes, Bulgaria

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Abstract. Copulatory behavior has been reported in many raptor species, possibly because their copulations are typically conspicuous and frequent. Vultures are single-brooded and long-lived birds in which pair bonds may be maintained from one year to the next. The copulatory behavior was studied in three out of the four vulture species that breed in Europe. However, a more detailed explanation of the exclusive copulatory behavior of the Egyptian vulture regarding the frequency of copulation is missing. Here we provide the first data from the Balkans on the different aspects of the copulatory behavior of the Egyptian vulture and compare it with previous studies on the species and other conspecifics. The study was carried out in the Eastern Rhodopes mountain, Bulgaria, the stronghold of the species in the Balkans. We observed 4 pairs from vantage points and 5 with trail cameras. We recorded 137 copulation attempts in total. The majority of the copulation attempts were successful (86%, n=68) with a mean duration of 17.6±11.8 sec. The daily pattern of copulation frequencies we found suggests a bimodal distribution. Regarding the seasonal pattern of copulation frequencies, two peaks were observed – at the beginning of the breeding season and before the laying. The presence of the male around the female increased with time and before the laying. In terms of conspicuousness, the number of copulation attempts increased with the linear distance from the nests of the observed pairs to the copulation site which was always visible and exposed.

Key words: Egyptian vulture, copulations, sperm competition, territory ownership signals, paternity assurance.

Introduction

The behavior of raptors during the breeding season is way more studied in comparison to any other period of their lives. The breeding cycle in the raptor species starts with the pre-laying period when birds mate, occupy a territory, build a nest, display and defend their territory, copulate actively (Newton, 1979). Copulatory behavior has been reported in many raptor species, possibly because their copulations are typically conspicuous and frequent

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(Negro & Grande, 2001). Vultures are single-brooded and long-lived birds in which pair bonds may be maintained from one year to the next (Cramp & Simmons, 1980). They exploit unpredictable trophic resources and thus need to spend a lot of time away from the nest foraging, which might affect the copulation behavior of the species (López-López, 2014). In three of the four vulture species that breed in Europe, the copulatory behavior was studied in terms of frequency, rate, pattern and conspicuousness (Negro & Grande, 2001). The Griffon vulture, a colonial species, differs in some main copulatory aspects compared to the Egyptian and the Bearded vultures, namely the low copulation frequencies and the lack of mate-guarding (Margalida & Bertran, 2010). In contrast, in both the Egyptian vulture and the Bearded vulture, a high frequency rate has been observed with an intensive presence of the male in the nest before the laying (Donázar et al., 1994; Bertran & Margalida, 1999). However, a more detailed explanation of the exclusive copulatory behavior of the Egyptian vulture regarding the different hypotheses about the frequency of copulation is missing. In this paper (1) we provide the first data from the Balkans on the different aspects of the copulatory behavior of the Egyptian vulture and compare it with previous studies on the species and other conspecifics, (2) explain the copulation patterns of the species in the context of the different hypothesis interpreting the frequent copulations.

Material snd Methods

The study was carried out in the Eastern Rhodopes mountain, Bulgaria which is the stronghold of the species in the Balkan peninsula (Velevski et al., 2015). The observations were carried out between 2008 and 2020. Four pairs were observed for 386 h during the pre-laying period from vantage points to detect copulations outside of the nest and in the breeding territory (Bibby et al., 1992). A breeding territory was defined as the territory actively defended by the pair and presumably it can't be fully covered from vantage points. However, this approach is considered relevant (Donázar et al., 1994). Observations were made in good weather conditions, in the majority of the cases lasted from early morning until dusk, and binoculars 10 x and 20 x 60 telescopes were used at a distance of at least 600 m away of the nest. This distance is considered appropriate for observation in order to avoid disturbance over the pair and eventually affect the behavior of the birds (Zuberogoitia et al., 2008). To detect copulations in the nest that are considered rare (Donázar et al., 1994), we monitored the other five pairs in their nests with mounted trail cameras (n = 5) for 2656 ± 104.83 h. Trail cameras were mounted at the beginning of March, before the return of the birds from Africa. Natural materials such as stones, leaves and branches were used as camouflage to prevent disturbance over the birds. The trail cameras were set to take 1 still image when activated by movement, with an interval of 30 seconds between shots. To avoid disturbance of the birds and to save battery voltage, trail cameras were not set to take a video and thus the duration of the detected copulations was not registered. Sex identification of the observed individuals was based on the slight difference in the pigmentation of the head at the beginning of the breeding season - more orange in most males and yellowish in the females (Cavallo et al., 1997). Furthermore, we also used the plumage patterns of individuals to identify the sex in a given year (Zuberogoitia et al., 2018). We recorded the time, duration (in sec), and location of copulations, the duration of the time when adults were present together or separately in the breeding territory (in mins). A copulation was considered successful when a cloacal contact was registered (Hunter et al., 1996). All others were considered unsuccessful. The data for the copulations were pooled together for all years. The daily pattern of copulation frequency was estimated as the number of copulation attempts divided by 60 for each time frame of 1 hour between 6 AM and 7 PM (copulation attempts per hour) for both the directly observed and those pairs monitored with trail cameras. In addition, to describe the seasonal variation in the copulation frequency, the number of copulations was combined in time in periods of 7 days dating backwards from egg-laying (day 0). Then, for each 7 days period, the copulation frequency was estimated as the number of copulation attempts divided by the total hours of observation. The clutch size in this species is between one and three eggs, and incubation starts as soon as the first egg is laid (Mendelssonhn & Leshem, 1993). In four of these pairs, the beginning of incubation was identified by direct observations and in the five remaining pairs using the trail camera shots. To account for the conspicuousness, the location of copulation sites were identified precisely, plotted and the distance to the nests was measured with Measure Tool in ArcGIS 10.2 (ESRI, 2014). We used descriptive statistics to describe copulations, One-way ANOVA with an LSD test to compare the duration of copulations between the different pairs and χ2 test to compare the daily pattern of copulation frequencies between directly observed pairs and pairs monitored with trail cameras in the nests. All test were performed in STATISTICA for Windows, Release 7.0 (StatSoft, 2004). The statistical significance was set at $p < 0.05$.

Results

We observed 79 copulation attempts for the four pairs observed from vantage points. Almost all copulation attempts were registered in the breeding territory (94%, n = 74) and the rest were detected in the nest (6%, n = 5). The majority of the copulation attempts were successful (86%, n = 68) with a mean duration of 17.6 ± 11.8 sec. A significant difference in the copulation duration was observed between two pairs (F = 2.31, p = 0.04). In the five pairs observed with trail cameras, 58 copulation attempts were registered. Nevertheless, except for 1 copulation attempt, the rest were observed in only one pair. The first recorded copulation occurred on day -25 before the laying of the first egg and copulation attempts continued until day -2. Nonetheless, copulations were observed until day +3 in the pairs observed with trail cameras, before laying the second egg. The pre-laying period lasted for 18.5 ±8 .3 days in all observed pairs. The daily pattern of copulation frequencies between day – 25 and day 0 suggests a bimodal distribution (Fig. 1).

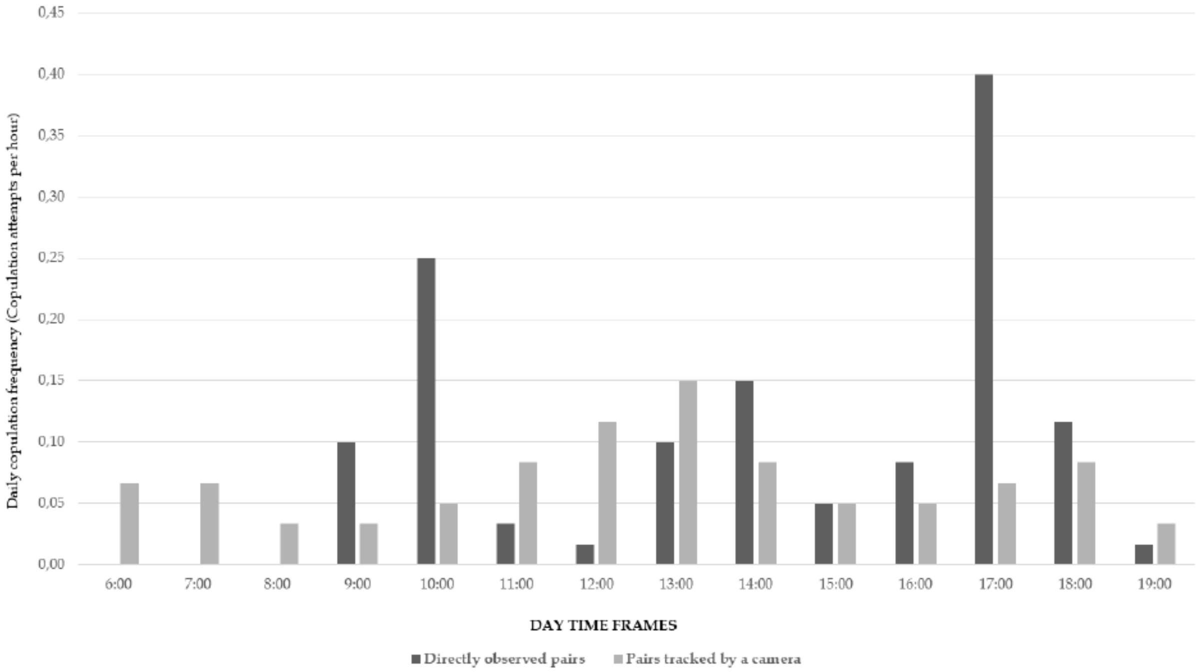

Fig. 1. The daily pattern of copulation frequencies of nine pairs of Egyptian vulture during the pre-laying period (day +3 to -25, n = 137 copulation attempts).

The copulation rate is high and two maximum peaks were observed just before noon (between 09:00 and 11:00), and in the late afternoon between 17:00 and 18:00 in the pairs observed from vantage points. We found a significant difference in the daily pattern of copulation frequencies between pairs observed from vantage points and pairs observed with trail cameras ($\chi 2$, $p < 0.05$).

During the pre-laying period, 125 copulation attempts were registered. Another 12 copulation attempts were registered between the laying of the first and the second egg. The copulation pattern of the observed pairs during the period suggest 2 peaks between days -1 – -7 and days -15 – -21 with values of 0.44 (n = 29.41 hours of observation) and 0.57 attempts hr-' (n = 82.16 hours of observation) accordingly. In contrast to the pairs observed from vantage points, the trail cameras detected copulations with high frequency at day 0, in the hours after laying of the first egg, and day +3, both beyond the fertile period. As a sequence, the frequency obtained during the post-laying period was 0.077 attempts hr-'(n = 26 hours of observation) (Fig. 2).

Mate attendance increased with time and was intensive prior to the laying. We didn't observe the females left unattended by their partners between day -10 and day 0. In terms of conspicuousness, the number of copulation attempts increased with the growth of the linear distance from the nests of the observed pairs. In addition, all observed pairs had concrete preferences towards the sites for copulation, choosing the same spot in most of the cases. Also, all sites were on top of a cliff or a hill and conspicuous (Fig.3).

Discussion

The Egyptian vulture is a territorial bird that starts copulating very soon after arrival in the breeding territory and like other raptor species copulations are frequent over extended period of time (Negro et al., 1992). Our research confirmed the results of previous studies on the species' copulatory behavior and found that the proportion of successful copulations is 86% and the rate of copulations is high (n = 137) (Donázar et al., 1994).

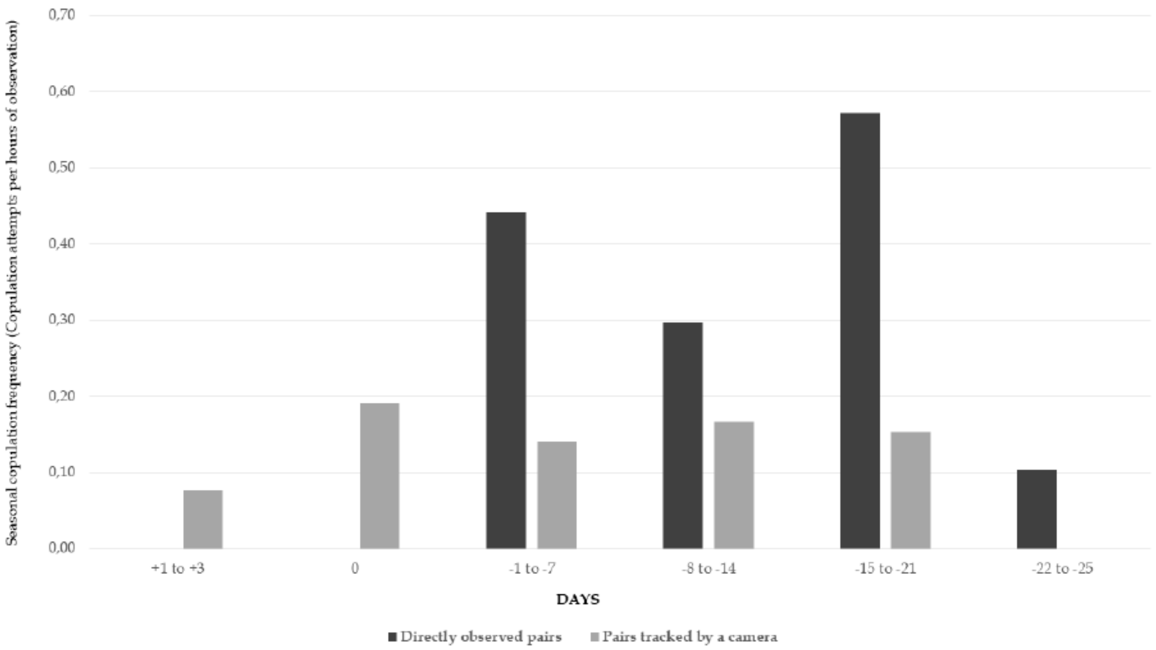

Fig. 2. Seasonal pattern of copulation frequencies of nine pairs of Egyptian vulture during the pre-laying period.

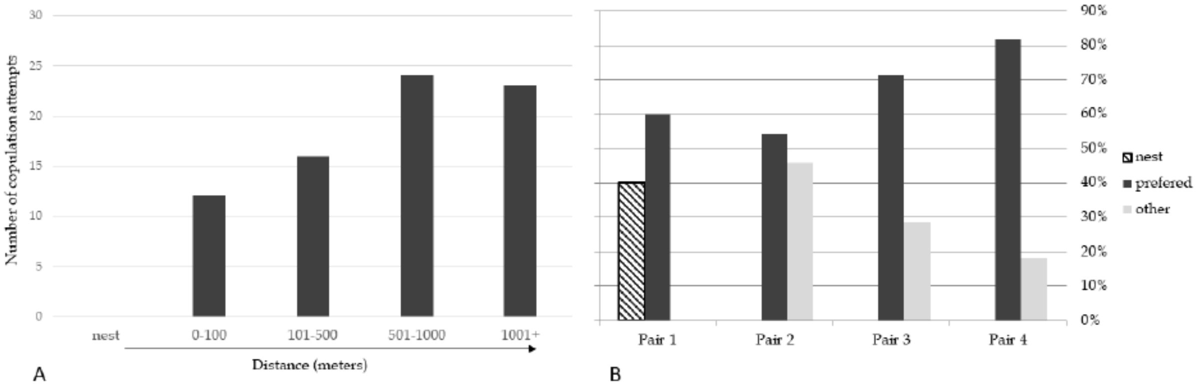

Fig. 3. A. Linear distances between nests of four Egyptian vulture pairs and their copulation sites; B. Proportion of copulation attempts at different copulation sites of the four Egyptian vulture pairs observed directly.

The proportion of successful copulations of the Egyptian vulture is similar across Europe and higher compared to sympatric species such as the Bearded vulture (Bertran & Margalida, 1999). Also, the duration of the successful copulation we report in our study is slightly higher than the reported for the species before (Donázar et al., 1994). In contrast with Donázar et al. (1994), we detected also copulation attempts after the onset of incubation. The fertile period of the species is unknown (Robetson, 1986). Nevertheless, copulations of the species exceeding the laying date and therefore beyond the fertile period of the Egyptian vulture should also play a role in strengthening the bond in the pair except for the fertilization itself (Birkhead & Møller, 1987). In addition, copulation attempts in the post-laying period, eve rare, are also observed in the Bearded vulture (Bertran & Margalida, 1999). In contrast with other studies on solitary and territorial vulture species, we recorded 63 copulation attempts of 3 different pairs that occurred in the nest (Donázar et al., 1994; Bertran & Margalida, 1999). Nevertheless, it seems that the majority of the pairs do not copulate in the nest, and it is most probably typical for some pairs and unusual in general. All pairs that we observed copulating in their nest had a large nest sites compared with the mean size of the nests described in the Egyptian vulture (Balaban & Yamaç, 2019). Furthermore, these pairs occupy high Egyptian vulture density areas where competition is more severe and territory defense and signaling should be more intensive (Newton, 1979; Harmata, 1982; Negro & Grande 2001).

We found that the daily pattern of copulation frequencies is bimodal in contrast to previous studies on the species and in line with sympatric species such as the Bearded vulture (Negro & Grande, 2001). Our results demonstrate a significant difference between the data on the daily copulation patterns in pairs observed by trail cameras and by direct visual observation. In the first case the bimodal distribution is very discrete. The analysis of the direct observations data indicates two very prominent peaks. The first peak in the bimodal distribution is observed in the late morning hours when the thermal uplifts start and when the activity of the scavengers is expected to increase (Mundy et al., 1992). The second peak in the copulations that we observed was in the late afternoon, just before dark. This finding supports the sperm competition hypothesis where the male bird should be more prone to copulate in the very last possible hours of the day to ensure paternity and to dilute the sperm of possible competitors (Birkhead &

Møller, 1987; Birkhead et al., 1988). During the pre-laying period raptors are predominantly mating and building the nest (Newton, 1979). We found high copulation rate of the Egyptian vulture in the days before the egg laying (from -7 to -1). We suggest this behaviour has to ensure the fertilization of the eggs on one hand and the paternity on other hand. As proved also in other species such as the Bearded vulture, extra copulations are possible. This would be more feasible in areas where the population of the Egyptian vulture is abundant and there are many neighboring pairs which would make the extra pair copulations possible. The Eastern Rhodopes mountain harbors the majority of breeding Egyptian vultures in Bulgaria (Arkumarev et al., 2018). Even though we didn't register extra-pair copulations in the recent study, such observations in the area exist.

Our results suggest that the Egyptian vulture performs a paternity guarding strategy based on frequent copulations and solid attendance of the male around the female during the whole pre-laying period similarly to other studies for the same (Donázar et al., 1994) or sympatric species (Bertran & Margalida, 1999). In our study, the time that both birds spend together prior to egg laying varied from 82.2% to 100%. Hence, the mate attendance reduces the risk of extra-pair copulations (Birkhead & Møller, 1987).

Copulation behaviour, however, could be some kind of advertisement (Ellis & Powers, 1982; Simmons, 2000; Village, 1990) or expression of territory ownership (Negro & Grande 2001). Usually, raptor copulations take place in prominent or conspicuous places (Bertran & Margalida, 1999). Our results support the hypothesis because all the pairs of Egyptian vultures we observed had a clear pattern in terms of conspicuousness – they were all mating on exposed places visible from far away and from their direct conspecifics and other competitors. The hypothesis is suggested by Negro & Grande (2001) to explain the high daily copulation rates of raptors over extended periods. Specifically, if repeated copulations have a function related to territoriality or the signaling of breeding status one would expect raptors to copulate preferentially in the defended breeding area, and copulations to be conspicuous, to convey information to potential signal receptors (Negro & Grande 2001). Furthermore, all the pairs we observed, used the same spot for copulation in the majority of cases. Raptors tend to copulate in the area where intruders (i.e. conspecifics and other birds) are not tolerated (Negro et al., 1992; Robertson 1986). As a pronounced territorial bird, the copulation behavior of the Egyptian vulture seems to serve as a signal of a territory ownership and to convey information to competitors (Negro & Grande, 2001).

In summary, our results suggest that the Egyptian vulture guarantees paternity using the strategy of high copulation rate and intensive attendance in the breeding territory, around the nest and the female during the pre-laying period. Furthermore, we suggest that copulatory behavior is related to the territorial displays and signaling of territory occupancy and used to convey messages to conspecifics.

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Assessment of the Environmental State of North-Western Black Sea Coast Territories, Ukraine using Indicators of Sustainable Development

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Abstract. The concept of "sustainable development" is interpreted as a process of development of countries and regions based on coordination and harmonization of social, economic, and environmental components in order to meet the needs of present and future generations. The concept of sustainable development aims at the socially desirable, economically viable and environmentally safe development of society. To assess the ecological state of the environment from the standpoint of sustainable development, certain indicators and parameters have been developed. The paper evaluates the state of the environment of the North-Western Black Sea region according to the indicators of sustainable development as factors in assessing the technogenic load. It is discovered that in the category «Ecological systems» of ecological policy the best conditions of sustainable development are noted in the Mykolayiv area, the least good - in the Odessa region. In the category "Environmental load" the best conditions are noted for the Kherson region, the worst – also for the Odessa region. In general, the most unfavorable environmental situation from the standpoint of sustainable development among the territories of the North-Western Black Sea Coast is observed in the Odessa region. Kherson region is characterized by the best conditions. Among all the analyzed indicators, the category of policy "Environmental load" plays a predominant role.

Key words: sustainable development, environmental assessment index, indicator.

Introduction

The inconsistency of economic development and environmental safety requirements, the dominance of industries with a high share of resource- and energy-intensive obsolete technologies, lack of rational consumption of natural resources all have led to the formation of technogenic economic development and environmental degradation (Wan et al., 2020).

Human awareness of the real danger of environmental catastrophe initiated the development of the concept of sustainable development, which should be understood not only in the context of changing human-nature relations to expand economic growth but also as a coordinated global survival strategy aimed at preserving and restoring the environment.

It should be noted that the sustainable development of any country is a controversial issue. Such development can be said only when economic growth, material production and consumption, and other social activities occur within the limits determined by the ability of ecological systems to recover (Geets, 2000).

Today there are many interpretations, and a single point of view on the essence of the concept of "sustainable development" has not been developed. Summarizing the achievements of scientists (United Nations, 2015; Doroguntsov & Ralchuk, 2001; Rudenko, 2001; Pidlisnyuk, 2001) it can be noted that the interpretation of the concept of "sustainable development" extends to the process of change in society, in which the exploitation of resources, scientific and technical development and institutional change are coherent and do not diminish the ability of future generations to meet human needs and aspirations.

The Decree of the President of Ukraine "On the Sustainable Development Goals of Ukraine until 2030" defines certain goals of sustainable development, a third of which relate to environmental security and preservation of a quality environment for future generations (President of Ukraine, 2019). In general, the peculiarities of the strategy of sustainable development in Ukraine in recent years have been considered by many researchers, in particular (Molokanova, 2018; Smirnova & Mykhailyuta, 2018; Haustova & Omarov, 2018). Particularly, the need to develop appropriate measures to consider the issue of further practical implementation, combining all levels of the socio-economic system with mandatory consideration of the environmental component is being given particular attention.

According to the research of leading scientists (Danilov-Danilyan & Losev, 2000; NAS of Ukraine, 2007; Zgurovsky, 2009; Prischepa & Klimenko, 2009; Shapar, 2009; Alexandrov et al., 2010; Klimenko & Klimenko, 2010; Kutsenko et al., 2013; Necos & Soloshych, 2014; Emas, 2015; Mensah, 2019; Powe, 2020), such an assessment should be made based on analysis of the dynamics of changes in certain indices and indicators that most broadly characterize all components of the ecological state of regions. According to the metrics for measuring the processes of sustainable development (MMSD) (Zgurovsky, 2009), sustainable development is assessed from the standpoint of economic, environmental and socio-institutional nature. According to the data (NISS, 2012), the UN Commission on Sustainable Development determines the level of development of countries by 96 indicators, 19 of which characterize the state of the environment. Experts from Yale University (USA) have proposed to determine the Environmental Performance Index, which characterizes the effectiveness of public policy in the field of environmental protection and ecosystem conservation. This index is calculated using 22 indicators, divided into 10 categories that characterize the quality of the environment and the viability of ecosystems. According to the value of this indicator in 2012, Ukraine ranked 102nd among 132 countries (NISS, 2012). This list of indicators largely repeats the parameters of the indicators of the environmental evaluation of sustainable development and from some points of view reflects the technogenic impact on certain components of the environment more objectively.

Materials and Methods

According to the metrics for measuring sustainable development processes proposed in the paper (Zgurovsky, 2009), the environmental evaluation index (Ie) can be determined taking into account three categories of environmental policy: 1) ecological systems (I_{SYS}); 2) environmental load (I_{STR}); 3) regional environmental management (I_{REG}). These categories contain 13 parameters and 44 indicators.

An indicator of sustainable development is an indicator that reflects economic, social, and/or environmental development in a particular region, and has such properties as ease of interpretation, wide range, sensitivity to change, quantification and allows to make forecasts and identify trends in time (Zgurovsky, 2009).

The category "Ecological systems" includes 6 indicators (air I_{AIR}, biodiversity I_{VIO}, land I_{LAN}, water quality I_{WQL}, water quantity I_{WQN}, radiation, and environmental hazard I_{RAD}), as well as 20 parameters in its composition. The category "Environmental load" contains 4 indicators (air emissions I_{EMS}, load on ecosystems I_{ECO}, generation and use of waste I_{WST}, water load I_{WAT}) and 19 parameters. The category "Regional environmental management" contains 3 indicators (participation in I_{COL} environmental projects, greenhouse gas emissions I_{GHG}, transboundary environmental pressure I_{GPC}) and 5 parameters (Zgurovsky, 2009).

It should be noted that these indicators and parameters are in fact indicators of technogenic load on individual components of the environment and the state of ecosystems in general. This applies, first of all, to indicators of emissions and discharges of pollutants, waste generation and accumulation, water consumption, and drainage, land use indicators, etc. Therefore, the assessment of the ecological condition of any territory with the use of the index of ecological measurement according to the MMSD can also be considered a result of the assessment of technogenic load.

The paper (Matviychuk & Sidoruk, 2018) proposes a model of a comprehensive system of environmental safety indicators in Ukraine. It should be noted that the environmental parameters included indicators of natural and technogenic hazards. Besides, the proposed model also includes economic and social indicators, i.e. the authors also consider the environmental safety of the state from the standpoint of sustainable development.

The analysis of literature sources (Zgurovsky, 2009; Prischepa & Klimenko, 2009; Harris, 2000; NISS, 2012) showed that there is no single methodological approach to assessing the ecological status of regions in terms of sustainable development. Thus, from the standpoint of assessing the technogenic load on the environment, the most optimal is the method proposed in the paper (Zgurovsky, 2009). Based on the above, the work aims to assess the state of the environment of the regions of the North-Western Black Sea Coast (NWBSC) on the indicators of sustainable development as factors in assessing the technogenic load.

Results and Discussion

The recommendations for calculating the environmental evaluation index (*Ie*) state that the parameters and indicators of the environmental evaluation are balanced averages (Zgurovsky, 2009). In this case, none of the parameters is assigned a weighting factor. Also, it is noted that assigning certain weights to environmental parameters is a difficult task. Statistical methods have shown almost equivalent weights of individual parameters within the indicators to which they are included.

Analysis of the initial information on the values of individual parameters of the environmental evaluation showed that the above statement (Zgurovsky, 2009) is somewhat erroneous. Thus, in some cases, the parameters have equivalent weights, such as air indicator (I_{AIR}), which is defined as the weighted average of the concentrations of nitrogen dioxide, sulfur dioxide and dust (in units of maximum permissible concentrations). But if the concentrations of pollutants are greater than the maximum allowable concentrations, then when calculating the I_{AIR} in the summation (according to the method of Zgurovsky, 2009) the result may exceed 1. In this case, it is not a confirmation of the best indicators of sustainable development. Similar conclusions can be made for other indicators, which sum up the values with quite different units of measurement. Such illustrative examples are indicators of land (I_{LAN}), load on ecosystems (I_{ECO}), where individual parameters are measured in points, square kilometers, percentages, hectares per capita, cubic meters per capita, etc.

For the analysis of 44 proposed parameters of the environmental evaluation, we used 27 to assess the sustainable development of the regions of the NWBSC. Note that the assessment was conducted based on analysis of individual parameters and in some cases without calculation of environmental measurement indicators. For analysis, the original data were normalized so that all parameters took values from 0 to 1 using the principle of linear normalization:

$$\tilde{x}_i = \frac{x_i - x_{min}}{x_{max} - x_{min}}. \quad (1)$$

The analysis of the obtained normalized values showed that the minimum indicators in the vast majority characterize the best conditions, and the maximum - the worst. That is, the best conditions for sustainable development indicators, in this case, are characterized by indicators close to 0. Only for some parameters that we took into account (I_{PZF}, I_{EF1}, I_{EF2}, $I_{EF3,}$ and I_{ORG}), it is fair to say that close to 1 values characterize the best conditions of sustainable development (Zgurovsky, 2009). Therefore, to unify the calculations, all parameters were reduced to such a form that their best values are close to 0.

The results of the ranking of the areas of NWBSC by individual indicators of the environmental evaluation of sustainable development are shown in Table 1.

As can be seen from Table 1, most often according to the list of the above parameters, the Odessa region is in the red zone (the one with the worst indicators of the ecological component of sustainable development), Kherson region, however, is in the green zone (the one with the best indicators). Although within certain categories of environmental policy on the worst indicators, such distribution differs.

Considering the principle of calculating environmental indicators and categories of environmental policy (Zgurovsky, 2009), we calculated indices for two categories of policy - environmental systems and environmental load. The spatial representation of these policy categories is shown in Fig. 1, 2.

The analysis of the presented figures shows that in the category of ecological policy "Ecological systems" (see Fig. 1) the best conditions of sustainable development are noted in the Mykolayiv region, the least good - in Odessa. Such an unfavorable situation in the Odessa region is due to such indicators as the content of sulfur dioxide and dust in the air, the spread of exogenous geological processes, high rates of disturbed and exhausted lands, nitrate content in the aquatic environment, the presence of a significant number of environmentally hazardous enterprises.

In the category "Environmental load" (see Fig. 2) the best conditions were observed for the Kherson region, the least good - also for Odessa. In this case, this situation is formed due to higher indicators of polluting emissions into the atmosphere, lower rates of arable land and forests, significant volumes of return water discharges. It was interesting to compare the results of the assessment of the categories of environmental policy for (Zgurovsky, 2009) for the territories of Ukraine, including the regions of the NWBSC with our results (Table 2).

The table shows that without taking into account the obtained numerical values, the general situation has not changed significantly. The most unfavorable situation from the standpoint of sustainable development in both categories of policy among the territories of the NWBSC is observed for the Odessa region. In the category of policy "Ecological systems" indicators for the Kherson area worsened and for the Mykolayiv area improved. In the category of policy "Environmental burden," the distribution of indicators has not changed.

The results of the general assessment of the index of ecological evaluation of sustainable development, considering all the indicators taken into account during the analysis are shown in Fig. 3. The analysis of the presented figure shows that the most unfavorable environ-

Table 1. Ranking of the territories of the North-Western Black Sea Coast according to the indicators of the ecological evaluation of sustainable development (2014 – 2018).

Index	**Odessa region**	**Mykolayiv region**	**Kherson region**
"Ecological systems" I_{SYS} policy category			
I_{NO2} (NO_2 concentration)	0.23	0	1
I_{SO2} (SO_2 concentration)	1	0.14	0
I_{TCP} (dust concentration)	1	0.33	0
I_{PZF} (NRF objects)	0.77	1	0
I_{EGP} (spread of exogenous geological processes)	1	0.79	0
I_{EXH} (worked and re-cultivated lands)	1	0.67	0
I_{NIT} (nitrate concentration)	1	0	0.23
I_{MIN} (mineralization)	0	1	0.45
I_{WAV} (water abstraction from natural sources per 1 person)	0.22	0	1
I_{GAV} (water intake from underground sources per 1 person)	0.16	0	1
I_{RHZ} (potential radiation hazard)	0	1	0
I_{IHZ} (environmentally hazardous enterprises)	1	0	1
"Environmental load" I_{STR} policy category			
I_{NOX} (N_{OX} emissions)	0.67	1	0
I_{SOT} (SO_2 emissions)	1	0.26	0
I_{CAR} (emissions from motor vehicles)	1	0	0.02
I_{EKM} (emissions per 1 km^2)	1	0.09	0
I_{EPC} (emissions per 1 person)	0.81	1	0
I_{EF1} (arable land)	1	0.29	0
I_{EF2} (hayfields and pastures)	0.775	0	1
I_{EF3} (forests and wooded areas)	1	0.64	0
I_{EF4} (built-up land)	0	1	0.23
I_{EF5} (use of freshwater per 1 person)	0	0.09	1
I_{ACC} (waste accumulation)	0.18	1	0
I_{WKM} (generation of wastes of I - III classes of danger on 1 km^2)	0	1	0.63
I_{WPC} (generation of wastes of I - III hazard classes per 1 person)	0	1	0.82
I_{REW} (discharge of return water into surface water entities)	1	0.11	0
"Regional Environmental Management" I_{REG} policy category			
I_{ORG} (public environmental organizations)	0	1	0.18

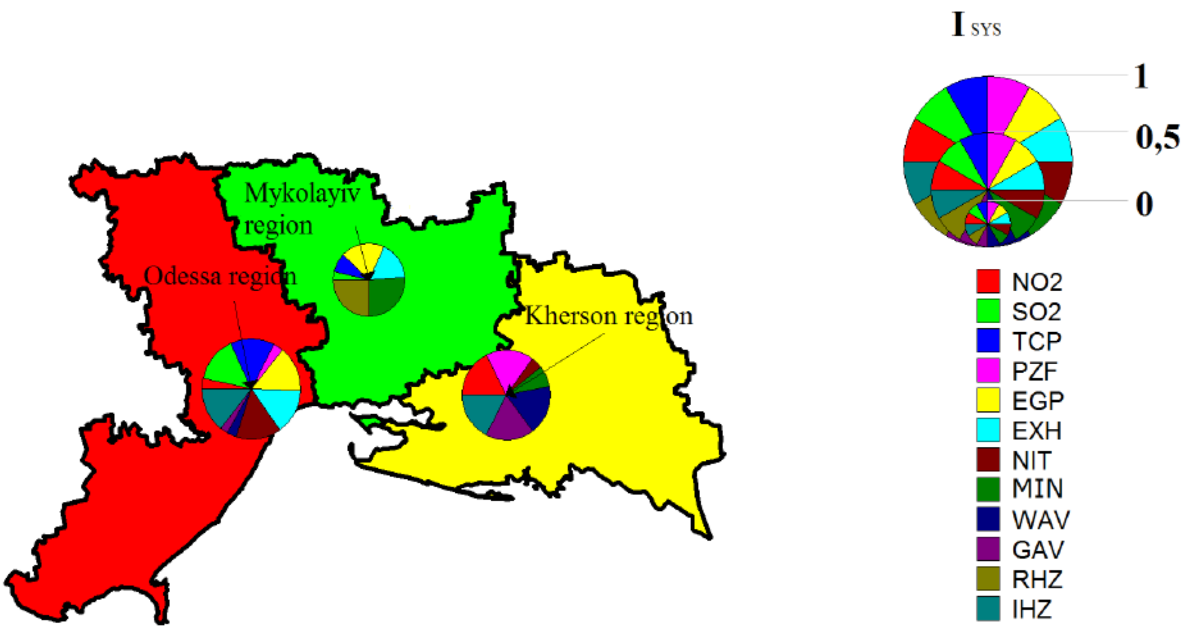

Fig. 1. Spatial distribution of the I_{SYS} index of the category "Ecological systems" for the territories of the North-Western Black Sea Coast (2014 – 2018).

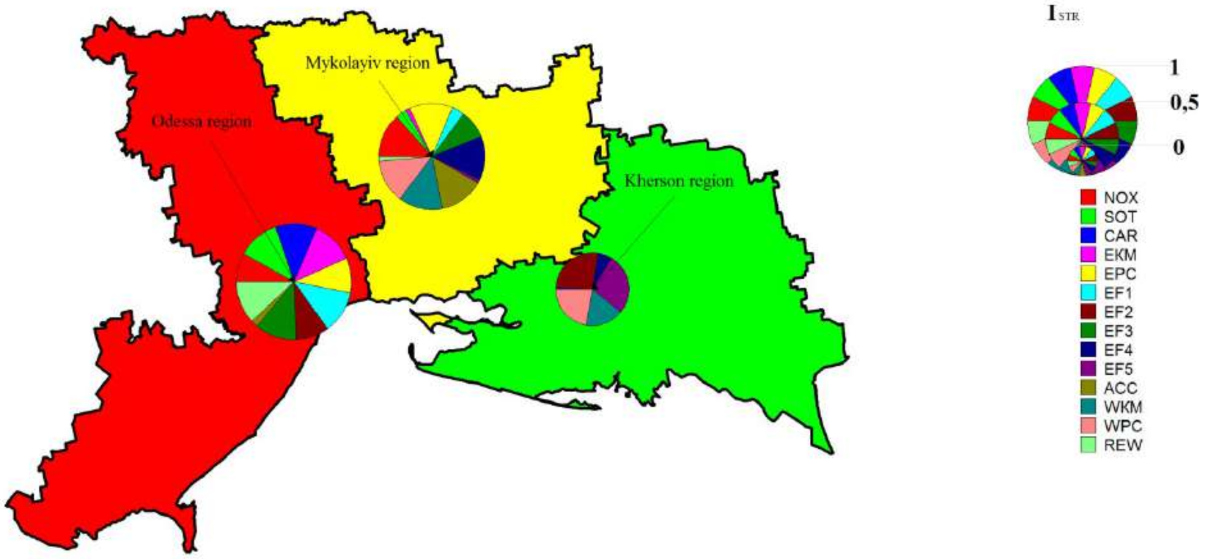

Fig. 2. Spatial distribution of the I_{STR} index of the category "Ecological load" for the territories of the North-Western Black Sea Coast (2014 – 2018).

mental situation from the standpoint of sustainable development is observed in the Odessa region. Kherson region is characterized by the best conditions for sustainable development of the region. It should be noted that among all the analyzed indicators, the category of policy "Environmental load" plays a predominant role.

One of the prerequisites for sustainable development is the minimization of technogenic load on certain components of the environment, the main directions of which can be considered the following measures:

Table 2. Comparative analysis of the assessment of environmental policy categories of the North-Western Black Sea coast.

Region	**Category "Ecological systems"**		**Category "Ecological load"**	
	2006 (20 indicators, Zgurovsky, 2009)	**2014 – 2018 (12 indicators, from authors)**	**2006 (19 indicators, Zgurovsky, 2009)**	**2014 – 2018 (14 indicatorss, from authors)**
Odessa region	0.418	0.699	0.753	0.603
Mykolayiv region	0.588	0.327	0.828	0.533
Kherson region	0.602	0.473	0.915	0.265

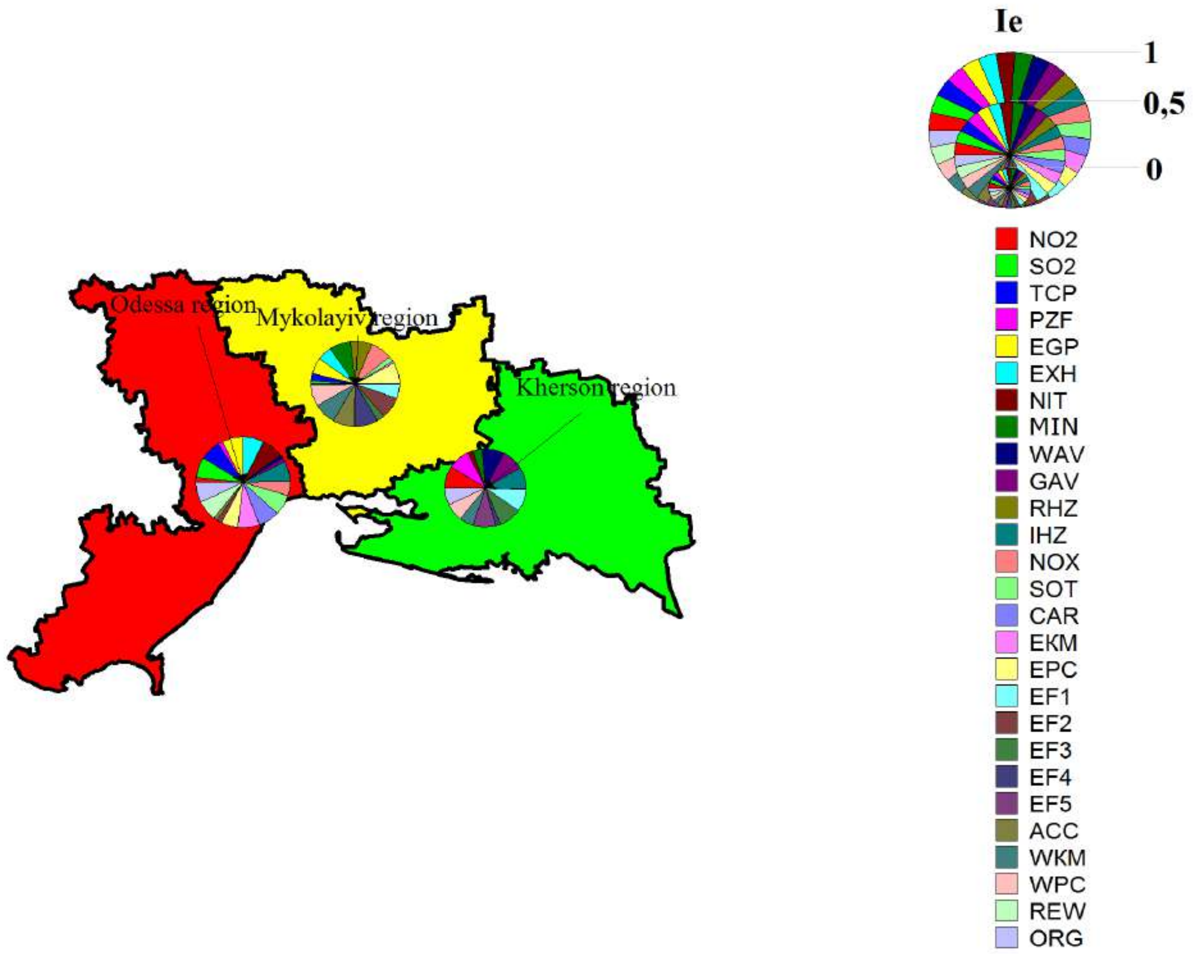

Fig. 3. Spatial distribution of the index of ecological evaluation of sustainable development *Ie* for the territories of the North-Western Black Sea Coast (2014 – 2018).

1) optimization of the monitoring network of the air basin, water entities, soil cover, and geological environment following modern EU requirements, organization of systematic observations of physical pollution under the influence of technogenic objects;

2) increasing the level of environmental safety of stationary sources of air pollution by improving technological processes, air protection measures, streamlining the configuration of sanitary protection zones, creating "green belts" around industrial and urban agglomerations, etc.;

3) given the dominance of mobile sources, the redistribution of traffic flows requires justification from the standpoint of logistics, the creation of a system of high-speed public transport, green boulevards with low traffic;

4) improving the efficiency of wastewater and other return water treatment, creating a system for drainage and treatment of stormwater, which is a powerful source of pollution of the coastal zone of the North-Western Black Sea, improving the technical condition of worn-out main and distribution networks that provoke high levels of secondary drinking water pollution;

5) limitation of the negative impact on the pollution of the soil cover and other natural environments of the accumulators of industrial toxic waste and numerous landfills of solid household waste;

6) creation of necessary capacities for processing, treatment of neutralization, and utilization of hazardous waste;

7) improvement of the system of management and treatment of solid household waste, which accounts for more than 90 % of the sum of all waste in the study area;

8) restriction of forms of economic activity that provoke the development of dangerous exogenous geological processes.

Conclusions

The conducted researches allow us to draw the following conclusions:

1) The analysis of indicators of the index of ecological measurement showed that most of them are used including for an assessment of a condition and technogenic loading on components of the environment;

2) Along with the indicators of the MMSD, the level of sustainable development of countries can be determined by UN recommendations, scientific developments of domestic and foreign scientists;

3) The analysis of the MMSD revealed that the authors' statement about the balance of parameters and indicators of the environmental measurement index is erroneous (analysis of the obtained normalized values showed that the minimum indicators characterize the best conditions and the maximum - the worst, in contrast to these conditions in the MMSD);

4) According to the results of ranking the territories of the North-Western Black Sea coast by individual indicators of ecological evaluation of sustainable development, it was found that most often the zones with the worst indicators of the ecological component of sustainable development included the Odessa region and the zones with the best indicators - Kherson region;

5) In the category of ecological policy "Ecological systems" the best conditions of sustainable development are noted in the Mykolayiv area, the least good - in Odessa region. In the category "Environmental load", respectively, the best conditions were noted for the Kherson region, the least - also for Odessa region;

6) In general, the most unfavorable environmental situation from the standpoint of sustainable development among the territories of the North-Western Black Sea Coast is observed in the Odessa region. Kherson region is characterized by the best conditions for sustainable development of the region, and among all the analyzed indicators, the category of policy "Environmental load" plays a predominant role.

7) In order to ensure the sustainable development of the study areas it is

necessary to implement a set of technical and technological solutions that will minimize the technogenic load on individual components of the environment.

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Data on the Stomach Content Analysis and Fat-body Morphology in the Agile Frog (Rana dalmatina Fitzinger in Bonaparte, 1838) during the Mating Season in Bulgaria

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Abstract. We present data on the stomach contents and the gross morphology of the fat body in Agile Frogs (*Rana dalmatina*) from a pond which is an object of a long-termed monitoring. We also provide information on the diet of one female of *Lissotriton vulgaris*. All of the specimens were found dead during the mating season, when sudden snowfalls and depression in the temperature covered the ponds with ice and snow layer for several days. The stomach content of the frogs was represented by some chitin rests, shed-skin, and plant material. The fat bodies of the frogs were dramatically reduced in size - an indicator of low level of energetic reserves. We conclude that on the basis of our findings, massive investigations by using stomach flushing methods during the mating season in the Agile Frogs have to be avoided.

Key words: Amphibia, diet, energetic reserves, reproduction success.

Introduction

The Agile Frog (*Rana dalmatina* Fitzinger *in* Bonaparte, 1838) is an explosive breeder (Wells, 1977). During the initial days of the breeding season, the frogs potentially expose themselves to risks, because of the low temperatures (see Hartel et al., 2007). The males may enter the water in large numbers - the sex ratio is strongly male-biased (Stojanov et al., 2011) and the sexual selection is a result of the direct competition. According to Hartel (2005), the pre-productive period of calling and 3-D positioning of the males is rather short. For the Agile Frog was described a unique behavior of “egg clutch piracy”, where some males inspect the freshly laid eggs and clasp them in the same way they would clasp a female. By doing so, these males may successfully fertilise the eggs, actually avoiding sexual selection, but contributing to the genetic variation (Vieites et al., 2004).

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Many aspects of the biology of *R. dalmatina* are well studied, however to date data on the diet of the Agile Frogs during the mating period are scarce (see Guidali et al., 2000; Mollov & Delev, 2020), with only one study from Bulgaria so far (Angelov & Batschwarov, 1972). In the present study we provide new data on the stomach content of the frogs during the reproduction time and also data on the gross morphology of their fat body – an indicator for the energetical status in frogs (Guidali et al., 2000).

The manuscript represents collateral findings made during a long-term monitoring on the habitat specifications of the Agile Frog larvae. Since February of 2014 we investigate the water quality of two water bodies in the Protected Site "Shumensko plato" BG0000382 (NE Bulgaria), because the larvae of this species are reported to be highly sensitive to certain changes in water chemistry - the mortality rate may reach 100% (see Andren et al., 1988). The region of the Protected site is rather dry, so we monitored the only two water basins which maintain permanent water volumes during the whole year. These two ponds are crucial for the survivability of the population of *R. dalmatina* in the region and are in the vicinity of a road with moderate traffic. The identified local threats are related to an increase in the acidity and conductivity of the water.

Material and Methods

We started the inspections of the ponds in the second half of February each year because these are the days when the initial seasonal activity of *R. dalmatina* can be detected in Bulgaria (Naumov, 1999, pers. comm.). In the range of our study we investigated 20 specimens of the Agile Frog. The frogs were found dead in the water basins they use as breeding spots at 43.2488°N, 26.8933°E, at about 480 a.s.l. (Datum: WGS84). The findings were performed in three different days in the period 2018-2020. All the frogs except one were males. On 29.03.2018 we found also one dead female Smooth newt (*Lissotriton vulgaris* Linnaeus, 1758) - for comparative analysis of the stomach content we included that specimen in our study. All dead animals were immediately fixed in Bouin solution and then conserved in Formol. The samples were transported to the laboratory for dissections, stomach contents analysis and observation of the fat body (only for the frogs). All frogs are now preserved in alcohol and are a part of the collection of the "Center for Natural Sciences" at the Shumen University.

We checked the stomach contents in all specimens and photographed the fat bodies of the frogs by using Sony RX 10 III. The contents of the stomachs which were not completely empty were preserved in fixative (Bouin solution) and investigated under a light microscope "Carl Zeiss Jena Amplival" microscope and "Carl Zeiss Stemi 2000" stereomicroscope. All particles were documented and we analysed the origin and the conditions of the materials. The photographs were made using a digital camera "Canon EOS 2000D" and "AxioCam ERc 5s". The preparations of the epithelium tissues were stained using "Methylene blue".

Results and Discussion

During our investigations, we detected a total of 48 nights in which the ponds were covered fully or partly with ice layer during the period the frogs were into the water. The ice coverage indicates that the water temperatures may drop dramatically even in the end of March. However, we were able to detect dead frogs only on three occasions: on 29.03.2018, on 31.03.2018 and on 30.03.2020. On 29.03.2018 were found 12 specimens (including the dead female *Lissotriton vulgaris*); on 31.03.2018 – seven and on 30.03.2020 – one specimen. In all three cases, the occurrence of dead specimens was not related to dramatic changes in the water chemistry (water pH was 6.25, 6.27, 6.84 and conductivity was 50, 64, 66 μs/cm), but presumably because of the ice and snow-covered the total surface of the basins.

In the stomach of the female *L. vulgaris* were obtained six specimens of *Asellus aquaticus* Linnaeus, 1758 (Fig. 1a) and six larvae of Chironomidae (Diptera) - most of them in an advanced stage of digestion. In this season of the year, *L. vulgaris* is in its aquatic stage (Stojanov et al., 2011; Heiss et al., 2016) and its feeding apparatus is in the "aquatic mode" (see Heiss et al., 2017). Obviously, there is plenty of potential prey in the water basins, however, the ranid frogs are constrained by their feeding morphology and may feed almost exclusively on land (see Nishikawa, 2000). The Agile Frog is reported to feed only terrestrially and preys on a variety of invertebrates (Aszalos et al., 2000; Guidali et al., 2000; Hodisan et al., 2010; Cicort-Lucaciu et al., 2011). Aszalos et al. (2000) reported an aquatic origin of some prey items found in the stomach content of Agile Frogs, but stressed also that the frogs have consumed them on land.

Fig. 1. Microscopical photographs of the stomach content of the investigated specimens: a. rests of *Asellus aquaticus* extracted from the gaster of a female *Lissotriton vulgaris* found dead together with 12 of the dead Agile Frogs on 29.03.2018; b. chitin remains found in frog specimen coded 07; c. chitin remains found in the stomach of frog specimen coded 08; d. plant material from the stomach of frog specimen coded 02; e. plant material from the stomach of frog specimen coded 07.

The male frogs start their activity in the ponds immediately after hibernation at the end of the winter and remain in the water for a prolonged time (Naumov, 1999 and pers. comm.; Guidali et al., 2000). It can be expected that the Agile Frogs (or at least the males) do not feed in the mating period, however, some data indicate on feeding activity during this time (Guidali et al., 2000). These authors report the presence of insects, insect eggs, diplopods, araneans, and oligohets in the diet of the frogs. In our investigation, we found that the stomachs of most specimens are completely empty (Fig. 2a). In five of the males we were able to detect some content in the stomach (Fig.1b,c,d,e): in one of the stomachs we found chitin remains and also some materials in a very late stage of digestion; in two we found material of plant origins (in one of them also chitin remains); in two there was shed-skin. This food particles indicate that the frogs attempt to feed during the mating period, but obviously the prey is scarce, because of the weather conditions. Similar situation is observed with another anuran species from Bulgaria – the Green Toad, *Bufotes viridis* (Laurenti, 1768), which is also an explosive breeder and breeding takes place in February-March. Mollov et al. (2020) registered a significant portion of empty stomachs in the toads captured, during the breeding season, which indicates, that most of the individuals do not feed during that time. The plants have apparently no food value for the frogs and the plant remains found within the stomachs can be regarded as material that may be ingested by a fail capture attempt or as collateral debris taken up during the predation (Whitaker et al., 1977; Mollov & Delev, 2020).

The shed-skin consumption in frogs was an object of some discussions. According to some authors, it has a random character (Ferenți & Covaciu-Marcov, 2009) and does not have any nutritious value, but Weldon et al. (1993) stated that this act is important for the recycling of the epidermal proteins. The rate of dermophagy is detected to increase in periods with low number of prey (Covaciu-Marcov et al., 2010).

The fat body is closely associated (both embryologically and anatomically) with the gonads and plays a very important role in the production of the sperms and the eggs (Chieffi et al., 1975). This is also the main deposit of fat in amphibians and the main energy deposit of the body for supporting the general metabolism and reproduction. As such, the fat body of the frogs may change dramatically in size during the year cycles and in animals which had starved for prolonged periods. That organ may undergo an almost full regression of the fat tissue to mesenchyme (Zancanaro et al., 1996). Girish & Saidapur (2000) report that even in frogs which are fed once a week, the fat body may be reduced to filiform shape, so the form and the volume of the fat body is a good indicator for the energy status of the particular individuals. In anurans, the annual fat body cycle and the cycle of the ovaries are in inverse correlation (Hoque & Saidapur, 1994). This is well supported by our finding represented in Fig. 2a - the female Agile Frog had developed eggs, but the fat body was reduced completely. Our sections revealed that the fat body in all males was dramatically reduced in size - the most prominent fat body we were able to found is represented in Fig. 2b. In three of all frogs we were not able to detect visually any fat body, in other three frogs the fat body was reduced in size, but had retained the typical star-formed shape of the organ and in all other frogs the fat body was filiform. The gross anatomy of the fat bodies indicates, that the frogs are on the limit of their energetic reserves. This is normal for the Agile Frogs and plenty of frog species are able to handle prolonged periods with low energetic income and high energetic demands (for an overview see Zancanaro et al., 1996), however the Agile Frogs do attempt to feed during the mating season and the potential income of food may be critical for the fitness of the animals prior to post-mating period in which more intense feeding begins (see Guidali et al., 2000). In our opinion, mass investigations by the use of stomach flushing techniques during the mating season are inappropriate for that species - potential loss of energy cannot be recovered by simply "eating again", because prey could be scarce during the late winter and the early spring.

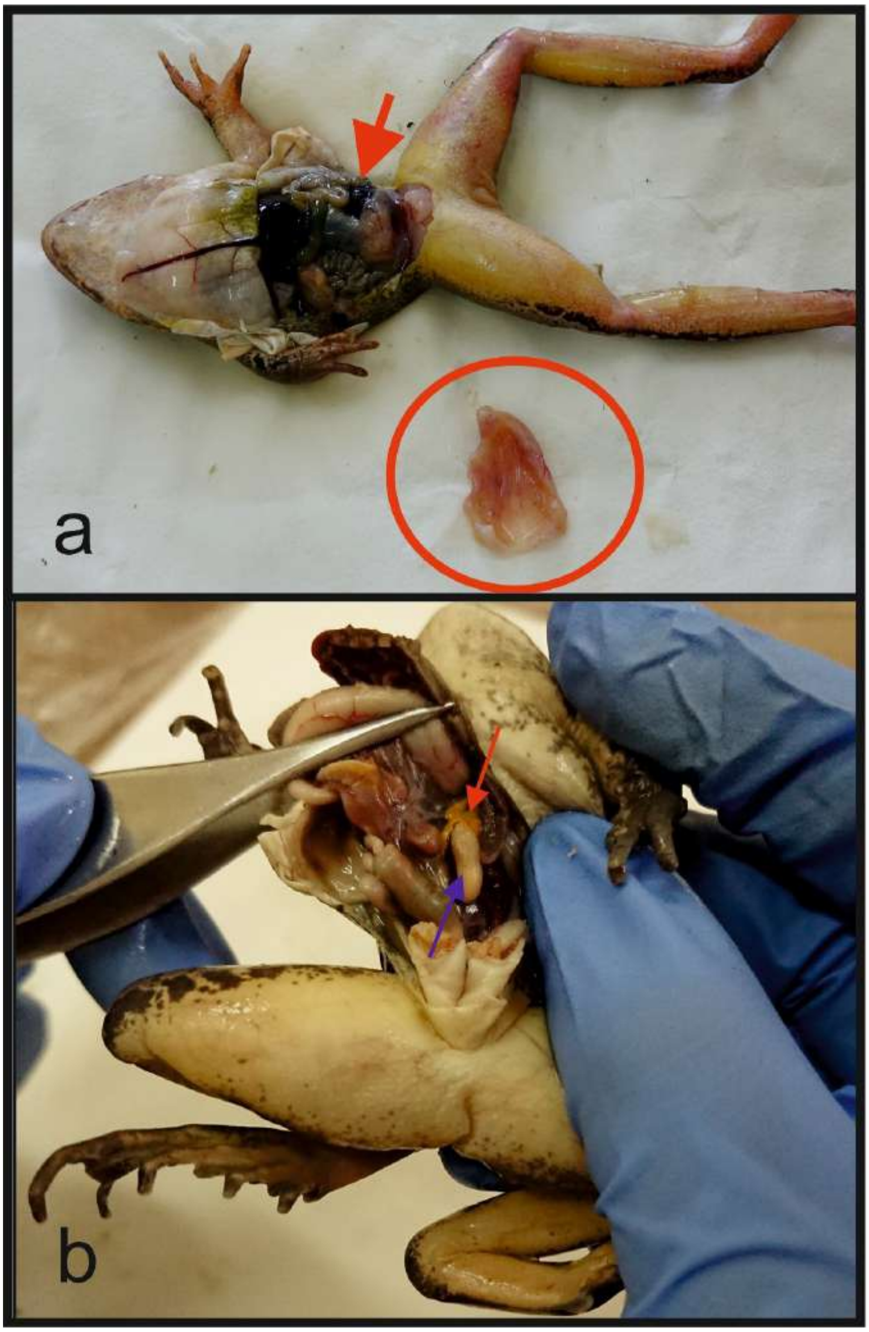

Fig. 2. Dissection of two Agile Frog specimens: a. female found dead on the 30.03.2020, the red arrow indicates the presence of eggs, the red circle is provided around the stomach - the dissection reveals lack of any food particles; b. male Agile Frog revealed the morphological condition of the fat body - the organ is dramatically reduced in size, but still had retained its general form (please note, that this was the largest fat body, which we were able to detect), the red arrow indicates the fat body associated with the left testes (blue indicator).

Acknowledgements

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Technogenic impact of acid tar storage ponds on the environment: a case study from Lviv, Ukraine

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Abstract. WA number of countries keep acid tar in the open air in spent quarries, barns, storage ponds (USA, UK, Netherlands, Belgium, Germany, Latvia, Slovenia, China) or near landfills (Zimbabwe). Storage of acid tars in open areas leads to an increase in regional environmental hazards. In Ukraine, acid tars are also stored in open spaces in storage ponds, in particular, in the city of Lviv near the municipal landfill. After the Lviv experimental petroleum-refining plant began to dispose of oil-refining waste at the Lviv city landfill, two ponds of acid tars were formed. Their total area is 6.8 ha. They were formed by constructing dams in the upper reaches of deep ravines, originating near the landfill. In total, about 300 thousand tons of acid tars have been accumulated in these earth basins. In 2004, the dams of the acid tar storage ponds got cracked. As a result, acidic water flows to the soil surface 1 km southwest of these storage ponds. Sampling for the study of acid tar migration was carried out from 4 sites of anthropogenic origin: 1 - acid tar storage pond No.1; 2 - acid tar storage pond No.2; 3 - technogenic water body at the foot of the landfill containing leacheate; 4 - technogenic water body on the north side of the landfill containing leacheate. The simulation was made by Surfer software. It was found that even after many years of their creation, a very high content of sulfuric acid is observed in the ponds (1,108-3,862 mg / kg), and they remain a major environmental hazard. The simulation results made it possible to assess the migration of toxic components contained in the tar to the components of the biosphere - the hydrosphere and ecotope. It was established that, unlike other investigated technogenic water bodies, acid tars in the storage ponds have high content of petroleum products, sulphates, phosphates, suspended substances, and high rates of chemical oxygen demand. The high content of petroleum products suggests that acidic tars can be processed into liquid fuel by freeing them from excess water and salts. At the same time, these large quantities of petroleum products pose an environmental threat to soil and groundwater, thereby reducing regional environmental security.

Key words: acid tar, civil protection, petroleum products, environment, landfill.

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Introduction

A number of countries keep acid tar in the open air in spent quarries, barns, and storage ponds (USA, UK, Netherlands, Belgium, Germany, Latvia, Slovenia, China) or near landfills (Zimbabwe, Ukraine). Storage of acid tars in open areas leads to an increase in regional environmental hazards (Popovych et al., 2018). Any storage of industrial waste has a detrimental effect on living organisms (Popovych et al., 2018; Kuzmenko et al., 2018). Many international research papers are devoted to this topic.

In particular, an analysis of the content of the main pollutants in the aqueous phase of a quarry with acid tar (petroleum products and sulphates) showed a strong pollution with petroleum products. The fluorimetric method revealed a vertical gradient of petroleum product content in depth - from 6.4 mg / dm^3 in surface to 1,995 mg / dm^3 in the depth of 1 meter in the bottom layers. The petroleum product content with depth increases by 312 times and significantly exceeds the maximum permitable concentration for water bodies (0.3 mg / dm^3) (Shilova et al., 2017). Soil samples were taken in the area near a quarry with acid tar, at a distance of 35-40 m and 150 m (at the border of the recreation area), taking into account the wind rose. Their analysis showed that with distance from the quarry the soil acidity decreased from pH 2.8 to pH 4.3. At a distance of 150 m, the acidity corresponded to the pH of brown forest soils (Shilova et al., 2017).

To date, dozens of copyright certificates and patents for acid tar processing have been received in the world. Most methods of disposal come to the regeneration of sulfuric acid from wastes or burning of neutralized tars. In Germany and the Netherlands, for example, waste is incinerated in enclosed boxes, while combustion products are not released into the environment (Popovich, 2016).

There are various ways of processing acid tars in the product that is not used, many scientists are trying to reduce the environmental problems, linking with recycling them in the lagoons (Družina, 2010). Some of the recycling processes considered included, among other things, the conversion of acid tars into asphaltenes, the production of pavement binders, energy generation. To reduce the negative impact on the environment, membrane technologies, ion exchangers and carbon adsorbents are used. However, these methods have not been fully investigated due to the complexity of acid tar structure. Accordingly, there remains a deep need for environmental stability (Danha et al., 2014).

Numerous studies have found that the addition of 10% additives by the total weight is sufficient to neutralize the free acids contained in acid tars. The resulting product is suitable for processing into bituminous mastic (Zharinov, 2015).

The work (Nadirov et al., 2019) presents the results of a study of the influence of technological factors on the saponification of fatty acids in cotton tars which are necessary for the development of a technology for the synthesis of new surfactants used in oil dehydration processes. Based on the data obtained, it was concluded that the effective concentration of alkali solution corresponds to 8-15%, while the amount of excess alkali does not affect the time of effervescence. Determined is the influence of temperature on the duration of the neutralization process. It was shown that fatty acids are a valuable and fairly affordable raw material used in various fields of the oil and gas industry; therefore, their extraction from waste, namely, from cotton tar, with their content of up to 65%, is appropriate and economically feasible.

In Ukraine, acid tars are stored in the open air in storage ponds, in particular in the city of Lviv near the municipal landfill. After the Lviv experimental petroleum processing plant began to discharge waste from the oil refining process at the Lviv city landfill, two ponds of acid tars were formed, which are still not managed in accordance with the requirements of the technological process (Popovych et al., 2015). It should be noted that in addition to storing garbage and acid tars, leacheates are released from deep within the landfill, which cause an additional technogenic load on the adjacent territory (Malovanyy et al., 2018; 2019; Popovych et al., 2015).

There are 2 acid tar storage ponds are in the landfill area. Their total area is 6.8 ha. They are formed by constructing dams in the upper reaches of deep ravines, originating near the landfill. In total, about 300 thousand tons of acid tars have been accumulated in these earth basins. In 2004, the dams of the acid tar storage ponds got cracked. As a result, acidic water flows to the soil surface of 1 km^2 southwest of these storage ponds (Khromyak et al., 2016).

The chemical composition of these tars in different years was studied by different organizations. Some of the most recent data (Khromyak et al., 2016) concerning the composition of acid tars are shown in Table 1.

In the zone of influence of the acid tar storage ponds, the soil, in terms of copper content, is the most polluted. In almost all samples, an excess of the MPC was recorded (Prykhodko, 2013). The minimum excess is 1.6, the maximum is 41.3 times. Nickel pollution is similar. However, both the average and maximum levels of soil contamination by these elements are significantly lower as compared to cadmium.

In some samples, the excess of MPC for zinc is recorded. For example, it reaches 9.5 times between the landfill and the dam of the tar pond. Soil contamination with chromium was detected in only two samples. The maximum excess reaches 1.7 times.

The content of cobalt and manganese in the soils does not exceed the MPC in any sample.

Particular attention is paid to soil contamination by leakage of acid tars which is traced in ravines south of the Lviv municipal landfill. The area of contamination only within one leakage exceeds 1 ha. No less large-scale leakages are recorded in another ravine, where the liquid phase of the tar forms a continuous flow. Slightly smaller leakage areas were discovered in the ravine at the top of which two acid tar ponds were created.

According to the analyses performed by the Ecology and Natural Resources Management Laboratory, the soils here have an acid reaction (pH 3.3–3.8), as well as an excess of MPC in lead by 34.2, copper - by 33, nickel - by 2.7, petroleum products - by 2 times (Leonard et al., 2010).

The object of the studies is acid tars which are stored in the open air. The subject of the studies is the hazardous components of acid tars and their migration into the biosphere. The purpose of work is to study physical and chemical properties of acid tars in the territory of a separate polygon to assess their technogenic threat to the environment.

Material and Methods

The following research methods were used in the work: methods of quantitative and qualitative analysis, of soil science, methods of chemical, physical, mathematical-statistical and system analysis.

Sampling for the study of acid tar migration was carried out from 4 sites of anthropogenic origin in the center of the pond from one spot (volume - 1.5 l). Sampling was carried out during July, 2019. Figure 1: 1 - acid tar storage pond No.1; 2 – storage pond of acid tars No. 2; 3 - technogenic basin at the foot of the landfill with the content of leacheates; 4 - technogenic basin on the north side of the landfill with the content of leacheates.

The samples were taken according to the recommendations given in the CPI 211.1.0.009-94. *Hydrosphere. Sampling to determine the composition and properties of wastewater and process water.* In the selected samples the following set of indicators (parameters) was determined:

Organoleptic:

- colour, transparency, odor - according to the reference book by Lurie (1984).

Physical:

- suspended substances, dry residue, mineral residue - by gravimetric methods (measuring instruments - analytical scales, laboratory thermometers).

Physico-chemical:

- pH value, total mineralization - by potentiometric methods in accordance with the instructions for the respective devices (devices - a pH-150I pH meter, a TDS universal salimeter).

Chemical:

- total hardness, alkalinity (carbonate hardness), content of carbohydrates by titrimetric methods with trilon B and hydrochloric acid;
- chloride content - by titrimetric method with silver nitrate;
- sulphate content - by gravimetric method (instrument - analytical balance);
- nitrite content – by photometric method with Griss reagent (device –a KFK-2 electro-photocolorimeter);
- nitrate content - by photometric method with Griss salicylic acid (device – a KFK-2 electro-photocolorimeter);
- ammonium ion content - by photometric method with Nesler Griss reagent (device – a KFK-2 electro-photocolorimeter);
- phosphate content - by photometric method with the combined *molybdenum reagent* and ascorbic acid (device – a KFK-2 electro-photocolorimeter);
- iron content – by photometric method with rhodanide (device – a KFK-2 electro-photocolorimeter);
- chemical oxygen demand (COD) - by titrimetric method with potassium dichromate;
- petroleum products content - by gravimetric method with chloroform and hexane (instrument - analytical balance).

Results and Discussion

The results of studies on the physicochemical parameters and chemical composition of acid tar samples from the storage ponds are presented in Table. 2.

As can be seen from Table 2, even after many years since the filling of tar ponds, their acidity is significant (pH < 3). This means that the content of acids (in particular, sulfuric acid) in the tars studied is very high. It is many times higher than the MPC for wastewater and constitutes a significant environmental hazard for both the soils surrounding the ponds and groundwater in the vicinity of the landfill. By the high solids content it can be judged that these tars contain a huge amount of salts. Moreover, the bulk of these salts are mineral salts, as evidenced by the ratio of dry residue (dried at + 90 ° C) and mineral (calcinated at + 800 ° C).

Table 1. Composition of tars stored at the Lviv city landfill (Khromyak et al., 2016).

Component	Content by weight,%
Carbohydrates	20.6
Resins, carbon, carboids, ash, oxidation products	78.2
Sulphuric acid and sulfonic acids	1.2
Acid number (mg KOH / g)	14

Fig. 1. Indicative map of the research areas.

Table 2. Physico-chemical properties of the samples.

No.	Indicator	Measurement units	Sample 1	Sample 2	Sample 3	Sample 4
1.	Odor at 20 ° C	points	1.5	1.5	2	1.5
2.	pH value	pH unit	2.2	2	8.1	2.1
3.	Suspended substances	mg/dm^3	154	198	46.2	54.8
4.	Dry residue (+90 °C)	mg/dm^3	7.248	6.857	11.554	7.130
5.	Mineral residue (+800 °C)	mg/dm^3	6.306	5.947	7.320	6.180
6.	Total hardness	mg-equi/dm^3	57.4	62.3	26.4	82
7.	Chlorides (Cl^-)	mg/dm^3	794	712	2.045	774
8.	Sulphates (SO_4^{2-})	mg/dm^3	3.625	3.862	1.108	3.716
9.	Nitrates (NO_3^-)	mg/dm^3	94.3	85.4	158.3	86.3
10.	Phosphates (PO_4^{3-})	mg/dm^3	12.7	9.5	12.8	3.6
11.	Ammonium salts (NH_4^+)	mg/dm^3	39.2	36.5	92.3	56.2
12.	Total iron (Fe_{total})	mg/dm^3	14.6	12.1	68.2	56.3
13.	The sum of (Na^+) + (K^+).	mg/dm^3	408	387	2.203	400
14.	Chemical oxygen demand (COD)	mgO/dm^3	332	491	265	132
15.	Petroleum products	mg/dm^3	523	488	1.69	23.6

The spatial distribution of odor (in points), pH, suspended substances, dry and mineral residues, and total hardness in the technogenic water bodies of the Lviv city landfill are shown in Fig. 2.

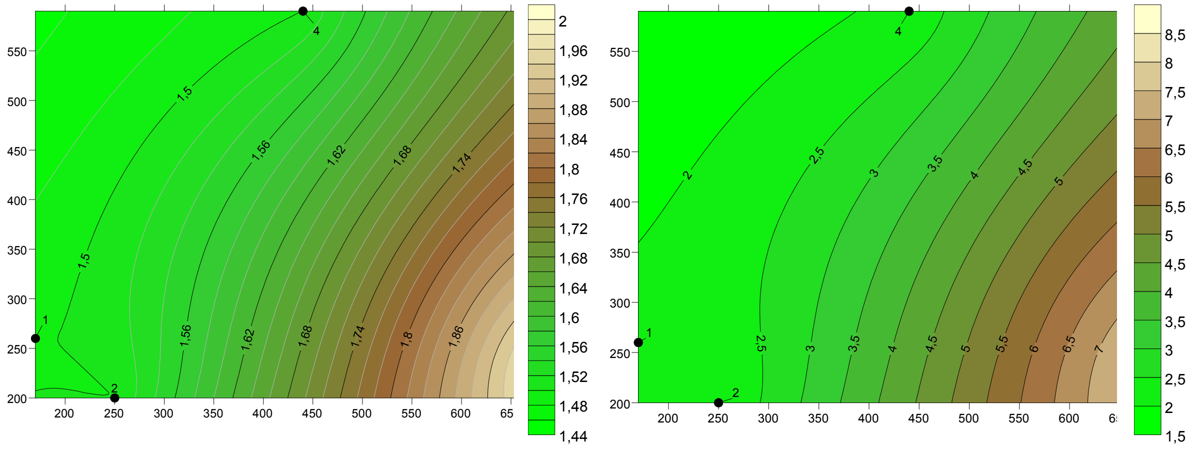
The difference between dry and mineral residues correlates well with the numerical values of petroleum product content and COD. That is, at + 800 ° C, it is the organic components of tars that burn out - petroleum products, organic acids and their salts. The same indicators (high content of petroleum products) suggest that these tars can be processed into liquid fuel by freeing them from excess water and salts. At the same time, these petroleum products in large quantities pose an environmental threat to soils and groundwater (as does sulfuric acid). The spatial distribution of petroleum products, COD and the amount of (Na^+) + (K^+) in technogenic basins of the Lviv city landfill is shown in Fig 3.

Very high sulphate content in the samples confirms the assumption of a significant amount of free sulfuric acid in the tar.

The complete absence of nitrites in the studied samples and the relatively low content of ammonium in them indicate aggressive oxidative conditions in these ponds, which excludes the existence of any living microorganisms in them. For the same reason, the leakage of these tars through the protective dams or soil banks can be a deadly threat to the plants and animals in the vicinity of the storage ponds. The spatial distribution of chlorides, sulphates, nitrates, phosphates, ammonium salts, and total iron is shown in Fig 4.

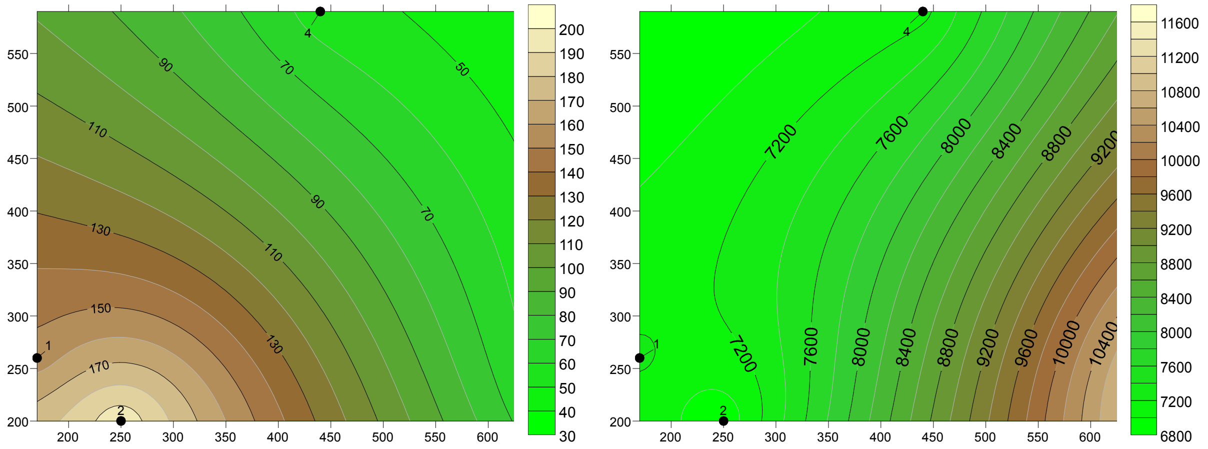
Table 3 presents values showing the multiplicity factors of exceeding the actual values of certain parameters determined in the investigated samples of acid tars, relative to the MPC for wastewater.

In fact, our studies confirm the data (Leonard et al., 2010) on the hazard of acid tars for the environment. Researchers found (Leonard et al., 2010) that the studied samples of acid tars contained aliphatic carbohydrates, cyclic carbohydrates, up to 12 primary polycyclic aromatic carbohydrates and many other organic groups, including organic acids (sulfonic acids, carboxylic acids and aromatic acids) phenyl, nitrile, amide, furan, thiophene, pyrrole and phthalates, many of which are toxic. An analysis of metals shows that Pb was present in significant concentration. The results show different transition peaks of the tested samples, which indicates their complexity and variability. The analysis further confirmed the presence of organic groups identified by GC/MS.



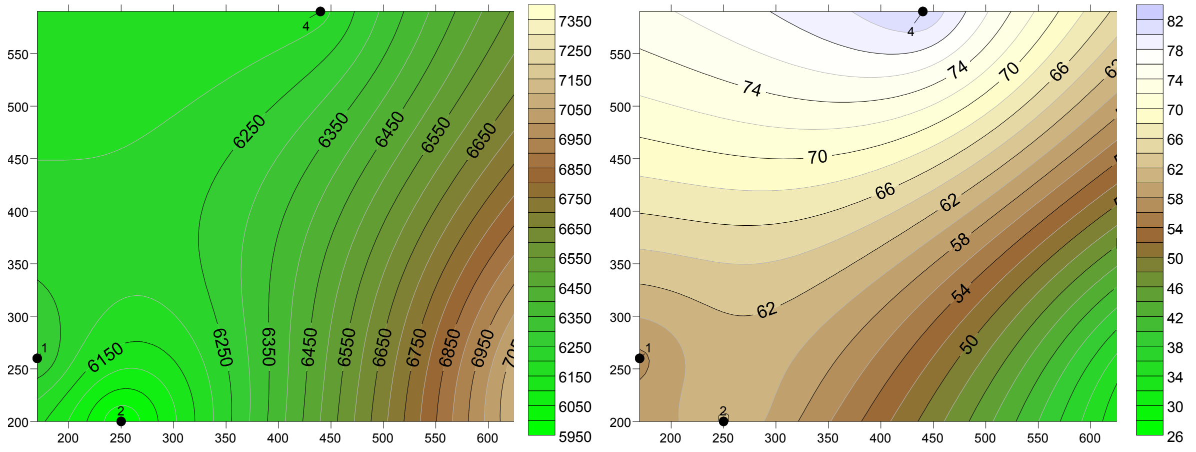
Odor at +20 °C (in points)

pH value



Suspended substances

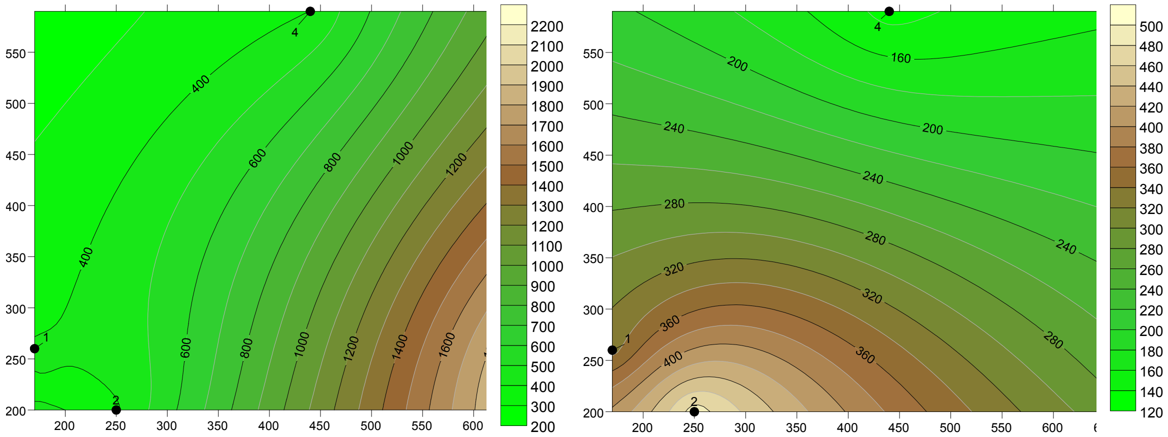
Dry residue (+90 °C)



Mineral residue (+800 °C)

Total hardness

Fig. 2. The spatial distribution of odor (in points), pH, suspended substances, dry and mineral residues, and total hardness in the technogenic water bodiesof the Lviv city landfill.



The sum (Na⁺) + (K⁺) (mg/dm3)

Chemical Oxygen Demand (COD)

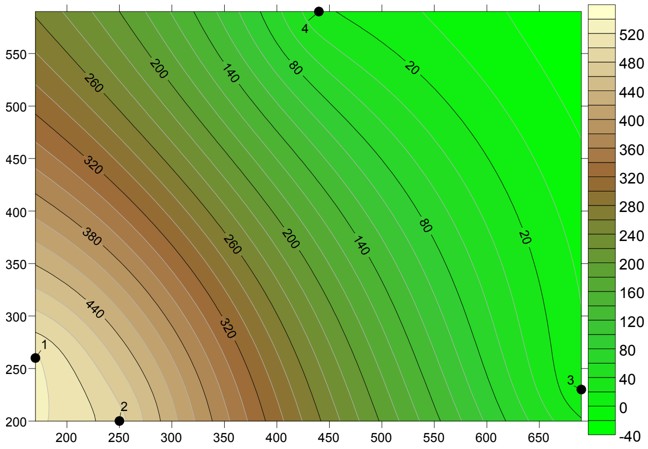
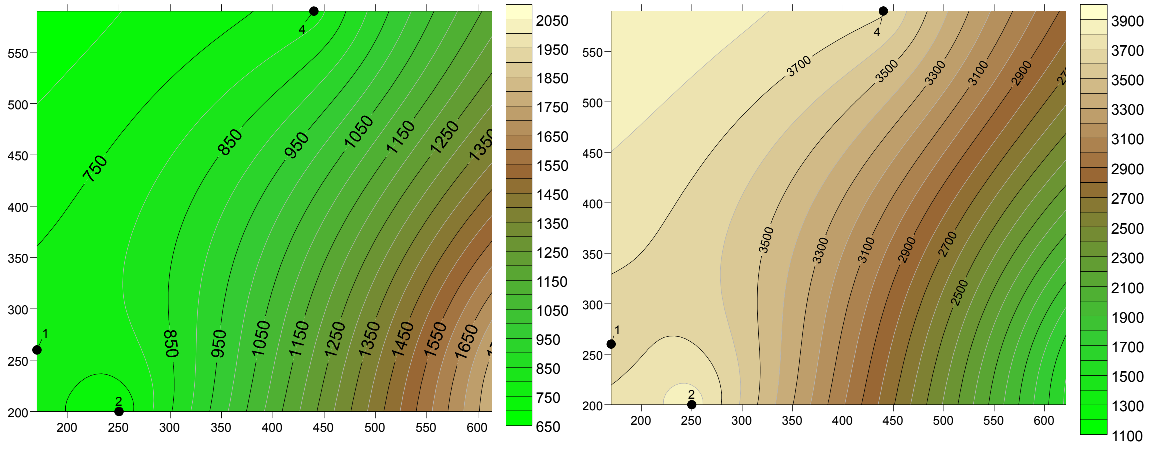


Fig. 3. Spatial distribution of petroleum products, COD and the amount of $(Na^+) + (K^+)$ in technogenic basins of the Lviv city landfill.

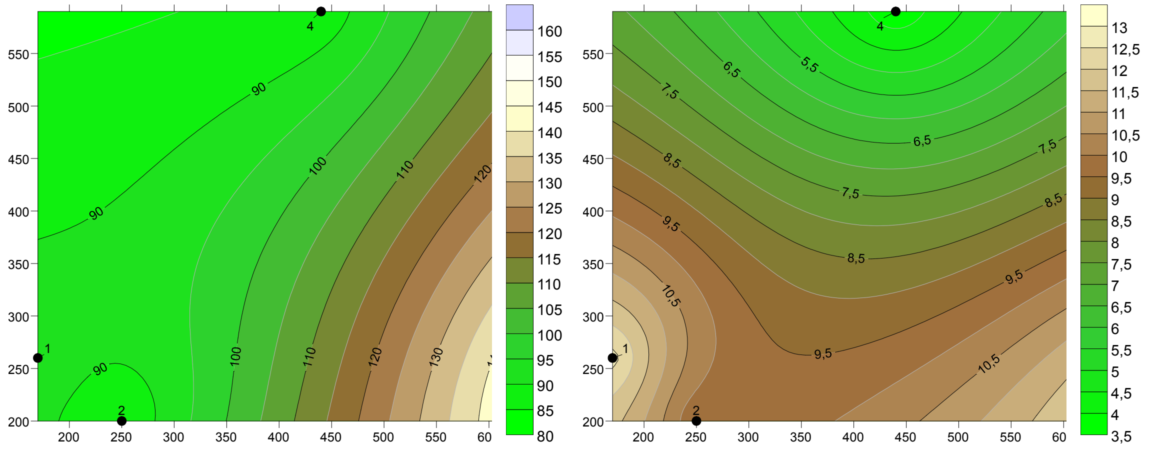
Table 3. Physico-chemical properties of the samples.

Item No.	Indicator	Measurement units	MPC	MPC excess	
				Sample 1	Sample 2
1.	Dry residue (+90 °C)	mg/dm^3	1,000	7.25	6.86
2.	Chlorides (Cl^-)	mg/dm^3	350	2.27	2.03
3.	Sulphates (SO_4^{2-})	mg/dm^3	500	7.25	7.72
4.	Nitrates(NO_3^-)	mg/dm^3	45	2.10	1.90
5.	Ammonium salts (NH_4^+)	mg/dm^3	38	1.03	0.96
6.	Total iron (Fe_{total})	mg/dm^3	2,5	5.84	4.84
7.	Chemical Oxygen Demand (COD)	mgO/dm^3	810	0.41	0.61
8.	Petroleum products	mg/dm^3	10	52.30	48.80



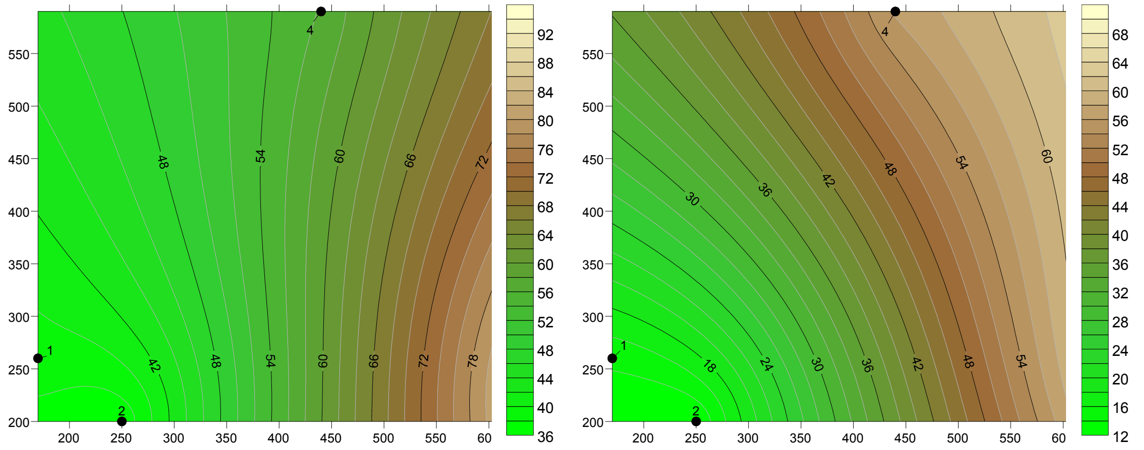
Chlorides (Cl^-) (mg/dm^3)

Sulphates (SO_4^{2-}) (mg/dm^3)



Nitrates (NO_3^-) (mg/dm^3)

Phosphates (PO_4^{3-}) (mg/dm^3)



Ammonium salts (NH_4^+) (mg/dm^3)

Total iron (Fe_{total}) (mg/dm^3)

Fig. 4. Spatial distribution of chlorides, sulphates, nitrates, phosphates, ammonium salts, and total iron

The results of micromechanical analysis made it possible to understand the surface characteristics of the samples and show that the distribution of pollutants is heterogeneous. The results obtained (Leonard et al., 2010) provide useful data on the composition, complexity and variability of acid tars, information on which is limited.

It was found that the most threatening pollution factor for territories adjacent to the acid tar storage ponds are petroleum products, sulphates and total iron content.

One of the most effective methods for cleaning soil contaminated with acid tars is phytomelioration (Popovich, 2016). In particular, in (Edenborn et al., 2015) the bio-coal from hard wood was studied for its potential use in phytostabilization of heavily contaminated soil in the territory of a sulfuric acid processing plant. The soil, which remained uninhabited for almost a century, contained a high concentration of lead, arsenic and antimony, and was both highly acidic and hydrophobic due to the presence of oil-based acid tar. Three approaches to application were tested with 10 and 20% (volume / volume) bio-coal: incorporation into the soil, top dressing on the surface, and layering in the soil. It was found (Edenborn et al., 2015) that uniform mixing of bio-coal from hard wood into the soil will facilitate rapid restoration in this area due to its low alkalinity with respect to the very high acidity of the existing soil. The surface application of bio-coal led to the most successful growth of *Elymus canadensis*.

Conclusions

Physicochemical analysis of acid tar samples from 2 storage ponds showed that even many years after their creation, a very high content of sulfuric acid in them remains a major environmental hazard. The simulation results made it possible to estimate the migration of toxic components contained in the tar into the biosphere components - hydrosphere and ecotope. It was found that, unlike other investigated technogenic water bodies, acid tars in the storage ponds have high indexes of petroleum products, chemical oxygen demand, sulphates, phosphates, and suspended substances.

The content of acids (in particular, sulfate acid) in the investigated acid tars is very high. It is many times higher than the MPC for wastewater and constitutes a significant environmental hazard for both the soils surrounding the ponds and groundwater in the vicinity of the landfill. The high sulfate content in the samples confirms the assumption of a significant amount of free sulfuric acid in the tars. The high content of petroleum products makes it possible to state that acid tar can be processed into liquid fuel by freeing it from excess water and salts. At the same time, these petroleum products in large quantities pose an environmental threat to soils and groundwater.

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Vascular flora of railway junctions in the Upper Tracian Lowland (Bulgaria)

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Abstract. Floristic studies have been carried out in the Upper Thracian Lowland in 14 railway stations: Dimitrovgrad, Knizhovnik, Malevo, Maslinovo, Most, Stara Zagora, Yabalkovo, Uzundzhovo, Harmanli, Skobelevo, Karadzhalovo, Stalevo, Plovdiv and Haskovo during the period 2017 to 2019. A total of 267 species, 174 genera and 53 families of higher vascular plants were identified. Alien and invasive species make up for a relatively high percentage of 9.4% in the railway flora. This indicates that the railway network plays an important role in the penetration and further dispersal of alien species in Bulgarian. The long-distance spread of railway areas is most common with passing trains and people moving. The largest number of alien species were found at stations with more intensive movement of passengers and cargo, where maneuvers are performed, stay of freight trains, such as Dimitrovgrad, Haskovo, Plovdiv, Stara Zagora. The alien species found at several of the studied railway stations were: *Convolvulus arvensis* L., *Erigeron canadensis* L., *Fallopia convolvulus* (L.) Á. Löve, *Amaranthus hybridus* L., *Amorpha fruticosa* L. Individual plants of strongly invasive *Ambrosia artemisifolia* L. have been identified in the railway station in the Haskovo City.

Key words: invasive alien species, railway flora, Tracian Lowland.

Introduction

The main feature of the flora in urban areas is the high participation of synathropic species, which have found conditions for establishment and secondary settlement there. The most common transport systems created by human activity are primary habitats and a corridor for the dispersal of several allochthonous and invasive species (Hayasaka et al., 2012). So far, in Bulgaria studies of the road and rail synanthropic flora are very fragmentary or refer only to a particular group of plants (Petrova & Vladimirov, 2009; Vladimirov & Petrova, 2009a; b). Litowski (1993) in a single inventory of the flora on the railway system in Plovdiv, reported 232 species, some of which were new to the area and a result of accidental transportation. In the last 10-15 years, more than 30 alien species of plants have settled in our antropophytes habitats (Petrova et al., 2012). These are the most common areas around rivers, railways and roads – generally anthropogenous heavily damaged areas. To date the invasive plants in Bulgaria were studied with numerous floristic notes (Petrova & Vladimirov, 2012), and summary information about 60 invasive and potentially invasive alien plant species in our flora has been given by Petrova et al. (2012). In this regard adventive and invasive species with the greatest impact are best studied, but more studies of species that are naturalized but not (yet) invasive are needed to improve understanding of the mechanisms leading to successful invasion (Pyšek et al., 2008).

The main purpose of this study was an inventory of vascular plants occurring at the railway stations of the Upper Tracian Lowland. The objectives associated with the main goal included and analysis of the railway flora and occurrence of alien and invasive species.

Material and Methods

The Thracian Lowland has developed as a crossroads for routes of goods and passenger transport. This study was carry out from spring to autumn in 2017- 2019, along with transect plots randomly selected 14 stations that fall on the territory of the Upper Thracian Lowland (Table 1). The sampling of study areas are represented (Fig. 1) on the BG-Mountains map (2019). At each site the investigated area was only the station proper (the part between the rails, between the lines, near railway buildings and platforms), up to the end of the stone embankments, but not only used but also abandoned lines were investigated.

For identifying the species was used standard keys, books and guides (Delipavlov & Cheshmedjiev, 2011; Tutin et al., 1964; Tutin et al., 1968-1980; Flora of Bulgaria (Yordanov, 1963-1979; Velchev, 1982-1989; Kozhuharov, 1995; Kozhuharov & Anchev, 2012). An inventory of the deposited available materials of Agricultural University-Plovdiv (SOA) from the studied area was made. In the course of this study, a 50 herbarium samples were collected and preserved, which is now stored at same herbarium. The origin of the adventive species is according to Assyov & Petrova (2012). Several species with cosmopolitan distribution were considered invasive, according to Petrova et al. (2012). In the analysis of species, the spectrum of life form was noted (Raunkiaer, 1934).

Results and Discussion

Flora of the investigated areas

The number of identified species families are provided in Table 1, for each of the stations, visited. On the railway network in the territory of the Upper Thracian Lowland a total of 267 species were identified, representing 174 genera in 53 families (Table 2). The analysis of the taxonomic structure of the railway flora in the studied area shows that the division Equisetophyta is represented by 1 species. In general, spore plants are poorly represented as a permanent element of the studied railway network. The species was found in the city of Plovdiv and the rail stations in the village of Malevo, where places with higher humidity have been registered. The lack of wet habitats is the reason for the absence of representatives of ferns (Pteridophyta). Quite logically, the seed plants (Magnoliophyta) take the largest share, of which the dicotyledons occupy 87.68%, and the monocotyledons are represented by 2 families, 23 genera and 33 species or 10.32%. The gymnosperms include 1 family, 1 genus and 1 species - *Cupressus sempervirens* L., introduced as an ornamental plant. In terms of taxonomic diversity, the largest number of species belong in the families: Asteraceae - 49 species, followed by Poaceae - 33 species, Fabaceae - 29 species, Caryophyllaceae - 11 species, Polygonaceae - 10 species, Lamiaceae - 9 species, Brassicaceae - 9 species, Apiaceae - 9, Rosaceae - 8 species, Chenopodiaceae - 8 species, Euphorbiaceae - 8, Scrophulariaceae - 7 species, Boraginaceae - 6, etc. 21 of the established families are represented by one genus and one species (Table 2).

The relatively high presence participation of species from the families Euphorbiaceae, Polygonaceae, Chenopodiaceae on the railway network. This is explained by the fact that these families include a large number of weed and ruderal species which grow in habitats with anthropogenic influence such as railway networks, roadside pavements, suburban habitats, landfills and others. As expected, the species richness was lowest in the relatively new and maintained rail stations - e.g. Stalevo, Yabalkovo, Karadzhalovo. Most species were found at the following stations: Most (68 species), Maslinovo (67 species), Knizhovnik (61 species), Plovdiv (59 species), Dimitrovgrad (54 species), Haskovo (48 species) - Table 1. This is explained on the one hand by the presence of more diverse habitats at these stations, and on the other hand by the reduced treatment of weeds in the end sections of the stations, where denser vegetation is found, including perennial herbaceous plants, sometimes shrubs and single tree species. *Tribulus terrestris* L. was found at all of the studied rail stations. Apart from this, the most frequently found species were: *Chenopodium album* L., *Centaurea solstitialis* L., *Cynodon dactilon* (L.) Pers., *Erigeron canadensis* L., *Convolvulus arvensis* L., *Crepis foetida* L., *Cichorium intybus* L., *Bromus sterilis* L., *Bromus tectorum* L., *Ballota nigra* L., *Avena fatua* L, *Amaranthus retroflexus* L., *Dasypyrum villosum* (L.) Borbás, *Eryngium campestre* L., *Fallopia convolvulus* (L.) Á. Löve and *Hordeum murinum* L. Species distribution over far linear distances by rail space takes place mostly with passing trains and people movement. At short distances, apart from trains and human activity, diasporas are carried by ants, rodents, wind, running water in heavy rain and more other. In some cases, the transfer of species takes place during construction and reparation works on railway lines and stations.

Ecological and biological structure of the floral elements

Among the most frequently noted plants were species that have a wide range of ecological preferences, such as: *Cynodon dactylon* (L.) Pers., *Poa bulbosa* L., *Galium aparine* L., *Bromus sterilis* L., *Stelaria media* (L.) Vill., *Capsella bursa-pastoris* (L.) Medik., *Thlaspi praecox* Wulfen, *Melilotus officinalis* (L.) Pall., *Trifolium arvense* L., *Euphorbia helioscopia* L., *Centaurea diffusa* Lam., *Chelidonium majus* L., *Eryngium campestre* L., *Cardaria draba* (L.) Desv., *Brassica nigra* (L.) K. Koch, *Raphanus raphanistrum* L., *Geum urbanum* L., *Cirsium ligulare* Boiss., *Phleum pratense* L., *Alopecurus pratensis* L., *Poa pratensis* L., etc. Most of these species usually occurred on abandoned railway tracks and therefore were not the result of strong anthropogenic influence.

Tree and shrub vegetation in the studied railway stations is poorly represented. There are single *Salix alba* L., *Sambucus ebulus* L., *Rosa canina* L., *Rubus sp.*, *Prunus spinosa* L., *Prunus cerasifera* Ehrh., *Pyrus communis* L., *Rubus caesius* L., seedlings of *Populus nigra* L., *Platanus orientalis* L., *Gleditsia triacanthos*, etc.

Analysis of the life cycle indicated therophytes were dominant in the life form spectrum – 44.36%. They are a typical element of the temperate zone. Hemicryptophytes comprised 27.44% in the investigated areas (Table 3). Most of these species were annuals plants. The high percentage of therophytes is mainly due to the specific regime of maintenance of the railway pavement - by mowing, treatment with chemicals, separation of plants from passing train compositions, etc., which gives an advantage to ephemeral species with a persistent seedbank. The development of annual species is also favored by large areas without vegetation, i.e. without competition. The least frequent were chamaephytes and geophytes – 0.36%. Altogether 17 species of phanerophytes were found, but most of these species were represented by seedlings or juveniles only. Anyway, the further growth of phanerophytes on active railway tracks is very difficult or even impossible.

Alien and invasive species

In the habitats of railways, 25 alien plant species were recorded (Table 4) and they constituted 9.4% of the total flora. This is not surprising as railway areas are considered both an entry for invasive species and the kind of habitats that accelerate the dispersal and migration of invasive plant species (Rutkovska et al., 2013). The natural region of origin of the major part of the alien species was North America (48%). Fewer species are from Asia (28%) and South America (20%). The remaining 4% includes species originating in cultivation and known from culture only. They are found on the railway embankments and near railways in most of the studied areas.

Table 1. List of the studied railway stations in the Upper Tracian Lowland.

No	Location (Train station)	Coordinates (begin and the end of studied area)		Alti-tude	No of Familia/ species
1	Dimitrovgrad	42.05569° N 25.58916° E	42.05407° N 25.59642° E	125 m	21/54
2	Knizhovnik	41.82726° N 25.59791° E	41.82585° N 25.59664° E	245 m	25/61
3	Malevo	41.86435° N 25.61650° E	41.86418° N 25.61532° E	158 m	18/43
4	Maslinovo	41.76920° N 25.54334° E	41.77008° N 25.54509° E	365 m	21/67
5	Most	41.74130° N 25.53047° E	41.74046° N 25.53036° E	450 m	21/68
6	Stara Zagora	42.41944° N 25.63429° E	42.41487° N 25.62784° E	196 m	24/47
7	Yabalkovo	42.07613° N 25.44704° E	42.07320° N 25.45202° E	199 m	16/28
8	Uzundzhovo	41.96711° N 25.63515° E	41.96827° N 25.63374° E	170 m	17/37
9	Harmanli	41.92371° N 25.92154° E	41.92142° N 25.92808° E	61 m	15/31
10	Skobelevo	42.09689° N 25.36891° E	42.09719° N 25.36519° E	130 m	12/24
11	Karadzhalovo	42.10309° N 25.31302° E	42.10230° N 25.32415° E	134 m	14/29
12	Stalevo	42.09615° N 25.39519° E	42.09610° N 25.39786° E	180 m	9/24
13	Plovdiv (st. Trakia)	42.12961° N 24.79967° E	42.14041° N 24.80346° E	160 m	30/59
14	Haskovo	41.93305° N 25.58411° E	41.93333° N 25.57601° E	194 m	22/48

Table 2. Number of genera and species by families.

Familia	Genera		Species	
	Number	% of investigated flora	Number	% of investigated flora
Equisetophyta				
Equisetaceae	1	0.57%	1	0.37%
Polypodiophyta	-		-	
Pinophyta				
Cupressaceae	1	0.57%	1	0.37%
Magnoliophyta				
Magnoliopsida				
Amaranthaceae	1	0.57%	3	1.74%
Apiaceae	9	5.2%	9	3.38%
Araliaceae	1	0.57%	1	0.37%
Asteraceae	34	19.65%	49	18.43%

Boraginaceae	4	2.31%	6	2.26%
Brassicaceae	6	3.47%	9	3.38%
Caesalpiniaceae	2	1.15%	2	0.75%
Cannabaceae	1	0.57%	1	0.37%
Caprifoliaceae	2	1.15%	2	0.75%
Caryophyllaceae	8	4.62%	11	4.13%
Chenopodiaceae	2	1.15%	4	1.5%
Convolvulaceae	1	0.57%	1	0.37%
Crassulaceae	1	0.57%	1	0.37%
Cucurbitaceae	1	0.57%	1	0.37%
Cuscutaceae	1	0.57%	1	0.37%
Dipsacaceae	2	1.15%	2	0.75%
Euphorbiaceae	2	1.15%	8	3.01%
Fabaceae	12	6.93%	29	10.9%
Fumariaceae	1	0.57%	1	0.37%
Geraniaceae	2	1.15%	5	1.88%
Hypericaceace	1	0.57%	1	0.37%
Lamiaceae	8	4.62%	9	3.38%
Malvaceae	3	1.73%	3	1.74%
Moraceae	1	0.57%	1	0.37%
Oleaceae	1	0.57%	2	0.75%
Onagraceae	3	1.73%	4	1.5%
Oxalidaceae	1	0.57%	1	0.37%
Papaveraceae	2	1.15%	2	0.75%
Plantaginaceae	1	0.57%	2	0.75%
Platanaceae	1	0.57%	1	0.37%
Polygonaceae	3	1.73%	10	3.75%
Portulacaceae	1	0.57%	1	0.37%
Primulaceae	1	0.57%	1	0.37%
Ranunculaceae	4	4.62%	4	1.5%
Resedaceae	1	0.57%	1	0.37%
Rosaceae	6	3.47%	8	3%
Rubiaceae	2	1.15%	4	1.5%
Salicaceae	1	0.57%	1	0.37%
Sapindaceae	1	0.57%	1	0.37%
Scrophulariaceae	2	1.15%	7	2.63%
Simaroubiaceae	1	0.57%	1	0.37%
Solanaceae	3	1.73%	4	1.5%
Tiliaceae	1	0.57%	1	0.37%
Ulmaceae	2	1.15%	3	1.74%
Urticaceae	1	0.57%	1	0.37%
Verbenaceae	1	0.57%	1	0.37%
Violaceae	1	0.57%	3	1.74%
Vitaceae	1	0.57%	1	0.37%
Zygophyllaceae	1	0.57%	1	0.37%
Liliopsida				
Cyperaceae	1	0.57 %	1	0.37%
Poaceae	22	12.14 %	33	12.03%
Total	**174**		**267**	

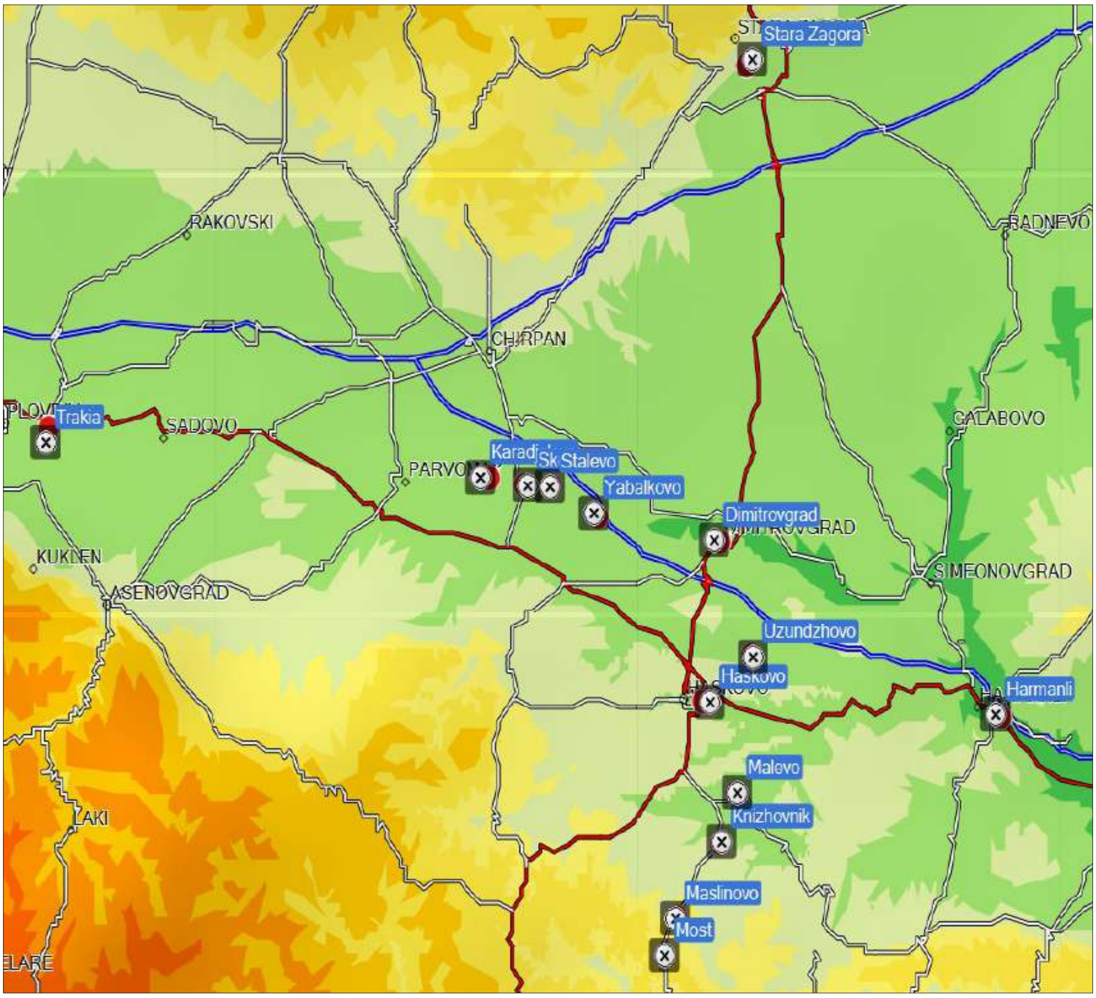

Fig. 1. Location map of the studied railway junctions of the Upper Tracian Lowland.

Ambrosia artemisiifolia has a native distribution range in North America. It is one of the most aggressive invasive alien species in SE Europe with a strong negative impact on human health and agriculture. In recent years, it has spread rapidly, also in Bulgaria, and its spread is facilitated by climate change (Thuiller et al., 2007). In our study, the species was found only at the city of Haskovo rail station. The species shows a tendency to spread in Tracian Lowland and has already been reported from the region (Petrova et al., 2012). It has previously also been reported from the Black Sea Coast and Northeast Bulgaria (Vladimirov & Petrova, 2009b), the Danubian Plain, Forebalkan, Valley of Mesta River, and the Valley of River Strouma floristic regions (Assyov & Petrova, 2012).

Settlers exclusively on the rails are *Bidens bipinnata*, *Erigiron acris*, and *Galinsoga parviflora*, which were found in a small number of stations. The invasive species *Euphorbia maculata* was found at Most Railway Station. *Xanthium spinosum* was represented by several individuals in three of the studied stations. At the end of the railway lines, seeds of *Robinia pseudoacacia*, *Amorpha fruticosa*, *Ailanthus altissima*, *Gleditsia triacanthos* successfully germinate, young individuals and seeds of *Morus alba*, *Platanus orientalis*, *Tilia* sp., and *Prunus cerasifera* are less common.

The highest number of alien taxa was found at the stations Haskovo, Dimitrovgrad and Plovdiv. This is related to the more intensive movement of passengers and freight at these stations, as well as to the performance of maneuvers and the stay of freight trains. The lowest share of alien species was found in Yabalkovo, Karazhdalovo and Stalevo, which is explained by the lower load of these stations, the lack of maneuvers, loading, and unloading activities, and better maintenance of the station area. Twelve species are included in the lists of dangerously invasive species in Europe (Daisie, 2009; Larsson et al., 2007). Although all stations carry out activities to control vegetation through mowing and herbicide treatment, in most places these activities are not very effective. This is due to both the one-time treatment of the species and the treatment in an inappropriate phenological phase e.g. often at the end of the growing season, when the seeds are ripe and invulnerable to the pesticides used, or the efficacy of mechanical control is limited by the ability of grasses to produce new stems after cutting).

Table 3. Divided of studied species by life form (by Raunkiaer).

Life form	Number of species	% of investigated flora
H	72	27.44%
Th	119	44.36%
Ph	17	6.19%
Th-H	51	19.17%
G	1	0.36%
H-Th	4	1.64%
Ha	1	0.36%
Ha-H	1	0.36%
Ha-G	1	0.36%
Total	**267**	

Table 4. Alien and invasive species of the investigated railway stations.

Species	Origin	Status	Occurrence (№ of stage in table 1)
Ailanthus altissima (Mill.) Swingle	Asia	Invasive	2, 6, 9, 13
Ambrosia artemisiifolia L.	N America	Invasive	14
Amaranthus hybridus L.	S America	Invasive	1, 2, 7, 8
Amaranthus albus L.	N America	Adventive	1, 5
Amorpha fruticosa L.	N America	Invasive	2, 9, 11, 13
Bidens bipinnata L.	N America	Potential invasive	3, 12, 14
Dysphania ambrosioides (L.) Mosyakin & Clemants	S America	Invasive	13
Dysphania pumilio (R.Br.) Mosyakin & Clemants	Australia	Invasive	10, 12, 14
*Erigeron annuus (*L.) Desf.	N America	Adventive	1, 10
Erigeron canadensis L.	N America	Invasive	4, 6, 8, 10, 13,14
Datura stramonium L.	S America	Invasive	7, 12
Euphorbia maculata L.	N America	Invasive	5
Galinsoga parviflora Cav.	N America	Invasive	10, 13
Gleditsia triacanthos L.	N America	Invasive	3, 13, 14
Bassia scoparia (L.) A.J.Scott.	Asia	Potential invasive	9
Koelreuteria paniculata Laxm.	SE Asia	Adventive	1

Lycium barbarum L.	Central Asia	Potential invasive	13
Medicago sativa L.	C Asia C Europe	Adventive	1, 6, 8
Morus alba L.	SE Asia	Adventive	1,13
Oenothera biennis L.	N America	Invasive	1
Portulaca oleraceae L.	S America	Potential invasive	6, 7, 9, 14
Robinia pseudoacacia L.	N America	Invasive	2, 4
Symphoricarpos albus (L.) S.F.Blake	N America	Potential invasive	7, 12
Helianthus annuus L.	N America (cultivar)	Adventive	1, 9
Xanthium spinosum L.	S America	Invasive	2, 4, 14

In general, the proportion of invasive species was high, indicating that the examined zones are highly antropophytic. As for the invasive spectrum, the richest group of species was the family Asteraceae – 7 species (25.9%) of identified alien species. A significant part was also made up of genera with invasive ruderal plants, e.g. *Chenopodium* - 2 species, *Amaranthus* - 2 species. Some species have been intentionally introduced and are widely used for landscaping and as ornamentals e.g. *Symphoricarbus albus, Koelreuteria paniculata, Oenothera biennis*.

Conclusions

The railway zones of the stations are artificial, relatively homogeneous ruderal habitats, which provide suitable conditions for secondary emergence and further resettlement not only of synanthropic but also of adventive and alien species, despite the regular management activities such as plant reaping, burning, and application of herbicides of the vegetation in the area of the railway stations. The plants typical of railway facilities and species strongly concentrated in operating railway areas are mainly therophytes with a short life cycle, comprising 44.36% of species in the present study. The participation of hemicryptophytes is relatively high (27.44%) and alien and adventive species make up for 9.4%. Among the most frequently found plants were species that have a wide range of ecological preferences. The above groups of species are dominated by plants adapted to dry habitats. Plant characteristics such as short life cycle and the ability to persist under dry conditions enable these plants to grow and thrive along operational rail tracks.

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Long-term Trends in Pseudo-nitzschia Complex Blooms in the Black Sea - is there a Potential Risk for Ecological and Human Hazards

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Abstract. The potentially toxic genus *Pseudo-nitzschia* is constantly present in phytoplankton community in the Black Sea often proliferating to bloom concentrations. Production of domoic acid (DA) has been confirmed by local *P. calliantha* strain and the presence of the neurotoxin has been detected in cultured molluscs and plankton samples from the Bulgarian Black Sea coast. This study aims to provide an overview of the long-term trends of *Pseudo-nitzschia* blooms in the Black Sea based on inventory of the available information for the period 1959-2019 and assess the relation with key environmental variables. *Pseudo-nitzschia* species diversity and identification constraints are critically analyzed along with data about the presence of DA in the basin. The results demonstrate that *Pseudo-nitzschia* spp. may bloom at any time of the year under different environmental regimes. Application of advanced identification techniques, technologically adequate regular monitoring, complemented by toxin analysis is essential to improve our understanding of *Pseudo-nitzschia* bloom dynamics in order to protect public and ecosystem health hazards.

Key words: *Pseudo-nitzshia,* harmful algal blooms, long-term trends, Black Sea.

Introduction

The marine planktonic genus *Pseudo-nitzschia* became a global concern after the first documented Amnesic Shellfish Poisoning (ASP) event in Canada in 1987 resulted in three deaths and 105 confirmed cases of acute intoxications (Bates et al., 1989). The clinical signs include gastrointestinal or neurological symptoms like vomiting, abdominal cramps, diarrhea, headache, and loss of short-term memory, and were related to the ingestion of mussels contaminated by neurotoxin domoic acid (DA) produced by *Pseudo-nitzschia* (Perl et al., 1990). Later findings that these microalgae can induce mass mortality in sea birds, fish and mammals further raised the interest to these diatoms (Work et al., 1993; Beltran et al., 1997; Scholin et al., 2000). Among the 52 species described so far in the literature within this genus, 26 were listed as toxigenic - capable to produce DA (reviewed in Bates et al., 2018). Many *Pseudo-nitzschia* species (including toxigenic) are widely distributed and of cosmopolitan nature (reviewed by Bates et al., 2018). In addition, both toxic and non-toxic species worldwide were reported to cause dense blooms under different environmental conditions, such as diverse temperature and salinity regimes, nutrients, pH, photoperiod, meteorological phenomena (reviewed in Lelong et al., 2012). Precise identification of *Pseudo-nitzschia* species is crucial in monitoring programs and ecological investigations but the high cryptic and pseudo-cryptic diversity reported in the genus hampered the determination by light microscopy (Quijano-Scheggia et al., 2010). Many of the species have morphological features in common (e.g. identical valve shape, length and width, equal overlap of cell length in chain). Their delineation needs detailed morphological observation (e.g. the structure of the poroid hymen, the density of band striae and the structure of the band striae) using transmission electron microscope (TEM) or scanning electron microscopy (SEM) and molecular methods (Lundholm et al., 2002, 2003, 2012; Quijano-Scheggia et al., 2009; Lim et al., 2012).

Data about *Pseudo-nitzschia* in the Black Sea are fragmentary, but the constant presence of these potentially toxic diatoms has been documented (Terenko & Terenko, 2012; Dzhembekova & Moncheva, 2014). *Pseudo-nitzschia* blooms are often observed basinwide (Nesterova et al., 2008; Moncheva et al., 2019). Coastal waters are the main ''hot spots'', where *Pseudo-nitzschia* blooms occur (Moncheva et al., 1995, 2001; Velikova et al., 1999; Türkoğlu & Koray, 2002; Vershinin et al., 2005; Terenko & Terenko, 2012) but high abundances have been recorded offshore as well (Mikaelyan, 1995). Although the available data on *Pseudo-nitzschia* toxicity in the Black Sea are scarce and fragmentary, DA has been detected in culture (Besiktepe et al., 2008) as well as in natural mussel and plankton samples (Peneva et al. 2011; Peteva et al. 2018).

Globally, the increased awareness and monitoring programs implemented effectively protect human health from intoxication and fatalities (Trainer et al., 2012). A special attention and more extensive study of the dynamics of the genus is crucial at a regional level, including the Black Sea. The aim of this study is to review the occurrence (spatial and temporal distribution) of *Pseudo-nitzschia* blooms in the Black Sea since 1959 to present and to diagnose potential ecosystem and human risk. *Pseudo-nitzschia* species diversity and identification problems are critically analyzed along with data about the presence of DA in the Black Sea. The relation between some environmental factors and growth of potentially toxic *Pseudo-nitzschia* species along the Bulgarian Black Sea is also discussed.

Material and Methods

The results are based on long-term own (published and unpublished) and literature data for the period 1959-2019. Significant part of the records used was built on species identification and cell counts by light microscopy (LM) on fixed samples. Available literature data produced by scanning and transmission electron microscopy (SEM and TEM) and/or molecular analysis were reviewed to explore the current knowledge on species diversity. Detailed information about the material and methods for the published data can be found in the original articles referenced in the text and Table 1. The unpublished own data set includes 1283 records from 205 stations in Bulgarian and Romanian waters collected between 1999-2019 under different research and monitoring programs and projects. Niskin bottle samples attached to CTD – Sea Bird Electronics 911 have been collected from various depths (0-100m) and 1 L aliquots were fixed with formaldehyde solution, buffered to pH 8-8.2 with disodiumtetraborate (4% final concentration). Taxonomic identification and cell counting were done under inverted light microscope by the Utermöhl (1958) method in Sedgwick-Rafter counting chambers. 400 cells were counted from each sample, while rare and large species were checked in the whole counting chamber.

Temperature (T) and salinity (S) were measured *in situ* at each station during the cruises by the CTD sensors. Seawater samples for chemical analyses were collected in parallel with phytoplankton samples at the stations located in the Bulgarian waters (1999-2015). Nutrients (nitrates - NO_3, phosphates - PO_4, and silicates – SiO_4) were analysed using standard methods (Grasshoff et al., 1999).

A generalized additive model (GAM) was used as a flexible modeling technique, which is useful to model more complex ecological responses and capture nonlinearities in data, to examine the effects of T, S, NO_3, PO_4 and SiO_4 on *Pseudo-nitzschia* abundance dynamics. Due to the difficult taxonomic identification under LM *Pseudo-nitzschia* species were clustered into two groups on the base of cell width - *P. delicatissima* group (< 3 μm) and *P. seriata* group (> 3 μm) (according to Hasle & Syvertsen, 1997). The association between *Pseudo-nitzschia* species abundance (cells l^{-1}) and *in situ* environmental variables was assessed on a matrix of 141 records of *P. delicatissima* group and 102 data of *P. seriata* group collected across the Bulgarian Black Sea coastal, shelf and open sea waters during 1999-2015. The statistical analyses and graphic representations were undertaken in R version 4.0.3 (2020-10-10) (R Core Team, 2020), CRAN package: mgcv (Wood, 2003, 2004, 2011, 2017; Wood et al., 2016), mgcViz (Fasiolo et al., 2018), gratia (Gavin, 2021), available through the CRAN repository (www.r-pro ject.org). QGIS2.18.10 and ArcGIS software version 10.2.2 (ESRI 2011) were used for mapping the *Pseudo-nitzschia* species spatial distribution and the reported blooms in the Black Sea.

Results and Discussion

Pseudo-nitzschia blooms in the Black Sea

Pseudo-nitzschia blooms proved to be a common phenomenon for different areas in the Black Sea. However neither clear spatial pattern of distribution or species identity involved, nor a recurrent specific seasonal occurrence during the year cycle was inferred from the analysis and mapping of available long-term data (Fig. 1, Table 1).

Among the first described outbreaks of these potentially toxic diatoms was the proliferation of *P. seriata* ($1x10^6$ cells l^{-1}) in March 1959 in Ukrainian waters (Odessa Bay) (Ryabushko, 2003) and in Bulgarian coastal waters (Varna Bay) lasting from February to May 1959 with a cell density exceeding $4x10^6$ cells l^{-1} (Petrova & Skolka, 1963). In the first half of 1966 (from January to August), a massive, long-lasting *P. delicatissima* outburst affected the entire Bulgarian coast and the trend continued in the next three years with *P. delicatissima* blooms occurring in spring 1968 and 1970, and in autumn 1969 (Petrova-Karadzhova, 1973). Since then, *Pseudo-nitzschia* blooms continuously occurred in Bulgarian waters more often dominated by *P. delicatissima* (Moncheva and Krastev, 1997; Velikova et al, 1999; Moncheva et al., 2001; Petrova et al., 2006; Petrova & Gerdzhikov, 2007, 2009, 2011). In May 2014 and 2016 bloom concentrations of *P. delicatissima* (between $1x10^6$ and $1.3x10^6$ cells l^{-1}) were registered at a couple of sites along the Bulgarian coast. Interestingly high abundances of *Pseudo-nitzschia* spp. were observed also in the brackish Varna Lake in September 2000 (Petrova & Gerdzhikov, 2007).

Between 1960 and 1970 a dramatic outburst of *P. delicatissima* ($21×10^6$ cells l^{-1}) was observed in Romanian waters (Bodeanu, 2002). Actually, all *Pseudo-nitzschia* blooms reported along the Romanian coast between 1960 and 2014 were caused by *P. delicatissima* mainly in spring (Bodeanu, 2002; Moncheva et al., 2019). Recently, in 2019, a spring widespread growth of *Pseudo-nitzschia* was registered along the Romanian coast (97.5% of the samples) with co-presence of *P. delicatissima* and *P. seriata* and maximum abundance > $4.39×10^6$ cells l^{-1} (unpublished own data).

In Ukrainian waters eight *Pseudo-nitzschia* outbreaks were recorded between 1959 and 2009 with *P. delicatissima* (in March 1959, June 1991, 1996, 2001 and May 2009, and in July 2006) and *P.*

pseudodelicatissima (in June 2008, max abundance 12x10^6 cells l^{-1}) and *Pseudo-nitszchia* spp. (December 2005, density 3x10^6 cells l^{-1}) cited as causal species, with the highest abundance found in Odessa Bay in the spring of 1996 when *P. delicatissima* reached 16.6×10^6 cells l^{-1} (Terenko & Terenko, 2012). *Pseudo-nitszchia* bloom in 2001 was found to cover a wide area of the Black Sea but the cell abundance exceeded 1x10^6 cells l^{-1} only in Ukraine (Bodeanu et al., 2004; Petrova & Velikova, 2004; Vershinin et al., 2004; Terenko & Terenko, 2008, 2012).

In 1996 two *Pseudo-nitzschia* blooms were reported in Turkish waters (Bay of Sinop) - in April of *P. delicatissima* (90×10^6 cells l^{-1}) and in July – of *P. pungens* (1.2×10^6 cells l^{-1}) (Turkoglu & Koray, 2002). Along the Georgian coast, a spring bloom of *P. delicatissima* in May 2009 was documented (Moncheva et al., 2019).

Interestingly, besides the numerous *Pseudo-nitszchia* outbreaks in the Black Sea coastal waters a massive spatially distributed winter bloom of *P. pseudodelicatissima* (4.19×10^6 cells l^{-1}) was detected in the open sea (Mikaelyan, 1995).

Generally, during the investigated period more bloom events have been registered in the coastal waters of Bulgaria, Romania and Ukraine with a large proportion reaching the highest cell abundances (> 5.5×10^6 cells l^{-1}) (Fig. 1). Blooms occurred in all seasons but more often they were registered in spring which is consistent with the general trend in the European waters (Hasle et al., 1996; Quiroga, 2006). On a decadal time scale, an increasing trend was observed in the second and third period in comparison with the pristine period (Table 1). However, it is difficult to generalize all these findings because as it was underlined the results are based on the accessible literature data for the different Black Sea regions.

Our long term data (1959-2016) in Varna Bay, Bulgarian waters show that even if the highest bloom density was reported in the period between 1959 and 1970 (13.5x10^6 cells l^{-1}) no clear trend in *Pseudo-nitzschia* blooms frequency during the decades was emerging (Fig. 2).

Out of the recounted 20 outburst events in Varna Bay, in the relatively pristine period for the Black sea region (up to 1970) there were 5 cases (4 caused by *P. delicatissima* and 1 - by *P. seriata).* During the highly eutrophic stage of the ecosystem (up to mid 1990) the number of these events was 8 (3 caused by *P. delicatissima* and 5 - by *P. seriata* with max density 5.3x10^6 cells l^{-1} in 1988) and thereafter (by 2016) – 7 blooms were registered with max density of 4.8x10^6 cells l^{-1} in 2009 due to *P. delicatissima*). After 1999 *P. seriata* group did not proliferate to bloom densities, even if some peaks were observed (frequently co-occuring with *P. delicatissima*).

Our dataset based on more frequent sampling campaigns between 2012 and 2018 in Varna Bay (Bulgarian waters) also confirmed the persistence of *Pseudo-nitzschia* throughout the year and in all seasons, except for spring 2017 when the genus was not detected (Figs. 3 and 4). As a common trend, *P. delicatissima* and *P. seriata* frequently co-occurred with the first species dominating in terms of frequency and abundance also reported for Ukrainian waters (Terenko & Terenko, 2012).

Pseudo-nitzschia species diversity and distribution

Presently, nine *Pseudo-nitzschia* species (*P. delicatissima*, *P. pseudodelicatissima*, *P. pungens*, *P. seriata*, *P. calliantha*, *P. inflatula*, *P. fraudulenta*, *P. prolongatoides*, *P. linea*) and one variety (*P. pungens* var. *aveirensis*) were reported in the Black Sea with eight of them precisely identified with electron microscopy and/or molecular methods (Fig. 5, Table 2).

One of the first records of *Pseudo-nitzschia* (*P. delicatissima* and *P. pungens*) in the Black Sea dates back to the middle of the 20th century (Morozova-Vodyanitskaya, 1954; Proshkina-Lavrenko, 1955). Spatially, among *Pseudo-nitzschia* species *P. delicatissima* and *P. seriata* were the most frequent, found in the coastal areas of all Black Sea countries. Both species occurred at many sites in Bulgarian waters as the most abundant too, while *P. pseudodelicatissima* and *P. pungens* appeared concurrently present along the Russian coast (Vershinin et al., 2005). *Pseudo-nitzschia pungens, P. pseudodelicatissima* and *P. delicatissima* were observed in Turkish waters in high concentrations (Turkoglu & Koray, 2002). In addition, *P. seriata* and *P. pseudodelicatissima* were found in open waters (Mikaelyan, 1995). Unlike the dominant *Pseudo-nitzschia*, some species have more restricted spatial distribution, spotted only at single areas - *P. inflatula* only in Ukrainian coast, *P. prolongatoides* only in Turkish waters, and *P. linea* only in Bulgarian waters (Fig. 5, Table 2). Interestingly, *P. delicatissima* was identified in surface sediment samples from the Black Sea via metabarcoding (Dzhembekova et al., 2018), although the information for the existence of resting stages in *Pseudo-nitzschia* spp. is rather contradictory and there is only one single report (Orlova & Morozova, 2009).

Generally, diversity within the genus is difficult to assess because of the complicated morphological identification under light microscopy, and the associated high uncertainty of the LM based data at a lower taxonomic level. Thus, the application of more sophisticated methods like scanning or transmission electron microscopy and/or molecular techniques is crucial for revealing the extant species diversity, including delineating cryptic and pseudo−cryptic species (Lelong et al., 2012). For example, on the basis of more advanced methods for identification (electron microscopy and molecular analyses) *P. calliantha* was defined in coastal waters of Turkey (Bargu et al., 2002), Romania (Lundholm et al., 2003) and Ukraine (Besiktepe et al., 2008; Ryabushko et al., 2008; Terenko & Terenko, 2012) (through the application of SEM and TEM) and in Bulgarian coast (via PCR amplification of LSU fragments with genus specific primers) (Dzhembekova et al., 2017a). The molecular technique has also allowed the determination of *P. pungens* var. *aveirensis* in Turkish (Baytut, 2013) and Bulgarian (Dzhembekova et al., 2017a) coastal waters. Worth to be noted, *P. calliantha* is comparatively newly described species within *P. pseudodelicatissima* complex (Lundholm et al., 2003). Altogether, the data reviewed suggest that the correct identification of the species reported earlier on the base of LM requires revision and re-examination as proved in other studies (e.g. McDonald et al., 2007; Terenko & Terenko, 2012; Rhodes et al., 2013; Ruggiero et al., 2015; Nagai et al., 2017).

Table 1. Number of *Pseudo-nitzschia* bloom events recorded within different periods and seasons in the Black Sea (1959-2019) (based on our own published and unpublished data and literature review data cited in the text).

Season	**up to 1970**	**1971-1999**	**after 2000**
Spring (April – June)	5	9	17
Summer (July – September)	0	7	1
Autumn (October - December)	1	1	1
Winter (January – March)	1	3	0

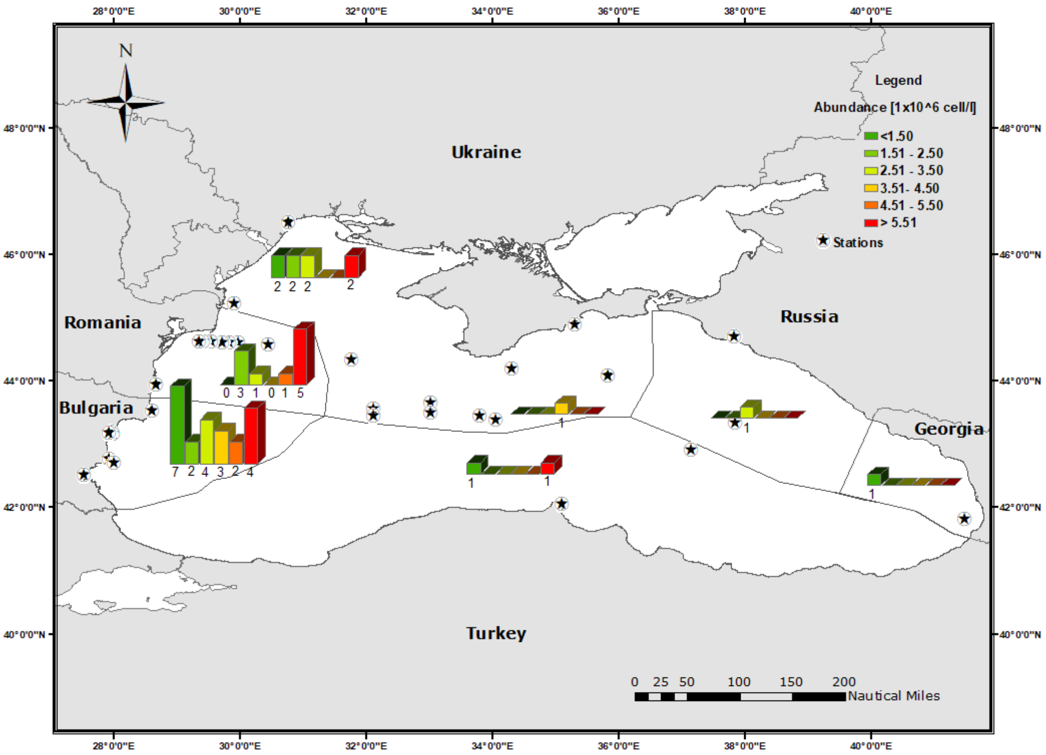

Fig. 1. Record of long-term *Pseudo-nitzschia* bloom events in the Black Sea countries marine waters (1959-2019) (based on our own published and unpublished data and literature review data cited in the text); the color denote cell abundance range (1x10^6 cells l^{-1}) and the columns - blooms frequency (number of events) within the corresponding abundance range; only the stations with bloom records are presented.

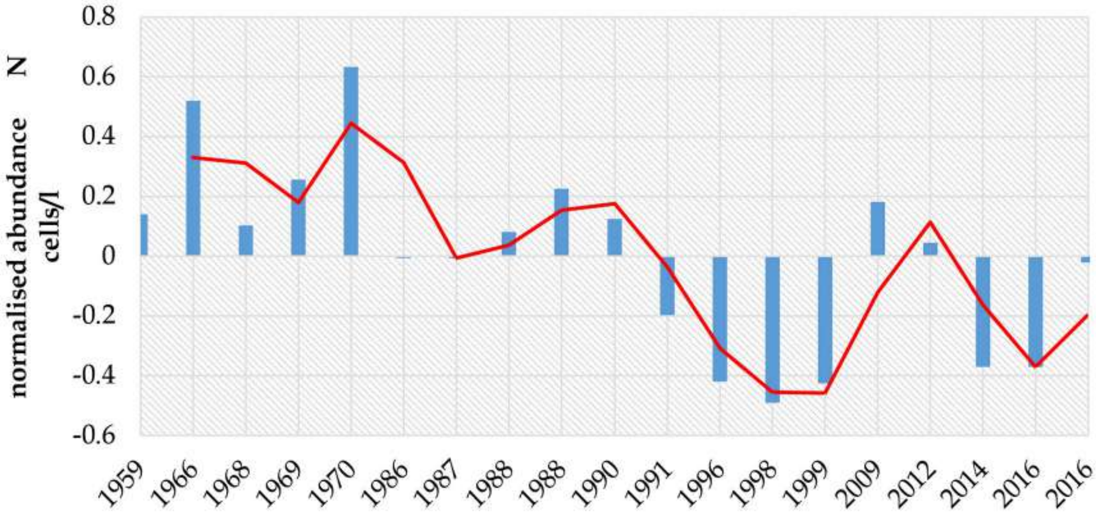

Fig. 2. *Pseudo-nitzchia* long-term (1959-2016) blooms variation (normalized abundance, N cells/l) in Varna Bay (Bulgarian waters); the red line denote the 2 period moving average trendline (own and literature data).

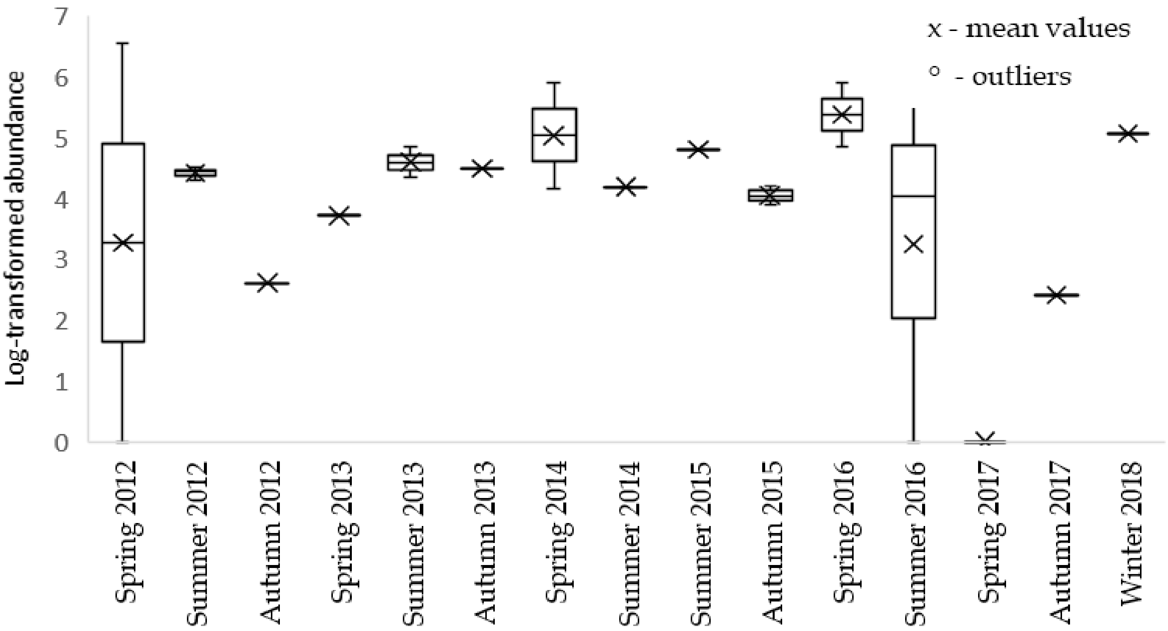

Fig. 3. Box plot - seasonal dynamics of *P. delicatissima* group abundance (cells/l, log-transformed) in Varna Bay (Bulgarian waters) in the period 2012-2018 (own data).

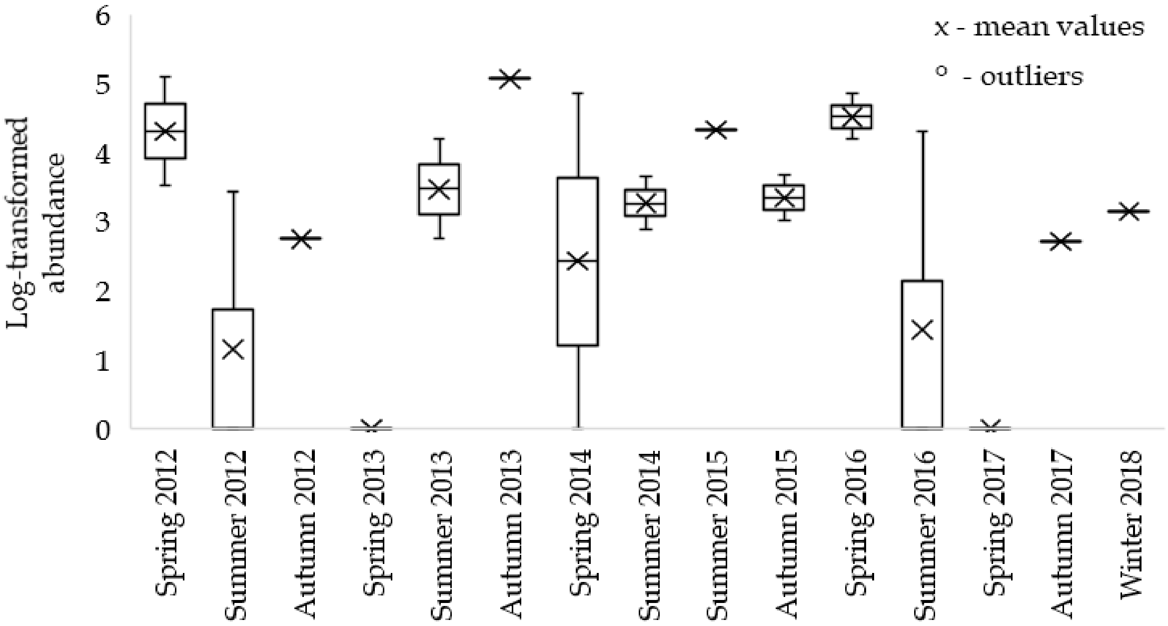

Fig. 4. Box plot - seasonal dynamics of *P. seriata* group abundance (cells/l, log-transformed) in Varna Bay (Bulgarian waters) in the period 2012-2018 (own data).

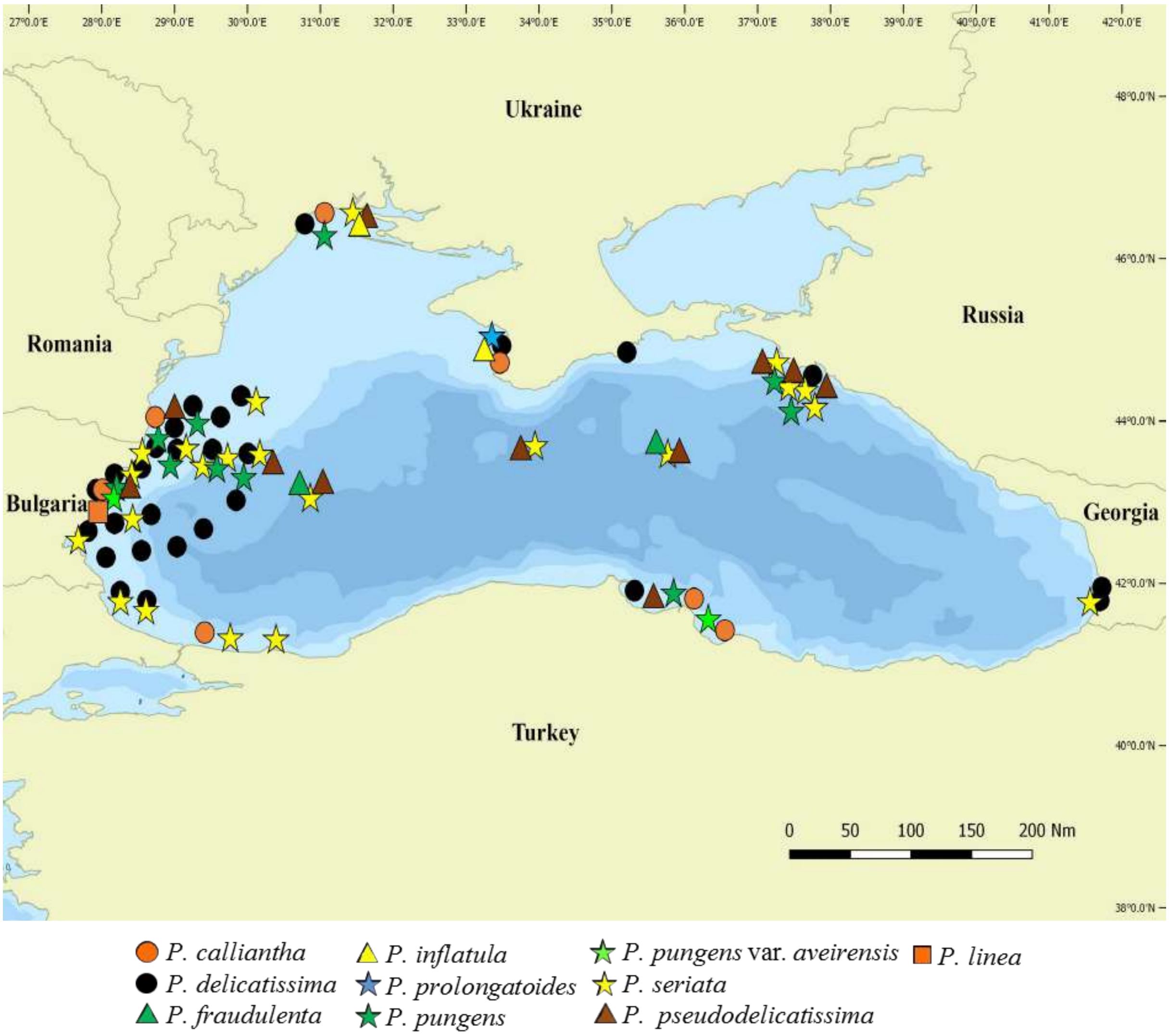

Fig. 5. *Pseudo-nitzschia* species distribution in the Black Sea.

Table 2. Inventory of Pseudo-nitzschia species, toxicity and distribution in the Black Sea. *Legend:* * Scanning Electron Microscopy (SEM); ** Molecular identification; [1] on the basis of cell width (according to Hasle & Syvertsen, 1997); BG – Bulgaria; GA – Georgia; RO – Romania; RU – Russia; TR – Turkey; UA – Ukraine.

Species	Group[1] (cell width)	Toxicity in the BS	Reference for toxicity	Distr.	Reference for distribution
***P. calliantha* Lundholm, Moestrup and Hasle, 2003** 🟠	*delicatissima* (1.4–1.8 µm)			BG	Dzhembekova et al., 2017a**
				RO	Lundholm et al., 2003*
				TR	Bargu et al., 2002*; Baytut et al., 2013*

		Yes maximum value of 0.95 pg DA cell^{-1}	Ryabushko et al., 2008 Besiktepe et al., 2008	UA	Ryabushko et al., 2008*; Besiktepe et al., 2008*; Terenko & Terenko, 2012*
P. delicatissima **(Cleve) Heiden, 1928** ●	*delicatissima* (ca. 2 µm)	Not tested	-	BG	Petrova-Karadzhova, 1973; Moncheva et al., 1995; Moncheva et al., 2001; Petrova & Gerdzhikov, 2012
				GA	Gvarishvili et al., 2010
				RO	Bodeanu, 2002; Cărăuş, 2012
				RU	Yasakova, 2013
				TR	Turkoglu & Koray, 2002
				UA	Kuzmenko, 1995; Terenko & Terenko, 2012*
P. fraudulenta **(Cleve) Hasle, 1993** ▲	*seriata* (4.5-6.5 µm)	Not tested	-	UA	Ratkova et al., 1989
P. inflatula **(G.R.Hasle) G.R.Hasle, 1993** △	*delicatissima* (1.5-2.5 µm)	Not tested	-	UA	Senicheva, 2002; Terenko & Terenko, 2012*
P. prolongatoides **(G.R.Hasle) G.R.Hasle, 1993** ★	*delicatissima* (0.5-2.5 µm)	Not tested	-	TR	Ozturk, 1998
P. pseudodelicatissima **(Hasle) Hasle, 1993** ▲	*delicatissima* (1.5-2.5 µm)	No	Vershinin et al. 2005	BG	Dzhembekova & Moncheva, 2014; Dzhembekova et al., 2017b**
				RU	Mikaelyan, 1995*; Vershinin et al., 2005*
				TR	Baytut et al., 2010
				UA	Ratkova et al., 1989; Terenko & Terenko, 2012*
P. pungens **(Grunow ex Cleve) G.R.Hasle, 1993** ★	*seriata* (3.0-4.5 µm)	Not tested	-	BG	Dzhembekova & Moncheva, 2014; Dzhembekova et al., 2017b**
				RU	Vershinin et al., 2005
				TU	Turkoglu & Koray, 2002; Baytut et al., 2010
				UA	Terenko & Terenko, 2012*
P. pungens **var.** ***aveirensis*** **Lundholm, Churro, Carreira and Calado, 2009** ☆	*seriata* (2.7-3.7 µm)	Not tested	-	BG	Dzhembekova et al., 2017a**
				TR	Baytut et al., 2013**
P. seriata **(Cleve) H.Peragallo, 1899** ☆	*seriata* (5.5-8.0 µm)	Not tested	-	BG	Petrova & Skolka, 1963; Petrova & Gerdzhikov, 2012; Dzhembekova & Moncheva, 2015
				RO	Cărăuş, 2012

				RU	Ratkova et al., 1989; Vershinin & Moruchkov, 2003; Mikaelyan, 1995*;Yasakova, 2013
				TR	Uysal, 2002
				UA	Ryabushko, 2003; Terenko & Terenko, 2012*
				GA	Komakhidze & Mazmanidi, 1998
***P. linea* Lundholm, Hasle & G.A.Fryxell, 2002** 🟧	*delicatissima* (1.8-2.2 μm)	Not tested	-	BG	Dzhembekova et al., 2017a**

Toxicity of Pseudo-nitzschia in the Black Sea

The available information on *Pseudo-nitzschia* toxicity in the Black Sea is very limited. DA detection was based either on single tests of *Pseudo-nitzschia* cultures (Besiktepe et al., 2008) or fragmentary studies on shellfish extracts and water samples (Peneva et al., 2011; Peteva et al., 2018). Toxic *P. calliantha* strains were isolated from Sevastopol Bay and DA was detected in batch culture with variable concentrations through the growth cycle and maximum (0.95 pg DA cell^{-1}) observed during the early exponential phase (Besiktepe et al., 2008). Among the other species, only *P. pseudodelicatissima* has been tested for toxicity but the examined strains proved non-toxic (Vershinin et al., 2005). The analysis of mussels tissue samples (*Mytilus galloprovincialis*) from aquaculture farms along the Bulgarian Black Sea coast (2009-2011) indicated that none of the tested 15 samples exceeded the regulatory level for DA of 20 mg kg^{-1} in shellfish (Peneva et al., 2011), the measured DA concentrations ranged from not detected to 0.55 mg kg^{-1}. During a later study (spring 2017) DA below the threshold was found in all tested samples of wild and farmed mussels, and plankton net samples (Peteva et al., 2018), however without targeting the source species. Globally, among all *Pseudo-nitzschia* species identified in the Black Sea, six (*P. delicatissima*, *P. pseudodelicatissima*, *P. calliantha*, *P. fraudulenta*, *P. pungens* and *P. seriata*) were reported as capable of DA production, two (*P. inflatula*, *P. linea* and *P. pungens* var. *aveirensis*) as non-toxic and one (*P. prolongatoides*) has not been tested yet. Some authors support the hypothesis that all species within the genus could be toxigenic under specific conditions (Parsons et al., 1999; Wells et al., 2005) and new reports confirmed the induction of DA production by different factors e.g, environmental conditions, zooplankton grazers, life cycle (Lema et al., 2017; Lundholm et al., 2018; Sauvey et al., 2019). Intraspecific diversity of toxin production requires more investigations to be carried out at a local level. Although globally the negative effects of *Pseudo-nitzschia* related toxicity have been prevented thanks to the increased research and effective implementation of monitoring programs and adequate management measures (Trainer et al., 2012; Bates et al., 2018) there is still limited institutional and medical awareness of shellfish poisoning events in the Black Sea countries (Vershinin & Moruchkov, 2003).

Environmental variables and Pseudo-nitzschia abundance

GAM was applied, to assess the significance of *in situ* environmental variables association with *Pseudo-nitzschia* group abundance (*P. delicatissima* group and *P. seriata* group) in the Bulgarian Black Sea waters. The *in situ* matrix was constructed based on data for temperature (T), salinity (S), nitrates (NO_3), phosphates (PO_4) and silicon (Si), covering the period 1999-2015 (statistical summary of the data is given in Table 3).

P. delicatissima group abundance was modeled first by the implementation of the following GAM:

$$P_{del} = \alpha + f_1(T) + f_2(S) + f_3(NO_3) + f_4(PO_4) + f_5(Si) + \varepsilon$$

GAM results (Table 4, Fig. 6) showed that nonlinear effects of salinity, temperature and nitrates concentrations have had statistically significant effects on *P. delicatissima* abundance. The model explained roughly 60% of total variations in abundance data.

Double penalty approach was applied to assess the terms effects on *P. dellicatissima* group abundance (by penalizing the null space), a process considered also as selection of model terms (Fig. 7). The results confirmed that PO_4 and SiO_4 have zero effect on *P. dellicatissima* group abundance.

Two-way interaction terms were also included as tensor products with the aim to study the interaction terms effects:

$$P_{del} s(T)+s(S)+s(NO_3)+s(PO_4)+s(Si)+ti(T,NO_3)+ti(Si,NO_3)+ti(Si,PO_4)+\epsilon$$

Their inclusion had not improved the model (deviance explained 56.2%), however underlined statistically significant interaction term effects (T in a combination with NO_3 concentrations, and SiO_4 interaction with NO_3 and PO_4 concentrations), which are of interest for further study and exploration.

PO_4 and SiO_4 concentrations were found to have statistically significant nonlinear association with *P. seriata* group abundance and linear association with salinity. The model:

$$P_{ser}=\alpha+f_1(T)+f_2(S)+f_3(NO_3)+f_4(PO_4)+f_5(Si)+\varepsilon$$

explained 31.5% of the deviance (i.e. the proportion of variance in *P. seriata* group abundance, explained by the predictor variables) (Table 4).

Stepwise model selection was implemented aiming at improving the model along with inclusion of linear terms for temperature salinity and nitrates, 2-way interaction terms were also included in the model, however neither of these model selection techniques provided better results. Double penalty approach was applied to provide insights on terms effects strength on *P. seriata* group abundance, showing that NO_3 concentration had zero effect and the effect of temperature remains unexplained by the model.

Worth noting, *Pseudo-nitzschia* abundance variations remained unexplained by the models could well be attributed to the uncertainty associated with the species identification, discussed above, as well as to the lack of systematic sampling frequency to follow the evolution of phytoplankton bloom dynamics during the outburst events. Unaccounted environmental noise could also have affected the results as it naturally contributes to data uncertainty, regardless of the robustness of GAM.

Worldwide, the growth and distribution of *Pseudo-nitzschia* has been linked to various environmental parameters and the relationship differs among species and regions. In Scottish waters, *P. seriata* group was linked to temperature, whereas *P. delicatissima* group was linked to salinity and the presence of ammonium (Bresnan et al., 2015) and seasonally *P. delicatissima* group dominate in spring, while *P. seriata* group dominate in the late summer/early autumn (Fehling et al., 2006). In the southern North Sea the highest abundance of *P. seriata* complex was observed at low nutrient levels (particularly DIN and $Si(OH)_4$), increasing surface solar irradiance and high pH, and the *P. seriata* complex seemed to tolerate higher variable environmental conditions (e.g. nutrients) as compared to the *P. delicatissima* complex (Delegrange et al., 2018). Ajani et al. (2013) found that in the coastal waters of south-eastern Australia *P. seriata* group abundance was temperature dependent, whereas *P. delicatissima* group development might be triggered by complex interactions between light availability and water temperature. In addition, the combination of factors related to *Pseudo-nitzschia* group abundance is species specific. For example, *P. pseudodelicatissima* growth was favored by high temperatures and salinities between 15 and 35‰ in Danish coastal waters (Lundholm et al., 1997). *P. calliantha*, *P. pseudodelicatissima* and *P.*

delicatissima abundance was positively correlated with nitrates in the Adriatic Sea and in the Bay of Fundy, and moreover in the Adriatic Sea a positive correlation was found between *P. delicatissima* abundance and salinity, whereas the densities of *P. calliantha* were negatively correlated with temperature (Caroppo et al., 2005; Kaczmarska et al., 2007). In the Marmara sea (Golden Horn estuary) for *P. calliantha* a significant negative correlation was found with temperature and a significant positive correlation with salinity, and in addition the species was correlated with $NO_3 + NO_2$ (Tas and Lundholm, 2017). *P. americana* and *P. australis* were positively correlated with high chlorophyll a, temperature and $Si(OH)_4$ concentrations, whereas *P. delicatissima* and *P. fraudulenta* were more abundant under high NO_3 and low $Si(OH)_4$ concentrations in samples from the Baie des Veys (Klein et al., 2010). *P. pungens* was more abundant in samples with higher concentrations of phosphates and lower concentrations of nitrates in the Bay of Fundy (Kaczmarska et al., 2007). In addition the association of any species to a single or a combination of environmental variables is further complicated by the reported intrinsic wide morphological and morphometrical variability as observed in the *P. pungens* clade I population from the Adriatic Sea (Accoroni et al., 2020). Although the relationships between environmental variables and *Pseudo-nitzschia* abundance are complex and vague, the knowledge at a regional scale is valuable allowing *Pseudo-nitzschia* bloom dynamics to be modelled and predicted (Anderson et al. 2009; Lane et al. 2009; Palma et al. 2010).

In the Black Sea, the exact mechanisms of *Pseudo-nitzschia* blooms are still uncertain and complex and *Pseudo-nitzschia* could proliferate under multiple environmental regimes (Terenko & Teremko, 2012; Dzhembekova & Moncheva, 2015). Suggesting that the *Pseudo-nitzschia* dynamics is controlled by a complex of environmental factors unique to a particular region (Trainer et al., 2012) future more comprehensive study could enable more robust conclusions for the effects of environmental parameters on growth and DA production at a local scale.

Table 3. Summary of descriptive statistics of environmental variables and *Pseudo-nitzschia* abundance (1999-2015) (own data).

Variable	**Mean**	**SE Mean**	**StDev**	**Min**	**Median**	**Max**
***P. delicatissima* group (cells l^{-1}), n=141**	87,643	30,297	359,751	5,101	27,549	3,495,680
T [C°]	22.07	0.34	4.03	7.67	23,005	26.27
S [‰]	16.87	0.08	0.96	13.34	17.02	18.53
NO_3 [μmol l^{-1}]	0.46	0.05	0.57	0	0.28	3.71
PO_4 [μmol l^{-1}]	0.14	0.02	0.19	0	0.09	1.76
Si [μmol l^{-1}]	3.27	0.38	4.5	0	2.48	46.4
Variable	**Mean**	**SE Mean**	**StDev**	**Min**	**Median**	**Max**
***P. seriata* group (cells l^{-1}), n=102**	19,152	3,307	33,397	1,031	9,311	211,355
T [C°]	22.1	0.39	3.91	8.21	22.76	26.27
S [‰]	16.9	0.09	0.89	14.17	17.04	18.15
NO_3 [μmol l^{-1}]	0.44	0.07	0.73	0	0.21	3.79
PO_4 [μmol l^{-1}]	0.15	0.02	0.2	0	0.09	1.56
Si [μmol l^{-1}]	3.38	0.22	2.2	0	2.93	9.26

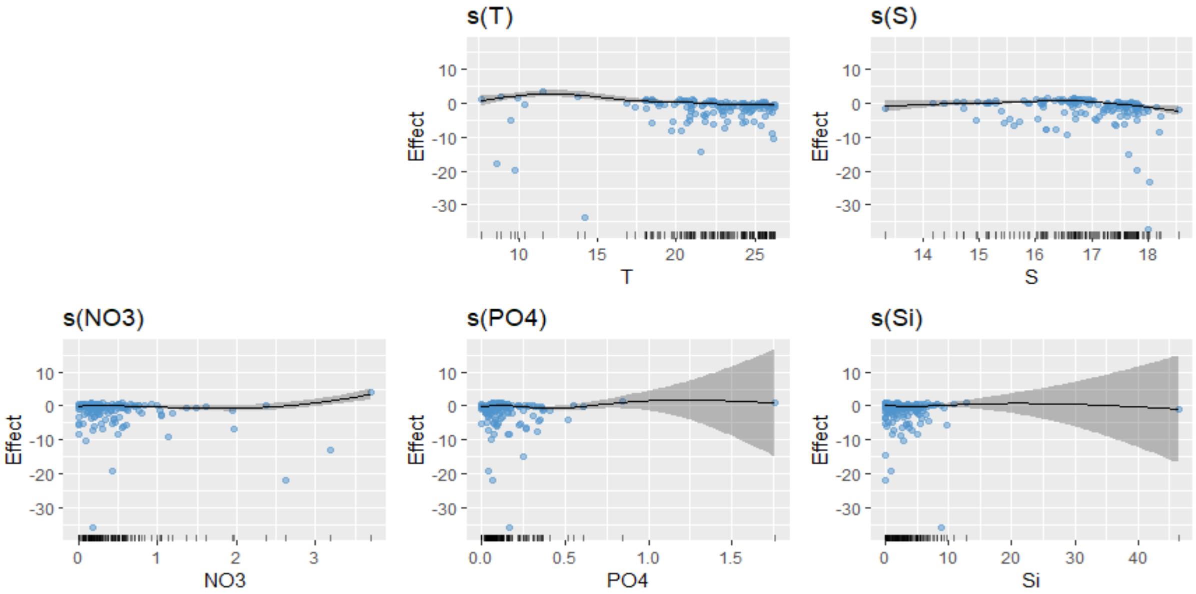

Fig. 6. Smooths of GAM terms showing the effect of various environmental variables on *P. dellicatissima* group abundance. Locations of observations are shown as vertical lines on the x-axes, the blue dots show the observations partial residuals. Solid lines are the estimates of the smooths; the dashed lines indicate 95% confidence intervals.

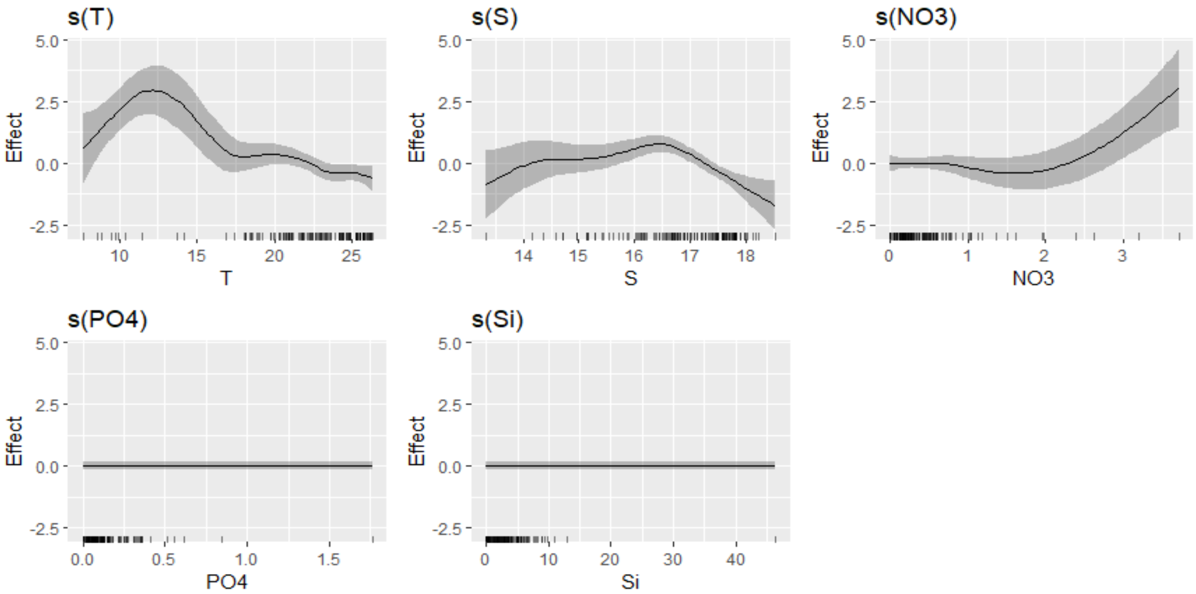

Fig. 7. *P. dellicatissima* group abundance GAM model terms effects evaluation after the implementation of double penalty approach.

Table 4. GAM model statistical summary (*P. delicatissima* group).

Family: Negative Binomial(1.096), Link function: log
Formula: Pdel ~ s(T) + s(S) + s(NO_3) + s(PO_4) + s(Si)
Parametric coefficients:
Estimate Std. Error z value Pr(>|z|)
(Intercept) 10.70098 0.08044 133 <2e-16 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(T)	4.888	5.899	39.117	3.77E-06	***
s(S)	4.118	5.09	32.012	7.48E-06	***
s(NO_3)	3.669	4.51	18.653	0.00165	**
s(PO_4)	4.003	4.818	10.027	0.09241	.
s(Si)	2.445	2.956	3.275	0.39121	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
R-sq.(adj) = 0.511 Deviance explained = 59.8%
-REML = 1681.3 Scale est. = 1 n = 141

Table 5. GAM model statistical summary (*P. seriata* group).

Family: Negative Binomial(1.096), Link function: log
Formula:Pser ~ s(T) + s(S) + s(NO3) + s(PO4) + s(Si)
Parametric coefficients:
Estimate Std. Error z value Pr(>|z|)
(Intercept) 9.64181 0.09489 101.6 <2e-16 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Approximate significance of smooth terms:

	edf	Ref.df	Chi.sq	p-value	
s(T)	1.500	1.817	2.646	0.18244	
s(S)	1.001	1.001	4.777	0.02888	*
s(NO3)	1.000	1.001	1.339	0.24727	
s(PO4)	2.209	2.661	8.207	0.03978	*
s(Si)	4.339	5.333	18.347	0.00347	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
R-sq.(adj) = 0.068 Deviance explained = 31.5%
-REML = 1100.6 Scale est. = 1 n = 102

Conclusions

In summary, *Pseudo-nitzschia* blooms are a common feature of Black Sea waters with four out of nine species proliferating in concentrations over 1×10^6 cells l^{-1}. *Pseudo-nitzschia* blooms are more likely to occur in spring but the genus representatives grow under different environmental conditions and may bloom at any time of the year. The regional differences in the *Pseudo-nitzschia* species diversity are likely to be related to the arduous morphological identification that could result in incorrect assessment of species composition. Despite the occurrence of high *Pseudo-nitzschia* spp. counts in some regions, no shellfish data exceeding the DA regulatory limit or ASP related health problems have been diagnosed. Given the uncertainties associated with the species identification, the scarcity of toxicity measurements during bloom events and the irregularity of environmental monitoring leave the answer to the question about the potential ecosystem and human risk pending. Hence, the application of advanced identification techniques, technologically adequate regular targeted monitoring of *Pseudo-nitzschia*, complemented by toxin analysis would be essential both to improve the understanding of *Pseudo-nitzschia* bloom dynamics in the Black Sea and for implementation of adequate HAB management to protect public and ecosystem hazards.

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On the Golden Jackal's (Canis aureus Linnaeus, 1758) Distribution During Snow Period in the Central Part of Stara Planina Mts, Bulgaria

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Abstract. The aim of the present study was to determine the frequency of occurrence of the Golden jackal according to snow cover changes in different elevations and slope exposure in the central parts of Stara Planina Mts, Bulgaria. To achieve the purpose, 15 camera traps were used, at both, south and north slopes of the mountain on 5 different elevations. The data revealed a significant difference in the number of jackals detected between the southern and the northern slopes as well as at different elevation of the Central Stara Planina Mts. The largest number of jackals was found at the lowest level (up to 600 m a.s.l.). With elevation increasing, the number of detected individuals decreased. The jackal presence at 1000 - 1200 m a.s.l. in both studied areas was sporadic, and over 1200 m a.s.l. the species was not detected. The Golden jackal from the region of Central Stara Planina Mts demonstrated clear preferences to the southern slopes up to 800 m a.s.l.

Key words: camera traps, elevation, snow, slopes, mountain, jackal.

Introduction

The Golden jackal (*Canis aureus* Linnaeus, 1758) is one of the most widespread canid species in Central and Southern Europe. It is numerous in the Balkan Peninsula, in Bulgaria particularly (Kryštufek et al., 1997). After the 1980s, the species' expansion began from Balkan Peninsula to the north and west, and nowadays it is found in Austria, Hungary, Italy, Ukraine, Poland, Belarus, Estonia, Latvia and Lithuania (Arnold et al., 2012; Trouwborst et al., 2015). Of all occupied European countries, however, the highest jackal population density remained in Bulgaria (Markov, 2012). There, the Golden jackal has a big economic impact (Stoyanov, 2012) and it is hunting object in order to regulate its number (SG, 2000). The species occupies almost the entire territory of the country, appearing sporadically in the highest parts of the mountains (Markov et al., 2018). The present study could clarify the Stara Planina Mts importance as a partial barrier for the jackal's spread which started from Strandzha Mountains to the north in the 1970s (Markov, 2012). The Stara Planina Mts has been overcome by the jackal in its lowest parts along the Black Sea coast (personal observation). It can be assumed that nowadays this long mountain chain isolates partially the jackal populations from Northern and Southern Bulgaria. During winter the conditions in the mountain habitats change drastically, especially after a snowfall. The behavioral response of the Golden jackal to the severe winter conditions is not studied in details, and the data on its distribution in Bulgaria according to different altitudes is scarce. The only information on its number in the highest parts of the Stara Planina Mts is obtained from the local hunters and from forestry officials. The aim of the present study was to determine the frequency of occurrence of the Golden jackal according to snow cover changes in different elevations and slope exposure in the central parts of Stara Planina Mts, Bulgaria.

Study Area

To reveal the Golden jackal's distribution related to the altitude in winter in the Balkan Mountains and to establish the influence of snow cover on its number, two areas with different exposure (southern and northern) were selected (Fig. 1). The first region is a part of the southern slopes of the Central Stara Planina Mts. The terrain is steep, difficult to access, with low anthropogenic influence (mainly hunting). The average January temperatures for the region range from - 1° C to + 1° C (Nikolova, 2002).

The second region is a part of the northern slopes of the Central Stara Planina Mts, more sloping and smoothly passing in hilly terrain to the north. The climate in this part of the Stara Planina Mts is harsher, compared to the first region, with an average air temperature in January varying from - 1 ° C to - 4 ° C (Nikolova, 2002), and the snow cover preserves longer. With altitude increasing, the snow cover in the highest parts of the region lasts up to 190 - 200 days a year (Mateeva, 2002). The anthropogenic influence is weak, as in the first region.

With elevation changing, the vegetation in both studied areas formes belts, that are same for both, the southern and northern slopes. The two studied areas in the Central Balkan Mountains comprise 5 vegetation belts, developed in all Bulgarian mountains: Mediterranean vegetation (up to 300 - 400 / 500 m a.s.l.); xerothermic oak forests (up to 600 - 700 m a.s.l.); hornbeam-gorun forests (from 600 - 700 to 900 - 1000 m a.s.l.); common beech forests (from 900 - 1000 to 1300 - 1500 m a.s.l.) and coniferous forests (from 1300 - 1500 to 2000 - 2100 m a.s.l.) (Velchev, 2002).

Material and Methods

For the purpose camera trapping was used which excludes modifying typical species behavior. To reveal the Jackal distribution depending on elevation, the devices were placed in 5 different levels: level A (up to 600 m above sea levels (a.s.l.); level B (600 - 800 m a.s.l.); level C (800 - 1000 m a.s.l.); level D (1000 - 1200 m a.s.l.) and level E (above 1200 m a.s.l.). After a preliminary investigation of the terrain, the most suitable places for setting the cameras were selected. The devices were mounted near the most useful predator trails. The criteria for their selection were: they need to be well trodden and with presence of predators' traces and feces. A total of 15 camera traps (Keep guard Cam, KG690NV) were set in each investigated area in two winter seasons (2016 - 2017 and 2018 - 2019). The devices were distributed in 3 at each elevational level. Each camera was mounted near a suitable animal path at an angle ranging from 45° to 90° to the leight of the trail. The devices were attached to a tree at 1.5 - 1.8 m height and at 3 - 5 m distance from the path, according to the instructions (Ancrenaz et al., 2012). The cameras were set to take 3 photos after triggering with 5 minutes delay. For each elevational zone, the days with and without snow cover were estimated within the studied period, from the obtained photos basis. Than the frequency of occurrence of the jackal was calculated according to the following equation:

$$F = \frac{number\ of\ jackals}{camera\ days} x 100.$$

Results and Discussion

Taking into account the first and the last snow day detected by the cameras, two periods: of 143 days (29.11.2016 - 20.04.2017), 2 145 camera days respectively for the southern slope and of 142 days (17.11.2018 - 07.04.2019), 2

130 camera days respectively for the northern one, were estimated (Table 1).

Data from the survey showed a significant difference in the number of jackals between the southern and northern slopes of Stara Planina Mts (179 photos for the southern slope against 39 - for the northern one). The largest number of jackals was registered at the lowest level (up to 600 m a.s.l.). For the same elevation range the big difference between the number of jackals detected in the two studied areas was found (152 for the southern slope and 14 - for the northern one). At this altitude the habitat type is determined by the main vegetation, typical for the xerothermic oak forests (Velchev, 2002). It provides various food base and shelters for jackals.

The jackal occurrence at 600 - 800 m a.s.l. was lower with more individuals detected on the southern slopes again. As a rule, the number of jackals registered decreased with an elevation increasing. The jackal presence from 1000 to 1200 m a.s.l. in the both studied areas was sporadic, and above 1200 m a.s.l. the species was not detected.

Despite the snow cover presence, in the highest parts of the studied areas, jackals have never been detected, and at lower altitudes individuals were registered. In the mountain basis, the snow cover did not negatively affect the jackals, as the number of individuals photographed on snow was significantly higher compared to those on snowless conditions. Snow influences animal behavior not only because its presence, but also via its characteristics (Novikov, 1981). Although the snow cover depth in different levels was not measured in the present study, it is well known that the snow at the mountain base, especially on the southern side, is shallow due to its frequent melting. It can be assumed that the shallow snow cover does not hinder the animals' movement. The dynamics in changes of snow periods with snowless ones at the lowest elevations, as well as the lesser number of snow days, compared to the other levels in the studied areas, are the probable reasons for the increased jackals presence at lowest places with snow cover. It can be assumed that such variable weather conditions facilitate the jackal in its vital functions (searching food and finding shelter). Among the medium-sized predators in Bulgaria, the Golden jackal is the least adapted for locomotion in deep snow (Raichev, 2010), which is a prerequisite for avoiding the high parts of the studied areas. The jackal has relatively short legs with narrow paws, which hinder its moving in deep snow. In addition, its fur is not adapted to severe winter conditions (Aliev, 1968; Taryannikov, 1974; Vereshchagin, 1959; Heptner & Naumov, 1998), typical for the high parts of the mountains. There are no researches on the critical snow depth for the jackal. For the fox, which has a similar anatomy, this depth is 30 - 40 cm (Formozov, 1946). It is very likely that the jackal will have difficulty even at less than the mentioned depth (Fig. 2), as the weight load of its paws is greater than that of the fox (Raichev, 2010). In addition, studies on the jackal's distribution revealed that the snow cover duration significantly affects its selection of new habitats (Ranc et al., 2017). Coyotes in North America, which occupy the same ecological niche as jackals in Europe, are most numerous in areas with low elevation and shallow snow cover (Murray & Boutin, 1991). The same as the Golden jackal, they have difficulties when moving in deep snow. They often move on trails left by snowmobiles, taking advantage of the pressed snow (Crete & Lariviere, 2003). High mountainous areas with deep and permanent snow cover, extremely low temperatures and steep terrain are not suitable for the Golden jackal (Spassov, 1989; Demeter & Spassov, 1993). Such conditions cause energy losses when moving, finding food and thermoregulating (Pacer et al., 1984; Root, 1988). Energy consumption increases with snow depth and density increasing (Withers et al., 2016). In domestic dogs, sinking of the limbs when moving on deep snow was found to accelerate heart rate more than accelerating movement does itself (Crete & Lariviere, 2003).

Fig. 1. Study areas located in the Central Stara Planina Mts, Bulgaria.

Table 1. Number of registered golden jackals during snow period according to elevation in northern and southern slopes of the Central Stara Planina Mts, Bulgaria.

	Southern slope			**Northern slope**		
Level	**Detected jackals / camera days with snow**	**Detected jackals / camera days without snow**	**Total**	**Detected jackals / camera days with snow**	**Detected jackals / camera days without snow**	**Total**
E	0 / 354	0 / 75	0 / 429	0 / 426	0 / 0	0 / 426
D	1 / 300	1 / 129	2 / 429	1 / 342	1 / 84	2 / 426
C	0 /204	1 / 225	1 / 429	2 / 324	8 / 102	10 / 426
B	10 / 159	14 / 270	24 / 429	8 / 204	5 / 222	13 / 426
A	117 / 153	35 / 276	152 /429	7 / 168	7 / 258	14 / 426

Fig. 2. Golden jackals detected moving in deep snow cover.

The declining jackal's presence with elevation rising (Fig. 3) underlines its preference towards the lowest levels (below 800 m a.s.l.) in the study areas.

The species prefers lowlands which are the natural habitats for its main prey (Nievergelt, 1981) avoiding the vast forest areas in the highest mountainous levels, described as a barrier for its distribution (Negi, 2014). In Greece, the Jackal inhabits mainly areas below 600 m a.s.l., being most numerous in wetlands overgrown with dense vegetation and at about 10 m a.s.l. (Giannatos et al., 2005). The small number of individuals photographed during the entire study period at levels of 800 - 1200 m a.s.l. (C and D) suggests the idea that this part of Stara Planina Mts is not preferred by jackals in winter. Habitats above 800 m a.s.l. comprise the beech forest belt (Velchev, 2002), offering limited food resources and scarce shelters for jackals.

Above 1200 m a.s.l., (level E) no jackals were detected in both areas throughout the studied periods. There, the snow cover was the most abundant and continuous, suggesting difficulties in finding food for the medium sized predators such as the Golden jackal. Many authors reported the jackal as a typical polyphagous species, feeding mainly on small prey (mostly rodents), plants (mostly fruit), carrion from wild and domestic animals and artificials (Cirovic et al., 2014; Markov & Lanszki, 2012; Tsunoda et al., 2017; Jaeger et al., 2007). Thus, it can be assumed that the limited food base in the highest parts of the studied areas is one of the main reasons for the small jackal number reported there. In the temperate latitudes of Europe, cervids make vertical migrations towards the lower parts of the mountains, as typical movement pattern (Mysterud et al., 2001). It is considered that the triggering mechanism for this behavior is the snow cover depth retention over 20 - 25 cm for a long time period (Schmidt & Gossow, 1991). Thus the hunting efforts in the lower parts of the mountain occur, ensuring additional food source for predators. The jackal was found to be an opportunistic species that feeds on leftovers from hunted animals (Lanszki et al., 2015). The presence of wolves in the higher parts of Stara Planina Mts (Fig. 4) could affect negatively the jackals' number at the levels C, D and E. The Grey wolf is one of the factors limiting jackal's distribution (Spassov & Acosta-Pankov, 2019; Ranc et al., 2017).

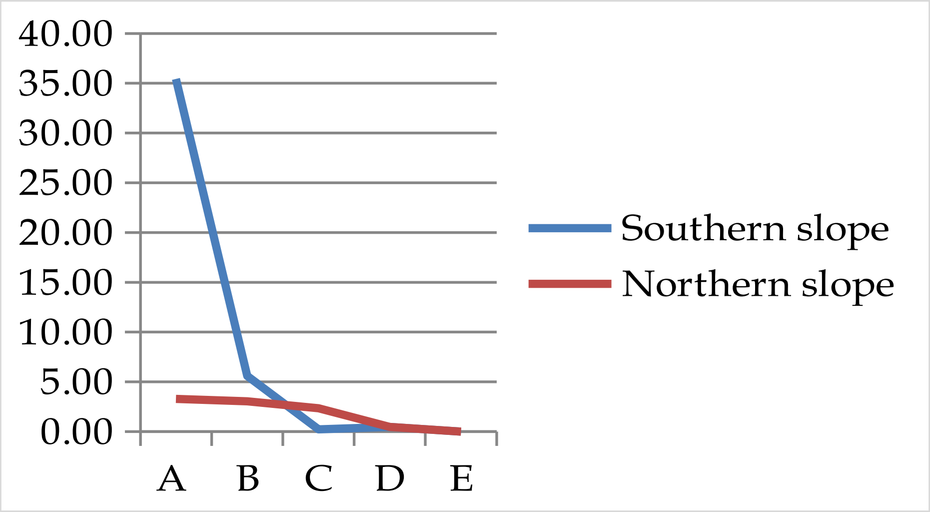

Fig. 3. Frequency of occurrence of the Golden jackal during snow period according to elevation (A - up to 600 m a.s.l.; B – 600 - 800 m a.s.l.; C – 800 - 1000 m a.s.l.; D – 1000 - 1200 m a.s.l.; E - above 1200 m a.s.l.) in northern and southern slopes of the Central Stara Planina Mts, Bulgaria, in %.

Fig. 4. Grey wolves detected at 1000 - 1200 m above sea level.

In order to clarify the influence of the factors mentioned above on the Golden jackal behavior and distribution in the mountains, detailed researches are needed.

Conclusions

During snow period, the Golden jackal from the region of Central Stara Planina Mts demonstrated clear preferences to the southern slope up to 800 m a.s.l. The elevation from 800 to 1000 m a.s.l. could be considered as a conditional border for the jackal's distribution in the Central Stara Planina Mts.

The following factors could be pointed out as probable reasons for the Golden jackal's uneven distribution in the Central Stara Planina Mts during snow period: the terrain exposure and elevation, the snow cover depth and the Grey wolf's presence.

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Biological Activity of Native Bacterial Isolates Against Aphids in Beans and Peas Field Production

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Abstract. Aphids (Hemiptera: Aphididae) are a major group of pests in legume crops. They cause direct damage to the host plants by sucking of plant sap. In addition to direct damage from their feeding, aphids also act as vectors for carrier of several viruses. The main method to control these pests is still the use of chemical insecticides. This practice hides risks due to harmful environmental effects, residual quantities in production, the emergence of resistance populations, destruction of beneficial species and many more. In recent years, there has been a growing interest in the discovery and development of new bioinsecticides as environmental friendly pest control tools to be integrated in combination or rotation with chemical pesticides in pest management programmes. Since the bacteria are relatively poorly studied as biocontrol agents for aphids control, the aim of this study was to evaluate the aphicidal effect of native bacterial isolates. Strains of *Bacillus amyloliquefaciens*, *Paenibacillus polymyxa* and *Providencia rettgeri* were used in experiments, conducted in the period 2019-2020. *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10 were tested against black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris) in the field growing of peas and beans. Among the studied microorganisms, *B. amyloliquefaciens* A1 and *P. rettgeri* K10 showed good aphicidal activity (E≥75%) against black bean aphid (*A. fabae*) and pea aphid (*A. pisum*) in the growing of beans and peas in the field.

Key words: bean, pea, aphids, biocontrol, *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3, *Providencia rettgeri* K10.

Introduction

Of the food legume crops grown in our country, bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) are the most preferred food for consumption due to their high nutritional value and taste qualities. Their proteins in a nutritional value are close to that of meat, fish and other animal products. Beans and peas also have important agro-technical importance, as they enrich the soil with nitrogen and release the areas early. They are an excellent precursor in crop rotation for many crops. The production of beans and peas is limited by certain environmental stress factors, particularly biotic and abiotic stresses. Among the biotic factors, the attack by pests causes a significant reduction in the yield of legumes.

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Aphids (Hemiptera: Aphididae) are a major group of pests that have a high degree of reproduction. They can cause direct damage to the host plants by sucking plant sap. In addition to direct damage from their feeding, aphids are vectors of various disease causing viruses (Shannag, 2007).

The black bean aphid, *Aphis fabae* Scopoli, is a polyphagous pest that attacks more than 200 legumes and can damage all the parts of the plant (Purhematy et al., 2013). In peas, pea aphid, *Acyrthosiphon pisum* Harris is considered a serious pest as it reduces both the mass and caloric value of young pea plants by 64% and 113%, respectively, depending on the number of aphids (Melesse & Singh, 2012). All stages of plant development are sensitive to direct damage to aphid feeding, but young pods are most susceptible. Feeding damage to flowers and pods is highly correlated with aphid density and show significant reductions in yield. Peas must be protected from aphids for a 2 to 3week period beginning when pods start to form (Maiteki & Lamb, 1985).

The main method of aphid control is still the use of chemical insecticides. This practice hides risks due to harmful environmental effects, residual quantities in production, the emergence of resistance in populations, destruction of beneficial species and many more.

In recent years, there has been a growing interest in the discovery and development of new bioinsecticides as environmental friendly pest control tools to be integrated in combination or rotation with chemicals in pest management programmes.

According to Gonzalez et al. (2016) the most effective control of aphids in integrated and biological production systems is the combination of microbiological plant protection products with bioagents.

Microorganisms namely bacteria are widespread in the environment and have developed various interactions with insects, including symbiosis. While many bacterial species inhabit the insect bodies, establishing different levels of relationships, a limited number of them behave as insectopathogens. The latter have developed a number of strategies for penetrating the host, overcoming its immune barriers and ultimately the ability to infect and kill it. The mechanisms leading to these interactions are thought to be of ancient origin and have evolved through a long process of coevolutionary development (Vilcinskas, 2010).

Such is the case with protein toxins produced by *Bacillus thuringiensis* Berliner (De Maagd et al., 2003). These toxins are usually very specific to a limited number of hosts, while in other cases the bacteria produce metabolites that show a wider insecticidal spectrum.

Entomopathogenic bacteria are traditionally well represented by members of the *Bacillaceae* family, such as *Bacillus* spp. and *Paenibacillus* spp.

Important information about the understanding of the mechanisms involved in the interactions between different pathogens and hosts is obtained as a result of modern research. However, many aspects have not yet been discovered and after several decades of microbial pest control dominated by *B. thuringiensis*, new bacterial species with innovative modes of action have been discovered and formulated as new biopesticide products (Ruiu et al., 2013).

Bacillus amyloliquefaciens commonly found in the soil. There is very little research on its properties as a biological agent to pest control. At this stage, *B. amyloliquefaciens* was studied for mosquito control (Geetha et al., 2010, 2011), tomato leaf miner (*Tuta absoluta* Meyrick) (Saoussen et al., 2015) and citrus mealybug (*Planococcus citri* R.) (Mohamedova et al., 2017). Experiments were performed to determine aphicidal effect of the strains *B. amyloliquefaciens* against *Myzus persicae* Sulzer. Received results propose that *B. amyloliquefaciens*, could function as a novel eco-friendly biopesticide for the control of *M. persicae* (López-Isasmendi et al., 2019).

Experiments with the bacterial isolates *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10, derived from soil samples were performed. They have been screened for their effectiveness in controlling citrus mealybug (*Planococcus citri* Risso). The studied bacterial strains show approximately the same effectiveness. It was found that the mortality rate of larvae from the first age caused by *B. amyloliquefaciens* A1, *P. rettgeri* K10 and *P. polymyxa* AB3 reached 84.29%, 82.62% and 90.37%, respectively (Mohamedova et al., 2017).

To date, several observations have been made on the effect of entomopathogenic bacteria on *Psedococcidae* species, as a result of extracellular production of enzymes or lipopolysaccharides, which destroys hemocytes and internal insect organs after the bacteria enter the hemocoele (*Pseudomonas aeruginosa*, *Serratia marcescens* and *Providencia rettgeri*) (Lysenko, 1985) or because of its ability to produce toxins that destroy the epithelial cells covering the intestines of insects (*Bacillus thuringiensis*) (Van-Rie et al., 1990).

Many authors have concluded that the mechanism of action of entomopathogenic bacteria is based on substances with contact action - enzymes and other metabolic products.

The aim of the study was to determine the biological activity of three native bacterial isolates *Bacillus amyloliquefaciens* A1, *Paenibacillus polymyxa* AB3 and *Providencia rettgeri* K10 against aphids in beans and peas grown in open field conditions.

Material and Methods

Microorganisms

The present study was performed with *Bacillus amyloliquefaciens* A1, *Paenibacilluspolymyxa* AB3 and *Providencia rettgeri* K10 from the collection of the Laboratory of Microbiological Technologies, Agricultural University, Plovdiv. The tested bacterial strains were isolated from the different natural soils. The bacterial isolates were maintained for long-term storage at -20°C storage temperatures in sterile glycerol. For the performed tests, the bacteria were activated by repeated sub-culturing on Bacto nutrient agar (Difco laboratories, Detroit, USA) at 28°C.

Cultivation

For biological tests, the microorganisms were cultured in a liquid medium. For this purpose, 500 ml flasks, containing 200 ml of sterile Triptic soy broth (TSB) (Biolife, Milano, Italy) were inoculated with 1 ml of bacterial suspension obtained by washing colonies of solid medium with sterile water. Inoculum cell density was adjusted to 10^9 CFU ml^{-1} before inoculation as determined by optical density. Cultivation was performed on a rotary shaker at 195 min^{-1} at 28°C for 48 h. Bacterial suspensions (10^7 CFU ml^{-1}) of each isolate were used as inocula for the treatment of experimental variants.

Entomological tests for aphids (Homoptera: Aphididae)

In aphid-infested plants are sprayed with the appropriate dose of test microorganisms. The number of live individuals before spraying and in intervals after 1, 3, 5, 7, 10 and 14 days was reported. Test pests: black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris). Test microorganisms: *B. amyloliquefaciens* A1, *P. polymyxa* AB3 and *P. rettgeri* K10. Total area of the experimental plot was 100 m^2 of bean variety Evros and 100 m^2 pea variety Skinado.

The effectiveness (%) was calculated by the formula of Henderson-Tilton (1955):

$$E\% = \left(1 - \frac{TaxCb}{TbxCa}\right).100,$$

where:

Ta - number of live aphids in the variant after treatment;

Tb - number of live aphids in the variant before treatment;

Ca - number of live aphids in the control after treatment;

Cb - number of live aphids in the control before treatment

A comparative analysis was made using Duncan’s multiple range test (1955).

Results and Discussion

Aphids are pests that have a high degree of reproduction. Successful control with them is associated with problems, as in populations often developed resistance to the used chemical plant protection products. In recent years, efforts have focused on exploring the possibilities of biological control - the use of phytopesticides, microbial products and products of mineral origin.

The biological activity of three microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros grown in the field was studied. Good effectiveness of *Bacillus amyloliquefaciens* A1 (E = 77.09% on the 5th day after treatment) and *Providencia rettgeri* K10 (E = 78.02% on the 7th day after treatment) was found. This trend was observed in both experimental years, as reported effectiveness in *Bacillus amyloliquefaciens* A1 was 73.42% (2019) and 80.76% (2020) on the 5th day after treatment, respectively. In *Providencia rettgeri* K10, the effectiveness was 76.55% (2019) and 79.49% (2020) on the 7th day after treatment. *Paenibacillus polymyxa* AB3 showed unsatisfactory biological activity (E <60%) against this pest (Table 1 and Fig. 1). The aphid density changes and it is significantly lower in the variants by the end of the experiment compared to the control. During the period 5 - 7 days after treatment in the variants *Providencia rettgeri* K10 and *Bacillus amyloliquefaciens* A1, the average number of aphids is less than 10 per plant (Fig. 2).

In pea variety Skinado, experiments to determine the biological activity of the same microorganisms included in the studies in bean variety Evros were made to control the pea aphid (*Acyrthosiphon pisum* Harris). The same trend for good biological activity of *Bacillus amyloliquefaciens* A1 (E = 75.25% on the 7th day after treatment) and *Providencia rettgeri* (E = 77.10% on the 7th day after treatment) was observed (Table 2).

Good effectiveness of the studied microorganisms during the experimental years was found in *Bacillus amyloliquefaciens* A1 74.52% (2019) on the 7th day after treatment and 78.74% (2020) on the 5th day after treatment, in *Providencia rettgeri* 76.03% and 78.17% on the 7th day after treatment, respectively. *Paenibacillus polymyxa* AB 3 showed unsatisfactory biological activity (E <60%) against this pest (Table 2 and Fig. 3).

Table 1. Biological activities of the studied microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros. *Legend:* a, b, c … – Duncan's multiple range test ($p < 0.05$).

Variants	**Days after treatment**	**Effectiveness**	
		2019	**2020**
Providencia rettgeri K10	1	40.24 b	43.00 c
Paenibacillus polymyxa AB3		28.22 b	31.55 n.s.
Bacillus amyloliquefaciens A1		33.00 b	43.29 b
Providencia rettgeri K10	3	50.70 b	61.68 b
Paenibacillus polymyxa AB3		39.82 ab	48.93 n.s.
Bacillus amyloliquefaciens A1		46.43 b	66.01 ab
Providencia rettgeri K10	5	57.23 b	67.32 ab
Paenibacillus polymyxa AB3		48.43 ab	58.26 n.s.
Bacillus amyloliquefaciens A1		**73.42** a	**80.76** a
Providencia rettgeri K10	7	**76.55** a	**79.49** a
Paenibacillus polymyxa AB3		58.70 a	49.58 n.s.
Bacillus amyloliquefaciens A1		49.92 b	68.91 ab
Providencia rettgeri K10	10	50.16 b	65.30 ab
Paenibacillus polymyxa AB3		49.67 ab	49.33 n.s.
Bacillus amyloliquefaciens A1		39.94 b	58.28 ab
Providencia rettgeri K10	14	40.54 b	54.81 bc
Paenibacillus polymyxa AB3		42.23 ab	46.47 n.s.
Bacillus amyloliquefaciens A1		33.49 b	53.90 ab

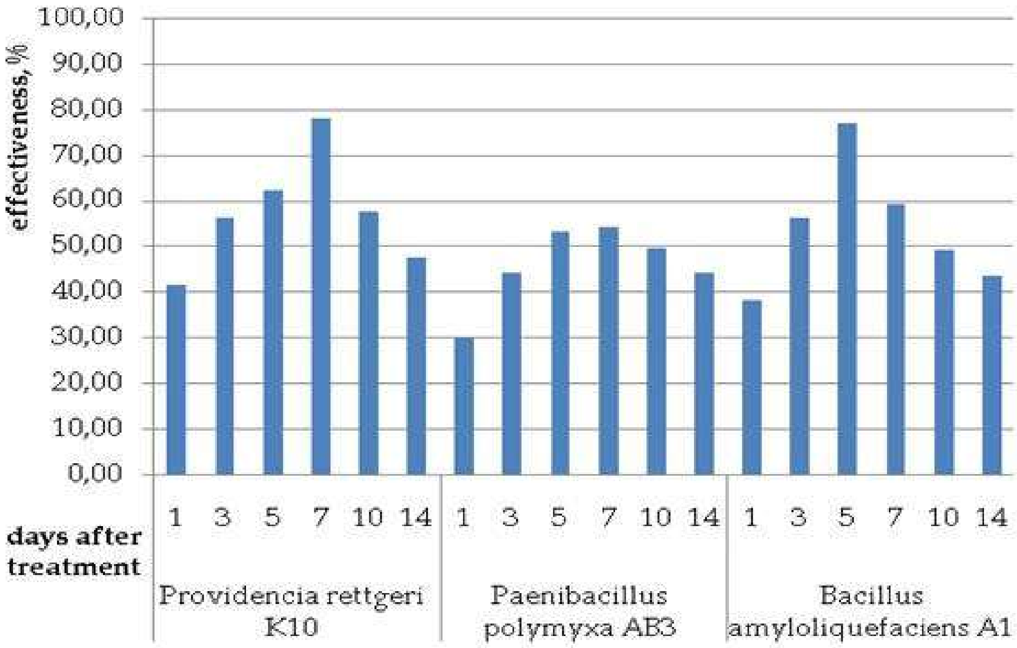

Fig. 1. Average effectiveness of the studied microorganisms against black bean aphid (*Aphis fabae* Scop.) in bean variety Evros.

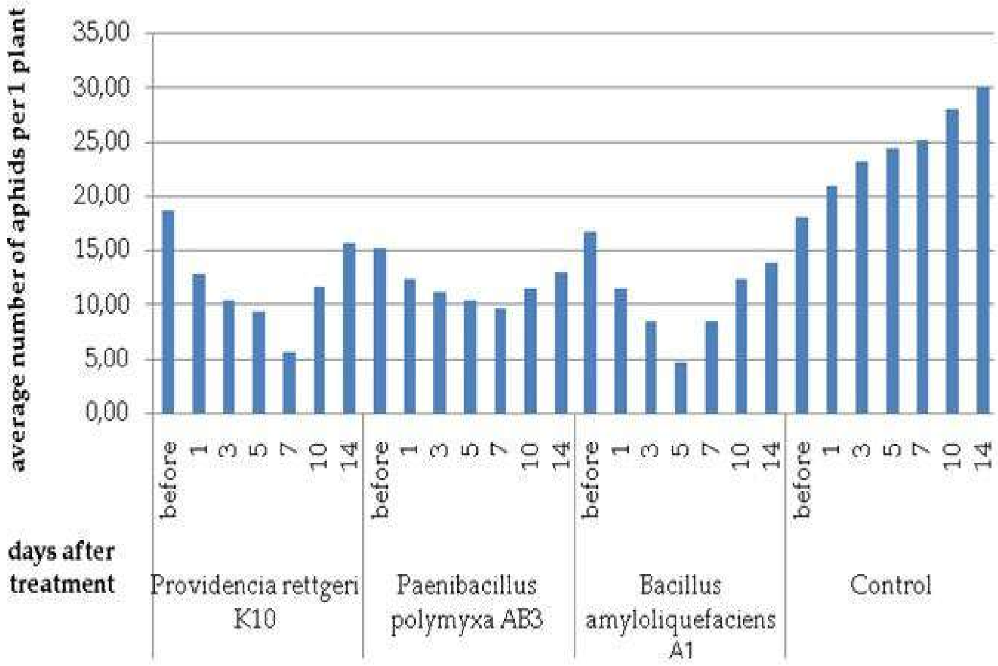

Fig. 2. Average population density of black bean aphid (*Aphis fabae* Scop.) in bean variety Evros.

Table 2. Biological activities of the studied microorganisms against pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado. Legend: a, b, c … – Duncan's multiple range test ($p < 0.05$).

Variants	Days after treatment	Effectiveness	
		2019	2020
Providencia rettgeri K10	1	34.84 c	47.89 b
Paenibacillus polymyxa AB3		25.33 d	45.56 n.s.
Bacillus amyloliquefaciens A1		29.67 b	46.21 c
Providencia rettgeri K10	3	48.96 b	66.57 a
Paenibacillus polymyxa AB3		32.47 cd	47.48 n.s.
Bacillus amyloliquefaciens A1		38.56 b	67.35 ab
Providencia rettgeri K10	5	57.08 b	70.99 a
Paenibacillus polymyxa AB3		41.48 bc	55.83 n.s.
Bacillus amyloliquefaciens A1		49.52 b	**78.74** a
Providencia rettgeri K10	7	**76.03** a	**78.17** a
Paenibacillus polymyxa AB3		52.46 ab	59.51 n.s.
Bacillus amyloliquefaciens A1		**74.52** a	75.98 ab
Providencia rettgeri K10	10	51.28 b	66.88 a
Paenibacillus polymyxa AB3		55.91 a	58.69 n.s.
Bacillus amyloliquefaciens A1		45.90 b	65.24 ab
Providencia rettgeri K10	14	46.46 b	61.62 ab
Paenibacillus polymyxa AB3		47.48 ab	49.13 n.s.
Bacillus amyloliquefaciens A1		41.00 b	61.40 b

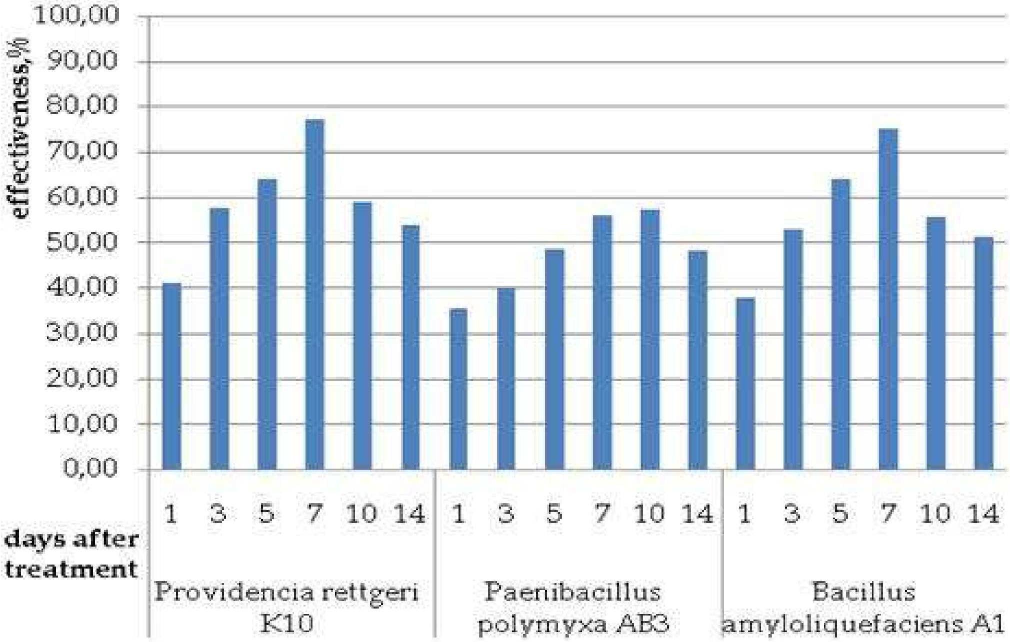

Fig. 3. Average effectiveness of the studied microorganisms against pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado.

The aphid density changes and it is significantly lower in the variants by the end of the experiment compared to the control. In the variants *Providencia rettgeri* K10 and *Bacillus amyloliquefaciens*, the density was lowest on day 7 after treatment, with an average number of aphids per stem 6.25 and 8.63, respectively (Fig. 4).

In the two crops included in the study, beans and peas, there was a decrease in the effectiveness of the microorganisms against aphids after the 7th day of treatment, which should be included in the treatment and re-introduction scheme after this period.

Of the studied microorganisms those that showed good biological activity against aphids in legume crops could be included in subsequent studies as effective bioagents to control these pests.

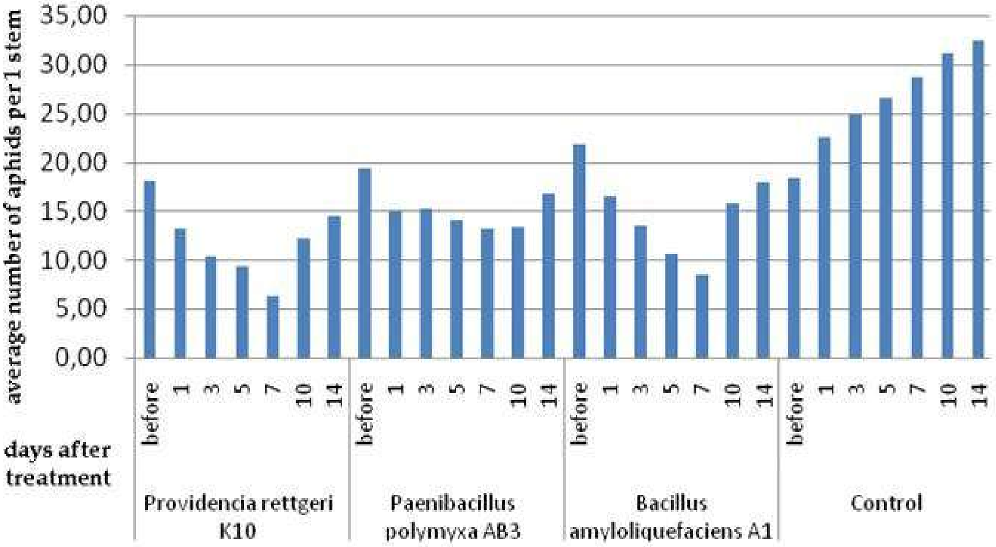

Fig. 4. Average population density of pea aphid (*Acyrthosiphon pisum* Harris) in pea variety Skinado.

Conclusions

Of the studied microorganisms, *Bacillus amyloliquefaciens* A1 and *Providencia rettgeri* K10 showed good aphicidal activity (E≥75%) against black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Harris) in growing of beans and peas in the field.

Due to the shown best effectiveness of the tested microorganisms *Bacillus amyloliquefaciens* A1 and *Providencia rettgeri* K10 on the fifth to seventh day after treatment, it can be included in the scheme and re-introduction after this period.

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Population Dynamics and Structure of Zooplankton Community of Mandra Reservoir, Bulgaria

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Abstract. The zooplankton community in Mandra Lake has been almost unstudied since it was turned into reservoir in 1963. Human intervention in the natural water regime of the lake has affected the species diversity of the aquatic system. This study was conducted in March 2017 and December 2018 in the reservoir. A total of 64 zooplankton taxa from the groups Protozoa, Rotifera, Cladocera and Copepoda were identified in the studied samples. The majority of these organisms (37%) belonged to the Rotifera group, with most common species in spring – *Keratella hiemalis* Carlin, 1943 and *Polyarthra major* Burckhardt, 1900. During the investigation period, the most frequent zooplankton components were *Chydorus sphaericus* (O.F. Müller, 1776) and Nauplii and Copepodites of Copepoda. 22 species from class *Branchiopoda* were established. Considerable changes in the quantitative composition of Rotifera at different sampling points and years are indicated by the low values of the Jaccard similarity index. When comparing the two spring seasons (1955 and 2017), the index has a value of less than 9 %. At the same time, the number of species increased 2.5 times.

Key words: zooplankton, Mandra Reservoir, modified coastal lake, human influence, eutrophication processes.

Introduction

Mandra was a natural Black Sea coastal shallow lake until the dam was built in 1963. The average annual fish production of the lake was approximately 170 tons per year (Mihaylova-Neykova, 1961). After the research of Mihaylova-Neykova (1961), no recent data about fish production in the reservoir exists. Nowadays reservoir is used only for recreational fishing. Main threats to the balance of the ecosystem and in particular for the zooplankton is internal loading and nuisance algal blooms with toxin presence, and the situation in industrial region (Stoyneva & Michev, 2007).

The zooplankton community in Mandra Lake has been almost unstudied since it was turned into reservoir. Human intervention in the natural water regime of the lake has affected the species diversity of the aquatic system. It is possible to trace the result of the changes of hydrological parameters after construction of a dam in the main characteristics of the formation and composition of the zooplankton (Naidenow, 1981; 1984). Many authors use zooplankton communities of the lakes as indicator for the ecological state of standing water bodies (Ismail & Adnan, 2016; Krupa et al., 2020).

The zooplankton of the reservoir Mandra was investigated by Pandourski in

1999 - 2000 (Pandourski, 2007). During that period the lowest biomass values were in the locality of the dam, which was determined by the high quality of a relatively small Rotifera species, and the central part of the reservoir was richest due to mass development of *Daphnia cucullata* G.O. Sars, 1862.

The recent data about population dynamics of the zooplankton in fresh and mixohaline lentic water bodies in relation to different environmental factors were given by many authors such as Løvik & Kjellberg (2003), Etilé, et al. (2009), Stanachkova et al. (2017), Su et al. (2019), Picapedra et al. (2020).

The aim of the present investigation was to determine the qualitative structure of the zooplankton complex of Mandra Reservoir in March 2017 and December 2018 in the changed conditions of the water body due to the anthropogenic impact and to compare the results with previous research data.

Material and Methods

This study was conducted in 25 March 2017 and 16 December 2018 in the Mandra Reservoir (Fig. 1). It is situated in Bulgarian coast of the Black Sea at geographic coordinates of 42° 24′ 30″ N, 27° 22′ 19″ E. The data about the area, surface and depth of the reservoir are given on Table 1. It also contains information about the area and maximum depth of the former Mandra Lake (Mihaylova-Neykova, 1961). During the two samplings, 12 quantitative samples were collected at 6 sites by using an Apstein plankton net 55 µm mesh size and via filtering of 100 dm^3 of water through the net. This method of direct filtering a certain amount of water through Apstein plankton net is widely used in the study of shallow holo-polymictic standing water bodies and ecotone river-reservoir zones (Kozuharov et al., 2007; Yakimov et al., 2016). The samples were fixed in 4% formalin. Under laboratory conditions, they were counted using the method of Hensen, modified by Dimoff (1959) and Naidenow (1981).

The biomass of the zooplankton organisms was calculated by using of their standart individual weights according to their average length after Jadin (1956) and Standard Operating Procedure for Zooplankton Analysis (2016).

The location of the sites can be seen on Fig. 1. The places of sampling points in the reservoir were chosen close to the inflowing rivers in the reservoir, to find out eventual influence on the zooplankton of the organic meter what inflow with the water of the rivers. One station is situated close to the dam and one in the central pelagic part of the reservoir as control point.

In the selection of the seasons for the study we have taken into account the climatic features of the region - warm and mild winters and cool summers, as well as the specific characteristics of Mandra Reservoir, which provides cooling water for local industries and after that warm water is returned to the reservoir. When the samplings were collected, the water temperature was also measured with an alcohol thermometer. The average water temperature measured on 25.03.2017 was 9.1℃, and on 16.12.2018 - 10℃.

The shallow polymictic freshwater ecosystems are particularly susceptible to climate changes also (Mooij et al., 2005; 2007; Jeppesen et al., 2014; Haberman & Haldna, 2017). It is well documented that the effect of climate change is more significant in spring and winter (Weyhenmeyer et al., 1999; Nõges, & Nõges, 2014; Haberman & Haldna, 2017).

Cluster analysis was used for comparing similarity of the zooplankton complexes in Mandra Reservoir and in the former Mandra Lake. The comparison was made by seasons - Spring 1955 / Spring 2017 and Winter 1955 / Winter 2018. Jaccard similarity index was used, with the unweighted pair-group average (UPGMA) algorithm. The calculation was made by statistical package PAST version 4.0 (Hammer et al., 2001). The RCC index presents the percent ratio between most common zooplankton groups Rotifera, Cladocera and Copepoda (Kozuharov et al, 2013).

Results

DA total of 64 zooplankton taxa from the groups Protozoa, Rotifera, Cladocera and Copepoda were identified in the studied samples. The majority of these organisms - (37%) belonged to the Rotifera group, with the most common species in spring – *K. hiemalis* and *P. major* (Table 2). In winter, the Rotifera abundance was significantly less, and no species from this group were found at sampling point 5. The most common Rotifera in winter were *Asplanchna sieboldi* (Leydig, 1854) and *Asplanchna priodonta* Gosse, 1850.

During winter, the most frequent zooplankton species were *C. sphaericus* and Nauplii and Copepodites of Copepoda. 22 species from class *Branchiopoda* were established. The most frequent were *Bosmina longirostris* (O. F. Müller, 1776), *Bosmina coregoni* Baird, 1857, *Bosmina kessleri* Uljanin, 1874 and *D. cucullata*. Microcrustaceans were represented by a total of 14 species, with greater species richness in winter.

Considerable changes in the Rotifera quantitative composition at different sampling sites and years of sampling were indicated by the low values of the Jaccard similarity index. When comparing the two spring seasons (1955 and 2017), the index has a value less than 9 %. At the same time, the number of species increased 2.5 times. The Jaccard similarity index for the winter was even lower – 2.2 %, and the number of species increased eight times.

Cluster analysis (Fig. 2 and Fig. 3) showed the similarity in species composition between the investigated periods. Four species were common for the spring of 1955 and the spring of 2017. The analysis included 46 taxa and 32 of them were newly registered in Mandra Reservoir. 10 species out of 14 in the past are absent. Only *C. sphaericus* was common for both periods.

Quantitative analysis showed the poorest zooplankton abundance and biomass at site 4, at the mouth of the Fakiyska River, while at the dam a relatively high abundance was observed (Fig. 4 and Fig. 5). In winter the highest zooplankton abundance and maximum biomass were measured at site 3.

Discussion

The reconstruction of costal lake to reservoir and lack of connection with the sea leads to considerable changes of community structure. Increasing number of rotifer species is a major sign to transformed environmental frame. Most of the rotifer species occurs mainly in freshwater habitats while in brackish water their number decline. The obtained results corresponded with the received data published by Stanchkova et al. (2015).

The high number of Rotifera species (34) is typical for advanced eutrophication process of the investigated water body (Hellawell, 1986; Karabin et al., 1997; Protasov, 2002; Kozuharov et al., 2013). Such high number of the rotifers was found in other Bulgarian shallow lakes and ponds (Pehlivanov et al., 2006). Most of the rotifers are from genus *Brachionus, Keratella, Notholca, Testudinella* and *Pompholyx,* wich is common for swamps and heavily loaded by organic meter basins (Hellawell, 1986; Erdoğan & Güher, 2012). The presence of many cladocerans from Chydoridae family also confirms that the studied reservoir is eutrophic water body. Most Chydorid crustaceans and specifically the small-bodied *C. sphaericus,* are typical for the eutrophicated water basins (Smirnov, 1971; Flossner, 1972; Frey, 1987; Dugan, 1992; Hofmann, 1996; Vijverberg & Boersma, 1997; Eyto et al., 2002). Species from genus *Chydorus* are generally found within lakes of higher eutrophication and on/or near the bottom sediments. Some scientists have suggested that these organisms migrate away from the bottom sediments during the night to feed within the general safety of low light (Evans & Stewart, 1977).

Table 1. Information about the area, surface and depth of Mandra Reservoir.

	Mandra Reservoir	**the former Mandra Lake**
Area	33 кm^2	10.8 кm^2
Max. length	11.8 km	
Max. width	3.8 km	
Max. depth	7 m	7 m
Surface altitude	2 meters above sea level	
Dam height	12 m	

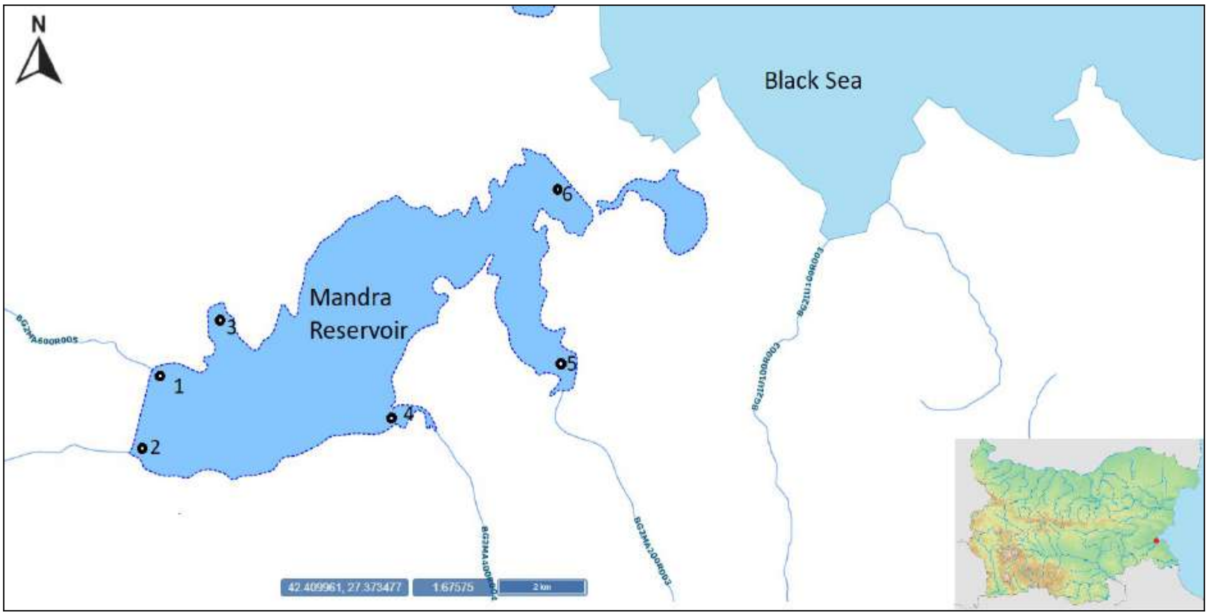

Fig. 1. Location of the worksites in Mandra Reservoir in Southeast Bulgaria.

Table 2. Presence (x) of zooplankton taxa during the two investigated periods in Mandra Reservoir compared to zooplankton in 1955 according to Mihaylova-Neykova (1961).

№	**Taxa**	**Spring 1955**	**Spring 2017**	**Winter 1955**	**Winter 2018**
	Protozoa				
1	*Arcella catinus* Penard, 1890				x
	ROTIFERA				
2	*Pompholyx complanata* Gosse, 1851		x		x
3	*Testudinella sp.*		x		x
4	*Testudinella truncata* (Gosse,1886)		x		
5	*Filinia longiseta/Triarthra longiseta* (Ehrenberg, 1834)	x			
6	*Monostyla cornuta* (O. F. Müller, 1786)	x			
7	*Euchlanis dilatata* Ehrenberg, 1832		x		
8	*Brachionus sp.*				x
9	*Brachionus angularis* Gosse, 1851	x			
10	*Brachionus mulleri* Ehrenberg, 1833	x			

11	*Brachionus urceolaris* (O. F. Müller, 1773**)**	x			
12	*Brachionus urceus* (Linnaeus, 1758)		x		
13	*Brachionus bakeri* O. F. Müller, 1786			x	
14	*Brachionus pala* Ehrenberg, 1838			x	
15	*Keratella cochlearis* (Gosse, 1851)		x		x
16	*Anuraea quadrata* Müller	x			
17	*Keratella quadrata* (Müller, 1786)		x		x
18	*Keratella hiemalis* Carlin, 1943		x		
19	*Keratella testudo* (Ehrenberg, 1832)				x
20	*Notholca squamula* (Müller, 1786)		x		
21	*Notholca acuminata* (Ehrenberg, 1832)		x		x
22	*Asplanchna sp.*	x			x
23	*Asplanchna sieboldi* (Leydig, 1854)				x
24	*Asplanchna priodonta* Gosse, 1850				x
25	*Trichocerca sp.*				x
26	*Synchaeta sp.*		x		x
27	*Polyarthra sp.*		x		x
28	*Polyarthra remata* Skorikov, 1896		x		
29	*Polyarthra dolichoptera* Idelson, 1925		x		x
30	*Polyarthra minor* Voigt, 1904		x		
31	*Polyarthra major* Burckhardt, 1900		x		
32	*Polyarthra luminosa* Kutikova, 1962		x		
33	*Polyarthra platyptera* Ehrenberg, 1832	x			
	CLADOCERA				
34	*Diaphanosoma lacustris* Korjinek, 1981		x		
35	*Bosmina sp.*		x		
36	*Bosmina longirostris* (O. F. Müller, 1776)		x		x
37	*Bosmina kessleri* Uljanin, 1874				x
38	*Bosmina coregoni* Baird, 1857				x
39	*Bosmina sp. Juv.*				x
40	*Daphnia cucullata* G.O. Sars, 1862		x		x
41	*Daphnia longispina typica* O.F. Müller, 1785		x		
42	*Daphnia pulex* (O.F. Müller, 1785)				x
43	*Daphnia sp. Juv.*				x
44	*Daphnia magna ephippium*				x
45	*Moina dubia* Guerne & Richard, 1892				x
46	*Macrothrix hirsuticornus* Norman et Brady, 1867		x		
47	*Alona affinis* Leydig, 1860				x
48	*Alona guttata* Sars, 1862		x		x
49	*Alona rectangula* Sars, 1861		x		
50	*Alonella nana* (Baird, 1850)				x
51	*Alonella exigua* (Lilljeborg, 1853)				x
52	*Chydorus sp.*		x		

53	*Chydorus sphaericus* (O.F. Müller, 1776)	x	x	x	x
54	*Chydorus ovalis* Kurz, 1875		x		
55	*Chydorus latus* G.O.Sars, 1862				x
	COPEPODA				
56	*Acartia clausi* Giesbrecht, 1889	x			
57	*Calanipeda aque dulcis* Krichagin, 1873	x			
58	*Eudiaptomus gracilis* (Sars, 1862)				x
59	*Eucyclops sp.*		x		
60	*Eucyclops macruroides* (Lilljeborg, 1901)				x
61	*Cyclops strenuus* Fischer, 1851		x		x
62	*Halicyclops neglectus neglectus* Kiefer, 1935				x
63	*Grateriella sp.*		x		
64	*Thermocyclops sp.*				x
65	*Thermocyclops crassus* (Fischer, 1853)				x
66	*Acanthocyclops sp.*		x		
67	*Acanthocyclops americanus* (Marsh, 1893)		x		
68	*Acanthocyclops robustus* (G. O. Sars, 1863)				x
69	*Macrocyclops albidus* (Jurine, 1820)				x
70	*Harpacticoida genus sp.* G. O. Sars, 1903	x		x	
71	*Cyclops sp.*	x	x	x	
72	*Copepodites - Copepoda*	x	x		x
73	*Nauplius*	x	x		x
74	*Gastrotricha g. sp.*				x

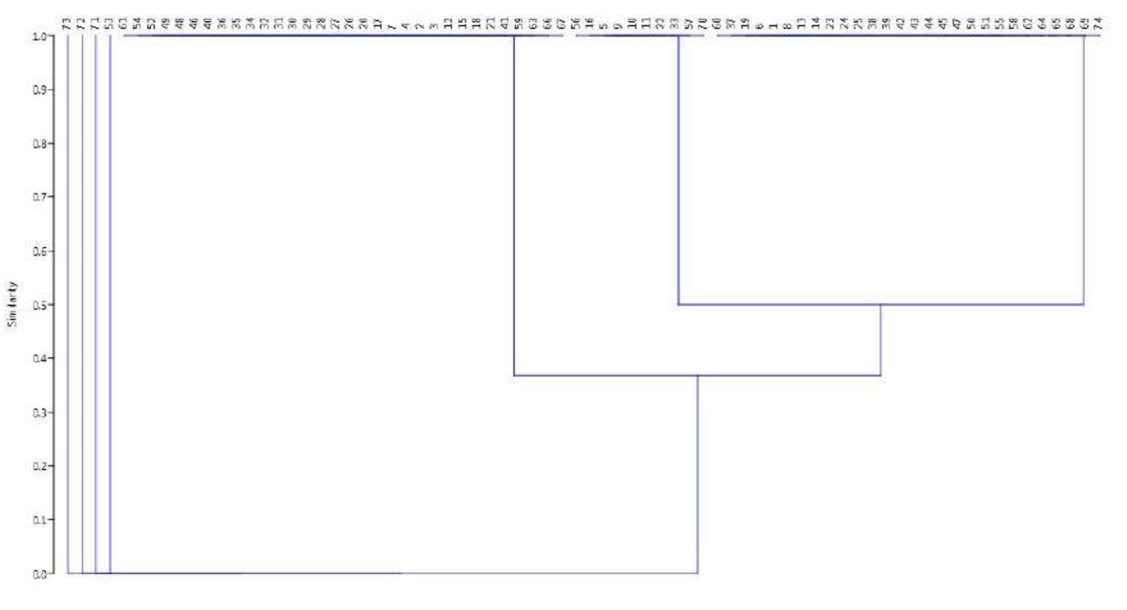

Fig. 2. Cluster analysis (Jaccard similarity index) - Spring. Corr. coph.: 0.9174. Numbers correspond to the zooplankton taxa sequence number in Table 1.

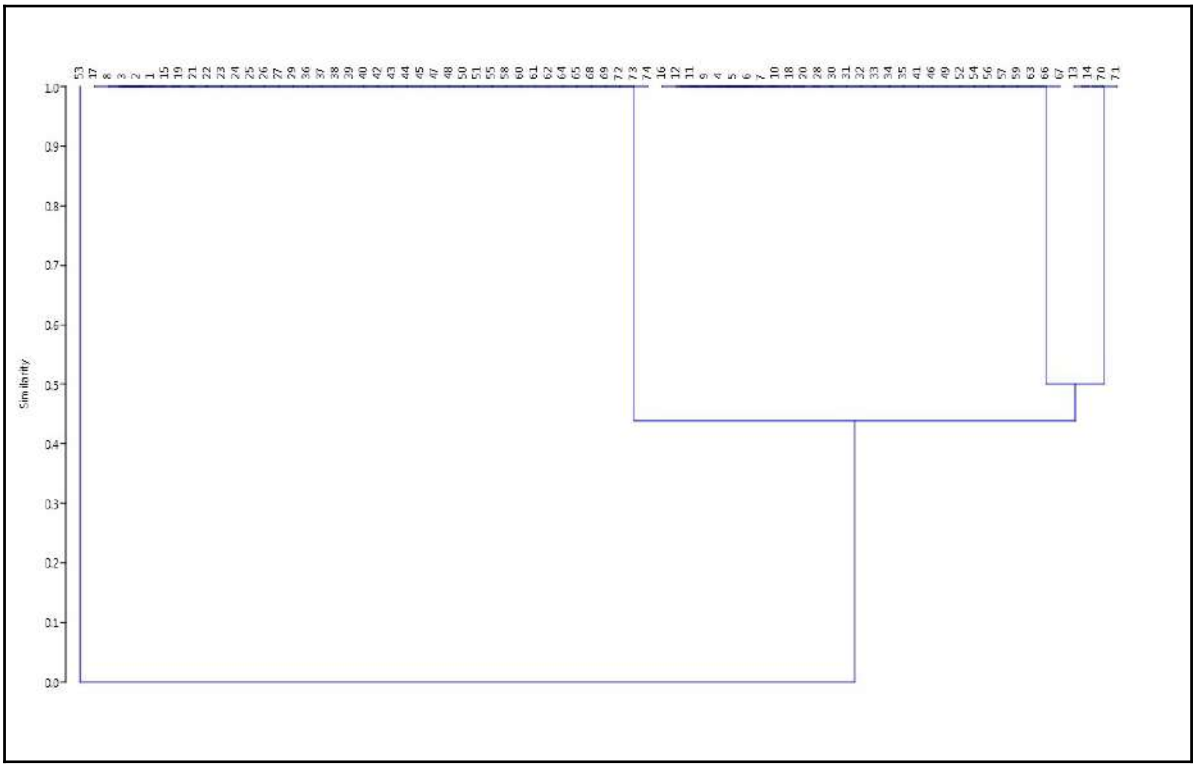

Fig. 3. Cluster analysis (Jaccard similarity index) - Winter. Corr. coph.: 0.9329. Numbers correspond to the zooplankton taxa sequence number in Table 1.

Halicyclops neglectus neglectus Kiefer, 1935 is brackish zooplankter that inhabits coastal parts of seas or reservoirs that are now or in the recent past connected with the sea, and *Acartia clausi* Giesbrecht, 1889 a typical marine species (Boxshall, 2001; Webber et al., 2010). Their presence indicate that in the past the former Mandra Lake was connected to the Black Sea.

Probably these species have the ability to survive in the modified water body with almost fresh water and permanent phytoplankton blooms that are characterized for Mandra reservoir.

The big differences in the RCC index of the different stations showed the influence of the flowing rivers and the nutrients they carry (Kozuharov et al., 2007). There are specific conditions in the ecotone zones, which lead to mass development of one zooplankton group or the complete absence of another, which is clearly visible at site 1 in the spring and at site 4 during the two studied seasons.

The maximum of zooplankton abundance and biomass measured at station 3 in the winter is probably due to the discharge of hot water from nearby businesses.

The wind effects could also be an explanation for the spatial distribution of zooplankton communities, as they are important factors in shallow water bodies. The effects of wind waves on the zooplankton was studied by many authors and they suggest that there might be a direct connection between the wind and the spatial distribution of the lighter Rotifera, or through implications in trophic interactions (Jenkins & Underwood, 1998; Blukacz et al., 2009; Zhou & Qin, 2018).

Probably the lower values of the total abundance and the biomass of the zooplankton in the littoral of the reservoir at sampling point 4 is also due to the press of young fishes (Fig. 4, 5). Higher fish predation of the young fishes shapes the general structure and dynamics of Cladoceran communities in the littoral of the shallow lakes (Meerhof et al., 2007).

Most of them, such as these from genus *Brachionus, Keratella, Notholca, Testudinella* and

Pompholyx, are common for swamps and heavily loaded by organic meter basins (Hellawell, 1986; Erdoğan & Güher, 2012; Guher, 2012). The increasing of the number of Rotifera species and their abundance (Fig. 4) indicates advancing of the eutrophication process in the shallow standing water bodies (Wallace et al., 2006; Guher et al., 2011; Kozuharov et al, 2013).

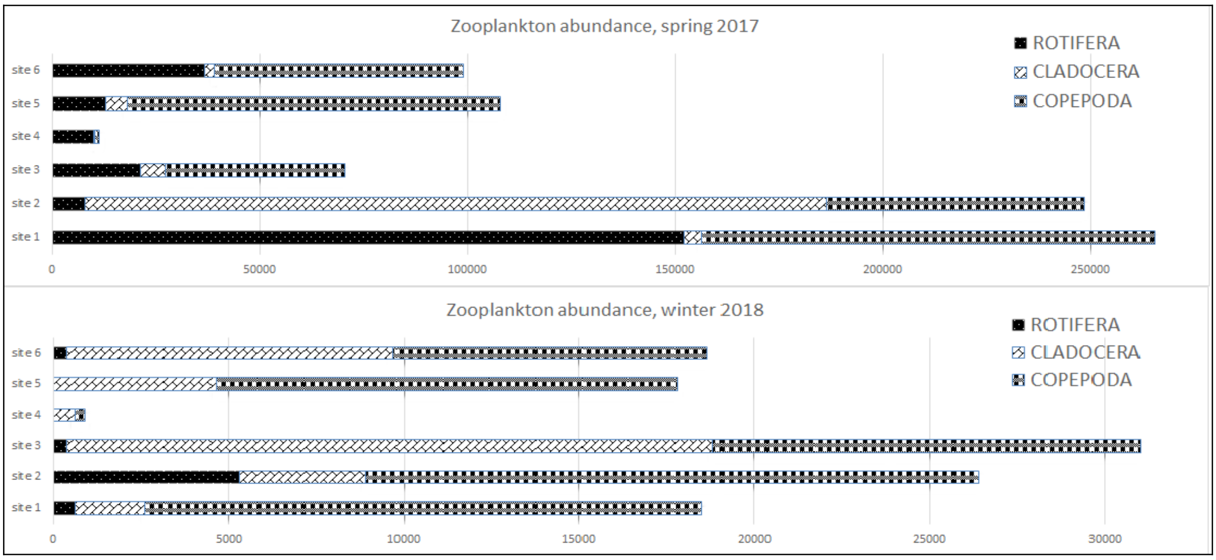

Fig. 4. Zooplankton abundance measured in spring 2017 and winter 2018.

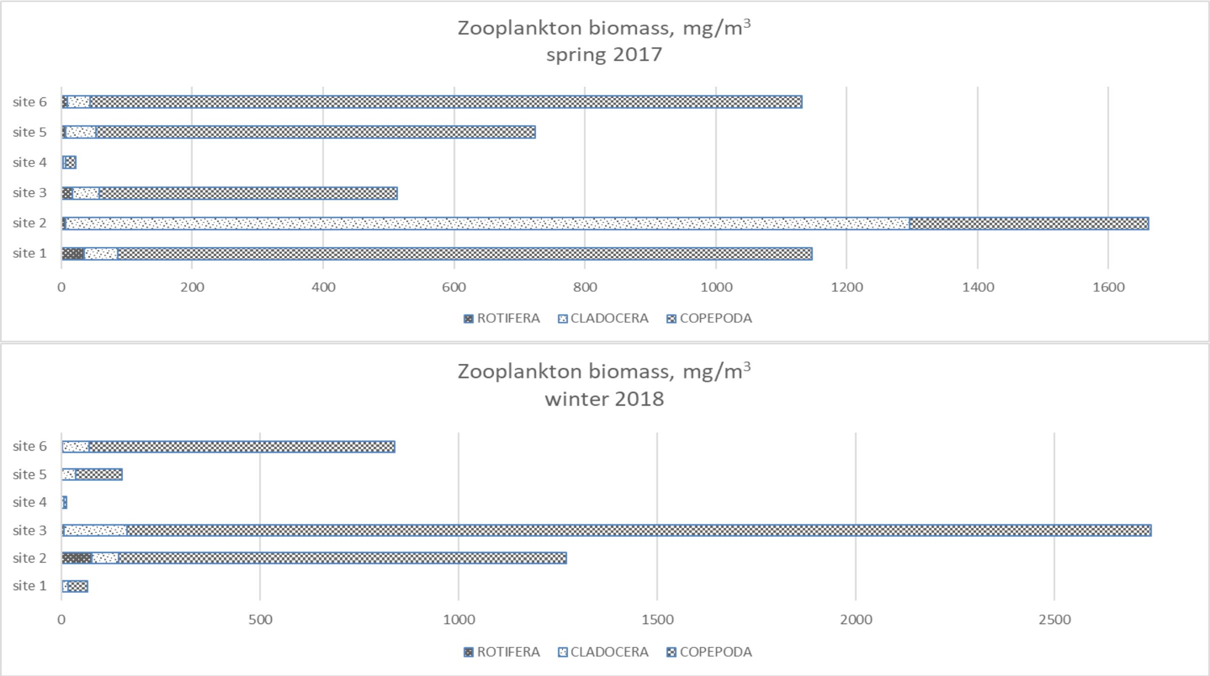

Fig. 5. Zooplankton biomass measured in the studied periods.

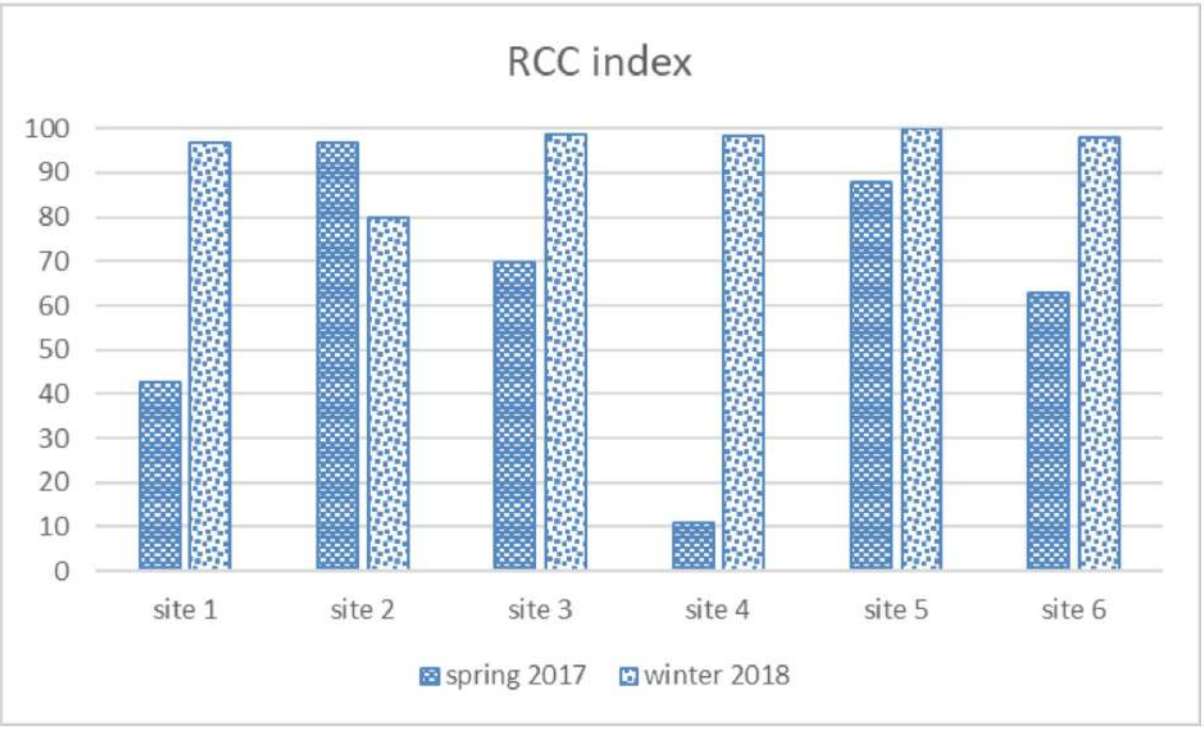

Fig. 6. Values of RCC index in the studied periods.

Conclusions

The obtained results concerning the zooplankton community in today's Mandra reservoir show a significant change in the composition of zooplankton compared to the communities in the former coastal lake Mandra. In addition, the results indicate the effects of eutrophication and provide a basis for further studies in this direction.

The increasing of the richness of the rotifers and their abundance, compared to the previous data, showed that zooplankton of the water body is now typical for the shallow holo-polymictic water bodies studied. Probably the modification from the shallow lake to the reservoir with bigger surface area give possibility to some newly found pelagic organisms to inhabit the water body. Such typical elements are Rotifera *A. priodonta, A. sieboldi, Polyarthra dolichoptera* Idelson, 1925 and Copepoda *Eudiaptomus gracilis* (Sars, 1862). The values of the used RCC index (Fig. 6) indicated that conditions in the reservoir are close to these in the other costal swamps and lakes in the region.

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Pollen Morphology and Pollen Production of Invasive and Native Impatiens Species Growing in Bulgaria

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Abstract. The purpose of this study was to establish baseline data concerning certain aspects of pollen biology of the invasive alien species *Impatiens balfourii* and *I. glandulifera,* and the native *I. noli-tangere* to the flora of Lozenska Mt. The aims of this study were: 1. To evaluate variation in pollen morphology, pollen production and fertility/sterility in the populations of *I. glandulifera* in order to obtain data for its invasive success and compare with the other two species; 2. To analyze differences in pollen biology in two flower color forms (pink and violet). The pollen morphology was investigated with light microscope and the pollen grains are 4(5)-zonocolpate oblate or peroblate in equatorial view, and rectangular-obtuse or suborbicular in most 4-zonocolpate grains in polar view, with a reticulate ornamentation. The mean pollen production per flower and stamen was very high in all three species comparable with anemophilous plants. The sterile pollen was between 8.9% and 17.54%. The variations in pollen biology can be related to different evolutionary adaptations of the flowers to be more attractive for pollinators and successful in reproduction and distribution. The results are of importance for taking measures to reduce the invasiveness of these alien species.

Key words: Bulgaria, invasive plant, Impatiens, pollen, reproduction.

Introduction

The genus *Impatiens* L. (Balsaminaceae) is the largest in the family, with c. 1000 species, and occurs mainly in the montane regions in the Old World tropics and subtropics (Grey-Wilson, 1980; Christenhusz et al., 2017). In Europe, most *Impatiens* species are neophytes from Asia and North America except *I. noli-tangere* L. (Maslo & Šarić, 2019) and some of them are worst invasive alien species threatening biodiversity in Europe (Petrova et al., 2013). The genus *Impatiens* contains several entomophilous species which vary in their invasive capacity (Perrins et al., 1993; Ugoletti et al., 2013) and represent a significant threat on the reproductive success of native plants. Among them are *Impatiens glandulifera* Royle, commonly known as Himalayan balsam, spread in almost all European countries, including Bulgaria (Petrova et al., 2013), and *I. balfourii* Hook.f.

The invasive species compete with native plants for nutrients, water, light, space and may also exert indirect pressure by competing for pollinators (Chittka & Schürkens, 2001). *Impatiens glandulifera* has been reported to effectively outcompete native species for pollination services in riparian sites and to represent a significant

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threat on the reproductive success of natives (Chittka & Schürkens, 2001; Vervoort et al., 2011, Cawoy et al., 2012). This plant has numerous attractive large size flowers producing large amounts of pollen and high-sugar content nectar (Vervoort et al., 2011; Ugoletti et al., 2013). These characteristics in the biology of the species attract many insects, and as visitation increases, so does the probability of pollen deposition and seed production (Titze, 2000). Studying the biology of invasive plant species is an essential component for understanding plant invasions in introduced regions (Tanner et al., 2014) and for its sustainable utilization. There are several reports in the literature concerning the pollen biology of *Impatiens* species (Emer et al., 2015; Jacquemart et al., 2015; Vinogradova et al., 2020).

Variations in pollen biology (i.e. pollen morphology, pollen production, pollen viability) can be related to different evolutionary adaptations of the flowers to make them more attractive to pollinators. The earliest description of Balsaminaceae (*Impatiens*) pollen morphology was done on light microscopy (LM) observations in several regional pollen morphological studies and in general surveys of the family (Erdtman et al., 1963; Erdtman, 1966; Huynh, 1968; Moore & Webb, 1978; Moore et al., 1991; Faegri & Iversen, 1989). Later, based on scanning electron microscopy (SEM), more precise characteristic of Balsaminaceae pollen morphology was done (Grey-Wilson, 1980; Lu, 1991). The information for separate *Impatiens* species was presented by different authors who accepted pollen aperture, shape, ornamentation, and density of columella in the lumina as the most important characters for distinguishing species (Perveen & Quiser, 2001; Pudjoarianto & Utami, 2002; Beug, 2004; Janssens et al., 2019; Vinogradova et al., 2020; Wang et al., 2020). Such information was provided for the populations of the species from different part of their area, but no data so far are reported for Bulgaria. Pollen morphology was used for classification of the taxa and decisions related to the systematic position in the family Balsaminaceae. A general survey of palynological variation in Balsaminoid Ericales was presented by Janssens et al. (2005) where LM, SEM and TEM results were combined to present a complete pollen description. Palynological characteristics of Balsaminaceae such as small to medium-sized pollen grains with three to four apertures, which can be either colpate or porate, and a sexine sculpturing varying from coarsely reticulate to almost microreticulate is mainly based on the pollen characters of the larger genus *Impatiens*. Despite the young evolutionary age of *Impatiens* and its rapid radiation, a palynological variability could be observed, as the ancestral pollen type in *Impatiens* being a triangular, tri-aperturate pollen grain with reticulate sexine ornamentation has evolved into a 4-aperturate, rectangular pollen grain with the same ornamentation (Janssens et al., 2012).

The production and the dispersal of pollen have both biological and genetical implications for the quality and genetic value of the seed produced (Khanduri & Sharma, 2002). Studies on integration of alien species into native communities emphasize the role of pollinators in dispersal of alien pollen and a "magnet effect" of the bigger and more colorful flowers, high amounts of nectar and pollen production (Chittka & Schürkens, 2001, Vervoort et al. 2011; Emer et al., 2015). The viability of pollen is important for species dispersal, fitness, and survival (Impe et al., 2020). Pollen mass and the nutritional value of pollen per flower are positively related to pollen viability. Conditions that lead to reduced pollen viability, especially early in pollen development, may greatly reduce the rewards to plant pollinators (Yeamans et al., 2014). Katiyar (2012) demonstrated differences in pollen fertility in five different flower color forms varieties of *Impatiens balsamina* from India.

The purpose of this study was to establish baseline data concerning certain aspects of pollen biology of the invasive alien and native *Impatiens* to the flora of Lozenska and Plana mountains. Our sampling represents a part of the geographic and taxonomic diversity in the family on the territory of Bulgaria, mainly Lozenska Mt. and Plana Mt., where three *Impatiens* species are present: the alien species *Impatiens balfourii* and *I. glandulifera* and the native *I. noli-tangere* which are sympatric in some places with the invasive alien species *I. glandulifera*. The aims of this study were: 1. to evaluate variation in pollen morphology, pollen production and fertility/sterility in the populations of *I. glandulifera* in order to obtain useful data for its invasive success and compare with the other two species; 2. to analyze differences in pollen biology in two flower color forms (pink and violet) of *I. glandulifera*.

Material and Methods

Sampling of material

The pollen samples were collected in June 2020 from five different populations of *Impatiens glandulifera* in Lozenska and Plana mountains at a distance of 1-3 km between them (Table 1). Three of them (№ 1, № 3 and №5) are situated at shadow places in riparian communities at the banks of Iskar river, while the other two (№2 and №4) are situated along the roads far from the river on dry and sunny places. In all sampling places, except for № 2 and № 4, the species appear with two different flower color forms (pink and violet). The population of the native for the flora of Lozenska Mt. species *I. noli-tangere* (№ 7*)* is very close to population №5 of *I. glandulifera. Impatiens balfourii* population (№ 6) is isolated from *I. glandulifera* situated under the *Acer pseudoplatanus* L. trees in the village Passarel.

Flowers (20-0) from 10 individuals with same color were collected before opening, placed in plastic bags, transported to the laboratory and separated for analyses. The material was collected and stored as described by Shivanna & Rangaswamy (1992). The voucher specimens of our study are stored in the Herbarium at the University of Sofia (SO).

Pollen morphology

The collected samples for pollen morphological studies were totally ten: eight for *I. glandulifera*, one for *I. balfourii* and one for *I. noli-tangere.* For each population a mixed sample from a minimum of five flowers with the same flower color from different individuals were prepared for light microscopy (LM) analysis. The pollen samples were acetolysed according to Erdtman (1966) and Skvarla (1966). For LM analysis slides were prepared by mounting pollen in glycerol jelly and the observations were made with an Olympus BX-51 (Tokyo, Japan) microscope under E40, 0.65 and oil immersion (E100, 1.25) magnification, using 10× eyepiece. Pollen grains (30) were measured for six features: polar diameter (P), equatorial diameter (E), apocolpium (A), distance between colpi edges in polar view (M), long diameter in polar view (Ld), and short diameter in polar view (Sd). The P/E ratio and Ld/Sd ratio were calculated to determine the pollen shape. The microphotographs were prepared using Zeiss Axiocam ERc5s (Zeiss, Jena, Germany) camera to show the pollen morphology of the species. The pollen terminology in general follows Faegri & Iversen (1989) and Hesse et al. (2009).

Pollen production and fertility / sterility

The analysis of pollen production and pollen fertility/sterility was conducted on the same samples prepared by following Godini (1981). Eighteen anthers from unopened flowers from 6 different individuals (three flowers per individual) per population were placed in separate vials and treated with 1 mL of 0.1% aqueous solution of a detergent and Alexander stain (Alexander, 1969) in the ratio 9 : 1. The Alexander stain is used for differential staining of aborted and non-aborted pollen (grains stained in red are considered fertile). A Fuchs-Rosenthal haemocytometer was used to count pollen grains and calculate pollen production following procedure described by Pavlova & Bani (2019).

Statistical analysis

Univariate and multivariate statistical procedures were applied to examine variation among the *Impatiens* populations. Data for pollen production and fertility/sterility were analyzed using analysis of variance (ANOVA) and the means were statistically grouped by Tukey's (HSD, honestly significant difference) test ($P < 0.05$). Means and standard deviations were calculated for measured pollen characters, pollen production and fertility/sterility.

Pearson correlations between flower pollen production, fertility and pollen morphological characters P, E, A, M, Ld, and Sd as variables were performed. Correlations among pollen production and fertile/sterile pollen were statistically evaluated by calculating the correlation coefficients (r) at different P-values. The results were considered significant at $P < 0.05$.

Cluster analysis using Euclidean distances and unweighted pair group average (UPGMA) was also used to express the similarities between studied populations of *Impatiens* species based on mean values of all measured pollen characters, pollen production and pollen fertility/sterility. Statistical analyses were carried out using Statistica 7.0 program (Statsoft Inc., Tulsa, OK, USA).

Results

Pollen morphology

Pollen description is presented for each species based on light microscopy (LM) observations and measurements of six characters. A summary of the results are presented in Table 2. *Impatiens glandulifera* (Fig.1A-E) pollen grains are 4(5)-zonocolpate, oblate or peroblate (P/E 0.42-0.6) in equatorial view and rectangular-obtuse or suborbicular in most 4-colpate grains in polar view (Ld/Sd 1.93-2.8), mean dimensions P × E = 16.5–18.54 × 34.88–39.38μm and Ld × Sd = 35.14–41.2 × 26.04–30.4 μm. The apertures are simple and consist only of an ectocolpus. The ectocolpi are very narrow. The colpus margin is uneven, the colpus membrane is usually invisible covered by the margins of the colpus. The exine is 1-2 μm thick. The ornamentation is reticulate with difference in shape of lumina (0.5-2.5 μm). The number and size of the granules inside the lumina are variable. The granules are solitary or fused in clusters of two or more. The populations of *I. glandulifera* can be divided into two groups (small and medium) considering the dimensions of the pollen grains, their shape in equatorial and polar view. The smallest pollen grains of *I. glandulifera* were observed in populations №2 and №4, both having pink flowers and differentiated from all other studied populations.

Impatiens balfourii (Fig. 1F-J) pollen grains are 4-zonocolpate, peroblate (P/E 0.34–0.48) in equatorial view and rectangular-obtuse in polar view (Ld/Sd 1.96–3.1), mean dimensions P × E = 15–22.5 × 40.5–49.5 μm and Ld × Sd = 39.5–52.5 × 19.5–34.5 μm. Ectocolpi are very short and narrow, not clearly visible. The exine is 1.5–3 μm thick. The ornamentation is reticulate with larger lumina and sometimes coalescences between adjacent lumina are visible caused by an incomplete surrounding by the muri (Fig. 1J). *Impatiens balfourii* pollen grains are morphologically well distinguished from the other two species because of the longer equatorial diameter (E), shortest and very narrow colpi and larger lumina. The density of the lumina is smaller compared to the other two species. Moreover, 5-zonocolpate pollen grains were not found this species.

Impatiens noli-tangere (Fig. 1 K-O) pollen grains are also 4-zonocolpate oblate or peroblate (P/E 0.39–0.63) in equatorial view and rectangular-obtuse in polar view (Ld/Sd 1.68–3), mean dimensions P × E = 15–25.5 × 37.5–46.5 μm and Ld × Sd = 35.5–49.5 × 21–36 μm. The ectocolpi are shorter compared to *I. glandulifera* and slightly wider with uneven margin. In LM observation in polar view margo is clearly visible around the ectocolpi area (Fig. 1K). It is differentiated from the rest of the sexine most probably by the ornamentation thickness which appears as a main difference from the other two species. The exine is up to 2 µm thick. The ornamentation is reticulate with larger lumina and more granules inside compared to *I. glandulifera*. The pollen grains of the only native to the flora of Bulgaria species *I. noli-tangere* are closer to *I. glandulifera* than to *I. balfourii* based on their dimensions, shape in polar and equatorial views and ornamentation. Similarly to the other species crystals of calcium oxalate (raphide) were found in all analyzed samples.

Table 1. List of the localities of the studied populations of *Impatiens* species.

Population №	Species	Locality	Site code	Geographical coordinates	Altitude [m]
1	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Devil's Bridge"; soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 1	42°34'58.674''N 23°25'39.212''E	650
2	*I. glandulifera*	Vitosha Mt. floristic Region, Plana Mt., near village Dolni Okol, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Pl 2	42°30'27.873''N 23°30'29.057''E.	880
3	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Fallen tree bridge" on the left bank of the Iskar River, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 3	42°34'5.006''N 23°25'42.95''E	610
4	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., along the road Sofia -Samokov, dry open terrain, soil type: Leptosols (WBR 2014), part of ruderal grass community.	Lz 4	42°34'06.8"N 23°25'50.1"E	680
5	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., screes on the right bank of the Iskar River; initial stage of soil formation, community of *Fagus sylvatica* L. close to the screes.	Lz 5	42°34'2.076''N 23°25'48.594''E.	630
6	*I. balfourii*	Sredna gora floristic Region, Lozenska Mt., Passarel village soil type: Leptosols (WBR 2014), with partial shade under the *Acer pseudoplatanus* trees.	Lz 6	42°32'29.0"N 23°29'52.9"E	700
7	*I. noli-tangere*	Sredna gora floristic Region, Lozenska Mt., soil type: Fluvisols (WBR, 2014), part of community *Alnus glutinosa and Alnus incana* community on the right bank of the Iskar River.	Lz 7	42°34'02.1"N 23°25'48.6"E	615

Table 2. Pollen morphological data of the examined populations of *Impatiens* species with measurements (µm) for each flower form with mean (in brackets) ± standard deviation and ranges of the pollen characters: polar (P) and equatorial (E) diameter, apocolpium (A), long distance between colpi in polar view (M), long diameter in polar view (Ld), short diameter in polar view (Sd), index P/E, and index Ld/Sd. Site codes correspond to data in Table 1. Abb. pink flower (p); violet flower (v).

Species	Site code	P	E	A	M	P/E	Ld	Sd	Ld/Sd
I. glandulifera	Lz 1p	16.5-19.5 (18.59±0.7)	33-39.8 (37.2±1.36)	22.5-30 (26.46±1.6)	18-28 (22.71±1.49)	0.5-0.6 (0.5±0.03)	33-40.5 (37.1±1.36)	25.5-31.5 (28.77±1.23)	1.7-2.3 (1.94±0.09)
	Lz 1v	16.5-22.5 (18.54±0.59)	34.5-42.0 (38.2±1.17)	24-37.5 (37.5±1.96)	18-28.5 (24.54±1.66)	0.4-0.6 (0.6±0.04)	36-42.0 (38.7±1.03)	24-30 (28.61±1.09)	1.8-2.4 (2.03±0.09)
I. glandulifera	Pl 2p	13.5-19.5 (16.5±0.82)	31.5-37.5 (34.88±1.24)	19.5-28.5 (25.55±1.29)	15-24 (20.57±1.41)	0.39-0.59 (0.47±0.05)	30-39 (35.46±1.52)	24-42 (27.7±2,3)	1.28-2.2 (1.94±0.15)
I. glandulifera	Lz 3p	15-22.5 (17.36±1.17)	31.5-43.5 (39.38±1.46)	22.5-31.5 (27.96±1.39)	19.5-28.5 (24.27±1.7)	0.34-0.62 (0.44±0.03)	34.5-43 (39.21±1.41)	24-30 (27.16±1.17)	1.8-2.6 (2.18±0.14)
	Lz 3v	15-19.5 (17.41±0.88)	34.5-45 (38.2±1.5)	15-30 (26.46±1.91)	12-27 (21.75±1.88)	0.4-0.52 (0.46±0.03)	30-39 (37.07±1.33)	25.5-33 (28.93±1.08)	1.67-2.16 (1.93±0.09)
I. glandulifera	Lz 4p	15-19.5 (16.77±0.94)	30-40.5 (35.41±1.75)	22.5-30 (25.88±1.29)	16.5-25.5 (20.89±1.33)	0.4-0.59 (0.48±0.04)	30-40.5 (35.14±1.64)	22.5-28,5 (26.04±1,06)	1,66-2,4 (2.03-0.13)
I. glandulifera	Lz 5p	16.5-19.5 (17.7±0.59)	36-45.5 (38.9±0.86)	22.5-33 (28.8±1.41)	21-30 (24.4±1.26)	0.41-0.52 (0.45±0.02)	36-48 (41.2±1.73)	25.5-36 (30.4±2.12)	1.75-2.45 (2±0.1)
	Lz 5v	15-18 (16.7±0.7)	34.5-40.5 (37.39±0.94)	22.5-31.5 (26.89±1.36)	18-28.5 (22.88±1.6)	0.37-0.52 (0.45±0.03)	30-40.5 (37.23±1.36)	22.5-30 (26.03±1.06)	1.81-2.5 (2.15±0.11)
I. balfourii	Lz 6	15-22.5 (19.23±0.92)	40.5-49.5 (45.99±1.31	30-37.5 (34.13±1.53)	34.5-42 (38.04±1.47)	0.34-0.48 (0.42±0.03)	39-52.5 (45.71±2.01)	19.5-34.5 (28.29±1.95)	1.96-3.1 (2.44±0.17)
I. noli-tangere	Lz 7	15-25.5 (20.19±1.38)	37.5-46.5 (41.36±1.48)	18-36 (28.55±2.06)	18-30 (24.43±1.66)	0.39-0.63 (0.49±0.05)	34.5-49.5 (42.08±2.08)	21-36 (29.87±2.3)	1.68-3 (2.13±0.14)

Pollen production and fertility/sterility

The pollen production per flower and stamen, and fertility/sterility percentage for studied *Impatiens* species and populations was calculated and the mean values are presented in Table 3. The lowest flower pollen production-was calculated for *I. noli-tangere* and the highest was calculated for *I. glanfulifera* population №4. The mean flower pollen production of *I. balfourii* was also low (mean 197639) and not significantly different from FPP of *I. noli-tangere* (mean 194444). All studied populations of the invasive alien species *I. glandulifera* show higher FPP compared to the other two species and flower pollen production varied between 772613 and 1350000 pollen grains. The lowest flower pollen production was recorded in population №5 and the highest in population №4. The flower pollen production was higher in flowers with pink color compared to violet flowers of the same population and between populations. The analysis of variance (ANOVA) performed on the flower pollen production show violet flowers from population №5 was statistically different from flower pollen production for the pink flowers from populations №№2, 3 and 4 (F = 4.637, P = 0.00017). Variation of pollen production was found for the flowers of the same individual as well. Statistically differences were proved by the post-hoc test (Tukey's HSD) (Table 3).

The percentage of fertile pollen varies between 82.49% and 91.09% (Table 3). The sterile pollen is above 5%, a limit considered as a normal abortion (Mičieta & Murin 1996), and is between 8.91% and 17.54%. The analysis of variance (ANOVA) performed on the percentages of the fertile and sterile pollen (Table 3) and proved by the post-hoc test (Tukey's HSD) demonstrated they were not significantly different (P = 0.05).

Correlations between variables such as flower pollen production and pollen morphological characters P, E, A, M, Ld, and Sd ($^{**}P< 0.01$; $^{*}P<0.05$, n=10) were calculated (Table 4). Positive correlation coefficients were found between polar diameter (P) and equatorial diameter (E), long and short diameters (r=0.72, r=0.74, and r=0.63, respectively, P<0.05); equatorial diameter (E) and apocolpium (A), distance between colpi edges in polar view (M), and long diameter in polar view (Ld) (r=0.69, r=0.94, and r=0.96, respectively, P<0.05 and P<0.01); apocolpium and distance between colpi edges in polar view (M) from one hand and with distance between colpi edges in polar view (Ld) from the other (r=0.80, and r=0.68, respectively, *P*<0.01 and *P*<0.05). Correlation between M and Ld were statistically significant and positive as well. Significant negative correlations (*P*<0.05 and *P*<0.01) were found only between flower pollen production and all measured pollen characters except apocolpium (A) and pollen short diameter in polar view (Sd). Pollen fertility/sterility as a variable does not show significant correlations with all other variables and is excluded from Table 4.

The relations between species populations based on pollen morphological characters, flower pollen production and fertility/sterility were examined using cluster analysis (Fig. 2). The populations of *I. balfourii* and *I noli-tangere* demonstrate the shortest Euclidean distance and they were separated from *I. glandulifera* populations. Very close to each other were populations № 2, 3 and 4 of *I. glandulifera*, all with pink color of their flowers. The individuals with violet flowers from population №3 were separated from them because of significant differences in pollen fertility and Ld:Sd ratio. The populations № 2, 3 and 4 are clustered separately from populations №1 and 5 at linkage distance 100000. The last two populations are separated from each other based on the pollen characters, mainly by the polar diameter (P).

Table 3. Mean pollen production, fertile and sterile pollen ± standard deviation of three replicates for each flower form of the *Impatiens glandulifera* populations, *I. balfourii,* and *I. noli-tangere*. Significant differences (based on one-way ANOVA and Tukey HDS-tests) are indicated with different letters, *P* < 0.05. Site codes correspond to data in Table 1. Abb. pink flower (p); violet flower (v).

Site Code	**Pollen production**		**Fertile pollen (%)**	**Sterile pollen (%)**
	flower	**stamen**		
		Impatiens glandulifera		
Lz 1p	1022951 ± 82405abc	204590 ± 16481abc	89.3 ± 4.53a	10.7 ± 4.53a
Lz 1v	997413 ± 245688abc	199483 ± 49138abc	85.82± 10.39a	14.18± 10.39a
Pl 2p	1358602 ± 469551a	271720 ± 93910a	87.95± 9.57a	12.05 ± 9.57a
Lz 3p	1350000 ± 126997a	270000 ± 25399a	88.27 ± 5.53a	11.73 ± 5.53a
Lz 3v	1239392 ± 227536ac	247878 ± 45507ac	82.46 ± 3.87a	17.54 ± 3.87a
Lz 4p	1368637 ± 378471a	273727 ± 75694a	91.09± 1.58a	8.91± 1.58a
Lz 5p	885677 ± 79850bc	177175 ± 15970bc	88.6 ± 4.1a	11.4 ± 4.1a
Lz 5v	772613 ± 181566b	154523± 36313b	82.49 ± 6.89a	17.47 ± 6.89a
		Impatiens balfourii		
Lz 6	197639 ± 53351d	39528 ± 10670d	87.59 ± 5.85a	12.41 ± 5.85a
		Impatiens noli-tangere		
Lz 7	194444 ± 62765d	38889 ± 12553d	88.45 ± 7.23a	11.55 ± 7.23a

Table 4. Pearson correlation coefficients between measured flower pollen production (FPP) and pollen morphological characters P, E, A, M, Ld, and Sd (***P* < 0.01;**P* < 0.05, n=10).

Variable	**FPP**	**P**	**E**	**A**	**M**	**Ld**	**Sd**
FPP	ns	-0,79**	-0,81**	ns	-0,72*	-0,83**	ns
P	-0.79**	ns	0,72*	ns	ns	0,74*	0,63*
E	-0,81**	0,72*	ns	0,69**	0,94**	0,96**	ns
A	ns	ns	0,69*	ns	0,80**	0,68*	ns
M	-0,72*	ns	0,94**	0,80**	ns	0,91**	ns
Ld	-0,83**	0,74*	0,96**	0,68*	0,91**	ns	ns
Sd	ns	0,63*	ns	ns	ns	ns	ns

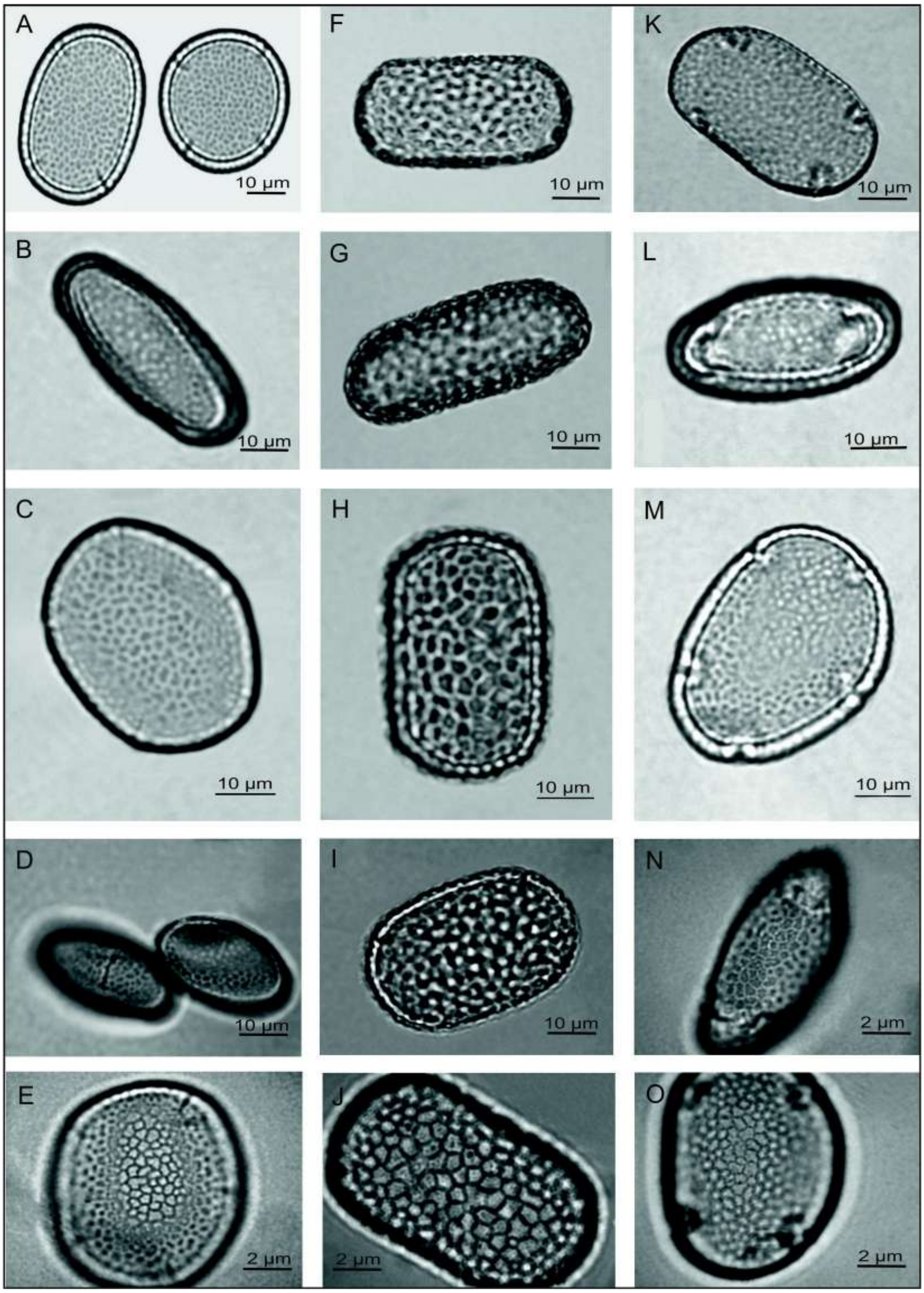

Fig. 1. LM micrographs of *Impatiens glandulidera* (A-E), *I. balfourii* (F-J), and *I. noli-tangere* (K-O) pollen grains: (A) rectangular-obtuse and subcircular pollen in polar view; (F, I, K) polar view; (B, G, L) peroblate pollen in equatorial view; (C, M) 5-colpate and (H) 4-colpate pollen; (D, N) colpus; (D, J, O) reticulate ornamentation. (Photo: D. Pavlova).

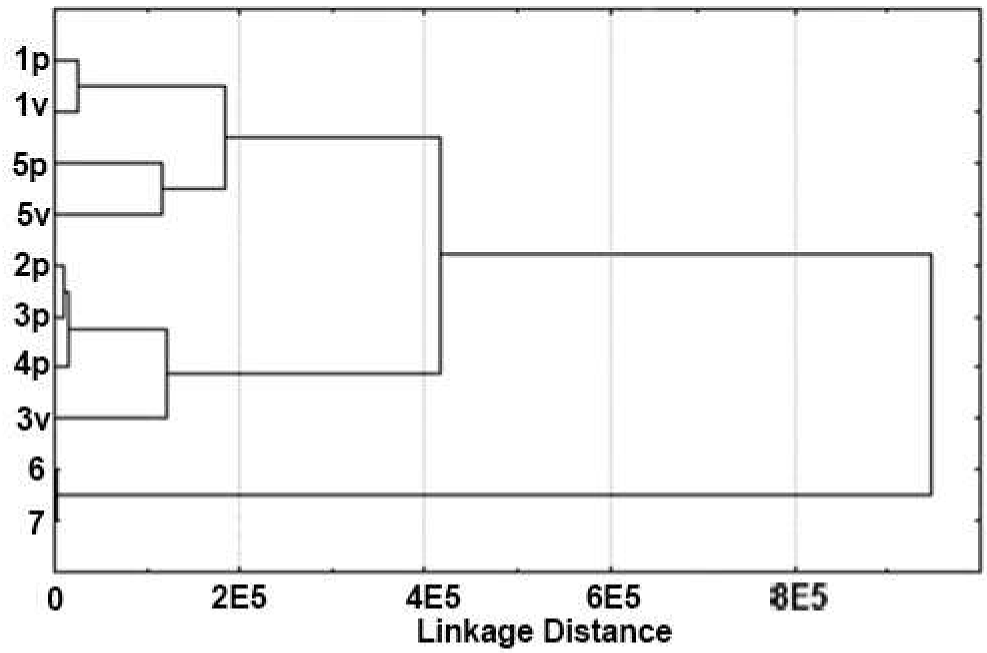

Fig. 2. Similarity dendrogram obtained by cluster analysis (Euclidean distances) applied to data of the measured pollen characters and calculated flower pollen production and fertility.

Discussion

The pollen morphological data for the studied *Impatiens* species confirm previous information about these species (Huynh, 1968; Moore et al., 1991; Beug, 2004; Janssens et al., 2005; Vinogradova et al., 2020). The three studied species are 4(5)-zonocolpate, except *I. balfourii* where no 5-zonocolpate pollen grains were found. According to Janssens et al. (2005) the main *Impatiens* pollen type is 4-aperturate, rectangular pollen grain with reticulate sexine ornamentation which originated from a triangular, 3-aperturate pollen grain with reticulate sexine ornamentation, considered as ancestral pollen type in *Impatiens*.

The apertures variation between 4 and 5-zonocolpate pollen grains confirms data presented by many authors and do not agree with Janssens et al. (2005). Studying pollen morphology of African *Impatiens* species Grey-Wilson (1980) mentions that the presence of 5-aperturate pollen in *Impatiens* is typical for hybrids. Bearing in mind that there are no described native hybrids for *I. glandulifera, I. balfouri* and *I. noli-tangere* till now, we can conclude that this phenomenon is more widely distributed between species in the genus and it is probably related to the pollination success of these plants. Similarly, high pollination success has been observed for other generalist and attractive alien species, such as *Rhododendron ponticum* and *Senecio inaequidens* (Vervoort et al., 2011). Pollen aperture number heteromorphism is considered as an evolutionary advantage if there is a trade-off between germination speed and life expectancy of the pollen grain, and if pollination conditions vary unpredictably (Nadot et al., 2000). The apertures provide exits for recognition proteins (Moore et al., 1991) as well and this is related to the distribution success of the species. As pollen of *Impatiens* species is rich in proteins, it is one of the most abundant pollen in bee breads (Donkersley et al., 2017).

The abundance of pollen and nectar in the invasive species *I. glandulifera* attracts potential bee pollinators from other native plants resulting in reduced seed set and fitness among other plants (Chittka & Schürkens, 2001).

Differences in pollen size and shape are found not only between species but also at the population level. The size of the pollen grains decreases in the order *I. balfourii* > *I. noli-tangere* > *I. glandulifera*. Our results confirm data presented for *I. noli-tangere* and *I. glandulifera* by Vinogradova et al. (2020), for *I. noli-tangere* by Beug (2004), and differ from Janssens et al. (2005) where pollen of *I. glandulifera* was bigger from the pollen of *I. noli-tangere*. The relation between studied species is proved by the performed cluster analysis (Fig. 2), where *I. balfourii* and *I. noli-tangere* are separated from *I. glandulifera* populations. The pollen size in *I. glandulifera* is quite variable between populations and two groups are formed. One of the factors influencing pollen size proved in our study, is the flower pollen production which negatively statistically correlates with polar and equatorial diameters, apocolpium, and distance between colpi edges in polar view. The second factor can be soil moisture and soil nutrients. The populations of *I. glandulifera* that are at distance from the rivers demonstrate smaller pollen size. Infraspecific pollen variation is frequently linked to the size of the species area and then in turn, to the ecological diversity (Guinet & Ferguson, 1989). The growth under unusual ecological conditions and changes in soil mineral nutritive elements also increase the amplitude in the variation of pollen characters (Guinet & Ferguson, 1989; Katiyar et al., 2012). However, such a relationship should be proved in future studies under experimental conditions.

Variation in pollen shape and outlines in polar and equatorial view found in *Impatiens* pollen confirm Janssens et al. (2005). The rounded pollen for *I. noli-tangere* reported by Vinogradova et al. (2020) is not confirmed. The most variable in shape, oblate or peroblate (P/E 0.42-0.6) in equatorial view and rectangular-obtuse or suborbicular in most 4-zonocolpate grains in polar view, were the pollen grains of *I. glandulifera*. Because of the variation found this pollen character should be used carefully in the identification of the species. The pollen grains are quite variable in shape and size even in the same individual and in the same anther. The smallest pollen grains in the anthers were sterile. Our results demonstrated a higher proportion of sterile pollen in *I. glandulifera* with violet flowers compared to pink flowers (Table 3) and could be a result of genetic and environmental factors. Katiyar et al. (2012) suggest that differences in *I. balsamina* pollen are due to meiotic abnormalities as a result of hybridization and polyploidy. A genetic reason for variation in pollen size and shape can be the diverse reproductive systems in studied *Impatiens* species. Their reproductive systems are prepositions for distribution success as well. According to Vervoort et al. (2011) some alien species such as *I. glandulifera* are successful because of their autogamous breeding system or because of their ability to attract pollinators successfully. The self-compatible species *I. glandulifera* does not suffer from inbreeding depression while this is not true for the native species *I. noli-tangere* (Vervoort et al. 2011). Studies on genetic diversity in *Impatiens* species confirm a low rate of DNA polymorphism in plants from natural populations of *I. noli-tangere* (7.3%) and higher DNA polymorphism in invasive populations of *I. glandulifera* (45.6%) (Kupcinskiene et al., 2015).

Pollen ornamentation in studied species is reticulate but differences in size and number of lumina, granules inside the lumina, and muri shape and height are enough informative to differentiate each of them. The differences in ornamentation between species should be related to their adaptation to specific pollinators as it was previously pointed out for different genera (Guinet and Ferguson, 1989; Pavlova et al., 2016). The reticulate ornamentation is considered ancestral for the *Impatiens* species but variation in ornamentation along with other pollen characters outlines phylogenetical lineage in the genus and Balsaminaceae as well (Janssens et al., 2005, 2012).

High flower pollen production, comparable to anemophilous plants, was found in the studied species and confirmed previous data (Vervoort et al., 2011). The highest amount of pollen produced per flower and stamen was found in the invasive *I. glandulifera*, followed by *I. balfourii* and the native *I. noli-tangere*. As pollen production is genetically and physiologically controlled and species-specific (Ferrara et al., 2007) this result and the high percentage of fertile pollen in invasive species directly can be referred to its pollination and distribution success, but future investigations are needed to elucidate the specifics in this aspect.

Conclusions

Although the pollen morphology of *I. glandulifera* is similar to the native *I. noli-tangere* some adaptations in pollen biology can favor the capacity of invasion. Pollen heteromorphism is more widely distributed in *I. glandulifera* related to the pollination success and distribution of the species. The abundance of pollen and nectar in the invasive species *I. glandulifera* attracts much more pollinators from other native plants, including *I. noli-tangere* in their populations, resulting in reduced seed set and fitness among other plants. Variation in pollen size and shape is found both at species and population level, being the highest in the invasive species *I. glandulifera*. This variation is directly correlated with flower pollen production, soil moisture, and soil nutrients. Variation in pollen size and shape is found between the pink and violet flowering forms of *I. glandulirera* altogether with the variation of the percentage of fertile/sterile pollen and could be a result of genetic and environmental factors. The high percentage of fertile pollen in invasive species is referred as its pollination and distribution success.

As a result of the study, we propose a careful use of pollen character in the identification of the species because of the variation established. Further studies related to the pollination biology of *Impatiens* species are needed to evaluate the invasive success of the alien species.

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Rumex kerneri Borbás (Polygonaceae) in the Bulgarian flora – Morphology, Leaf Epidermis, Pollen Morphology, and Karyology

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Abstract. The current study provides detailed information about *Rumex kerneri* Borbás in the flora of Bulgaria. The data about this species in the floristic literature is contradictory and uncompleted. The morphologically closely allied species *R. cristatus* DC. и *R. patientia* L. misleads in the determination of the species. Both species are used as a referent to establish the discrete morphological characters and metrics. The current study provides detailed information about pollen and fruit morphology, leaf epidermis, and karyology of *Rumex kerneri* Borbás in the flora of Bulgaria. The data about this species in the floristic literature is contradictory and uncompleted. The most reliable characters in the differentiation between *R. cristatus* are *R. patientia* the reduced number of flowers in a cluster of *R. kerneri*, the development of only one tubercle in the mature valves, and the abaxial surface of the leaf blade. Also, the pollen morphology (light and scanning electron microscopy) was confirmed in this study. The somatic chromosome number of the species 2*n* = 80 is also given. This is the first chromosome number for the taxon from Bulgaria and confirms the earlier reports.

Kew words: chromosome number, *Rumex kerneri*, morphology, leaf epidermis, SEM pollen.

Introduction

Subgenus *Rumex* (*Rumex* L., Polygonaceae Juss.) is taxonomically a very difficult group. This fact is caused by the high range of morphological variability, infraspecific variation, widely presented hybridization, and introgression - typical for the European species from section *Rumex* (Rechinger, 1990). As mentioned by Campderá (1819) and Mosyakin (2005), the species of this genus are forming groups with similar morphological descriptions, hampering their diagnostics. Furthermore, according to Rechinger (1932), the hybrids of*Rumex* too often have been determined and reviewed as single species by many authors. An example: originally, the hybrid between *R. confertus* and *R. obtusifolius* was described under the name *R.* × *kerneri* Błocki (Błocki, 1888). However, the name was corrected when Błocki (1889) found out that the epithet had already been applied to another *Rumex* species by Borbás (1884), namely *R. kerneri* Borbás, to honor of Austrian botanist Anton Joseph Kerner von Marilaun (1831-1898).

Rumex kerneri Borbás (subg. *R.*, subsectio *R.*), reviewed as endemic for the

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Balkans (Akeroyd, 1986), have been migrated outside the Balkan Peninsula and is recently known as a neophyte in Austria and Hungary (Rechinger, 1933; 1964), reaches in the east to Southern Ukraine (Eremko, 1997), west to Italy (Prosser, 2000), and in the north to Belgium (Verloove, 2013). From the phytogeographic viewpoint, *R. kerneri* is South-European floristic element with Illyrian-Pannonian areal-diagnosis (Meusel *et al.,* 1965). Due to similar morphological characteristics with *R. cristatus*, many authors include *R. kerneri* as its infraspecific taxon, most often as subspecies (Akeroyd & Webb, 1991).

In Bulgarian flora, before Rechinger (1933), who reported the species for the first time from the Balkan Range, Rila and Rhodopi Mts., the taxon has been not recognized. Probably, this is the reason why in the subsequent floristic editions Stojanov & Stefanov (1948), Stojanov *et al.* (1966) the species is reported for Vitosha Region and Rhodopi Mts. (Western, Central) and is under question for the Balkan Range. Possibly, that is why Delipavlov (2003) treats the species as not confirmed for the flora of Bulgaria. Until recently, no specimens from *R. kerneri* have been deposited in the Bulgarian herbaria (Raycheva, 2009).

The morphology of *R. kerneri* is incompletely described in the Bulgarian literature, and this causes series of wrong determinations. We did not find data about the leaf anatomy and pollen morphology of *R. kerneri.* The data about the chromosome number area few – based on materials from California 2n=80 (Degraeve, 1975; Löve, 1986) and Greece 2n=c. 80 (Löve, 1981). There are no recent studies from other areas of distribution.

In this regard, we undertook a morphological and anatomical study of *R. kerneri,* considering to distinguish it from the related, and most confused in the determination, *R. cristatus,* and *R. patientia*.

Materials and Methods

Plant material collected by the authors from naturally growing populations (Table 1) has been used for the morphological, anatomical, and karyological study. Voucher specimens have been deposited in the herbaria SOA (Agricultural University - Plovdiv) and SOM (Institute of Biodiversity and Ecosystem Research).

Mature specimens with well-developed inner tepals – valves, have been carefully collected for reliable identification of *Rumex* species, according to Rechinger (1932) and Snogerup (1991) and confirmed by our observations.

All relevant literature data have been assembled and checked for additional information on the distribution and taxonomy of the species. The herbarium collections of *R. kerneri* W and WU (acronyms according to Thiers, 2020+) have been studied and revised. The metric data for the height of the plants have been taken in vivo (Table 2).

Leaf epidermis. Fresh basal leaves from 20 individuals have been conserved in 75% ethanol for the epidermal observations. The sections have been prepared manually using a razor, in the middle part of the leaf, on both sides of the half-blades. The epidermis was peeled manually from the leaf blade using pincers. The material was mounted in glycerine slides. The observations have been taken on a Carl-Zeiss Amplival microscope with a 5MPix camera mounted to the eyepiece. The measurements in the microscopic slides have done using a mechanical micrometer, attached to the eyepiece of the microscope. Fifty measurements for each observed character were taken randomly. The parameters of the adaxial (ad) and abaxial epidermis (ab) have been compared – shape and disposition of the common epidermal and stomatal cells. The stomatal shape in the studied species has been compared using the proportion length/with. The count of the stomata has given in magnification 16×16. The obtained values have analyzed using basic descriptive statistics.

Pollen observations

Light microscopy. The observations have been done following the methodology of Swietlinska (1960). Dry pollen grains, slightly heated in a drop of acetocarmine, have been prepared for temporary microscopic slides. The characteristics diameter (µm) and the correlation between fertile and sterile pollen (%) for 1000 pollen grains have been taken 50 times for each evaluated population.

Scanning electron microscopy. Pollen grains collected by the authors have been used for scanning electron microscopy (Table 1). The objects have been observed directly, without any physical or chemical treatment. The pollen has been prepared for observation before scanning, following the methodology of Terziisky & Atanasov (1977) and Terziisky (1983). The observations have been conducted with a JEOL scanning electron microscope, with scanning adaptor JSM-5500.

Karyological studies Root tips of dry nuts (SOA 059243) after germination were pretreated with 0.05% colchicine for 2 hours, fixed in ethanol: glacial acetic acid (3:1) at least for 2 hours at room temperature or 24 h in the refrigerator, and stored in 96% ethanol until required. Hydrolysis has conducted in 1N HCl at 60°C for 9 min. Then the root tips have transferred into HCl:di-ethyl ether (1:1), washed thoroughly in distilled water and stained with hematoxylin after Gomori (Melander & Wingstrand 1953) for 20-25 min at 60°C. Finally, the root tips have squashed in 45% acetic acid and mounted in Canadian balsam. Chromosomes have counted on more than 10 plates to define their numbers. The photos of the best mitotic metaphase cells have been used as a basis for the drawings.

Result and Discussion

Genus *Rumex* L.
Subgenus *Rumex*
Sectio *Rumex*
Subsectio *Rumex*

= Subsect. *Patientiae* Rech. f., Repert. Spec. Nov. Regni Veg. 31 (1933) 230.

R. kerneri Borbás, Fl. Comit. Temesiensis 60 (1884) 34; Rech. f., Repert. Spec. Nov. Regni Veg. 31 (1933) 240 et Fl. Eur. 1 (1964) 86; Prodán, Fl. Reipubl. Popularis Romanicae 1 (1952) 412; Stoj. & Stef., Fl. Bulg. ed. 3 (1948) 346; Stoj., Stef. & Kitan., Fl. Bulg. ed. 4, 1 (1966) 312; Valev, Fl. R. Bulg. 3 (1966) 207; Snogerup & B. Snogerup, Fl. Hellenica 1 (1997) 99; ≡*R. cristatus* subsp. *kerneri* (Borbás) Akeroyd & D.A. Webb, Bot. J. Linn. Soc. 106 (1991) 104; Rech. f. & Akeroyd, Fl. Eur. ed. 2, 1 (1993) 104; =*Rumex confertoides* Bihari, Magyar Bot. Lapok 27 (1829) 73.

Lectotypus: WU-Generale 34260(**!**)**.** Flora comit. Veröcensis Hungariae. Infra cacumen montis Papuk ad pagum Zvecrova, coll. Borbás, 1879.07.14 (orig.: *Rumex patientia*; rev. K.H. Rech. 1936: *Rumex kerneri*).

Specimens of *R. patientia* and *R. cristatus* have found as incorrectly determined as *R. kerneri*. The close characteristics of these species are the reason for the difficulties and mistakes in the determination. That's why we have included *R. patientia* and *R. cristatus* as referent species in the comparison of the morphological and anatomical data.

Revised specimens: W: 00104 - Jugoslavia: Rechinger f. 1956; 01225- Iter Graecum: Rechinger f. 1956; 1935-1643 - Austria: Rechinger 1931; 6333 - Graecia, Phtiotis, in alpestris silvarum et pteridetis jugi Zacharaki circa 36 km ad Macrokomi versus confines Thesaliae, sustr. arenaceo corca 1500 m.: Rechinger f. 1958; 6679 - Iter Aegeum, Creta, Macedonia, Jugoslavia: Rechinger f. 1955; (Originali *R. kerneri*, rev. *R. patientia*, Raycheva 2007): 16111 (Herb. Ernst Vitec 1987); 1997-04628 (Austria: Rechinger 1966); Originali *R. kerneri*, rev. *R obtusifolius*, Raycheva 2007): 05600 - Albania, 1980, Krendl, (Originali *R. kerneri*, rev. *R. patientia*, Raycheva 2007): WU: 1916-32 - Nord Albanien: leg. Dőrfler, det. Rechinger 1916. (Originali *R. kerneri*, rev. *Rumex crispus* L. x *R. patientia* L., Raycheva 2007). Specimens of this hybrid have been determined as *R. kerneri*, because of the similar morphological and metric parameters of the valves and tubercles. The high sterility of the nuts, as well as the different size of the valves and also crumbly fruits, demonstrate the hybrid origin of the specimens below: W: 1965-7592 - Banatus: Prodán 1914, sub *R. kerneri*; 1956-8091 - Standort: Korb 1926; 1980-04007 - Turcia: Rechinger 1974, sub *R. kerneri*; 2006-21578 - Austria: Barta 2005, sub *R. kerneri*; 2005-15615; 15614; 15613 - Austria: Barta 2003, sub *R. kerneri*; 1974-06916 -Austria: Melzer 1973, sub *R. kerneri*.

Distribution and ecology

General distribution. Native to Southern Europe (Balkan peninsula), sporadic in Austria, Hungary, Italy, South Ukraine, and Belgium. Introduced in за North America (California), from Europe.

Distribution in Bulgaria. At the foothill and mountain meadows, around deciduous forests and populated areas. Rhodopi Mts., between 200 and 1250 m above sea level.

Rumex kerneri differs with a later blooming, according to the inhabited in Bulgaria ecological niches. It is localized mainly at the foothill semi-natural meadow communities, but also represented, by small populations, in anthropophilic localities – around the margins of the villages. According to personal observations on natural populations for 10 years, the Bulgarian populations of this species don't show a tendency of expansion and increasing of the number of individuals. While the *R. kerneri* number of populations in its secondary distributional area, as Italy and other parts of Europe, this species is characterized as aggressive, invasive, with fast expansion (Galasso, 2008).

Rumex cristatus is a lowland species, occurred between 0 and 477 m altitude, with Mediterrano – West Asian origin (Rechinger 1959). In Bulgaria, this species is found mainly in ruderal places, forms dense populations. Similar ecological characteristics shows also *R. patientia,* a Caucasian-Pontico-Poannonian floristic element, with a moderate continental European - West Asian areal-diagnosis (Meusel *et al*., 1965). The primary boundaries of its range are not clear. It is often cultivated as a leafy vegetable, and its high ecological tolerance allows its successful resettlement in ruderal and anthropogenically influenced communities, with a large number of individuals in the populations.

Morphological characteristics

The members of the subgenus *Rumex* are characterized with the shrub-like habit, high and thick stems, branched panicles with dense and close leafless flower clusters, large basal leaves – entire, or wavy to irregularly shallowly serrate margin, valves in the fruit with 1, sometimes 3 non-equally developed tubercles. In the occupied area, the species are polyploids, like hexa- and octoploids ($2n = 60, 80$, further studies for *R. patientia* and *R. cristatus* from Bulgarian populations, Raycheva, 2005).

Table 1. List of accessions studied species of subg. *R.*, subsectio *Rumex*: *R. kerneri, R. cristatus, R. patientia* in Bulgarian origin. Legend: [a] The floristic regions follow the standard accepted in *Flora R Bulgaria*. [b] SEM– scanning electron microscopy of the pollen; K– karyological studies; LA – leaf anatomical studies; M–gross-morphological studies; P – pollen analysis. [c] New chorological data from Bulgaria.

Species, voucher number	**Floristic region, locality, UTM, voucher number**	**Applied methods** [b]
R. kerneri Borbás		
SOA 059605	Rhodopi Mts. (central). 35TLG03, 700 m, Meadows above village of Hvoyna, 22.07.2005, coll. Ts. Raycheva	M, K, LA, P
SOA 56414	Rhodopi Mts. (central) 35TLG13, 780 м, Grass places around the village of Jougovo, 10.07.2003 coll. Ts. Raycheva	M, LA, P
SOA 059243	Rhodopi Mts. (eastern), 35TMF28, 200 m, Between the villages	M, K,

	of Siv Kladenets and Mandritsa, 18.06.2008, coll. Ts. Raycheva.	SEM
SOA 56414	Rhodopi Mts. (western)[c] – Near the village of Youndola, on the road to Belmeken Dam Lake, 34TGM36, 1250 m, 15.07.2016, coll. Ts. Raycheva, K. Stoyanov	M, LA
R. cristatus DC.		
SOA 056842	Rhodopi Mts. (eastern), 35TLF99, 272 m, The village of Rogach, Krumovgrad district, 14.07.2005, coll. Ts. Raycheva	M, LA, P
SOA 56939	Black Sea Coast (northern), 35TNJ90, 10 m, Near city of Varna, between Albena and town of Balchik, 18.06.2004, coll. Ts. Raycheva	M, SEM
SOA 057065	Black Sea Coast (south), 35TNJ76, 20 m, The town of Tsarevo, 3.07.2014.	P
SOA 059247	Strandja Mt., 35TNJ75, 56 m, Grass places, near the village of Brodilovo, 23.06.2007, coll. Ts. Raycheva	M, LA
SOA 056941	Pirin Mt. (south), 34TGL19, 477 m, In the village of Gorno Spanchevo, 18.06.2011, coll. Ts. Raycheva	P
R. patientia L.		
SOM 163984	Znepole region, 34TFN43, 916 м, Near to the village of Paramoun, 05.07.2006, coll. Ts. Raycheva	LA, M, SEM
SOA 059477	Balkan Range (central) 35TKH63, 710 м, Near to village of Anton (town of Pirdop), 03.07.2005, coll. Ts. Raycheva	M, LA, P
SOA 059235	Sredna Gora Mts. (western), 35TKH60, 530 m, Near to town of Panagjuriste, 03.07.2005, coll. Ts. Raycheva	M, LA, P
SOA 059478	Rhodopi Mts. (central), 35TLG03, 695 m, Grasslands near to village of Hvoyna. 18.07.2016, coll. Ts. Raycheva, K. Stoyanov	P

Fig. 1. *Rumex kerneri* Borbás: A – flower clusters; B – mature fruits.

Table 2. Comparative morphological parameters of *R. kerneri, R. patientia,* and *R. cristatus.*

Character	**Values x±Sx** (min-max)		
	R. kerneri **Borbás**	***R. cristatus*** **DC.**	***R patientia*** **L. subsp. *patientia***
Stem height, cm	(75) 90-120 (160)	(125)135-180 (200)	(80) 165-190(210)
Valve length, mm	**5.86**±0.05 (5.2-6.5)	**6.57**±0.05 (5.3-7.8)	**7.98**±0.1 (6.8-10)
Valve width, mm	**5.46**±0.04 (4.9-6)	**6.18**±0.05 (5-7.6)	**7.48**±0.08 (5.9-9.6)
Tubercle length, mm	**2.06**±0.02 (1,9-2,3)	**2.78**±0.03 (1-3,4)	**1.53**±0.,02 (1.1-1.9)
Tubercle width, mm	**1.48**±0.02 **(**1,1-1,8)	**2**±0.02 (1.4-2.8)	**0.95**±0.01 (0.5-1.3)
Nut length, mm	**2.66**±0.04(2.1-3.1)	**2.92**±0.02 (2.3-3.4)	**3.24**±0.03 (2.8-4)
Nut width, mm	**1.54**±0.02 (1.4-1.8)	**1.82**±0.02 (1.5-2.2)	**1.91**±0.02 (1.5-2.3)
Fruit stalk, mm	**7.63**±0. (6.4-9.1)	**8.09**±0.17 (5.8-11.3)	**10.28**±0.21 (7-14.2)
Number of flowers in a cluster	(12)**15–20**(26)	(21)**30-60**(68)	(17)**20-50**(55)

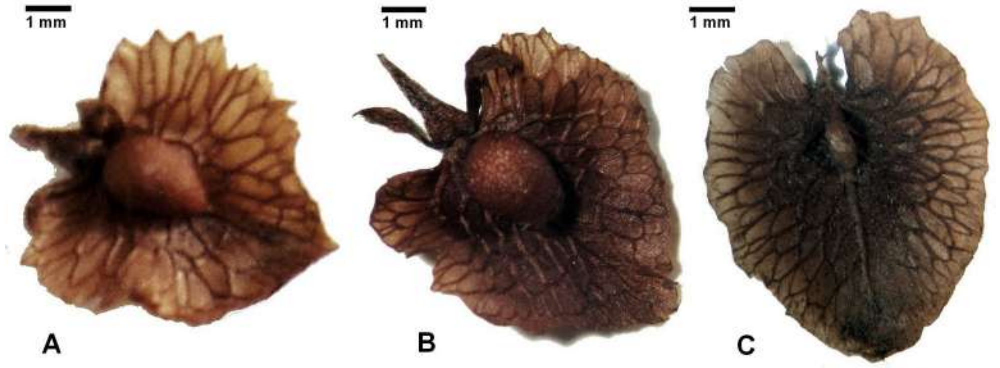

Fig. 2. Valves of A – *Rumex kerneri*; B – *R. cristatus;* C – *R. patientia.* Scale bar: 1 mm.

A character with a diagnostic value for the determination of *R. kerneri* is the presence of sparse hairs on both surfaces of the basal (cataphylls) leaves, and their absence in *R. patientia* and *R. cristatus*. The stems of *R. kerneri* a shorter approximately 90-120 cm, and the number of the flowers in a cluster is the lowest – about 20 (Fig. 1). The stems of the other two related species reach a height between 135 and 200 cm and have densely clustered flowers– between 30 and 60 flowers in a cluster. The metric values of the fruiting stalk are definitive too – shorter in *R. kerneri*, as well as the sizes of the mature valves and tubercles (Table 2). The mature fruits of *R. kerneri* (Fig. 2A) and *R. patientia* (Fig. 2C) have one smaller tubercle (about 2×1.5 mm). The tubercles of the other two valves lack, or are reduced to nodes. Compared to them, *R. cristatus* has three unequally developed (by size) tubercles, the biggest one about 2.8 × 2 mm, i.e. has the larger sizes as compared to the remaining two species (Fig. 2B). The nut's sizes correspond to those of the valves, and the longest are those of *R. patientia* (3.2 mm) and respectively the smallest are those of *R. kerneri* (2.7 mm).

Karyology

Karyology of *R. kerneri* in Bulgaria has shown stable chromosome numbers 2n = 8x = 80 (Fig. 3). The our study confirmed the octopoid number 2n = 80 (Degraeve, 1975; Löve, 1986) и 2n = c. 80 (Löve 1981). Karyologically, the species is studied for the first time in Bulgarian specimens.

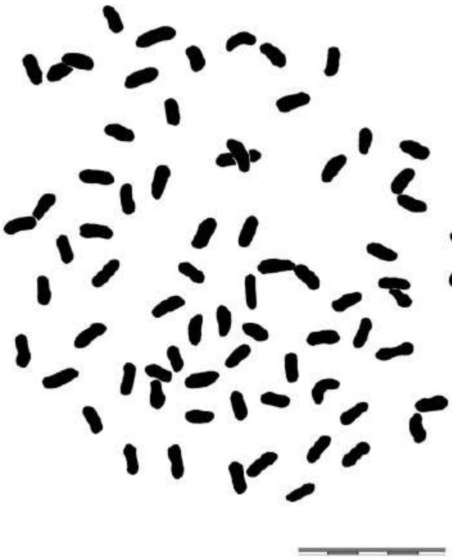

Fig. 3. Drawing of mitotic metaphase plate of *Rumex kerneri*, 2n=80. Scale bar = 5 µm.

Leaf epidermis

The leaf has a dorsoventral type in cross-section. The palisade parenchyma in the studied species has 2 rows. The spongy parenchyma has 4-5(6) rows. The geodes of calcium oxalate, reported by Metcalfe & Chalk (1950) were found in all three studied species.

The epidermal complex of the evaluated taxa contains 4 types of cells: common epidermal cells, stomatal guard cells, subsidiary cells, and trichomes. The leaves are amphistomatic – with stomata on both of the surfaces. The density of the stomata is regularly higher on the abaxial surface of the leaf. The stomata on both surfaces are messily oriented.

The indumentum of the leaf and stem of *R. kerneri* is represented by unicellular papillae on the abaxial surface on the abaxial leaf surface, finely distributed on the stem base. The leaves and the stems of the other referent species have not simple trichomes. All three taxa showed the presence of unicellular and multicellular vesicular glands, similar to the papillae but differing by their spheric shape, without basis, containing 1 to 4-5 cells, and formed by swelling of the apical part of the epidermal cells. According to Metcalfe & Chalk (1950), the glands are typical for genus *Rumex*.

According to Aneli (1975), the variability of the shape and the degree of curvature of the anticlinal walls in the common cells have defined limits. The shape of the cells varies from isodiametric to oblong-amoebic. The anticlinal cells of the adaxial epidermis are straight in *R. patientia* (Fig. 4E) and *R. cristatus* (Fig. 4C), while in *R. kerneri* (Fig. 4A) they are moderately curved (Fig. 4A). This feature is less differentiated on the abaxial epidermis – the studied taxa have more or less curved walls (Fig. 4E, 4D, 4F).

Stomatal type. All three species frequently showed the presence of anisocytic type of stomata, which is typical for order *Polygonales* (Inamdar, 1969). Single stomata are surrounded by symmetrically arranged subsidiary cells, directed along the long and short axes of the guard cells, which is a sign of an actinocytic type (according to the classification of Metcalfe & Chalk, 1950). Actinocytic stomata with radially oriented periosteal cells, which are difficult to distinguish from the common epidermal cells, are also observed. Besides, tetracytic stomata are found. All these types occur within the same species. Although the studied species from the *Rumex* subsection show a wide range of stomata types, the main anisocyte type for the family is dominant in all three studied species (Fig. 3).

Shape, size, and count of the stomata. On the adaxial epidermis, the stomatal index is between 1,38-1,45 (*R. cristatus*) and 1,51-

1,61 (*R. kerneri*) and describes the stomata as oval-elliptical. The metrics of the stomatal guard cells have a low amplitude of variation (Table 2). The sizes (length and width) are higher on the abaxial side. The number of the stomata is also variating parameter, correlating to the irregular distribution of the stomata and their sizes. In the investigated Bulgarian populations, the number of stomata on the adaxial is about 32 to 52 for mm^2, and on the abaxial side– between 52 and 73 per mm^2 (Table 3). Lower values of variations are found in *R. cristatus* and *R. kerneri.* However, no significant difference is recorded in the widths of the stomata in all the three investigated species. Their lengths are adequately related to the level of the ploidy. The stomata of the octoploids *R. cristatus* and *R. kerneri* are longer than those of the hexaploid *R. patientia*.

Pollen analysis. The microscopic analysis of the pollen fertility is a good indication for the non-hybrid origin of the investigated samples of species (Table 4). We consider this indicator to be an important feature when working with species from this group, due to widespread hybridization and the existence of introgressive populations. Between the species of the typical subsection were not found discrete differences of the pollen size, but, according to the high levels of ploidy in the group (6*x* to 8*x*), the sizes correspond to the classification of Erdtman (1952). *Rumex patientia* and *R. cristatus* fall into the group with medium (31-37.5 µm), while *R. kerneri* has large pollen grains (38-41 µm).

The performed scanning electron microscopy analysis of *R. kerneri* (Fig. 4A), together with the related *R. patientia* (Fig. 4B) and *R. cristatus* (Fig. 4C) pollen grains displayed uniform structural organization of the submicroscopic surface of the exine. The observation confirms the results in previous studies (Romanova, 1993; Zhou et al., 2000). The sculpture of the exine is perforate-microechinate, without differences in the observed taxa. The pollen grains have 3(4)-colpate apertures, with shallow ends of the furrows, and as result – with difficult to measure exact sizes. The submicroscopic structure of the sporoderm in the investigated species did not show discrete differences and could be valued as a feature with a low taxonomic value.

Table 3. Metric characteristics of the epidermal stomata – mean ± standard deviation.

Voucher specimen (see Table 1)	**Stomata**					
	Adaxial epidermis			**Abaxial epidermis**		
	Length, µm	**Width, µm**	**Count, mm²**	**Length, µm**	**Width, µm**	**Count, mm²**
	R. kerneri Borbás					
SOA 056414	52.8±0.47	32.67±0.44	42±1.75	48.53±0.67	28.05±0.18	56±1.77
SOA 059243	52.07±0.9	34.34±0.64	42±2.27	50.16±0.58	32±2.27	54±2.35
	R. cristatus DC					
SOA 056939	48.24±1.78	33.21±0.93	49.1±2.28	46.98±0.67	34.56±2.12	53.21±1.8
SOA 059247	50.12±2	36.1±2.18	47±2.22	48.5±1.26	32.02±1.94	52.5±2.92
	R. patientia L.					
SOA 059235	42.67±2.43	32.4±2.73	52±2.46	42.06±4.31	31.85±2.27	73.33±2.4
SOA 059477	46.51±3.16	30.21±1.63	32.33±2.28	46.03±2.21	34.9±2.64	50±1.86
SOA 163984	37.99±2.87	34.81±2.86	48.3±2.6	33.93±1.66	29.34±1.34	67.3±1.9

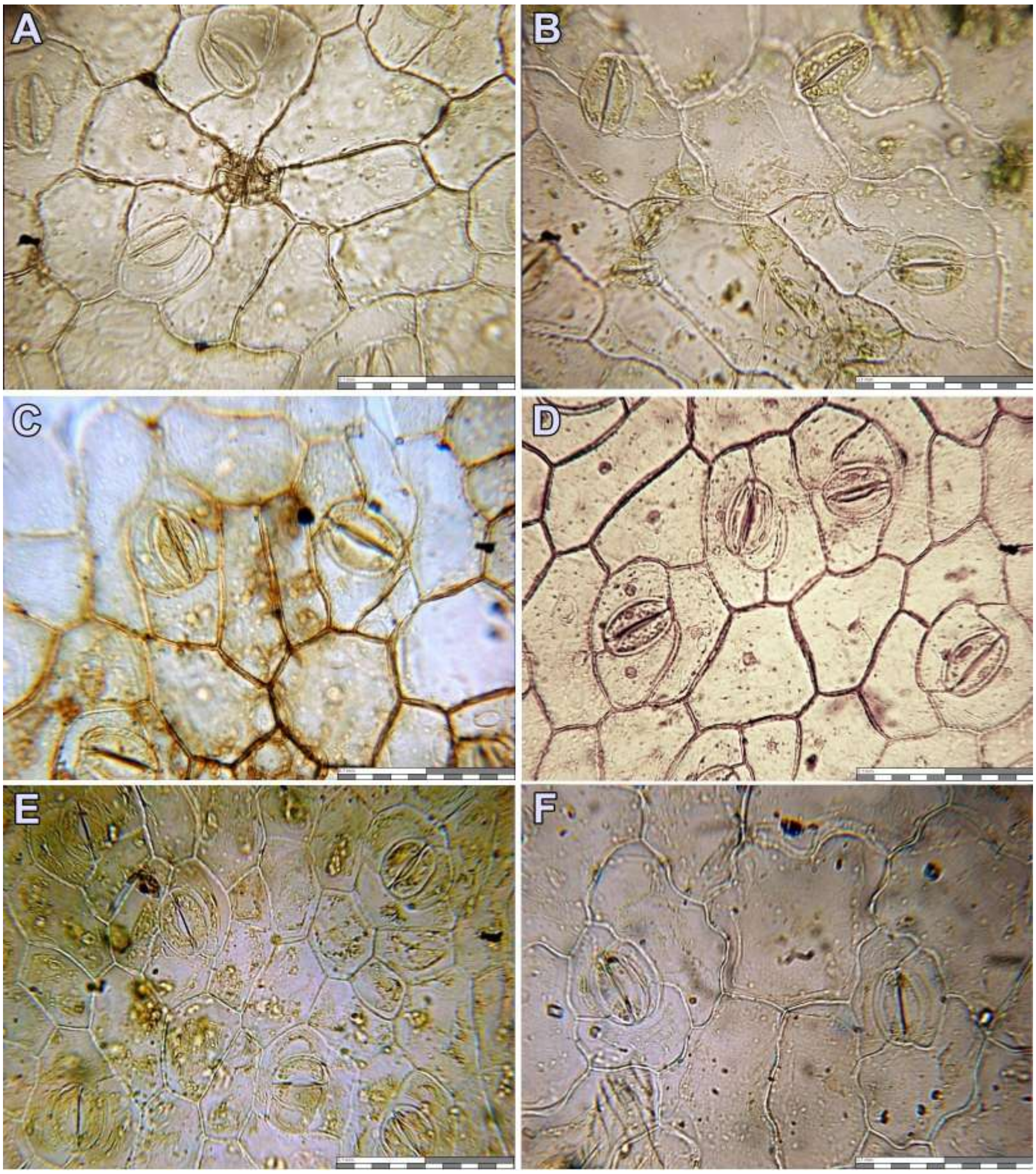

Fig. 4. -Leaf epidermis in frontal view: *Rumex kerneri* – adaxial epidermis with 4-celled peltate trichome in the middle (A) and abaxial epidermis (B); *R. cristatus* – adaxial (C) and abaxial (D) epidermis; *R. patientia* – abaxial (E) and adaxial (F) epidermis. Scale bars A-F = 100 µm.

Table 4. Metric features and fertility of the pollen grains of Bulgarian representatives of subsectio *Rumex*.

Taxon	**Voucher specimen**	**Diameter, µm mean ± standard deviation**	**Fertility, %**
R. kerneri Borbàs	SOA 056414	38.55±2.28	96
	SOA 059605	40.03±1.59	92
R. cristatus DC	SOA 056939	36.47±2.24	90.7
	SOA 057065	37.45±1.57	96
	SOA 056941	36.99±1.98	93
R. patientia L.	SOA 059478	36.97±1.94	95.3
	SOA 059477	33.75±1.84	97.6
	SOA 059235	37.03±1.87	94

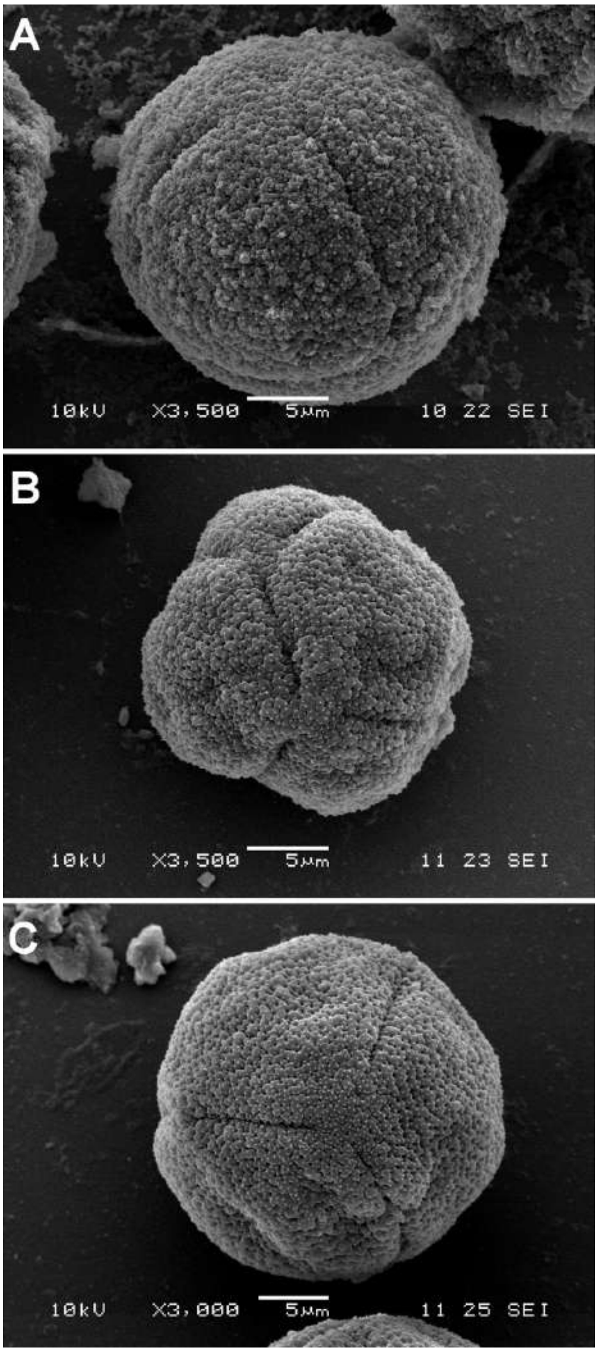

Fig. 5. Scanning electron microphotographs of the pollen grains from:
A – *R. kerneri*, B- *R. cristatus*; C – *R. patientia*.

Conclusion

Our observations showed that most of the taxonomically reliable morphological features for *Rumex*, sect. *Rumex*, subsect. *Rumex* can be found in the valves of mature plants. The evaluated morphological differences are stable and reliable in the distinction of *R. kerneri* from both compared species from the typical subsection. The differentiation of *R. kerneri* is expressed in a reduction of the flower count in a cluster, as well as the one developed tubercle, presence of indumentum on the stem base and the abaxial surface of the leaf blades, and the sporadic distribution on the mountain foothill belt. The leaf epidermis is not decisive for the closely related species of subsect. *Rumex*, but some of the qualitative parameters, as the shape of the common cells and the stomatal index, are useful for the delimitation of the species. The pollen grains of the studied species have a similar type of apertures and sculpture surface of the exine, but they tend to size increase related to the ploidy level.

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Aquatic Macroinvertebrates Diversity in the Upper Stretch of Una River (Una National Park, SW Bosnia and Herzegovina)

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Abstract. The karstic Una River is one of the larger tributaries of the Sava River. Although being a part of Balkan biodiversity hot-spot, aquatic macroinvertebrates of the Una are rarely studied. The aquatic macroinvertebrates sampling was performed in the summer of 2015 throughout the upper river stretch covering the Una and its tributaries situated in the Una National Park (Una NP). A rich community consisting of 130 macroinvertebrate taxa, with dominance of Trichoptera and Ephemeroptera, was detected. A snail *Sadleriana* sp. and endemic gammarid *Echinogammarus acarinatus* were found to be dominant taxa in terms of percentage participation and frequency of occurrence. Mollusca and Crustacea were found to be the most abundant groups, with higher abundance in the Una River than its tributaries. Although a higher overall taxa richness was found in tributaries, calculated biotic indices and evenness pointed to greater diversity in the Una River. Additionally, BMWP and ASPT scores were also higher in the Una River than its tributaries. Calculated Jaccard index value pointed to moderate faunistic similarity between investigated communities. Obtained results suggest that investigated upper stretch of the Una River harbours a high diversity and rather homogenous macroinvertebrate community.

Key words: tributaries, benthic community, western Balkans.

Introduction

On global scale aquatic macroinvertebrates are well studied component of aquatic animal diversity. Fairly diverse freshwater habitats in rivers allow the existence of high variety of endemic, relict or rare macroinvertebrate species. In Europe, the Balkan Peninsula is recognized as one of the main hot-spot of animal diversity (Griffiths et al, 2004; Savic,

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2008). It is particularly true in the case of its karstic parts, such as Dinaric region (Western Balkan), which harbours rich communities of aquatic macroinvertebrates (Previsic et al, 2009; Klobucar et al, 2013). The most recognizable macroinvertebrates representatives in Dinaric karstic rivers are aquatic insects (i.e. Matonickin & Pavletic, 1960b; Habdija et al, 1997; Radja & Puljas, 2008; Płóciennik et al, 2016; Savic et al, 2017; Vilenica et al, 2017).

Una is karstic river, one of the largest tributaries of the Sava River. Although a part of biodiversity hot-spot, aquatic macroinvertebrates compositions of the Una, particularly its upper part are rarely studied. To date, apart from study of complete macroinvertebrates communities at few waterfalls (Matonickin & Pavletic, 1960b) researches were conducted with amphipods (Zganec et al, 2010; 2016), water quality assessment (Trozic-Borovac and Skrijelj, 2000; Kerovec et al, 2005; Nogic et al, 2016) and fish-diet (Trozic-Borovac. 2002).

Being aware of the gaps in published data we focused our research on the upper river stretch in the area which nowadays is part of the Una national park (located in Bosnia and Herzegovina), aiming to contribute to the knowledge of its aquatic macroinvertebrates fauna and to raise awareness of importance of preserving this Dinaric hot spot. Furthermore, we intend to assess overall aquatic macroinvertebrates diversity (based on biotic indices) and habitat quality (based on BMWP and ASPT scores) between the Una and its studied tributaries.

Material and methods

Study area

The upper Una stretch is situated in the south-western part of Bosnia and Herzegovina (Fig. 1). From the 2008, this area is under protection as a national park. Investigated area belongs to inland water ecoregion Dinaric Western Balkan (Illies, 1978).

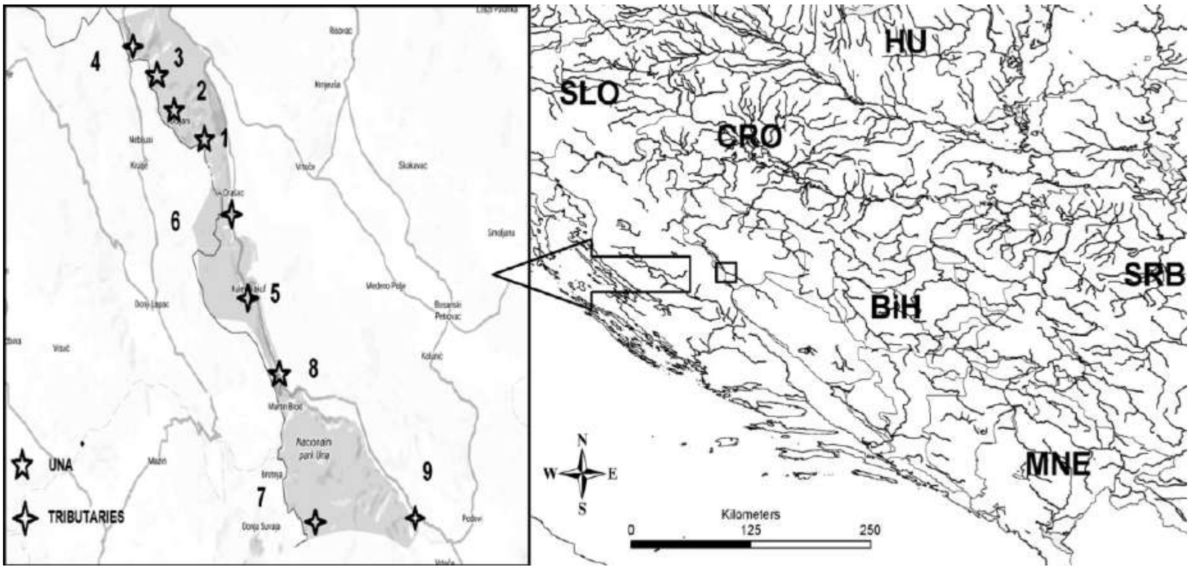

Fig. 1. Investigated localities at the upper Una stretch (the Una NP): 1- Una (Strbacki Buk) N44°39'21.72" E 16° 0'39.53"; 2- Una-(Bjelila) N44°40'11.46" E15°58'49.48"; 3- Una (Loskun) N Una (Loskun) N44°41'39.60" E15°57'29.05"; 4- Una (Martin Brod) N44°29'47.23" E16° 8'0.63"; 5. Djukanov potok N44°42'54.81" E15°56'3.38"; 6. Ostrovica N44°33'32.51" E16° 5'19.71"; 7. Toplica (Klisa) N44°35'45.25" E16° 3'59.78"; 8. Krka (Radjenovici) N44°25'21.34" E16° 8'38.72"; 9. Unac (Bastasi) N 44°23'41.69" E 16°19'39.08".

Situated in Dinarids, a karst mountain range, the Una River water is characterized by high alkality and carbonate solidity, harboring favorable conditions for formation of calcareous sinter, although due to lower water temperature this process is slower, excluding parts with better aeration (such as bigger (tectonic) slaps – Martin Brod and Strbacki buk) (Matonickin & Pavletic, 1960a; Bognar 2005).

The investigated upper part of the Una (Una national park - NP) is situated in not so inhabited area. Bottom sediments at sampling localities were characterized by dominance of lithal, mesolithal and psammal. Primary producers consisting of aquatic macrophytes mosses (Bryophyta) and epiphytic algae were common in localities along the Una, and also present in the Ostrovica Stream.

In total samples from 9 localities were taken, of which four were situated on the Una, while five were situated on various Una tributaries (Fig. 1).

Among five tributaries investigated, two were larger (the Unac and the Krka), and three were smaller streams, of which two (Ostrovica and Toplica) in Kulen Vakuf were with partially captivated springs (Dzankic et al., 2006).

Sampling and data analysis

The sampling was performed by the standard benthological hand-net (25x25 cm, 500 µm mesh size) in the Summer of 2015. One cumulative sample per locality was taken and multi-habitat procedure was applied (Hering, 2004). All samples were preserved with 60-80% ethanol solution and further processed in the laboratory. Identification of macroinvertebrates was done by using appropriate taxonomic keys.

The main physical and chemical parameters (temperature, dissolved oxygen, conductivity, pH) were measured by WTW Multiline IDS field set, while for an additional physical and chemical parameters (nitrates, nitrites, chlorides, fluorides, total N, total P, Chemical oxygen demand (COD), total dissolved solids (TDS)), a 1L of water was taken at each site, preserved in cold chamber and transported in the laboratory of Biotechnical Faculty, Bihac, were further processing and measurements were done by applying standardized chemical protocoles.

To better describe diversity in the Una River and its tributaries a following biotic indices were used: Shannon Wienner index (SWI; Shannon & Weaver, 1949), Evenness (Pielou, 1969; Hill, 1973) and Jaccard similarity coefficient (S_J; Jaccard, 1908). The first two were calculated by ASTERICS software (version 3.1; AQEM, 2002), while Jaccard index was calculated manually by using formula $S_J = a/(a + b + c)$, where „a“ is number of species common to entire community, „b“ is number of species unique to the first community (Una River) and „c“ is number of species unique to the second community (tributaries). In order to assess water quality a BMWP and ASPT scores (Chester, 1980; Armitage et al, 1983) were calculated by ASTERICS (AQEM, 2002). Kruskal-Wallis nonparametric test (Kruskal & Wallis, 1952) was used to compare differences between calculated indices of the Una and tributaries. This analysis was performed in STATISTICA software ver. 7 (Statsoft, 2006).

Results

On Table 1 are presented values of some water parameters measured at localities of Una River and its tributaries (Table 1).

A rich community consisting of total of 130 macroinvertebrate taxa, with insects being the most diverse group (Trichoptera and Ephemeroptera with 27 taxa each; Fig. 2a) was recorded.

A higher taxa richness was found in tributaries compared to the Una River itself (108 vs 82 taxa), mainly due to higher number of taxa from groups Trichoptera, Ephemeroptera, Diptera and Odonata. On the other hand, in the Una River a higher diversity of Mollusca was recorded, while regarding Oligochaeta and Crustacea, no notable difference in number of taxa between the Una River and the tributaries was found.

Regarding calculated biotic indices (H and Evenness) a somewhat different situation occurred, with higher values of these indices in the Una River than in its tributaries (Fig. 3a,b). However, the performed nonparametric test (Kruskal-Wallis) showed that found differences were not statistically significant (at the p= 0.05 level). The obtained value of the Jaccard index (S_J=0.41) pointed to moderate similarity between tested communities (Samples from the Una River and samples from its tributaries. Values of BMWP and ASPT indices (Fig. 3c,d) were higher and more variable in the Una River than its tributaries. In respect to abundances/relative percentage of community, somewhat different picture arises (Fig. 2b). Mollusca and Crustacea were found to be the most abundant groups overall. Diptera, the third group regarding abundances overall, was the most abundant group in the tributaries, followed by the Mollusca and Crustacea.

A snail *Sadleriana* sp. was the dominant component of recorded macroinvertebrates community overall. It was omnipresent (found in all samples) and occupied 28% of the overall community recorded during our investigation. The highest abundance was recorded in samples 1 (78% of total community) and 4 (52%).

Table 1. Main values of selected physico-chemical parameters.

UNA	**MU**	**Minimum**	**Maximum**	**Median**	**Mean**	**SD (n-1)**	**SE of the mean**	**Median AD**
Temperature	°C	10.9000	11.7000	11.1500	11.2250	0.3403	0.1702	0.1500
pH		7.7500	8.0400	7.8200	7.8575	0.1271	0.0636	0.0450
Conductivity	µS	328.0000	412.0000	392.0000	381.0000	36.7967	18.3984	12.5000
COD	mgO$_2$/l	3.2000	7.2000	6.4500	5.8250	1.8410	0.9205	0.6500
Total P	mg/l	0.1400	0.4800	0.2200	0.2650	0.1484	0.0742	0.0450
Sulphates	mg/l	26.1530	35.1160	30.9055	30.7700	4.3230	2.1610	3.5120
Nitrates	mg/l	0.8410	2.0360	1.0300	1.2343	0.5419	0.2709	0.0975
Chlorides	mg/l	4.1270	5.2740	4.8835	4.7920	0.5605	0.2803	0.3725
Fluorides	mg/l	0.2570	0.4280	0.3265	0.3345	0.0792	0.0396	0.0570
TRIBUTARIES	**MU**	**Minimum**	**Maximum**	**Median**	**Mean**	**SD (n-1)**	**SE of the mean**	**Median AD**
Temperature	°C	9.4000	11.3000	11.2000	10.6000	0.8860	0.3962	0.1000
pH		7.2500	7.9600	7.6400	7.5980	0.2769	0.1238	0.2300
Conductivity	µS	324.0000	386.0000	354.0000	354.4000	21.9727	9.8265	2.0000
COD	mgO$_2$/l	2.9000	6.2000	3.3000	3.8000	1.3601	0.6083	0.2000
Total P	mg/l	0.0100	0.3600	0.0310	0.1302	0.1609	0.0720	0.0210
Sulphates	mg/l	21.3050	36.3490	22.7830	26.5270	6.4210	2.8710	1.4780
Nitrates	mg/l	0.1260	1.7640	0.7620	0.687	0.941	0.420	0.6360
Chlorides	mg/l	5.0430	8.0920	6.0340	6.2734	1.1776	0.5266	0.6160
Fluorides	mg/l	0.2130	0.3150	0.2310	0.2408	0.0424	0.0190	0.0170

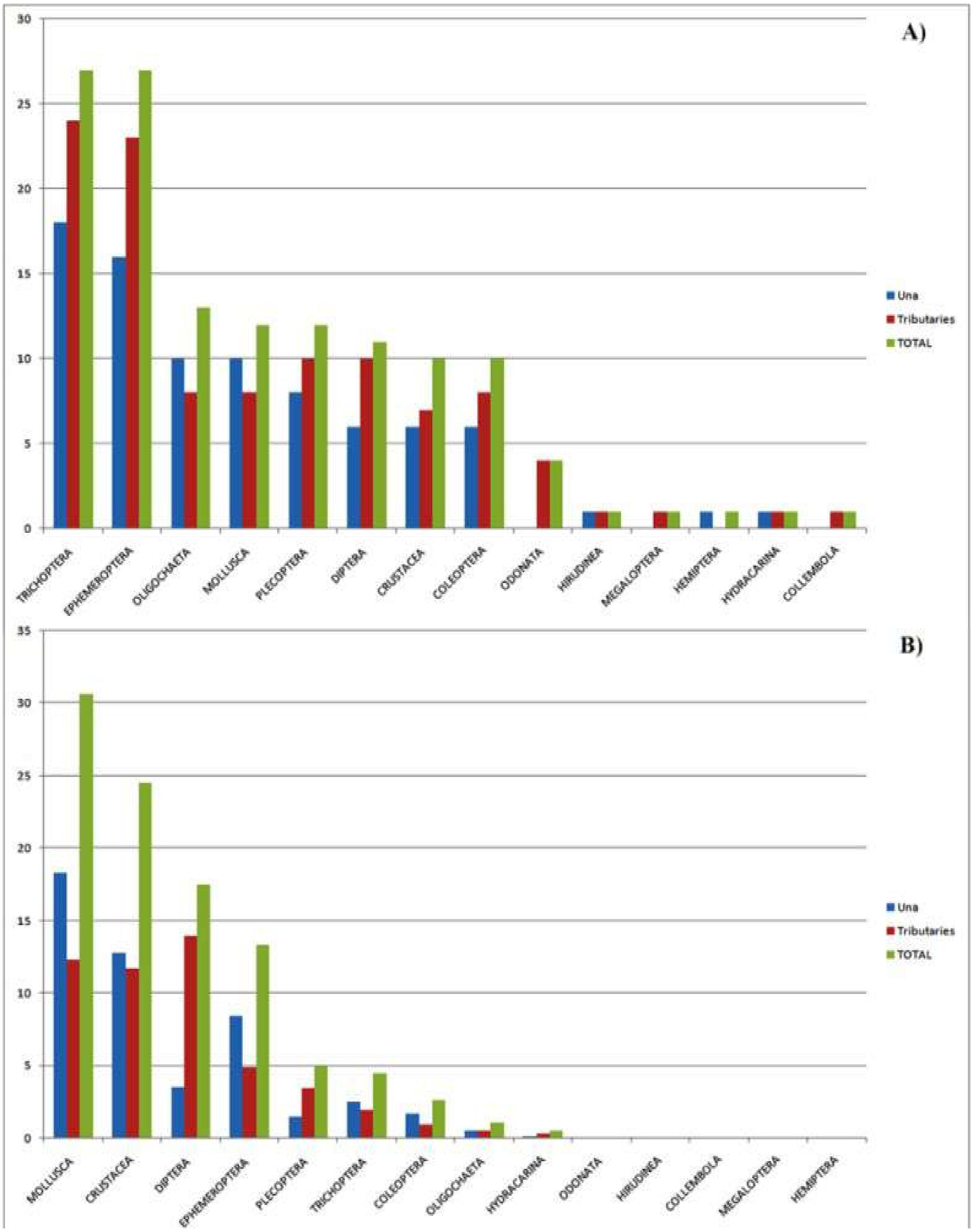

Fig. 2. (a) Taxa richness and **(b)** percentage participation of main taxonomic groups recorded at the upper Una stretch; x axis - main taxonomic groups, y axis - number of taxa (a) and percentage participation of taxa in the sample (b).

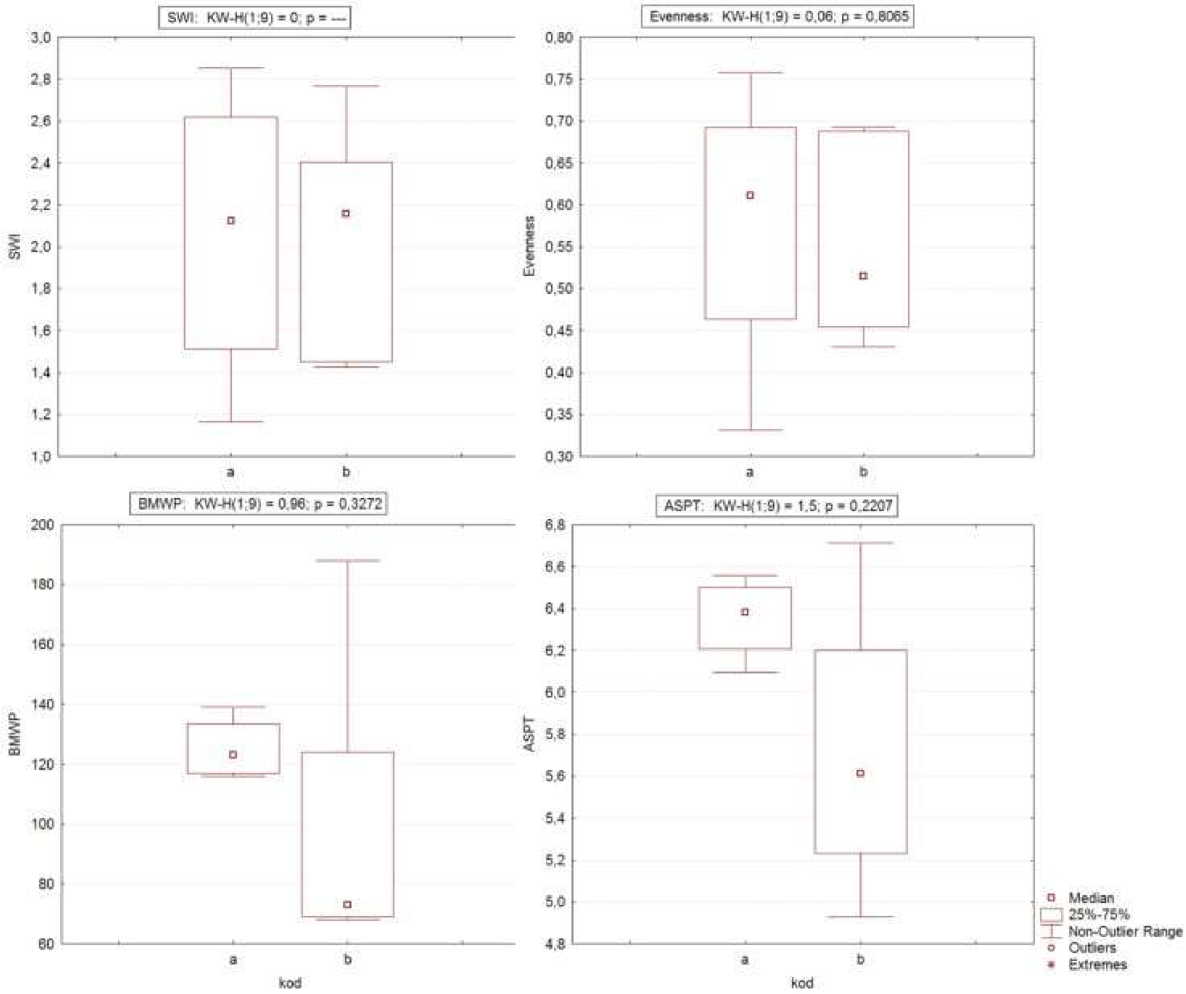

Fig. 3. A comparison of values of calculated indices of diversity (SWI), evenness, BMWP and ASPT between the Una River (code a) and its tributaries (code b).

On the other hand, in samples 2 and 6, this gastropod was represent with only 4% and 2% in the community, where in, the highest abundance of the *Echinogammarus acarinatus* (17% of overall community, with frequency of occurence (F) of 0.89) was observed. Both of these dominant taxa overall were more abundant in the Una (than in tributaries) where were comprised 35% and 20% of the community (versus 21% and 13% in tributaries), respectively.

Among the Diptera fauna the most dominant was the group Chironomidae (17% of overall community). These dipteres were more abundant in tributaries, particularly in samples 7 and 4, where they consist 58% and 29% of the total community. The found *Niphargus* spp. group probably consisted of two taxa, one of which could be a hypogean, while the other one based on morphological characters could belongs to one of a few epigean niphargids, apart from *Niphargus hrabei*, S. Karaman, 1932 and *Niphargus valachicus*, Dobreanu and Manolache, 1933. It should be mentioned that in the same samples with these *Niphargus* specimens, an abundant gammarid population (dominated by *E. accarinatus* and *G. balcanicus*) was recorded. In our research *E. accarinatus* was dominant amphipod and one of the most frequent species. Especially abundant it was in the sample 8 (the Una downstream Martin Brod). Among coleopterans, *Limnius* *volckmari* (both adults and larvae) was the most numerous/abundant taxon. In the main stream/Una it was much more present and numerous than in tributaries. Other numerous and common beetle found was *Elmis* sp. (posibly *E. bosnica*, but due to lack of taxonomic keys for larvae it could not be identified to species level) and *E. latreillei* were evenly numerous in Una/tributaries. Finally *Esolus angustatus*, one of common coleopterans recorded in this investigation was more abundant in tributaries. Among Ologochaetas naidids *Nais* sp./*N. bretscheri* were more abundant in tributaries than in the main course of the Una. Regarding molluscs, *Sadleriana* sp. was found to be dominant taxon in this investigation, as mentioned before. Other molluscs were less numerous, and except *Bythinella* sp. and *Pseudosuccinea columella* were more common in the Una River itself. In case of succinid this could be explained by the fact that they are mostly semiaquatic and commonly found in waters with dense riparian vegetation, as in case of a smaller Una tributaries here. Although mayflies were more abundant in the Una, in tributaries a greater diversity was recorded. Among more common (abundant and frequent) taxa here only *B. rhodani* was evenly numerous and frequent in Una and tributaries. A more common presence of *Ephemerella ignita* in the Una (than in tributaries) was recorded.

Discussion

High macroinvertebrates taxa richness found (130 taxa) and the dominance of insect could be expected for mountainous rivers in the region (Habdija et al, 1997; Radja & Puljas, 2008). Diversity in karstic rivers/streams is influenced by specificity of habitats and geological (limestone) substrate (Radja & Puljas, 2010). A higher taxa richness found in tributaries was due to higher number of taxa from groups Trichoptera, Ephemeroptera, Diptera and Odonata. Higher abundances of mollusks and crustaceans recorded in the Una, could be expected due to more stable environmental conditions, which favor these fully aquatic groups. An explanation, mentioned in case of the diversity of groups (regarding its presence in the Una vs tributaries) could be implemented here, as well – Diptera, as insects distinguish shorter aquatic life-phase and thus they should be more adapted to less stable habitat conditions, especially in case of streams running dry (which is common in case of karstic, especially smaller watercourses). An occasionally running dry is common characteristic of some smaller carstic watercourses (Matonickin et al, 1972). In our investigation intermittent watercourses were present (localities 4, 5 and 6), though during our investigiation those were not dry, yet with a fairly low water levels (personal communication). Unstable conditions inevitable cause adaptation of its biota (macroinvertebrates included), leading to higher presence of more adapted taxa, such as insects with semiaquatic shorter life cycles and faster colonization abilities (Williams & Feltmate, 1992). Additionally, more pronounced differences in physical and chemical water conditions present in tributaries could influence found macroinvertebrates species, as well (Dzankic et al, 2006). Although, higher diversity (reflecting as taxa richness and H index) was present in tributaries, a higher values of the evennes, along with higher values of BMWP and ASPT indices, pointed to importance of more stable water conditions, which allow more persistent habitat variety and simultaneously more suitable environmental condition for aquatic macroinvertebrates communities in such karstic environment.

In respect to abundances/relative percentage of investigated community, a different picture arised, with Mollusca and Crustacea found to be the most abundant macroinvertebrates. However in tributaries, Diptera (the third most abundant group overall) was the most abundant group, followed by Mollusca and Crustacea. Higher abundances of mollusks and crustaceans recorded in the Una/mainstream, could be expected due to more stable environmental conditions, which should favor these fully aquatic groups (for example Mollusca). An explanation, mentioned in case of the diversity of groups (regarding its presence in the Una vs tributaries) could be implemented here, as well – Diptera, as not entirely aquatic insects and usually with relatively short aquatic phase should be more adapted to more variable habitat conditions (Williams & Feltmate, 1992), especially in a case of streams running dry, which is common in case of karstic, especially smaller, watercourses (Bonacci et al, 2013).

A snail *Sadleriana* sp. was omnipresent and dominant component of recorded macroinvertebrates community, followed by an endemic gammarid *Echinogammarus acarinatus*. *E. accarinatus* is a rather small (up to 10.5 mm max length) endemic amphipod of western karstic Balkan (Karaman, 1970; Pinkster 1993; Zganec et al, 2010; 2016). Although it is considered as mesohaline species (Stock, 1968) it apparently could well adapt to oligohaline conditions. The species *Gammarus balcanicus* is dominant gammarid in colder Croatian waters (bellow 20 °C; Zganec, 2009; 2010), but in our investigation although it was present and frequent it was not abundant as previously mentioned *E. accarinatus.*

The finding of *Niphargus* spp. coexisting with rich and abundant gammarid population is particularly interesting. Fisher et al. (2007) analyzed the coexistence of two species (one niphargyd and one gammarid) in Slovenia and recorded dominance of gammarids in the mainstream/permanent stream and the presence of niphargids in the fissure system where niphargid species could survive desiccations and gammarid competition. A one endemic gammarid present in the investigated region (*Fontogammarus dalmatinus*) could not be confirmed in our investigation. A recent investigation (Zganec, 2016) states presence of this endemic gammarid species, only in the most upper Una part (spring and upstream Martin Brod), along with *Echinogammarus accarinatus* which was the most common gammarid in this investigation, so our findings could indicate somewhat wider distribution and more abundant presence of *E. accarinatus* in this region.

The taxa which were not identified to lower/species level, particularly among Diptera and Gastropoda, could hide some interesting and endemic taxa as well.

The investigated upper part of the Una River (Una NP) harbours high aquatic macroinvertebrates diversity, with few rare taxa. A pronounced difference in community structure between tributaries and the Una itself could not be confirmed, although tributaries were more taxa rich, mainly due to the greater diversity of smaller insects such as caddisflies and mayflies. A continued research should shed more light on aquatic macroinvertebrates and its community structure in this protected part of the Una River.

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Changes in the Composition of Natural Grassland (Chrysopogon gryllus type) in Grazing and Haymaking Mode of Use

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Abstract. The objective of the experiment was to observe the impact of the different management practices applied on natural meadow grassland (*Chrysopogon gryllus* L.) under mountain conditions and their impact on the qualitative composition of the formed biomass. The share of grasses in the composition of natural grassland decreased in grazing (up to 10.3%) and haymaking (up to 14.3%) mode of use from the first to the fifth experimental year. During the last two experimental years, there was a higher share (39.8-55.5% - in grazing and 38.6-52.0% - haymaking mode) of the representatives of genus *Fabaceae*, which is a prerequisite for higher quality and nutritional value of the formed mass for grazin g and hay. The amount of crude protein exceeded by 10.1% (in grazing mode) and by 10.8 to 25.4% (in haymaking mode) the average annual values of the indicator. The amount of crude protein in the dry matter of grassland with pasture management practice was 5.1% higher than that in grassland with haymaking mode. A high positive correlation was found between the percentage share of legumes in the grassland in the pasture mode of use with the concentration of crude protein (r = 0.82) and the gross energy value (r = 0.95) of the feed mass. In hay-making variants, the weight percentage of legumes correlated positively with the indicators characterizing the energy nutritional value (GE - r = 0.81; EE - r = 0.79; FUM - r = 0.77 and FUG - r = 0.69) of the formed aboveground mass.

Key words: *Chrysopogon gryllus* L., natural grassland, chemical composition, yield of crude protein.

Introduction

The quality and nutritional value of pasture and hay are subordinated to the species composition and the quantitative ratio of the individual biological groups in the natural grasslands (Tomović et al., 2014; Aćić et al., 2015). Natural and economic factors have an impact on the changes in floristic composition and reflect differences in yield and quality characteristics of grass biomass in first mowing, early grazing, or late grazing (Tenikecier & Ates, 2018; Iliev et al., 2020). The grass cover of natural meadows and pastures, as a set of diverse plant species, affects the mode of use and taste of the aboveground mass (Sanderson, 2010; Hegedüšová & Senko 2011; Woodward et al., 2013). The development of perennial grasses and legumes for meadows (a major component in the natural grass association), as well as the period of their mowing is a significant factor in obtaining good quality hay (Mitev et al., 2010; 2011; Yavuz & Karadağ, 2016). In the conditions of the Central Balkan Mountain, the maximum accumulation of nutrients in

Chrysopogon gryllus meadow coincides with the phase of tasseling-beginning of flowering, which determines the optimal harvesting period of the grassland (Iliev et al., 2017). Species of local origin form highly productive and long-lasting grasslands (Naydenova & Mitev, 2008). The chemical composition of the feed mass (the content of proteins, carbohydrates, vitamins, minerals and nitrogen-free extracts) affects the nutritional value of feed and its full absorption by ruminants (Jeranyama & Garcia, 2004; Enchev, 2013; Butnariu et al., 2016; Bozhanska, 2017; Bozhanska et al., 2018).

Grazing use of mode is an economical and environmentally friendly way to meet the food needs of adolescent and highly productive animals (Woyessa et al., 2013; Shapiro et al., 2015). The grazing mode allows the obtained animal production (milk, meat, wool, etc.) to be at a lower cost (Thomas et al., 2010; Pringle et al., 2014). When grazing, animals show selectivity, which leads to changes in the botanical composition and quality of the formed aboveground mass (Luscher et al., 2014). Changes in the values of the main quality indicators in the chemical composition of grassland determine its nutritional value, the degree of digestibility by animals (Getachew et al., 2004; Cho et al., 2012; Tenikecier & Ates, 2019) and their productivity.

The objective of the study was to evaluate the effect of different management practices on the chemical composition of mountain meadow grassland, *Chrysopogon gryllus* L. type.

Material and Methods

The experiment was conducted on a natural grassland, of *Chrysopogon gryllus* type, in mountain conditions (515 m above sea level). The experimental period was five years (2013-2017). The grazing and hay productivity of the grassland in the first regrowth was monitored, with two modes of use and three terms of harvesting (early, medium-early and late at the respective dates).

Variants of pasture harvesting (PH):

1. PH1 - (Control) - from 31st May to 9th June;
2. PH2 - from 10th June to 19th June;
3. PH3 - from 20th June to 29th June.

Variants of hay-making harvesting (HH):

1- HH1 - (Control) - from 30th June to 09th July;
2- HH2 - from 10th July to 19th July;
3- HH3 - from 20th July to 31st July.

During the first three experimental year was used stockpile fertilization with N_6P_6 for the variants. The terms of grazing and mowing were at an interval of a ten-day period (considering the climatic conditions). Fertilization with triple superphosphate (containing 44–48% P_2O_5) was applied once in autumn (September-October), and fertilization with nitrogen fertilizer (NH_4NO_3) once in spring (April).

Climatic characteristics of the area in the experimental period

The highest amount of vegetation and autumn-winter precipitation (1164.9 mm) was in 2014 (second experimental year). The relative difference in the amount of precipitation in 2015 (922.7 mm) and 2017 (983.2 mm), 2013 (807.3 mm) and 2016 (837.0 mm) compared to the maximum value of the characteristic varied from 18.5 to 26.2% and from 39.2 to 44.3%, respectively. The highest average air temperature (11.9°C) and the highest average temperature for the months of July, August and September (22.4°C) was registered in 2015, and the lowest average annual temperature (10.5°C) in 2017 year.

Research indicators:

- Botanical composition of grassland (%) - determined by weight analysis of grass green mass samples taken at each mowing of each variation. Their weighing is carried out in an air-dry state, by weighing the percentage of sown grass species and motley grasses (in total).
- The chemical composition of the dry feed is analyzed according to *Weende* analysis: Crude protein (CP, g kg^{-1}) according to *Kjeldahl* (according to BDS/ISO-5983); Crude fiber (CFr, g kg^{-1}); Crude fat (CF, g kg^{-1}) (according to

BDS/ISO-6492) - by extraction into a *Soxhlet* extractor; Ash (g kg^{-1}) - (according to BDS/ISO-5984) degradation of the organic matter by gradual burning of the sample in a muffle furnace at 550°C; Dry matter (DM, g kg^{-1}) - empirically calculated from % moisture; NFE = 100 - (CP, % + CFr, % + CF, % + Ash, % + Moisture, %) converted to g kg^{-1}; Calcium (Ca, g kg^{-1}) - Stotz (Complexometric) and Phosphorus (P, g kg^{-1}) - with vanadate-molybdate reactive according - spectophotometer (*Agilent 8453 UV - visible Spectroscopy System*) measuring in the area of 425 ηm.

- The nutritional value of the feed was assessed by the Bulgarian system as Feed Unit for Milk (FUM) and Feed Unit for Growth (FUG) and calculated on the basis of equations according to the experimental values of CP, CFr, CF and NFE, recalculated by the coefficients for digestibility by Todorov (2010): Gross energy (GE, MJ/kg DM) = 0,0242*CP + 0,0366*CF + 0,0209*CFr + 0,017*NFE - 0,0007*Zx and Exchangeable energy (EE, MJ/kg DM) = 0.0152*DP (Digestible protein) + 0.0342*DF (Digestible fat) + 0.0128*DFr (Digestible fibers) + 0.0159*DNFE (Digestible Nitrogen-free extractable substances) - 0.0007*Zx.

Statistical data processing includes the analysis product Analysis Toolpak for Microsoft Excel 2010 and analysis of variance (ANOVA).

Results and Discussion

Botanical composition of natural grassland Chrysopogon gryllus L. type in pasture and haymaking harvesting

The mode of use has an impact on the quantitative share and the ratio of plant species in the botanical composition of the grassland (Šantrůček et al., 2002) while genotypic factor successfully determines abiotic restriction and specific adaptation of legumes to environmental conditions (Naydenova & Vasileva, 2019).

The applied mineral fertilization, as well as the modes of use have an impact on the percentage of plant species in the main functional groups in the grassland (Table 1). On average for the first three years (2013, 2014, 2015) of the experimental period, the share of cereal meadow grasses is from 17.4 to 68.7% (for grazing) and from 21.9 to 66.8% (for hay-making harvesting). During the period 2016-2017 (last two experimental years) the percentage share of grass species marked a decreasing trend (up to 10.3% - in the grazing mode and up to 14.3% - in the hay-making mode) in the composition of the studied grassland.

Proper management of pastures and meadows is a key element in maintaining biodiversity and optimal forage productivity in natural grasslands (Talmaci & Miron, 2016; Butnariu, 2018). The data on the species diversity and the quantitative share of legumes in the amount of the formed above-ground mass are opposite to those of grasses. In the period 2013-2015, a slight increase was observed in the average values (1.0-6.6% in the pasture mode and 0.0-9.2% in the hay-making mode) regarding the percentage share of the species of genus *Fabaceae*. In the last two years of the experimental period, legumes registered a higher presence (39.8-55.5% in pasture and 38.6-52.0% - hay-making) in the natural grassland, which is a prerequisite for higher quality and nutritional value of grass biomass used for pasture and hay. The percentage share of the group of weeds varied from 24.7 to 81.7% (in pasture mode) and from 24.0 to 78.1% (in hay-making) in the composition of the grassland.

Basic botanical composition of natural grassland of Chrysopogon gryllus L. type in pasture and hay-making harvesting

Changes in the botanical composition of grassland are associated with changes in the chemical composition and digestibility of feed (Garden et al., 2000). When the experiment was set, grasses predominated in the composition of grasslands with pasture and hay-making mode, and the feed mass had the highest carbohydrate content (PH - 509.3 g kg^{-1} and HH - 470.9 g kg^{-1}) - Tables 2 and 3.Grassland age, species and phenological identity of plants are a prerequisite for the quality and relative forage value of grass areas (Andueza et al., 2010; Grant et al., 2014). The grassland tested in the fifth experimental year (in both modes of use) had the highest average values regarding the amount of crude fiber (PH - 435.8 g kg^{-1} and HH - 437.4 g kg^{-1}).

Table 1. Botanical composition (%) of natural grassland of *Chrysopogon gryllus* L. type in pasture and hay-making harvesting (over the years).

Species	**Pasture harvesting**				**Hay-making harvesting**			
Groups	**PH1**	**PH2**	**PH3**	**Average**	**HH1**	**HH2**	**HH3**	**Average**
2013								
Grasses	20.5	13.2	18.4	17.4	20.8	21.1	23.9	21.9
Legumes	3.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0
Motley grasses	76.8	86.8	81.6	81.7	79.2	78.9	76.1	78.1
2014								
Grasses	49.0	50.5	50.6	50.0	60.9	44.4	46.4	50.6
Legumes	2.3	6.7	5.4	4.8	6.1	13.2	4.9	8.1
Motley grasses	50.1	42.8	44.0	45.6	33.0	42.4	48.7	41.4
2015								
Grasses	77.1	71.0	58.0	68.7	79.2	70.1	51.1	66.8
Legumes	0.0	6.7	13.2	6.6	0.0	5.2	22.3	9.2
Motley grasses	22.9	22.3	28.8	24.7	20.8	24.7	26.6	24.0
2016								
Grasses	19.6	9.9	21.4	17.0	16.0	24.3	17.6	19.3
Legumes	28.6	46.8	44.0	39.8	46.0	34.7	35.2	38.6
Motley grasses	51.8	43.3	34.6	43.2	38.0	41.0	47.2	42.1
2017								
Grasses	15.0	11.6	4.3	10.3	11.1	16.2	15.7	14.3
Legumes	54.5	56.0	56.0	55.5	43.6	59.7	52.8	52.0
Motley grasses	30.5	32.4	39.7	34.2	43.6	59.7	52.8	52.0

Table 2. Chemical composition (g kg^{-1}) of natural grassland of *Chrysopogon gryllus* L. type over the years (pasture harvesting).

Indicators **Variants**	**CP**	**CF**	**CFr**	**Ash**	**NFE**	**Ca**	**P**
2013							
PH1	101.9	18.9	264.1	83.6	536.5	14.0	2.1
PH2	76.9	32.5	307.2	86.7	496.6	21.2	2.3
PH3	71.0	19.4	334.9	80.1	494.6	14.0	1.8
Mean±SD	***83.3±16.4***	***23.6±7.7***	***302.1±35.7***	***83.5±3.3***	***509.3±23.6***	***16.4±4.2***	***2.0±0.2***
2014							
PH1	55.4	9.2	407.3	71.6	456.5	10.0	2.2
PH2	78.9	24.5	399.8	70.0	426.8	8.8	2.0
PH3	80.0	15.1	403.0	66.1	435.8	7.6	2.2
Mean±SD	***71.4±13.9***	***16.2±7.8***	***403.4±3.8***	***69.3±2.9***	***439.7±15.2***	***8.8±1.2***	***2.1±0.1***
2015							

PH1	56.7	20.2	363.9	84.1	475.2	10.9	2.8
PH2	69.5	16.0	344.8	82.9	486.9	12.1	3.0
PH3	71.3	22.6	371.0	87.4	447.7	13.3	2.1
Mean±SD	***65.8±8.0***	***19.6±3.3***	***359.9±13.6***	***84.8±2.3***	***469.9±20.1***	***12.1±1.2***	***2.7±0.5***
2016							
PH1	120.8	28.9	352.3	67.3	430.6	13.8	1.8
PH2	157.4	34.4	381.8	57.2	369.2	16.3	1.8
PH3	150.5	28.0	390.1	63.8	367.7	15.0	1.7
Mean±SD	***142.9±19.5***	***30.4±3.5***	***374.7±19.8***	***62.8±5.1***	***389.2±35.9***	***15.0±1.3***	***1.8±0.1***
2017							
PH1	132.9	26.9	430.0	74.2	336.0	15.0	1.4
PH2	157.5	23.4	437.9	72.3	308.9	18.5	1.8
PH3	138.5	26.4	439.5	73.3	322.2	17.4	1.5
Mean±SD	***143.0±12.9***	***25.6±1.9***	***435.8±5.1***	***73.3±1.0***	***322.4±13.5***	***16.9±1.8***	***1.6±0.2***
2013-2017							
PH1	93.5	20.8	363.5	76.2	447.0	12.7	2.1
PH2	108.0	26.2	374.3	73.8	417.7	15.4	2.2
PH3	102.3	22.3	387.7	74.1	413.6	13.5	1.9
Mean±SD	***101.3±37.8***	***23.1±6.8***	***375.2±49.2***	***74.7±9.1***	***426.1±70.1***	***13.9±3.7***	***2.0±0.4***

In the first experimental year (2013), grassland harvested as a pasture at an earlier date (31.05-09.06.) and fodder used as hay-making in the period - 10.07.-19.07., marked the highest content of crude protein (PH1 - 101.9 g kg^{-1} and HH2 - 86.7 g kg^{-1}). The values of the indicator exceed the average (PH - 83.3 g kg^{-1} and HH - 71.9 g kg^{-1}) by 22.3% and 20.6%, respectively (in both modes of use). In the years with the highest amount of vegetation precipitation (2014) and the highest average annual air temperature (2015), the grassland, which was formed during the third decade of the pasture (20.06.-29.06.) and haymaking (20.07.-31.07.) modes of use, marked maximum values in terms of the amount of crude protein.

Table 3. Chemical composition (g kg^{-1}) of natural grassland of *Chrysopogon gryllus* L. type over the years (hay-making harvesting).

Indicators **Variants**	**CP**	**CF**	**CFr**	**Ash**	**NFE**	**Ca**	**P**
2013							
HH1	69.0	31.4	335.9	81.5	482.1	16.3	2.0
HH2	86.7	32.8	366.6	74.0	439.9	17.4	1.9
HH3	60.1	16.4	355.9	77.0	490.7	11.3	1.4
Mean±SD	***71.9±13.5***	***26.9±9.1***	***352.8±15.6***	***77.5±3.8***	***470.9±27.2***	***15.0±3.3***	***1.8±0.3***
2014							
HH1	53.9	10.2	419.0	64.4	452.5	6.3	2.3
HH2	71.2	19.7	402.5	68.7	437.9	8.9	1.9
HH3	87.7	14.7	372.1	70.2	455.3	6.4	2.4
Mean±SD	***70.9±16.9***	***14.9±4.8***	***397.9±23.8***	***67.8±3.0***	***448.6±9.3***	***7.2±1.5***	***2.2±0.3***

2015							
HH1	50.5	15.5	399.2	67.3	467.5	14.5	2.2
HH2	58.7	20.3	462.7	72.8	385.5	13.5	2.9
HH3	81.9	24.0	380.7	76.4	437.0	12.1	2.0
Mean±SD	***63.7±16.3***	***19.9±4.3***	***414.2±43.0***	***72.2±4.6***	***430.0±41.4***	***13.4±1.2***	***2.4±0.5***
2016							
HH1	225.7	33.7	415.3	63.5	261.8	21.3	2.7
HH2	155.8	26.7	384.8	62.8	369.8	15.1	1.5
HH3	158.5	29.1	392.7	65.3	354.4	16.2	1.6
Mean±SD	***180.0±39.6***	***29.8±3.6***	***397.6±15.8***	***63.9±1.3***	***328.7±58.4***	***17.5±3.3***	***1.9±0.7***
2017							
HH1	100.7	23.5	431.0	73.7	371.2	12.3	0.8
HH2	105.9	22.8	425.4	69.0	376.9	13.7	1.1
HH3	80.3	24.1	455.9	71.6	368.0	14.9	0.7
Mean±SD	***95.6±13.5***	***23.5±0.7***	***437.4±16.2***	***71.4±2.4***	***372.0±4.5***	***13.6±1.3***	***0.9±0.2***
2013-2017							
HH1	100.0	22.9	400.1	70.1	407.0	14.1	2.0
HH2	95.7	24.5	408.4	69.5	402.0	13.7	1.9
HH3	93.7	21.7	391.5	72.1	421.1	12.2	1.6
Mean±SD	***96.4±48.5***	***23.0±7.0***	***400.0±35.7***	***70.5±5.4***	***410.0±61.5***	***13.3±4.0***	***1.8±0.6***

The excess in the values of the trait compared to the averages for the vegetation period (PH - 71.4 g kg^{-1} and HH - 70.9 g kg^{-1} in 2014; PH - 65.8 g kg^{-1} and HH - 63.7 g kg^{-1} in 2015) was 12.0% (in pasture mode) and 23.7% (in hay-making mode) in 2014 and by 8.4% (in pasture mode) and 28.6% (in hay-making mode) in 2015. The data of the studied indicators show that the mineral fertilization (with N_6P_6) applied in the first years of the experimental period, together with the practices of grassland use, had a positive effect on the composition and quality of the aboveground mass.

Mineral fertilization significantly increases the share of legume component (*Trifolium pratense, Trifolium repens*) in natural grasslands (Kacorzyk & Głąb, 2017).

According to the results obtained in 2016 and 2017, the amount of crude protein in dry matter is significantly increased in all variants of pasture and hay-making harvestings, namely:

In *pasture harvesting,* the values of the indicator varied from 120.8 to 157.4 g kg^{-1} (2016) and from 132.9 to 157.5 g kg^{-1} (2017). With maximum values of crude protein, the grassland was harvested in the period 10.06.-19.06 The excess in the values of the indicator compared to the averages for the fourth and fifth experimental year was by 10.1%.

In *hay-making harvesting,* the values of the indicator varied from 155.8 to 225.7 g kg^{-1} (2016) and from 80.3 to 105.9 g kg^{-1} (2017). The grassland, with maximum values of crude protein, was harvested in the period 30.06 - 09.07 (in 2016) and 10.07 - 19.07 (in 2017). The excess in the values of the indicator compared to the averages for the fourth and fifth experimental year is from 10.8% to 25.4%.

On average for the period (2013-2017), the highest amount of crude protein (108.0 g kg^{-1}) in grassland with *pasture management practice* was registered in the second decade of June (10.06.-19.06.), and in *hay-making* in the grassland formed in the period from June 30 to July 9 (100.0 g kg^{-1}). Given the average values of the indicator for a five-year period (PH - 101.3 g kg^{-1} and HH - 96.4 g kg^{-1}), we found that grass biomass from the pasture mode had 5.1% higher amount of protein fraction than in grassland with hay-making mode of use.

Correlation and regression dependences between the botanical composition of the grassland and the quality indicators

A high positive correlation was found between the percentage share of legumes in the grassland in the pasture mode of use with the concentration of crude protein (r = 0.82) and the gross energy value (r = 0.95) of the feed mass in *pasture mode* of use (Table 4).

The share of grasses shows a relatively high negative dependences (r = -0.89 - CP and r = -0.74 - GE) to the quantitative form of both indicators. The dependence of the percentage share of the representatives of *Poaceae* family with the amount of minerals (r = 0.69), nitrogen-free extracts (r = 0.73) and phosphorus (r = 0.99) is expressed by high correlation coefficients.

In the variants with *hay-making harvesting,* the weight percentage of legumes correlates positively with the energy nutritional value (r = 0.81 - GE; r = 0.79 - EE; r = 0.77 - FUM and r = 0.69 - FUG) of the formed aboveground mass, and that of cereals is in such a negative dependence (r = -0.65 - GE; r = -0.82 - EE; r = -0.85 - FUM and r = -0.84 - FUG) - Table 5.

For both modes of use, the presence of legume components in the studied natural grassland (type *Chrysopogon gryllus* L.) is negatively correlated with the carbohydrate content (r = -0.89 - PH and r = -0.94 - HH) and in a relatively high correlation with the amount of crude fiber (r = 0.69 - PH and r = 0.79 - HH) in the dry matter.

The theoretical regression line and the equation of the regression dependence between the crude protein content and the weight percentage of legumes (in pasture and hay-making harvesting) are shown in Fig. 1 and 2, namely:

- For pasture harvesting - y = 68.8059 + 1.5057x, with coefficient of determination - R = 0.925 (P <0.00001);
- For hay-making harvesting - y = 68.8059 + 1.5057x, with coefficient of determination - R = 0.925 (P <0.00001).

Conclusion

The applied management practices in natural grassland of *Chrysopogon gryllus* L. type affected the quantitative share of grasses and legume. From the first to the fifth experimental year, the percentage share of grass species in the volume of the formed grassland decreased by up to 10.3% - in the pasture mode of use and by up to 14.3% - in the hay-making mode of use. During the last two years of the experimental period, the presence of the legume component marked an increasing trend (39.8-55.5% in pasture and 38.6-52.0% in hay-making), which is a prerequisite for higher quality and nutritional value of feed.

For the experimental period, the amount of crude protein in the dry matter of grassland with pasture management practice was 5.1% higher than that in grassland with hay-making mode.

In the *pasture mode* of use there is a high positive correlation between the quantitative share of legumes with the concentration of crude protein (r = 0.82) and the gross energy value (r = 0.95) of the feed mass.

In hay-making harvesting, the weight percentage of legumes is positively correlated with the values of gross energy (r = 0.81), metabolic energy (r = 0.79), feed units for milk (r = 0.77) and feed units for growth (r = 0.69).

Table 4. Correlation dependences between botanical composition and some qualitative indicators of a natural grassland of *Chrysopogon gryllus* L. type (in pasture harvesting). Legend: FUM - feed unit for milk - number in kg of dry matter; FUG - feed units for growth - number in kg of dry matter.

	Grass.	Legum.	CP	EE	CF	Ash	NFE	Ca	P	GE	ME	FUM	FUG
Grasses, %	1.00												
Legumes, %	-0.54	1.00											
CP, g kg^{-1}	-0.89	0.82	1.00										
EE, g kg^{-1}	-0.70	0.61	0.86	1.00									
CF, g kg^{-1}	-0.42	0.69	0.46	-0.03	1.00								
Ash, g kg^{-1}	0.69	-0.56	-0.63	-0.41	-0.55	1.00							
NFE, g kg^{-1}	0.73	-0.89	-0.83	-0.46	-0.87	0.62	1.00						
Ca, g kg^{-1}	-0.56	0.31	0.66	0.79	-0.17	0.11	-0.30	1.00					
P, g kg^{-1}	0.99	-0.42	-0.82	-0.61	-0.38	0.61	0.67	-0.57	1.00				
GE, MJ/kg	-0.74	0.95	0.89	0.62	0.76	-0.75	-0.95	0.29	-0.64	1.00			
ME, MJ/kg	0.25	-0.43	-0.20	0.30	-0.92	0.24	0.70	0.21	0.27	-0.48	1.00		
FUM	0.42	-0.60	-0.41	0.09	-0.97	0.40	0.84	0.10	0.41	-0.66	0.98	1.00	
FUG	0.42	-0.66	-0.45	0.04	-0.98	0.40	0.86	0.06	0.40	-0.70	0.96	1.00	1.00

Table 5. Correlation dependences between botanical composition and some qualitative indicators of a natural grassland of *Chrysopogon gryllus* L. type (in hay-making harvesting). Legend: see Table 4.

	Grass.	Legum.	CP	EE	CF	Ash	NFE	Ca	P	GE	ME	FUM	FUG
Grasses, %	1.00												
Legumes, %	-0.54	1.00											
CP, g kg^{-1}	-0.64	0.66	1.00										
EE, g kg^{-1}	-0.52	0.31	0.69	1.00									
CF, g kg^{-1}	-0.16	0.79	0.09	-0.29	1.00								
Ash, g kg^{-1}	0.35	-0.62	-0.72	0.00	-0.40	1.00							
NFE, g kg^{-1}	0.64	-0.94	-0.88	-0.52	-0.54	0.72	1.00						
Ca, g kg^{-1}	-0.31	0.39	0.63	0.95	-0.14	0.01	-0.54	1.00					
P, g kg^{-1}	0.79	-0.48	-0.19	-0.36	-0.37	-0.13	0.40	-0.25	1.00				
GE, MJ/kg	-0.65	0.81	0.98	0.62	0.30	-0.76	-0.96	0.60	-0.27	1.00			
ME, MJ/kg	-0.82	0.79	0.89	0.77	0.27	-0.48	-0.91	0.71	-0.59	0.92	1.00		
FUM	-0.85	0.77	0.88	0.77	0.25	-0.46	-0.89	0.69	-0.62	0.90	1.00	1.00	
FUG	-0.84	0.69	0.89	0.83	0.13	-0.43	-0.85	0.74	-0.58	0.89	0.99	0.99	1.00

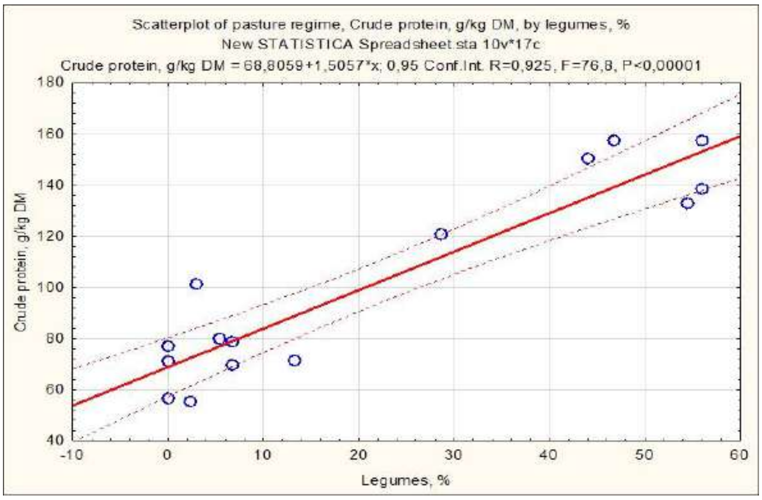

Fig. 1. Regression model for Crude protein (g/kg DM) determination by Legume species participation, % in pasture regime of utilisation of *Chrysopogon gryllus* grass type.

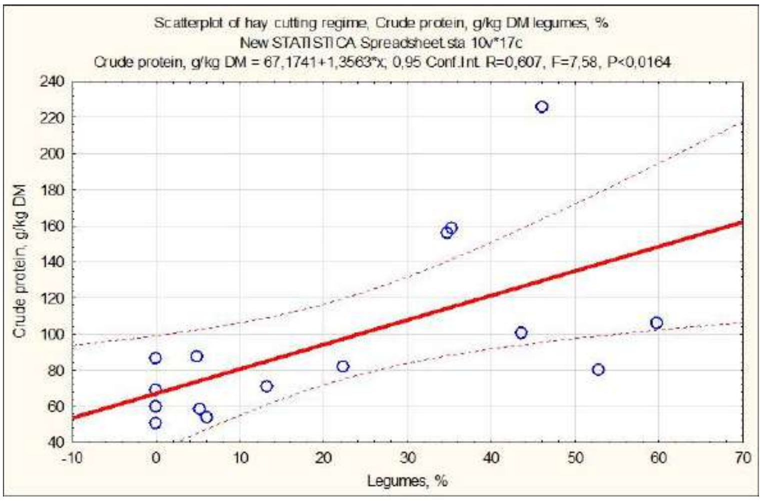

Fig. 2. Regression model for Crude protein, (g/kg DM) determination by Legume species participation, % in hay cutting regime of utilisation of *Chrysopogon gryllus* grass type.

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Short note

Cadmium Induced Ultrastructural Changes in Chloroplasts of Elodea nuttallii (Planch). H. St. John Leaves

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Abstract. The effect of cadmium (Cd) on the ultrastructure of plastid apparatus in leaves of *Elodea nuttallii* (Planch). H. St. John was investigated in experimental conditions using transmission electron microscopy (TEM). The experimental plants were cultivated in a green house environment after preliminary adaptation. The plants were exposed to Cd during 5 days period with concentrations 1 mg/l and 3 mg/l, consistent with previous studies. Cultivated plants under condition of the experiment without Cd were used as the controls. The results showed that at concentration 1 mg/l Cd chloroplasts have a well organized internal membrane system relative to the control, but fragmentation and weak swollen thylakoids were observed. Increasing of cadmium concentration at 3 mg/l Cd show hardly affected plastid ultrastructure, as swollen thylakoid membrane and reduction of grana stacks. The established structural changes of photosynthetic apparatus of *E. nuttallii* are analyzed and compared to previous researches of cadmium toxicity on *E. canadensis* under the same conditions.

Key words: cadmium, chloroplast ultrastructure, thylakoid membranes, *Elodea nuttallii*.

Introduction

The problem of water contamination with heavy metals is important in ecological aspect. Aquatic plants actively accumulate heavy metals in polluted freshwater basins through their roots, stems and leaves (Jackson, 1998). Many techniques have been developed and applied in the removal of pollutants from the aquatic environment especially phytoremediation with the use of aquatic macrophytes (Rai, 2009). *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch). H. St. John, a cosmopolitan aquatic macrophytes with an important role in the ecology of many littoral zones, have high sensitivity to a wide range of contaminants. Therefore, it was interesting to investigate the accumulation of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in this species (Cegłowska et al., 2016). Cadmium (Cd) is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto et al., 2004). Cd appears to cause more marked ultrastructural changes in *E. canadensis* such as destruction of the protoplast (Stoyanova & Tschakalova, 1993; 1999). The most frequently observed ultrastructural effect to this toxic metal is damage to chloroplasts and decreased photosynthetic activity. Ultrastructural investigations indicated that Cd, applied at toxic concentrations, disorganizes the chloroplast membrane system, leading to changes in the ratios of the main structural components of thylakoid membranes and their lipid composition (Dalla Vecchia et al., 2005). During investigation after toxic metal accumulation in *E. canadensis* and *E. nuttallii*, Thiébaut et al. (2010) established higher accumulated amounts of Cd for *E. nuttallii*. From this point of view, it is of interest to compare the structural reaction to cadmium relative to both species. A study under the same experimental conditions would make it possible to evaluate *E. nuttallii* as phytoremediator.

The aim of the present study is to establish the ultrastructure changes in the organization of photosynthetic apparatus of *E. nuttallii* after dose concentration of Cd and make comparative analysis of earlier data for *E. canadensis* under the same experimental conditions.

Material and Methods

Methodical settings were compliant with previous results for structural organizations of plastid apparatus of *E. canadensis* under the influence of stated cadmium concentrations (Stoyanova & Tchakalova, 1999). The experimental plants were cultivated in a green house environment after preliminary adaptation. Cultivated plants under condition of the experiment without Cd were used as controls. The heavy metal was incorporated into the aquatic environment as $CdSO_4$ in concentrations of 1 and 3 mg/l. After 5 days treatment with Cd *E. nuttallii* leaf samples were taken for transmission electron microscopy. For transmission electron microscopy (TEM) specimens were fixed in 3% glutaraldehyde (pH 7.4) in 0.1 M sodium phosphate buffer (pH 7.4) for 12 h at 4°C temperature and post-fixed with 2% $KMnO_4$ for 4 h, dehydrated with ethanol and acetone and embedded in Durcopan (Fluka, Switzerland). Ultra-thin sections were cut by ultramicrotome Reichert-Jung and stained lead citrate (Reynolds, 1963). The observations were examined with a JEOL1200 EX transmission electron microscope.

Results and Discussion

The plastid apparatus in the leaves of the control plants is composed of chloroplasts with a typical organization of the internal membrane system that consists of granal and stromal thylakoids. The granas are structured by 6-17 thylakoids and their height varies. Stromal thylakoids are long and numerous. Osmophilic plastoglobules and no presence of starch grains were established in the stroma (Fig. 1A).

After five days treatment of plants with a low concentration of Cd 1mg/l changes in the structural organization of the internal membrane system were observed. (Fig.1B). The inner membrane system with granal and stromal thylakoids was normally structured but partially swelled. Single larger plastoglobules were found in the stroma that do not generally disturb the shape of the plastids.

In plants exposed to a higher concentration of Cd 3 mg/l the observed changes are more strongly expressed. Impaired orientation of the inner membrane system was observed in comparison with the control plants. In some of the chloroplasts significantly higher and wider grana with strongly swelled thylakoids were observed (Fig. 1C-D). The shape of these chloroplasts was more rounded and their internal volume was larger than those of the control plants. An increase in the number of plastoglobules was observed in the stroma. The outer chloroplast membrane was significantly fragmented.

The structural analysis of the changes of the plastid apparatus registered under conditions of low concentrations of Cd in leaves of *E. nuttallii* shows that they are similar to those observed in *E. canadensis* by Stoyanova (1998) and Dalla Vecchia et al. (2005). Both species have a close resistance range. The most commonly observed change in the structure of chloroplasts is fragmentation and mild swelling of the thylakoid membranes and this transformation is not associated with thylakoid destruction. Similar changes of the plastid apparatus of *E. canadensis* have been reviewed by Esposito et al. (2007).

In previous studies on the effect of higher concentrations of cadmium on changes in the ultrastructure of chloroplasts in *E. canadensis* serious destructions has been reported (Stoyanova & Cshakalova, 1990). The authors observed a strong swelling of the stromal and granal thylakoid membranes that made them difficult to differentiate. Most likely this is due to the destructive effect of cadmium on the lipids structuring the inner membrane system. Deformed and dilated thylakoids have been observed at high cadmium concentrations (Esposito et al., 2007). A similar thylakoid degradation has been established in chloroplasts in the leaves of aquatic plants treated with other heavy metals such as Pb, Cu, Zn, Ni (Stoyanova & Tschakalova 1997; Stoyanova 1999; Stoyanova & Balgjiev, 2002; Nyquist & Greger, 2007; Stoyanova-Koleva & Tchakalova 2008; Chandra & Yadav, 2011; Ceglowska et al., 2016).

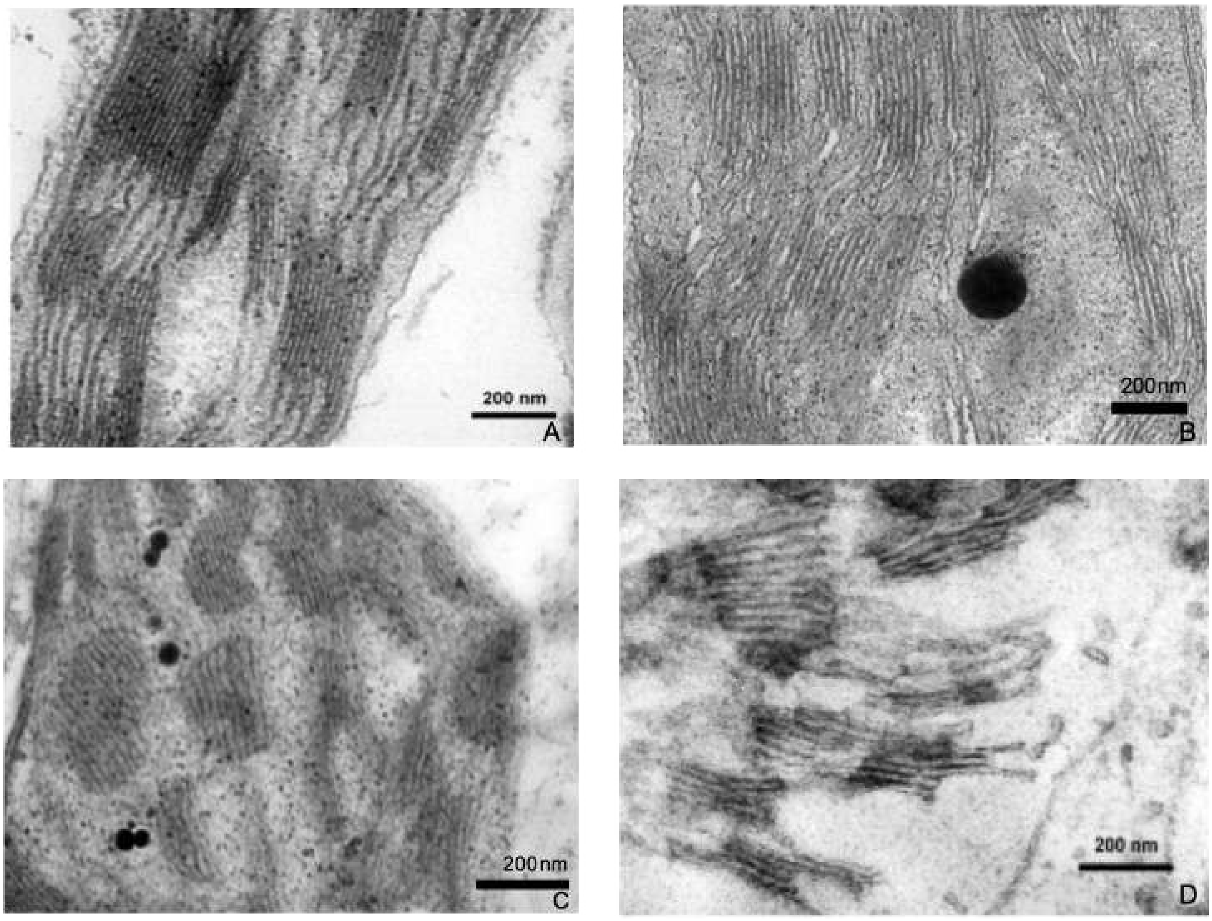

Fig. 1. A: TEM micrograph of chloroplast in a cell of *E. nuttallii* control plants. **B:** TEM micrograph of chloroplast in a cell of *E. nuttallii* treated with Cd 1mg/l. **C-D:** TEM micrograph of chloroplast in a cell of *E. nuttallii* treated with Cd 3 mg/l.

In the stroma of the studied plastids at higher concentrations of Cd there was an increase in the number of plastoglobules. This is related to the destructive effect of heavy metal on the lipid components of thylakoid membranes (Ouzounidou et al., 1997). Our observations are consistent with the results of a study by Stoyanova & Tchakalova (1999), where the authors reported the presence of multiple plastoglobules in the chloroplasts of *E. canadensis* leaves exposed to high concentrations of lead. It has been shown that their size and number increase under conditions of stress, simultaneously with the destruction of thylakoid membranes.

Conclusions

In the present study of the structural organization of chloroplasts in *E. nuttallii* in response to exposure to Cd 1 mg/l, observed changes in thylakoids are associated with less noticeable swelling of granal thylakoids and preservation of stromal functional integrity and activity. The higher concentration of Cd 3 mg/l induces destructions of the photosynthetic apparatus of *E. canadensis* that leads to loss of their functional ability. In contrast the changes in the structure of thylakoid membranes caused by the same Cd concentration in *E. nuttallii* do not affect their function so hard and the chloroplasts retained their functionality close to the control plants. Based on a comparison of the structural organization of chloroplasts it can be said that *E. nuttallii* has a greater capacity to accumulate heavy metal and will therefore be a more efficient species in the biological treatment of water contaminated with cadmium.

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Short note

Long-distance Exploratory Dispersal of an Immature Egyptian Vulture (Neophron percnopterus) from the Balkans

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Abstract. Dispersal, a behavior typical of several raptor species has important implications for population genetics and demographic processes, including the capacity for recolonization, (meta) population persistence and breeding output. Many raptor species are migratory and return to reproduce in a territory close to their natal area where they have fledged (natal philopatry). The Egyptian vulture is the smallest of the four vulture species breeding in Europe and the only one that migrates. Here we describe the first case of long-distance exploratory dispersal of an immature Egyptian vulture from the Balkans across three continents. To accomplish this extreme long-distance exploratory dispersal, Dobromir crossed 23 countries in 168 days, covering more than 30,000 km (mean 179km/day).

Key words: Egyptian vulture, philopatry, site fidelity, migratory, scavenger.

Dispersal behavior is one of the fundamental features of an organism. It plays an important role in geographical distribution, population structure, and population dynamics (Walters, 2000). Generally, the evolution of dispersal is associated with a tradeoff between fitness-related costs and benefits. Dispersal is defined either as the movement of juveniles from the natal site to the site of first breeding (natal dispersal) or as breeding dispersal (but see Pearce, 2007). The later one is the movement of individuals that have previously reproduced, between subsequent breeding attempts (Terraube et al., 2014). Dispersal is a behavior typical of several raptor species and has important implications for population genetics and demographic processes, including the capacity for recolonization, (meta) population persistence and breeding output (Grande et al., 2008; Elorriaga et al., 2009; Di Vittorio et al., 2016). Many raptor species are migratory and return to reproduce in a territory close to their natal area where they have fledged (natal philopatry)(Newton, 1979). However, some typical long-distance migrants before recruiting to a breeding territory might explore and disperse to locations far from their natal areas. Even rare, these events might have important genetic implications and role in population dynamics as long-distance dispersal can be underestimated (Grande et al., 2008; Di Vittorio et al., 2016).

The Egyptian vulture is the smallest of the four vulture species breeding in Europe and the only one that migrates to Africa (Cramp & Simmons, 1980). Due to the rapid and steady decline in most of its range, the species is declared Endangered (BirdLife International,

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2020). Across Europe, the population of the species in the Balkans has experienced one of the steepest declines due to increased mortality in the breeding grounds (Velevski et al., 2015), on migration (Oppel et al., 2015) and in the wintering grounds (Buechley et al., 2018).

The Egyptian vulture spatial and temporal variability in migration movements are well studied. It is known that it exhibits a high level of variability at the subpopulation level and flexibility at the individual level in respect to basic migration parameters (Phipps et al., 2019). As a long-distance migratory bird, the Egyptian vulture remains in Africa where explores wide areas with different centers of activity for at least 1.5 years before returning to the breeding grounds (Oppel et al., 2015). Thus, the extent of juvenile dispersal and recruitment into the breeding population when sexual maturity is reached, is crucial for the survival of the species and at an evolutionary level (Greenwood, 1980; Grande et al., 2008). Once birds reach maturity they disperse close to their natal areas, facilitating the replacement of dead individuals and/or recolonization of abandoned territories (Carrete et al., 2007; Grande et al., 2008). Furthermore, the behavior of individuals in small populations is relatively more important than that in larger populations (Gosling, 2003). Here we describe the first case of long-distance exploratory dispersal of an immature Egyptian vulture from the Balkans across three continents.

Dobromir is a juvenile Egyptian vulture tagged with a satellite transmitter in northern Bulgaria in 2012. We used a solar-powered 45-g GPS satellite transmitters (Microwave Telemetry) attached to the birds' back with a Teflon ribbon harness in a backpack configuration. The entire transmitter equipment did not exceed 3% of the birds' body mass, which is considered safe for the bird and unlikely to influence its migratory performance and life spam (Bodey et al., 2018). The satellite transmitter was set to record the location of the bird with GPS accuracy every 2 h between 04:00 and 22:00 h on a daily basis. Data were downloaded via the ARGOS satellite system and deposited in Movebank.

The bird migrated successfully in the Autumn of 2012 to Darfur region, Sudan, a wintering ground for birds originating from the Balkans, Middle East, the Caucasus (Buechley et al., 2018). As other juvenile and immature birds wintering in the Sahel, Dobromir had different centers of activity in the two consecutive years before returning to the north in the spring of 2014 (Oppel et al., 2015). On the 18th of April, 2014 Dobromir started its first migration to the north, from Sudan towards the Balkans. The bird used the Suez flyway bottleneck, then traversed to the north and reached Turkey where it stayed between 4th and 23rd of June. On the 23rd of June, Dobromir crossed the Dardanelles and reached Bulgaria. In one month (between 23rd of June and 23rd of July) the bird was wandering across the east of the country and made two long trips reaching as far as northern Romania. The bird flew to the west of the Balkan peninsula visiting former breeding grounds of the species in North Macedonia and Serbia (Dobrev, 2017). Between 23rd and 27th of July, Dobromir returned to the its natal territory and was feeding and perching close to its nest. On the 27th, the bird left and started its movement to the north, traversing the Black sea and the sea of Azov to the east. Dobromir reached the region of Rostov, Russia on the 8th of August where it turned south and crossed the Caucasus mountains on the 18th. Then it resided for 6 days in Azerbajdzhan and for 22 days in northeastern Iraq. Afterwards, it continued moving slowly to the south, following known migratory flyway through Saudi Arabia and Yemen (Buechley et al., 2018). Finally, the bird reached the wintering grounds in Ethiopia on the 2nd of October crossing Bab el Mandeb. To accomplish this extreme long-distance exploratory dispersal across the three continents, Dobromir crossed 22 countries in 168 days, covering more than 30,000 km (mean 179km/day) (Fig. 1).

The Egyptian vulture is a long-distance migrant and adult birds are faithful to the migratory ways and to the timing during the migration (Phipps et al., 2019). Home ranges of wintering adult birds significantly differ compared to juvenile and immature birds, with the latest being much larger (Oppel et al., 2015). Younger birds do exploratory flights before reaching maturity. They stay at least 1.5 years in Africa and then start moving to the breeding grounds and their natal areas (philopatry). Thus, the phenomena acts as a mechanism for recolonization of abandoned breeding territories assisting population recoveries or colonization of new areas (Grande et al., 2008). Reagrdless that birds originating from different regions might form congregations at communal roosts or at the wintering grounds, due to the high level of natal philopatry the probability of effective dispersal and recruitment in places different from their natal is low (Sará & Di Vittorio, 2003; Donázar, 2004). However, as evidenced in some cases the social attraction might prevail over the natal philopatry, and birds might disperse far away from their natal areas (Elloriaga et al., 2009; Di Vittorio et al., 2016). Some important ecological processes may operate at the population level as a result of behavioral decisions made by individuals (Carrete et al., 2007), and dispersal events even if anecdotal, may play an important role in the species' population dynamics and persistence (Elorriaga et al., 2009). This might have happened with Dobromir when wandering in the Balkans during the movement in 2014 and visiting former breeding grounds of the species. The social attraction by other conspecifics and the network of vulture feeding stations across the Balkans might have bridged his movements. Natal philopatry was probably the reason why Dobromir returned to its natal area. Naturally as evidenced (see Oppel et al., 2015), juveniles tend to make very long dispersals when residing in Africa and shifting their activity centers for different reasons (i.e. food availability, habitat alterations). Dobromir was still only 2 years old and the ranging behavior in combination with other factors (i.e. social attraction, migration instincts) might explain partially the extreme long-distance dispersal across the steppes of Russia, the Caucasus and the Middle East.

This case, even a single, reveals mechanisms that might affect population dynamics and genetics and demonstrate that animal movement and dispersal could go beyond explanation. It also provides evidences on Egyptian vulture movements and connections between different subpopulations of the species.

Fig. 1. Long-distance exploratory route of the tagged vulture (Dobromir) in 2014 and days per countries where the bird spent more than 5 days during the exploratory dispersal.

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Short note

Hydnotrya michaelis – an Uncommon Fungus from Unexpected Habitat

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Abstract. The paper introduces the first records in Southeastern Europe of *Hydnotrya michaelis,* rare ascomycete, collected in artificial plantation with Macedonian pine (*Pinus peuce*) and compared with specimens found with other Pinaceae. The species was studied for the first time on living materials and description and illustrations of the Bulgarian specimens are provided. The first nLSU sequence of this rare and little-known species is also released.

Key words: Bulgarian mycota, Discinaceae, hypogeous fungi, Pezizales, truffle-like fungi.

Introduction

Despite the vast array of diverse habitats, the knowledge on hypogeous fungi was until very recently surprisingly limited in the Balkan Peninsula (Chavdarova et al., 2011; Kaounas et al., 2011; Polemis et al., 2019). While the studies have become more intensive in the last decade due to the interest in species of commercial potential, the number of species has notably increased, including with some previously undescribed (see e. g. Kaounas et al., 2015, 2016; Milenković et al., 2016; Polemis et al., 2019; Vidal et al., 2019). *Hydnotrya* Berk. & Broome is a genus of hypogeous ascomycetes, with eleven species so far described from across Europe, some of them pending taxonomic reassessment as suggested by recent molecular studies (Stielow et al., 2010). Of these only two have been recorded so far in some Balkan countries: *H. cerebriformis* Harkn. and *H. tulasnei* (Berk.) Berk. & Broome from Bulgaria (Dimitrova & Gyosheva, 2008), and *H. tulasnei* in Greece (Konstantinidis & Kaounas, 2014). Our collecting efforts yielded one particularly interesting *Hydnotrya* specimen from habitats with the Balkan endemic pine *Pinus peuce* Griseb., followed by another collection from boreal coniferous forests. Their study revealed that these are the first collections of the rare and less-known *H. michaelis* (E. Fisch.) Trappe in Southeastern Europe. As far as it is a species seldomly described in the mycological literature, detailed morphological characterization is provided herein.

Material and Methods

The fungus was retrieved with the aid of trained dogs. The specimens were photographed and documented in the field, as well as *ex-situ*. The microscopic study was held

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on fresh specimens on slides in tap water (Baral, 1992). Melzer's reagent was used for additional observations. All measurements of microscopic structures in the description are reported from slides in water. The colours of the different parts of ascomata as closely as possible refer to the "*Flora of British Fungi Colour Identification Chart*" (Anonymous, 1969). Air-dried voucher specimens are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

DNA extraction, amplification, and sequencing were handled by ALVALAB (Spain) by the following protocol. Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54ºC. The primers LR0R and LR3-Asc (Cubeta et al., 1991, Tedersoo et al., 2008) were used to amplify the 28S rDNA region (LSU). The PCR product was checked in a 1% agarose gel, and the amplicon was sequenced with primer LR3-Asc.

Results and Discussion

Hydnotrya michaelis (E. Fisch.) Trappe, Mycotaxon 2(1): 113 (1975).

Macroscopic features. Ascomata hypogeous, up to 6 cm across, initially subspherical, with rounded opening, then with maturation more or less irregular, wrinkled, lobulate, with numerous invaginations. Peridium irregularly coloured in buff, clay pink to chestnut brown, with pronounced vinaceous or purple tinges, finely pubescent to smooth. Gleba labyrinthoid, with large, sinuous cavities, separated by folded inwards portions of ascomatal wall; trama clay pink to pale vinaceous, with distinct, narrow, livid vinaceous subhymenial line; hymenial layer continuous, in surface view off-white to vinaceous pink or clay buff, in section whitish. Odour very strong, somewhat pungent, rather persistent.

Microscopic features. Peridium following the ascomatal surface and the respective side of the invaginated parts of the wall, 80–150 µm thick, composed of 5–8 layers of almost spherical, ovoid or polygonal elements and sometimes slightly elongate and narrow apical elements, 17.5–40.0 × 12–35 µm; with numerous spherical lipid bodies, yellowish to brownish in water and in places with extracellular granules of brown pigment; walls not thickened or by exception thickened up to 3 µm, encrusting particles not seen. Trama composed of strongly interwoven, thin-walled, branched, septate, hyaline hyphae 2–7 µm wide, with numerous lipid bodies; some inflated up to 17 µm hyphae present, occasionally with walls up to 2 µm thick and with somewhat granular appearance. Hymenium lining the cavities, composed of regularly arranged asci and paraphyses. Asci cylindrical, 200–220 × 30–35 µm, including up to 30 µm narrowed basal part, 8-spored, thin-walled, inamyloid. Paraphyses considerably longer than asci (by some 100–150 µm), narrowly cylindrical, 5–6 µm wide, sometimes slightly widened (up to 8 µm) at the apex, thin-walled, septate, hyaline, with numerous lipid bodies. Ascospores (21.2–)24.9–29.6(–32.2) × (18.8–)19.8–22.4 (–24.9) µm; Q = (1.1–)1.2–1.4 (–1.5) , on average 27.5 × 21.3 µm ; Qav = 1.3 (n=60, ornamentation excluded), broadly ellipsoid, honey-yellow, thick-walled, with one large central guttule; ornamentation 2–6 µm, consisting of large, dense, mostly irregular to ameboid warts with usually rounded or truncate apices, forming somewhat incomplete reticulate pattern.

Specimens examined. Bulgaria: Western Stara Planina Mts: Petrohan Pass (Sofia distr.), 43°06'53.6"N 23°07'34.6"E, artificial plantation, under *Pinus peuce* Griseb., 04.07.2016, B. Assyov (SOMF 30345, GenBank MW879528); Western Rodopi Mts, Atoluka resort (Pazardzhik distr.), forest with *Picea abies* (L.) H. Karst. and *Abies alba* Mill., 17.06.2018, M. Slavova (SOMF 30346).

The Bulgarian collections of *H. michaelis* (Fig. 1a–g) correspond well, both macro- and microscopically to the existing descriptions of the species in the mycological literature (Fischer, 1878; Pegler et al., 1993; Vidal, 1994; Montecchi &

Sarasini, 2000; Gori, 2005; Kříž et al., 2017). The specimen from *P. peuce* stand agrees in all aspects to the second studied collection, associated with other Pinaceae. As far as possible to judge from the few descriptions in literature, the Bulgarian specimens are the first to be studied microscopically in living state. Among the European species of the genus, *H. michaelis* is recognized by ascomata with large, irregular cavities, combined with ellipsoid spores with ornamentation of broad, irregularly-shaped warts. An attempt was made to obtain barcoding sequences of one of our specimens. An nLSU sequence was successfully acquired and appears to be the first publicly available of this species (GenBank MW879528). Sequence of the nrITS region could not be obtained for the moment.

Fig. 1. Morphological features of *Hydnotryia michaelis*: A,B – ascomata, C – section of ascoma, D – parts of asci and paraphyses, E,F – ascospores, G - peridium. Scale bars: A,B = 1 cm, C = 100 µm, D,E,F,G = 30 µm.

Hydnotrya michaelis was described by Fischer (1878) and for a long time after its description remained relatively little-known, with more records starting to emerge only in the late XXth century. In Europe until now it has been found in Austria, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Norway, Poland, Spain, Sweden and the United Kingdom (Kers, 1989; Ławrynowicz, 1990; Vidal, 1994; Montecchi & Sarasini, 2000; Spooner, 2003; Gori, 2005; Dieker, 2010; Huntinen, 2010). The closest known localities are apparently those in the Italian Alps (Montecchi & Sarasini, 2000). The Bulgarian findings are among the southernmost so far known and represent an outpost in the disjunct range of the species, which is mostly confined to Central and Northern Europe. *Hydnotrya michaelis* is known to be mycorrhizal species, linked to mountain coniferous trees, primarily *Picea abies* (L.) H. Karst., but also to *Abies alba* Mill., *Pinus* spp. and *Larix decidua* L. (Vidal, 1994; Ławrynowicz, 1990; Montecchi & Sarasini, 2000). The first Bulgarian locality is an old artificial plantation of mixed conifers with scattered trees of *Fagus sylvatica* L. *Pinus peuce* is the dominant coniferous species on the spot and *Picea abies, A. alba* and *Pinus sylvestris* L. are also present in the stand. Ascomata of *H. michaelis* were however only recovered under trees of *P. peuce* and far from other conifers, making the mycorrhizal relation to this tree a plausible assumption. The species was consequently collected also in another distant locality, where it seemed associated with *A. alba* or *P. abies.* In both above places the ascomata were found at shallow depth, slightly buried in the top soil layer and only covered by 1–5 cm thick layer of leaf-litter. *Hydnotrya michaelis* appears to be the first record of a hypogeous fungus allegedly associated with *P. peuce,* unequivocally showing that the subterranean mycobiota of this peculiar and restricted to the Balkan Peninsula mycorrhizal host is so far underexplored and merits further attention.

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Short note

They are Back: Notes on the Presence and the life Activities of the Eurasian Beaver (Castor fiber L. 1758) from the Territory of Bulgaria

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Abstract. This scientific note reports on the recently detected presence of the Eurasian beaver (Castor *fiber* L. 1758) on the territory of Bulgaria. The species was previously a part of the Bulgarian fauna, however it disappeared from the territory of the country more than 150 years ago. Currently, one site in North Bulgaria was an object of long term monitoring and our team was able to detect the life activity of the Eurasian beaver in that particular habitat. The main goal of the present publication is to inform both the scientific community, as well as the public for the presence of the species in Bulgaria, but also to encourage the competent authorities to undertake initiatives for the protection of the species and its habitat.

Key words: faunistics, zoogeography, range, rodents, mammal, vertebrate.

In the past, the European beaver (*Castor fiber*) was widely distributed on the territory of Bulgaria and inhabited the low streams of many rivers (see Boev & Spasov, 2019). The species' populations declined dramatically in the XVIII century (Boev, 1958) and vanished from the Bulgarian fauna presumably in the middle of the XIX century (Boev & Spasov, 2019). According to the information provided by Wrobel (2020), the beaver population in Europe is growing during the last decades and had reached over 1.2 million specimens. However, the same author reported that data on the occurrence of beavers are missing for Bulgaria.

With the present short note, we would like to inform the scientific community, the public, the competent authorities and the government on our findings concerning the presence and the detected life activity of *C. fiber* in a particular habitat on the territory of Bulgaria. We were able to document bite marks on tree logs - predominantly from *Salix* sp. and *Populus* sp. (Fig. 1a), as well as fresh footprints of adult beavers (Fig. 1b). The data were collected from a territory under protection of the NATURA 2000 network, however we received some alarming information from the local residents related to negative attitude toward animals in the local society. That was the main reason to release this short note before the publication of the data from our long term monitoring on the Eurasian beaver's activities from the location.

Fig. 1. Indications of the presence of the Eurasian beaver (*Castor fiber*) on the territory of Bulgaria: a. marks from gnawing activities of the beaver on a fallen tree and its log rests – note the typical serration traces on the wood material (indicated by a red arrow) and the abundant wood raspings at the base of the log; b. foot prints from an adult beaver in the mud shore besides the river – the violet arrows indicate the typical shape of the beaver traces; both photographs were performed by the use of "Sony RXIII" (Sony corporation, Minato, Tokyo, Japan).

The construction activities of *C. fiber* impact the morphology of their habitats to large scale and these changes may concern other vertebrates that live in the same territory (Collen & Gibson, 2001; Herr & Rosell, 2004; Hartman & Tornlov, 2006). The Eurasian beavers demand some special characteristics of the sites they inhabit (see Kiss et al., 2012). Our data (and also data of N. Kodzhabashev and T. Teofilova, pers. comm.) revealed, that *C. fiber* had occupied a defined territory in Bulgaria for prolonged time (since January 20-th, 2020 in our case), which indicates on the high suitability of that particular habitat for the species.

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Synopsis

Syntaxonomical and Ecological Diversity of Class Artemisietea vulgaris Lohmeyer et al. in Tx. ex von Rochow 1951 in Bulgaria

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Abstract. Class *Artemisietea vulgaris* includes thermophilic and (sub)xerophytic ruderal vegetation, which consists mostly of biannual and perennial seminitrophilous to nitrophilous species, widely distributed in the temperate and the submediterranean zones of Europe. The study presents a comprehensive review of its ecology and syntaxonomy on the territory of Bulgaria, based on 160 relevés stored in the Balkan Vegetation Database and selected using the "EuroVegChecklist Expert System". Numerical classification and ordination were performed by hierarchical agglomerative clustering and Detrended Correspondence Analysis. The diagnostic species were determined by calculating the Phi-coefficient. The syntaxonomical diversity of the class *Artemisietea vulgaris* on the territory of Bulgaria is presented by 2 orders (*Agropyretalia intermedio-repentis, Onopordetalia acanthii*), 3 alliances (*Convolvulo arvensis-Elytrigion repentis, Dauco-Melilotion* and *Onopordion acanthii*), 10 associations (*Convolvulo arvensis-Elytrigietum repentis, Falcario vulgaris-Elytrigietum repentis, Convolvulo arvensis-Brometum inermis, Cardarietum drabae, Tanaceto vulgaris-Artemisietum vulgaris, Melilotetum albo-officinalis, Berteroetum incanae, Poo compressae-Tussilaginetum farfarae, Potentillo argenteae-Artemisietum absinthii* and *Carduo acanthoidis-Onopordetum acanthii*) and 1 plant community (*Achillea pannonica-Elytrigia repens*). One order, two alliances and nine associations were discovered for the first time in the country. All of the syntaxa are well separated floristically and ecologically in the ordination space. The associations of the alliance *Dauco-Melilotion* represent initial stages of succession of antropogenic habitats and has higher species diversity, whereas those of the alliances *Convolvulo arvensis-Elytrigion repentis* and *Onopordion acanthii* represent more stable antropogenic vegetation types and have lower species richness.

Key words: ruderal vegetation, Braun-Blanquet approach, *Artemisietea vulgaris*.

Introduction

The *Artemisietea vulgaris* class unites thermophilic and (sub)xerophitic ruderal vegetation consisting mostly of biannual and perennial seminitrophilous to nitrophilous species. Typically, it develops in sunny

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anthropogenic places affected by different levels of human impact. The soils are sandy and may contain a lot of gravel. This vegetation thrives within human settlements and their surroundings. In case of full absence of human or animal caused disturbances, such vegetation changes by secondary succession into grassland or shrub vegetation (Mucina, 1993; Jarolímek et al., 1997; Láníková, 2009).

The *Artemisietea* class is widely distributed in places with warm local microclimate in the temperate and the sub-Mediterranean regions of Europe, while in the Mediterranean region it occurs at higher altitudes. The class is presented by 5 orders and 20 alliances in Europe, including the heavily disturbed semi-ruderal and ruderal grasslands of *Agropyretalia intermedio-repentis* and *Elytrigio repentis-Dittrichietalia viscosi* (Mucina et al., 2016).

On the territory of Bulgaria *Artemisietea vulgaris* class has been invesitigated by Kolev (1965), Mucina & Kolbek (1989) and Dimitrov et al. (2005). According to Tzonev et al. (2009) it is presented by 2 orders, 3 alliances, 7 associations and 2 plant communities. Following the most recent scheme about syntaxonomical division of high-rank syntaxa in Europe (Mucina et al., 2016) currently the alliances *Artemisio-Kochion prostratae* Soó 1964 and *Arction lappae* Tx. 1937 are moved to the classes *Festuco-Brometea* and *Epilobietea angustifolii*. Up until now the syntaxonomical diversity of the *Artemisietea vulgaris* class in Bulgaria has been presented by 1 order (*Onopordiethalia acanthii*), 1 alliance (*Onopordion acanthii*), 3 associations (*Carduo acanthoidis-Onopordetum acanthii*, *Onopordetum acanthii*, *Echio-Melilotetum*) and 2 plant communities (*Ballota nigra - Artemisia absinthium* and *Achillea pannonica-Elymus repens*).

The aim of this study is to investigate the distribution, ecology and syntaxonomical diversity of the *Artemisietea vulgaris* class in Bulgaria.

Material and methods

Study area

The *Artemisietea vulgaris* class occurs in a vast array of geographical territories with diverse features (Fig. 1). The Thracian, Sofia and Kostenets-Dolna Banya Valleys are among the lowest lying areas where the investigated class was observed. The hilly and plateau relief include the features of the Dobrudzha Plateau and the Chirpan Highlands, ranging from 300 m to 600 m a.s.l. Low mountainous territories include the areas of the Western and Central Forebalkan, the mountains of Mala Planina, Ponor, Lyubash, Chepun and Sredna Gora that mainly fall within the hypsometric zone between 600 m and 1200-1300 m a.s.l. and reach over 1600 m a.s.l. The highest mountains within the studied area are Belasitsa, the Balkan Range, Pirin and Rila.

The climate is mainly temperate. Nevertheless, significant territories are located in the transitional climate zone and some even have typical submediterranean features. The latter are found around the city of Petrich and along the Black Sea coast. The rivers of Iskar, Maritsa and Yantra, accompanied by some their most prominent tributaries, are watering the zone.

The class is distributed in territories with high soil diversity with a prevalence of the different subtypes of Cambisols, Vertisols, Luvisols, Fluvisols and Leptosols.

Data collection and statistical analysis

Vegetation sampling. During 2017-2020 were sampled 650 vegetation plots (relevés) presenting synantropic vegetation from the territory of Bulgaria, following the Braun-Blanquet approach (Westhoff & van der Maarel, 1973). The sample plots were square-shaped with size in the range 10-100 m^2, as recommended for synantropic vegetation (Chytrý & Otýpková, 2003). Altitude, slope, inclination and location were measured with a Garmin eTrex Vista device for all reléves whereas the slope aspect was determined by a compass. Soil depth was estimated in three degrees as (1) shallow (<10 cm depth), (2) moderately deep (10-20 cm) and (3) deep (>20 cm). For each relevé full species composition was recorded as well as the total vegetation cover, the cover of the shrub, the herb and the cryptogam layers. All of the relevés were stored in the Balkan Vegetation Database (Vassilev et al., 2020). Then they were exported in the JUICE 7.0 (Tichý, 2002) software. We applied the "EuroVegChecklist Expert System" function of JUICE, which uses diagnostic species lists according to the EuroVegChecklist (Mucina et al., 2016) and were selected 160 relevés, which were classified to the *Artemisietea vulgaris* class (19 relevés from digitized literature sources and 141 new collected relevés).

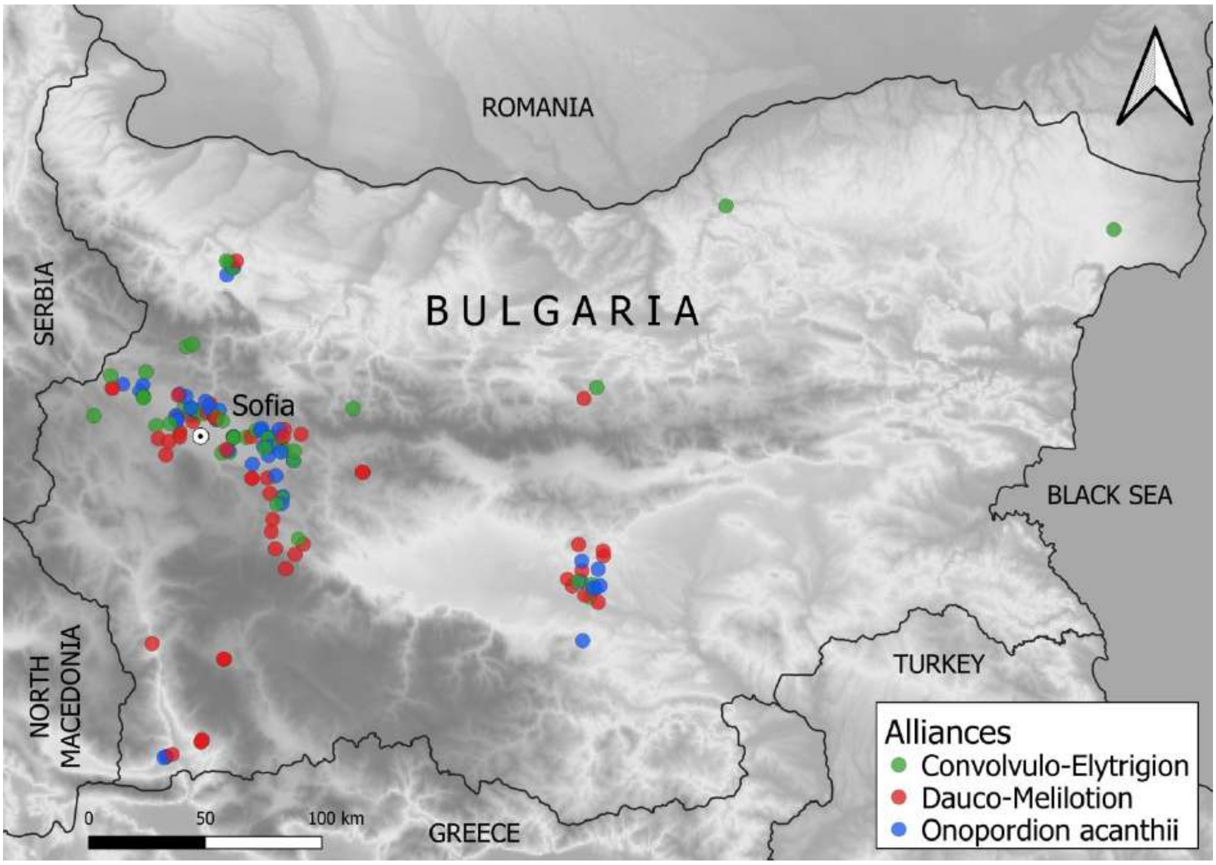

Fig. 1. Map of the studied areas. Here all collected relevés are given classified to alliance levels because on association level they are overlapping.

The selected relevés were analyzed by the PC-ORD (McCune & Mefford, 1999) and the JUICE 7.0 (Tichý, 2002) software packages. Sørensen (Bray-Curtis) was used as a distance measure and similarity was calculated by flexible beta clustering method. The species values were square-root transformed and three cut levels (0, 5, and 25) were used. The clusters were standardized to equal size (Chytrý et al., 2006). The diagnostic species were determined by calculating the Phi-coefficient (Chytrý et al., 2002). Only the statistically significant Phi-coefficient values evaluated by Fisher's exact test (*P<0.05) were considered. In the synoptic table the threshold value for a species to be considered a diagnostic one was set up at a Phi-coefficient ≥ 0.3 (multiplied by 100). They were coloured in light-green in Table 1. The species with a Phi-coefficient ≥ 0.5 were considered highly diagnostic (coloured in darkgreen in Table 1). The species in the synoptic tables were ordered by decreasing of their fidelity value. The diagnostic role of the species was also considered on the basis of available literature sources. Two values were presented for each species in the synoptic table: "Fidelity" - expressed as a Phi-coefficient and "Frequency"expressed in percentage. Species with coverage above 50% in at least in 20% of the relevés in any cluster were considered as dominants, whereas constant species were those having at least 50% presence in a cluster.

Detrended Correspondence Analysis (DCA) was used as indirect ordination technique using the CANOCO 4.5 software package (ter Braak & Šmilauer, 2002) to reveal the major environmental gradients determining vegetation distribution. Square root transformation and downweighting of the rare species were applied. The habitat's ecological conditions were assessed using the "Ellenberg Indicator Values" (Ellenberg et al., 1992) passively projected onto the ordination space.

The nomenclature of the vascular plants followed Delipavlov & Cheshmedzhiev (2003) and was subsequently standardized according to the Euro+Med PlantBase (2006-2020). The floristic elements were interpreted according to Assyov & Petrova (2012). The species life forms were determined according to the classification of Raunkiær (1934). The nomenclature of the high-rank syntaxa was harmonized with Mucina et al. (2016). The species, identified to the genus level, were deleted. In addition, bryophytes and lichens were removed from species composition because they were still not identified for all relevés. We also merged the following taxa into aggregates: *Ajuga chamaepitys* agg. (*Ajuga chamaepitys, A. chamaepitys* subsp. *chia*), *Cerastium fontanum* agg. (*Cerastium fontanum, C. fontanum* subsp. *vulgare*), *Consolida regalis* agg. (*Consolida regalis, C. regalis* subsp. *regalis*), *Crepis foetida* agg. (*Crepis foetida, C. foetida* subsp. *rhoeadifolia*), *Euphorbia seguieriana* agg. (*Euphorbia seguieriana, E. seguieriana* subsp. *niciciana*), *Heracleum sphondylium* agg. (*Heracleum sphondylium, H. sphondylium* subsp. *sibiricum*), *Lamium purpureum* agg. (*Lamium purpureum, L. purpureum* var. *hybridum*), *Ononis spinosa* agg. (*Ononis spinosa, O. spinosa* subsp. *hircina*), *Polygonum rurivagum* agg. (*Polygonum rurivagum, P. aviculare*), *Tragopogon pratensis* agg. (*Tragopogon pratensis, T. pratensis* subsp. *orientalis*), *Trifolium michelianum* agg. (*Trifolium michelianum, T. michelianum* var. *balansae*), *Vicia sativa* agg. (*Vicia sativa, V. sativa* subsp. *cordata*), *Vicia villosa* agg. (*Vicia villosa, V. villosa* subsp. *varia*).

Results

Based on the numerical analysis the syntaxonomical diversity of *Artemisietea vulgaris* class is presented by 2 orders, 3 alliances, 10 associations and 1 community. The syntaxonomical scheme proposed is:

Cl. *Artemisietea vulgaris* Lohmeyer et al. in Tx. ex von Rochow 1951
Ord. *Agropyretalia intermedio-repentis* Müller et Görs 1969*
All.*Convolvulo arvensis-Elytrigion repentis* Görs 1966*
Ass. *Convolvulo arvensis-Elytrigietum repentis* Felföldy 1943*
Ass. *Falcario vulgaris-Elytrigietum repentis* Müller et Görs 1969*
Ass. *Convolvulo arvensis-Brometum inermis* Eliáš 1979*
Ass. *Cardarietum drabae* Tímár 1950*
Ord. *Onopordetalia acanthii* Br.-Bl. et Tx. ex Klika et Hadač 1944
All. *Dauco-Melilotion* Görs ex Rostański et Gutte 1971*
Ass.*Tanaceto vulgaris-Artemisietum vulgaris* Sissingh 1950
Ass. *Melilotetum albo-officinalis* Sissingh 1950*
Ass. *Berteroetum incanae* Sissingh et Tideman ex Sissingh 1950*
Ass. *Poo compressae-Tussilaginetum farfarae* Tüxen 1931*
All. *Onopordion acanthii* Br.-Bl. et al. 1936
Ass. *Potentillo argenteae-Artemisietum absinthii* Faliński 1965*
Ass. *Carduo acanthoidis-Onopordetum acanthii* Soó ex Jarolímek et al. 1997
Achillea pannonica - Elytrigia repens plant community

The new syntaxa for vegetation of Bulgaria are marked with *.

Ass. *Convolvulo arvensis-Elytrigietum repentis* Felföldy 1943 (Table 1, cluster 1)

Constant species: *Elytrigia repens, Convolvulus arvensis*

Dominant species: *Elytrigia repens*

Distribution and ecology: This association has a wide distribution on the territory of Bulgaria along roads, park alleys, near buildings, parking lots and other anthropogenic areas. It is presented by 34 relevés (Fig. 1). It was found between 70 and 1250 m a.s.l. predominantly on flat terrains or with inclination up to 5-8°. The vegetation was sampled from the Sofia lowland (Sofia city, Elin Pelin, Gorna Malina, Slivnitsa, Bozhurishte municipalities), the Thracian plain (Chirpan municipality), Sredna Gora Mt (Ihtiman, Kostenets municipality), the Danubian plain (Nisovo municipality), the Znepole region (Breznik municipality), West Balkan Range (Etropole and Godech municipalities) and the northern Black Sea coast (Balchik municipality). The soils are moderately deep and the bedrock is diverse. Habitats are characterized by extensive sunlight.

Vegetation description and syntaxonomy: Its communities have closed horizontal structure with a total cover of 90-100%. The dominant species is *Elytrigia repens*. Species richness is between 4 and 39 species (an average of 18 species). *Elytrigia repens* is a geophyte, which has vegetative propagation with stolones. Its cover determines the different successional stages of development. In stands with cover of *Elytrigia repens* > 60% it has very strong shade effect. Other specific feature is high accumulation of litter as aboveground layer. *Convolvulus arvensis* is a constant species, with a different cover in the different samples of the association. Other ruderal species such as *Cirsium arvense, Carduus acanthoides, Berteroa incana, Cyanus segetum*, etc. are found in the species composition. Some diagnostic species for *Festuco-Brometea* (such as *Galium verum, Trifolium striatum, Potentilla argentea, Eryngium campestre*) and *Molinio-Arrhenatheretea* classes (e.g. *Agrostis capillaris, Poa pratensis, Alopecurus pratensis, Dactylis glomerata*) are also typical for the species composition.

The hemicryptophytes (H – 50.3%) prevailed in the species composition, followed by the therophytes (T – 32.4%), the biannuals (B – 7%) and the therophyte-biannuals (T-B – 6.6%). Euro-Asiatic (Eur-As – 19.5%), Euro-Mediterranean (Eur-Med – 13.7%), Euro-Siberian (Eur-Sib – 9.4%) and sub-Mediterranean (subMed – 8.6%) are dominant floristic elements in the species composition.

The ecology and the species composition of the association in Bulgaria are similar to its communities in other European countries – the Chezh Republic (Opálková & Cimalová, 2011; Láníková, 2013), the Russian Federation (Golovina, 2015), Romania (Sanda et al., 2008). According to Sanda et al. (2008) 4 subassociations are known for Romania - *convolvuletosum arvensis*, *lepidietosum drabae*, *cirsietosum arvense* and *rubetosum arvalis*.

Ass. *Falcario vulgaris-Elytrigietum repentis* Müller et Görs 1969 (Table 1, cluster 2)

Constant species: *Elytrigia repens*

Dominant species: *Falcaria vulgaris*

Distribution and ecology: This vegetation type is presented by 6 relevés (Fig. 1), which are distributed in the Sofia lowland (Sofia city, Slivnitsa municipality), West Forebalkan (Krivodol municipality) and Znepole region (Dragoman municipality). It covers an area up to 100 m^2 around arable fields and along roads. The altitudional range is between 147 and 665 m a.s.l. The terrains are flat or with inclination of up to 5°. The soils are moderately deep and frequently dry. This vegetation sometimes is mown.

Vegetation description and syntaxonomy: The communities have closed horizontal structure with a total cover of 100%. In the species composition dominant species is *Falcaria vulgaris* and subdominant is *Elytrigia repens*. In some stands other species with cover up to 25-30% are *Festuca pseudodalmatica, F. dalmatica, Rubus caesius, Poa angustifolia* and *Salvia amplexicaulis*. The number of species is between 12 and 24 (average 17). Frequently the communities of the *Falcario vulgaris-Elytrigietum repentis* association form mosaic with other antropogenic vegetation types of classes *Artemisietea vulgaris*, *Stellarietea mediae* and dry grassland of class *Festuco-Brometea* (alliance *Festucion valesiacae*). Other ruderal species in the species comoposition are *Rumex crispus*, *Lactuca serriola*, *Cichorium intybus*, *Lepidum draba*, *Artemisia absinthium*, *Carduus acanthoides* and *Lathyrus tuberosus*.

The hemicryptophytes (H – 47.7%) prevailed, followed by the therophytes (T – 26.2%), the biannuals (B – 12.3%) and the therophyte-biannuals (T-B – 7.7%). Chamaephytes are presented by 3.1%.The floristic elements dominant in the species composition are Euro-Asiatic (Eur-As – 18.5%), sub-Mediterranean (subMed – 16.9%), Euro-Mediterranean (Eur-Med – 13.9%), Euro-Siberian (Eur-Sib – 9.2%) and Boreal (7.7%).

The syntaxonomical diversity of *Elytrigia repens* communities in Bulgaria is poorly studied. According to Apostolova & Slavova (1997) 7 associations are described following the Dominance approach in the country. The *Falcario vulgaris-Elytrigietum repentis* association is well-know from other European countries (Mucina, 1993; Jarolímek et al. 1997; Láníková, 2013).

Ass. *Convolvulo arvensis-Brometum inermis* Eliáš 1979 (Table 1, cluster 3)

Constant species: *Convolvulus arvensis*

Dominant species: *Bromopsis inermis*

Distribution and ecology: It is found near arable fields, along roads and in proximity of shrub vegetation in different stages of succession. It forms horizontal narrow stripes. Terrains are flat or slightly inclined (up to 5°). So far it is found in the lowlands and the semi-mountainous regions in Western Bulgaria – Sredna Gora Mts, Western Balkan range and Sofia lowland. The altitudional range is 626 and 730 m a.s.l. (average 677 m). The habitats are shiny and during the summer there are long dry periods. The soils are moderately deep, whereas the bedrock type is predominantly silicate.

Vegetation description and syntaxonomy: This vegetation has closed horizontal structure with a total cover of 100%. There is a well-developed herb layer, but in some stands there are also single shrubs (such as *Prunus spinosa*, *Crataegus monogyna*, *Rubus* spp.). This is a species poor community dominated by grasses. The dominant species is *Bromopsis inermis* with a cover of 50-75%, which leads to a strong shade effect. The litter is accumulated above the ground and its cover is up to 70-80%, which increased the shading effect. The number of the species per plot varies between 3 and 24 species (average 14 species). Some xerophytic species of *Festuco-Brometea* class are found in the species composition, such as *Botriochloa ischaemum*, *Achillea collina*, *Poa angustifolia*, *Medicago falcata*, *Eryngium campestre*, *Euphorbia seguieriana* agg., and *Festuca pseudodalmatica*.

The hemicryptophytes (H – 53.9%) prevailed, followed by the therophytes (T – 20%), the therophyte-biannuals (T-B – 7.7%) and the biannuals (B – 6.2%). The chamaephytes are presented by 4.6%. The floristic elements that are dominant in the species composition are Euro-Asiatic (Eur-As – 29.2%), Euro-Mediterranean (Eur-Med – 20%), sub-Mediterranean (subMed – 10.8%), Euro-Siberian (Eur-Sib – 4.7.

The phytocoenoses of *Bromopsis inermis* have not been investigated in Bulgaria so far. *Convolvulo arvensis-Brometum inermis* is a well-known association of the *Artemisietea vulgaris* class in the European vegetation. The ecological preferences and the species composition of our stands is very similar to those given by Láníková (2009) for Czech Republic. In comparison to the Bulgarian stands, the Czeck ones have a higher number of ruderal species. This association is also known for Austria (Mucina, 1993) and Slovakia (Jarolímek et al., 1997).

Ass. *Cardarietum drabae* Tímár 1950 (Table 1, cluster 6)

Constant species: *Convolvulus arvensis, Elytrigia repens, Anisantha sterillis*

Dominant species: *Lepidium draba*

Distribution and ecology: This vegetation type inhabited diverse antropogenic habitats. It was found as stripe communities along roads (incl. such in arable areas), railways, as a mosaic with other ruderal vegetation types in proximity to arable fields. It is widely distributed in the country but in our data set it is presented by 4 relevés and was studied on the territory of the Thracian plain (Chirpan municipality), Western Sredna Gora (Elin Pelin municipality) and the Sofia lowland (Sofia-town municipality). It grows on flat and sunny terrains in the altitudional range 141-921 m a.s.l. (average 667 m). The soils are moderately-deep and the bedrock type is quite diverse.

Vegetation description and syntaxonomy: *Cardarietum drabae* includes monodominant communities of *Lepidium draba* with a cover of 50-75%. Other species with a cover up to 20-25% in some stands are *Anisantha sterillis, Convolvulus arvensis, Elytrigia repens*. The total vegetation cover is 80-100%. The horizontal structure is semi-open to closed. *Lepidium draba* does not have a strong shade effect and thus some annual termophylous plants such as *Holosteum umbellatum, Alyssum alyssoides, Trifolium striatum, Arenaria serpyllifolia* are also found in the species composition. Some diagnostic species for *Stellarietea mediae* class are also distributed, such as *Veronica polita, Lamium amplexicaule, Erodium cicutarium, Cota austriaca, Torilis japonica*.

This vegetation develops as a spring and early summer ruderal vegetation type. After mid-June it is replaced by other ruderal vegetation syntaxa mainly dominated by high herbs (such as *Artemisia vulgaris, Tanacetum vulgare*, etc.) and grasses (such as *Elytrigia repens, Dasypyrum villosum*, etc.).

The therophytes (T – 33.3%) prevailed, following by the hemicryptophytes (H – 29.8%), the therophyte-biannuals (T-B – 17.5%) and the biannuals (B – 8.8%). The chamaephytes are presented by 5.2%. The dominant floristic elements in the species composition are Euro-Asiatic (Eur-As – 29.8%), Euro-Mediterranean (Eur-Med – 19.3%), Euro-Siberian (Eur-Sib – 7%), sub-Mediterranean (subMed) and Boreal with 5.3%.

Mucina (1993), Jarolímek et al. (1997) and Coldea (2012) classified the communities of *Lepidium draba* to the association *Lepidio drabae-Agropyretum repentis* Müller er Görs 1969, which is a synonym of *Cardarietum drabae* Tímár 1950 (Láníková, 2009). Generally its communities in other parts of Europe are similar to those in Bulgaria but more data is required for comparison.

Ass. *Tanaceto vulgaris-Artemisietum vulgaris* Sissingh 1950 (Table 1, cluster 4)

Constant species: *Elytrigia repens, Artemisia vulgaris, Dactylis glomerata, Lactuca serriola, Cichorium intybus*

Dominant species: *Tanacetum vulgare, Artemisia vulgaris*

Distribution and ecology: Occurs in quite diverse habitats – abandoned arable fields and gardens, forestbelts, landfills, along roads and alluvial terrace of rivers, edges of fences and parking slots. From the territory of Bulgaria this community was recorded from the Sofia lowland, West Sredna Gora Mts, Lyulin Mt., the Dolna Banya-Kostenets lowland, the Danubian plain. It is distributed in the altitudional range 151-928 m a.s.l. (average 666 m) and predominantly on flat and sunny terrains. The soils are moderately deep, clay and dry during the summer period.

Vegetation description and syntaxonomy: This is a moderately species-poor community with closed horizontal structure and a total cover of 95-100%. The dominant species are high herbs *Tanacetum vulgare* and *Artemisia vulgaris*. There are 2 well-formed layers - a 1-2 m high herb layer presented by *Tanacetum vulgare, Artemisia vulgaris, Elytrigia repens* and a lower herb layer formed by other herbs (such as *Ballota nigra, Galium aparine, Tragopogon pratensis* agg., *Vicia villosa* agg., etc.) with up to 60-80 cm heigh. A dense litter cover (up to 60-80%) prevents the formation of a cryptogam layer.

The hemicryptophytes (H – 47.2%) prevailed, followed by the therophytes (T – 28.4%), the therophyte-biannuals (T-B – 8.7%) and the biannuals (B – 7.9%). The dominant floristic elements in the species composition are Euro-Asiatic (Eur-As – 22.8%), Cosmopolitan (Kos – 10.3%), Boreal (9.5%) and Euro-Siberian (Eur-Sib – 8.6%).

This association has been reported for the Bulgarian vegetation for the first time by Kolev (1965). Tzonev et al. (2009) classified it to the *Arction lappae* alliance, but we placed it in the *Dauco-Melilotion* alliance following the classification scheme suggested by Mucina et al. (2016). In the species composition we also found 2 variants – with *Artemisia vulgaris* and with *Tanacetum vulgare*, with concides with Láníková (2009) for Czech Republic.

Ass. *Melilotetum albo-officinalis* Sissingh 1950 (Table 1, clusters 5, 11)

Constant species: *Cichorium intybus*, *Plantago lanceolata*, *Elytrigia repens*, *Convolvulus arvensis*, *Carduus acanthoides*, *Anisantha sterillis*

Dominant species: *Melilotus albus*, *Melilotus officinalis*

Distribution and ecology: This vegetation type is presented by 17 relevés and is found on fully sunlit habitats between 115 and 1117 m a.s.l. (average 406 m) and predominantly on flat terrains. Its communities cover areas from 50-60 m^2 up to 200-250 m^2. This vegetation is formed in athropogenic areas – around parkings, arable fields, vineyards, industrial areas, quarries, along roads. It is distubuted in the Thracian plain (Bratya Daskalovi and Chirpan municipalities), the valley of the Struma river (South) (Petrich municipality), the Sofia lowland (Sofia-town and Bozhurishte municipalities), the Znepole region (Dragoman municipality), Sredna Gora Mts (Panagyurishte municipality) and the central Forebalkan (Gabrovo municipality). The soils are shallow to moderately deep but during most of the vegetation season they are dry. In some stands they are are also sandy. The bedrock type is quite diverse.

Vegetation description and syntaxonomy: From an ecological point of view this vegetation was separated in 2 clusters dominated by *Melilotus alba* (cluster 5) and *Melilotus officinalis* (cluster 11). The *Melilotus alba* group is found in xero-mesic habitats, which retain water for a longer period during the raining season. The soils are clay and moderately deep. Plants in the species composition with a cover up to 10-15% are *Dactylis glomerata*, *Dipsacus laciniatus*, *Rumex crispus*, *Lotus corniculatus*. *Melilotus officinalis* prefers warmer habitats with shallow to moderately-deep sandy soils. Most stands are also distributed at a lower altitude (between 115 and 400 m a.s.l.). This thermophilic community is richer of annual species such as *Sorghum halepense*, *Dasypyrum villosum*, *Vulpia ciliata*, *Scolymus hispanicus*, *Setaria viridis*, *Medicago minima*, *Anisantha sterillis*.

This association includes species-rich vegetation (average number of species is 22) and has semi-open to closed horizontal structure with a total cover of 80-100%. Two layers are well-formed in the vertical structure – a high herb layer (1-2 m high) formed by *Melilotus alba* and *M. officinalis* and a lower herb layer (up to 60-80 cm high) with quite diverse species compostion. Some species from neighbouring vegetation types are found in the species composition. In the submontane and mountainous regions species such as *Dactylis glomerata*, *Verbascum longifolium*, *Fagus sylvatica* (low shrub), *Scrophularia canina*, *Plantago major* which come from different mesic vegetation types (*Carpino-Fagetea* and *Molinio-Arrhenatheretea* classes) are also found.

The hemicryptophytes (H – 46.9%) prevailed, followed by the therophytes (T – 28.6%), the therophyte-biannuals (T-B – 10.2%) and the biannuals (B – 8.2%). The dominant floristic elements in the species composition are Euro-Asiatic (Eur-As – 22.5%), Euro-Mediterranean (Eur-Med – 18.4%), European (Eur), subBoreal and Euro-Siberian (Eur-Sib) presented with 7.1%.

Kolev (1965) reported the association *Echio-Melilotetum* Tüxen 1947 for the first time for the territory of Bulgaria. According to Jarolímek et al. (1997) and Láníková (2009) it is a synonym of the ass. *Melilotetum albo-officinalis* Sissingh 1950. For Slovakia the association is presented by 2 subassociations - *brometosum tectori* and *tussilaginetosum farfarae* (Jarolímek et al., 1997), which are not found in our data. In the Czech Republic the association has 3 variants with *Potentilla argentea*, *Carduus acanthoides* and *Agrostis capillaris* (Láníková, 2009).

Ass. *Berteroetum incanae* Sissingh et Tideman ex Sissingh 1950 (Table 1, cluster 9)

Constant species: *Cichorium intybus*, *Plantago lanceolata*, *Convolvulus arvensis*

Dominant species: *Berteroa incana*

Distribution and ecology: This plant community occurs on abandoned fields, near roads, parking slots and railways. It is presented by 9 relevés distributed in the Sofia lowland, the Valley of Struma River (South), Bansko and Kostenets-Dolna Banya lowlands. The habitats are sunny and arid and this vegetation is developed during the spring to early summer period. It is found on flat to moderately inclined terrains (up to 25°). The soils are shallow to moderately-deep.

Vegetation description and syntaxonomy: It has semi-closed to closed horizontal structure with total cover 75-100%. In the vertical structure there is one well-developed herb layer, dominated by *Berteroa incana*, which has cover 50-80%. The number of species in the vegetation plots is between 15 and 46 (an average of 26 species). The species composition includes some diagnostic species for ass. *Melilotetum albo-officinalis* (such as *Melilotus alba*, *Plantago lanceolata* and *Echium vulgare*) and ass. *Carduo acanthoidis-Onopordetum acanthii* (such as *Onopordon acanthium*, *Hordeum murinum* and *Cynoglossum officinale*), which shows floristical similarity between the communities.

The hemicryptophytes (H – 45.4%) prevailed, followed by the therophytes (T – 27.7%), the therophyte-biannuals (T-B – 10.9%) and the biannuals (B – 10.1%). The dominant floristic elements in the species composition were Euro-Asiatic (Eur-As – 18.5%), Euro-Siberian (Eur-Sib – 11.8%), Euro-Mediterranean (Eur-Med – 10.9%), Cosmopolitan (Kos – 10.1) and Boreal (7.6%).

Berteroetum incanae presents termophylic vegetation distributed mainly at a lower altitude and in more open and sunny habitats. In our data set 7 out of all 9 classified relevés are published by Mucina & Kolbek (1989) to association *Centaureo diffusae-Berteroetum* Oberd. 1957 (ord. *Eragrostietalia* Tüxen in Poli 1966). Tzonev et al. (2009) placed it in alliance *Amarantho-Chenopodion* Morariu 1943, which according to Mucina et al. (2016) is a synonym of all. *Eragrostion* Tx. in Oberd. 1954. Based on results from analysis the *Berteroa incana* dominated communities were classified to the class *Artemisietea vulgaris* and the *Berteroetum incanae* association. Its species composition and ecology are very similar to its communities from other parts of Europe (Jarolímek et al., 1997; Láníková, 2009; Coldea, 2012). For the territory of Romania Coldea (2012) also proposes 2 subassociations – *typicum* and *carduetosum thoermeri*.

Ass. *Poo compressae-Tussilaginetum farfarae* Tüxen 1931 (Table 1, cluster 10)

Constant species: -

Dominant species: *Tussilago farfara*

Distribution and ecology: This community is disturbed in ruderal habitats around quarries, embankements, construction sites, road edges and landslides. It is distributed in the mountainous regions of western Bulgaria (West Balkan Range, Lulin Mt., West Sredna Gora Mt. and Viskyar Mt) in the altitudinal range between 517 and 1844 m a.s.l. (average 905 m). It inhabits predominantly moderately-steep and steep terrains (45-80°) with different exposition. The habitats are sunny, eroded, with unstable soil substrate. The soils are shallow to moderately-deep, rich in skeleton materials and rocks.

Vegetation description and syntaxonomy: Moderately species-poor community (average number of species is 12) with open horizontal structure and total cover 60-85%. Only in 2 stands, where soils substrate is stable and terrains are flat or with inclination up to 5° the total cover is 95-100%. The dominant species is *Tussilago farfara*. The species composition is quite diverse. It is formed by infiltration of species from neigbouring habitats and represents pioneer vegetation, often with a very heterogeneous species composition. Other species with a higher cover (10-15%) in some stands are *Epilobium lanceolatum*, *E. dodonaei*, *Plantago subulata*, *Melilotus officinalis*. Some other ruderal species found in the communitites are *Torilis arvensis*, *Dipsacus laciniatus*, *Cephalaria transsylvanica*, *Lactuca serriola*, *Cichorium intybus*, *Cirsium arvense*, *Polygonum rurivagum* agg., *Bromus arvensis*.

The hemicryptophytes (H – 57.8%) prevailed, followed by the therophytes (T – 17.2%), the therophyte-biannuals (T-B – 10.9%) and the biannuals (B – 6.3%). The dominant floristic elements in the species composition are Euro-Asiatic (Eur-As – 20.3%), Boreal (12.5%), Euro-Mediterranean (Eur-Med) and European (Eur) presented by 9.4%.

The phytocoenoses of *Tussilago farfara* have not been investigated in Bulgaria so far. *Poo compressae-Tussilaginetum farfarae* is a well-know association in different European countries – Romania (Coldea, 2012), the Czech Republic (Láníková, 2009), Slovakia (Jarolímek et al., 1997), Austria (Mucina, 1993).

Ass. *Potentillo argenteae-Artemisietum absinthii* Faliṅski 1965 (Table 1, cluster 7).

Constant species: *Lactuca serriola*, *Elytrigia repens*, *Cichorium intybus*, *Rumex crispus*, *Convolvulus arvensis*, *Anisantha sterillis.*

Dominant species: *Artemisia absinthium*

Distribution and ecology: This association is distributed in warmer habitats, abondoned intensive pastures, along roads, the edges of arable fields, close to infrastructure buildings and other urban areas. In the analyzed data set it is presented by 7 relevés from the Sofia region (near Negovan and Kazichane villages), Western Sredna Gora Mts (near Golyama Rakovitsa village), the Western Balkan Range (close to Eleshnitsa and Dragovishtitsa villages). It occurs both on flat and slightly inclined terrains (up to 5°) with varying expositions. The soils are shallow to moderately deep and in some stands they are rich in gravel and skeletons.

Vegetation description and syntaxonomy: It is a moderately species-rich community (the average number of species is 23) with semi-closed to closed horizontal structure and a total vegetation cover of 85-100% (average 94%). The dominant species is *Artemisia absinthium* with a cover of 50-80%. Other high ruderal species included in the phytocenoses are *Carduus acanthoides*, *Lactuca serriola*, *Elytrigia repens*, *Rumex crispus*. Some annual ruderal species also occur such as *Anisantha sterilis*, *Papaver rhoeas*, *Torilis arvensis*, *Polygonum rurivagum* agg., *Arenaria serpyllifolia*, *Petrorhagia prolifera*. There are 2 well-developed herb layers – a tall herb and a ground floor layers.

The hemicryptophytes (H – 39.4%) prevailed, followed by the therophytes (T – 33%), the therophyte-biannuals (T-B – 12.8%) and the biannuals (B – 9.6%). The dominant floristic elements in the species composition are Euro-Asiatic (Eur-As – 22.3%), Euro-Mediterranean (Eur-Med – 13.8%), Euro-Siberian (Eur-Sib – 10.6%) and subBoreal (8.5%).

On the territory of Bulgaria the syntaxonomy of *Artemisia absinthium* phytocoenses has been investigated by Mucina & Kolbek (1989), who established the *Ballota nigra-Artemisia absinthium* plant community, which is classified to the order *Onopordietalia*. Tzonev et al. (2009) placed it in the alliance *Onopordion acanthii* but during done analyses those relevés were not classified to the class *Artemisietea vulgaris*. Dimitrov et al. (2005) also determined *Artemisia absitnhium-Elytrigia repens* (*Elymus repens*) from landfill near Dolni Bogrov village. *Potentillo argenteae-Artemisietum absinthii* is a well-known association of the alliance *Onopordion acanthii* in Europe (Mucina, 1993; Jarolímek et al., 1997; Láníková, 2009; Coldea, 2012).

Ass. *Carduo acanthoidis-Onopordetum acanthi* Soó ex Jarolímek et al. 1997 (Table 1, clusters 8, 13)

Constant species: *Lactuca serriola, Daucus carota, Galium aparine, Elytrigia repens, Convolvulus arvensis, Anisantha sterillis,*

Dominant species: *Carduus acanthoides, Onopordon acanthium*

Distribution and ecology: It is a widely distributed association on the territory of Bulgaria (presented by 40 relevés in the analyzed data set). It is found in the Sofia lowland, the Thracian planin, the Danubian plain, Eastern and Western Sredna Gora Mts, West Balkan Range and Viskyar Mt. This vegetation is found in sunny open habitats such as landfills, around farming houses, etc. It occurs in the altitiudional range 123-851 m a.s.l. (average 468 m) on both flat to slightly inclined with varying expositions terrains (up to 10°). The soils are moderately deep and nutrient rich soils. The bedrock type is rather diverse.

Vegetation description and syntaxonomy: This vegetation is presented by 2 clusters dominated by *Carduus acanthoides* or *Onopordon acanthium*. The species composition is very diverse. In the communities dominated by *Carduus acanthoides* (cluster 8) the average number of species is 23, whereas for the *Onopordon acanthium* (cluster 13) stands the average number of species is 13. The structure of both phytocenoses is predominantly closed with a total cover of 95-100%. The total cover is 70-75% only in 3 stands, which are in different stage of successions. The *Carduus acanthoides* phytocoenoses formed more heterogenous habitats, which included many annual and biennual species. *Onopordon acanthium* with its wider leaves has a stronger shade effect and the accumulation of litter in the stands is substantial, which determines a lower species diversity.

In the vertical structure 2 layers are formed – a high herb layer with ediphicators *Carduus acanthoides* and *Onopordon acanthium* and a low herb layer with quite diverse species composition. Other ruderal species participating in the species composition are *Lactuca serriola, Artemisia vulgaris, Lamium purpureum* agg., *Papaver rhoeas, Elytrigia repens, Erigeron canadensis, Dipsacus laciniatus*.

The therophytes (T – 42.3%) prevailed, followed by the hemicryptophytes (H – 28.6%), the therophyte-biannuals (T-B – 16.3%) and the biannuals (B – 9.2%). The dominant floristic elements in the species composition were Euro-Asiatic (Eur-As – 26.5%), Euro-Mediterranean (Eur-Med – 15.3%), subMediterranean (13.3%) and subBoreal (6.1%).

Carduo acanthoidis-Onopordetum acanthi was reported for the first time for the Bulgarian vegetation by Mucina & Kolbek (1989). Kolev (1965) classified the *Onopordon acanthium* phytocoenoses to the ass. *Onopordetum acanthii* Braun-Blanq. 1936. According to Mucina (1989) this association is tied to the continental deep valleys of the Alps and the Pyrenees and may secondarily occur in other parts of Western Europe, where it is tied to dry valleys in the rain shadow of the mountains. On the other hand *Carduo acanthoidis-Onopordetum acanthi* association has wider distribution in Europe with 2 geographical races – a more mesophilic western race occurring in the northern and western parts of Central Europe and a more drought-loving Eastern European race.

Achillea pannonica - Elytrigia repens plant community (Table 1, cluster 12)

Constant species: *Elytrigia repens, Cichorium intybus, Anisantha sterillis, Melilotus officinalis, Matricaria chamomilla*

Dominant species: *Elytrigia repens*

Distribution and ecology: This community has local distribution on the landfill near Dolni Bogrov village, closely to Sofia. All relevés are digitized from literature (Dimitrov et al., 2005).

Vegetation description and syntaxonomy: It has a semi-open to closed horizontal structure with a total cover of 70-100%. The dominant species is *Elytrigia repens*. It was described by Dimitrov et al. (2005) and it includes 2 subcommunities – a *Hordeum murinum-Poa annua* variant with *Descurainia sophia* and an *Artemisia absinthium-Elytrigia repens* variant with *Dipsacus fullonum*. Some species of other ruderal classes like *Sisymbrietea* are found in the species composition (such as *Crepis tectorum, Artemisia annua, Erodium cicutarium, Descurainia sophia*), *Polygono-Poetea annuae* (such as *Plantago major, Ochlopoa annua*), *Epilobietea angustifoliae* (e.g. *Dipsacus fullonum, Symphytum officinale*), *Digitario sanguinalis-Eragrostietea minoris* (e.g. *Amaranthus deflexus, Cynodon dactylon*). Additionally, some diagnostic species for neighbouring grasslands of classes *Festuco-Brometea* (such as *Inula britannica, Potentilla argentea, Medicago falcata, Eryngium campestre*) and *Molinio-Arrhenatheretea* (such as *Alopecurus pratensis, Poa pratensis, Mentha arvensis, Veronica chamaedrys, Lotus corniculatus*) also occur.

The hemicryptophytes (H – 44.6%), prevailed, followed by the therophytes (T – 32.3%), the biannuals (B – 9.2%) and the therophyte-biannuals (T-B – 6.2%). The dominant floristic elements in the species composition were Euro-Asiatic (Eur-As – 29.2%), Euro-Mediterranean (Eur-Med – 18.5%), Boreal and Cosmopolitan (Kos) presented by 7.7% and Pontic-Mediterranean (Pont-Med – 6.2%).

Vegetation – environment relationships

The studied associations are well separated in the ordination and the ecological space (Fig. 2). The first axis was related to the continentality and temperature conditions. Association *Poo compressae*–*Tussilaginetum farfarae* was found in steep and fully light habitats with high radiation. The soils were eroded and unstable. All the other syntaxa were predominantly distributed on flat or slightly inclined terrains and arid habitats, where the soil radiadion is lower. The variability expressed by the second axis may be associated with the soil conditions – soil depth, moisture and nutrient conditions. The stands of the alliance *Dauco-Melilotion* have semi-closed to closed horizontal structure, where soils are with a high content of gravel and skeleton materials, which are dry and poor of nutirients. This vegetation represents the initial stages of the succession of antropogenic habitats. On the other hand the associations of the alliances *Convolvulo arvensis-Elytrigion repentis* and *Onopordion acanthii* represent more stable antropogenic vegetation types. The soils are rich in nutrients and the soil moisture is higher because of the stronger shade effect and the higher litter accumulation. This determined the lower species richness in comparison to the *Dauco-Melilotion* associations.

Table 1. Synoptic table for class *Artemisietea vulgaris* Lohmeyer et al. in Tx. ex von Rochow 1951 in Bulgaria. In the table Fidelity is given as Phi, whereas Percentage frequency is given as PF. Were used following abbreviations for syntaxa: CE - ass. *Convolvulo arvensis-Elytrigietum repentis*, FE - ass. *Falcario vulgaris-Elytrigietum repentis* , CB - ass. *Convolvulo arvensis-Brometum inermis*, TA - ass. *Tanaceto vulgaris-Artemisietum vulgaris*, MAO - ass. *Melilotetum albo-officinalis*, CD - ass. *Cardarietum drabae*, PA - ass. *Potentillo argenteae-Artemisietum absinthii*, CO - ass. *Carduo acanthoidis-Onopordetum acanthii*, BI - ass. *Berteroetum incanae*, PT - ass. *Poo compressae*–*Tussilaginetum farfarae*, AE - *Achillea pannonica-Elytrigia repens* plant community. Legend: [1] - Diagnostic species for ass. *Potentillo argenteae-Artemisietum absinthii*, ass.*Tanaceto vulgaris-Artemisietum vulgaris*, cl. *Artemisietea vulgaris*; [2]- Diagnostic species for ass. *Tanaceto vulgaris-Artemisietum vulgaris*, all. *Dauco-Melilotion*, cl. *Artemisietea vulgaris*; [3] - Diagnostic species for ass. *Melilotetum albo-officinalis*, ass. T*anaceto vulgaris-Artemisietum vulgaris*, all. *Dauco-Melilotion*, *Onopordion acanthii &*cl. *Artemisietea vulgaris*; [4] - Diagnostic species for ass. *Berteroetum incanae*, ass. *Melilotetum albo-officinalis*, all. *Dauco-Melilotion*, *Onopordion acanthii &* cl. *Artemisietea vulgaris*; [5]- Diagnostic species for all.

Onopordion acanthii; [6] - Diagnostic species for ass. *Potentillo argenteae-Artemisietum absinthii*, all. *Onopordion acanthii* &cl. *Artemisietea vulgaris*; [7]-Diagnostic species for ass. *Carduo acanthoidis-Onopordetum acanthii*, ass. *Potentillo argenteae-Artemisietum absinthii*, ass. *Berteroetum incanae*, all. *Onopordion acanthii* &cl. *Artemisietea vulgaris*; [8] - Diagnostic species for ass. *Carduo acanthoidis-Onopordetum acanthii*, ass. *Melilotetum albo-officinalis*, all. *Dauco-Melilotion* & *Onopordion acanthii*; [9] - Diagnostic species for ass. *Potentillo argenteae-Artemisietum absinthii*, ass. *Carduo acanthoidis-Onopordetum acanthii* & all. *Onopordion acanthii*; 10 - Diagnostic species for ass. *Berteroetum incanae*, ass. *Falcario vulgaris-Elytrigietum repentis*.

Life forms	**Floristic elements**	**Number of cluster**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
		Syntaxa	**CD**	**FE**	**CB**	**TA**	**MAO**	**CD**	**PA**	**CO**	**BI**	**PT**	**MAO**	**AE**	**CO**
		Number of releves	**34**	**6**	**6**	**15**	**6**	**4**	**7**	**17**	**9**	**8**	**12**	**13**	**23**
		Phi/PF	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**	**Phi/ PF**
Diagnostic species for ass. ***Convolvulo arvensis-Elytrigietum repentis***															
H	Boreal	*Elytrigia repens*[1]	22.4^{100}	—^{100}	—^{33}	—^{73}	—^{67}	—^{75}	—^{86}	—^{53}	—^{33}	—	—^{33}	22.4^{100}	—^{57}
Diagnostic species for ass. ***Falcario vulgaris-Elytrigietum repentis***															
B	Eur-As	*Falcaria vulgaris*	—	91.2^{100}	—	—^{7}	—	—	—	—^{12}	—	—	—	—	—
H	Eur-Sib	*Centaurea scabiosa*	—	39.5^{17}	—	—	—	—	—	—	—	—	—	—	—
Diagnostic species for ass. ***Convolvulo arvensis-Brometum inermis***															
H	Eur-As	*Bromopsis inermis*	—	—	100^{100}	—	—	—	—	—	—	—	—	—	—
H	Kos	*Poa angustifolia*	9.3^{32}	—^{50}	34.2^{67}	—^{20}	—	—^{25}	—^{14}	—^{6}	—^{11}	—	—^{25}	—	—^{4}
H	Kos	*Convolvulus arvensis*[10]	17^{79}	—^{17}	—^{83}	—^{27}	—^{50}	—^{100}	—^{57}	—^{53}	—^{56}	—	—^{58}	—	—^{70}
Diagnostic species for ass. ***Tanaceto vulgaris-Artemisietum vulgaris***															
H	Eur-Sib	*Tanacetum vulgare*[2]	—	—	—^{17}	53.2^{67}	—^{17}	—	—	—^{12}	—^{11}	—^{13}	—	—	—
H	subBoreal	*Artemisia vulgaris*[3]	—^{3}	—	—^{17}	29.7^{73}	—^{50}	—	—^{43}	—^{24}	—^{44}	—	—^{17}	32.1^{77}	—^{9}
Diagnostic species for ass. ***Melilotetum albo-officinalis***															
T	subBoreal	*Melilotus albus*[4]	—^{3}	—	—	—^{7}	67.4^{83}	—	—^{14}	—	20.9^{33}	—	—	—	—
T	Eur-As	*Melilotus officinalis*[8]	—^{4}	—	—^{17}	—^{13}	—^{50}	—	—	—^{12}	—	—^{13}	57^{100}	23.9^{54}	—^{4}
H	Kos	*Plantago lanceolata*	—^{12}	—^{17}	—^{33}	—^{27}	—^{67}	—^{50}	—^{14}	—^{24}	18.6^{67}	—	13.6^{58}	34.1^{92}	—^{4}
B	Eur-As	*Echium vulgare*	—	—	—	—^{13}	30.1^{50}	—^{24}	—^{14}	—^{18}	25.5^{44}	—	—^{8}	—^{8}	—
Diagnostic species for ass. ***Cardarietum drabae***															
T-B	Kos	*Capsella bursa-pastoris*	—^{9}	—	—	—^{7}	—^{17}	—^{25}	—^{29}	—^{29}	—^{11}	—	—^{17}	55.4^{100}	—^{25}
H	Eur-Med	*Lepidium draba*	—^{6}	—^{33}	—^{17}	—	—^{33}	50.6^{100}	—^{29}	—^{12}	—^{22}	—	—^{17}	14.5^{46}	—^{4}
H	Boreal	*Bromus hordeaceus*	—^{3}	—	—	—	—	—	35.4^{29}	—	26.2^{22}	—	—	—	—
Diagnostic species for ass. ***Potentillo argenteae-Artemisietum absinthii***															
H	Pont-Med	*Artemisia absinthium*[5]	—^{6}	—^{17}	—	—^{20}	—^{17}	—	61.7^{100}	—^{6}	—^{22}	—	—	21.2^{46}	—
H	Eur-Med	*Ballota nigra*[6]	—	—	—	—^{21}	—^{18}	—	—	—^{7}	30.5^{56}	—	—	53.2^{85}	—^{30}
Diagnostic species for ass. ***Carduo acanthoidis-Onopordetum acanthii***															
T	Eur-Med	*Onopordum acanthium*[9]	—^{6}	—	—	—^{7}	—	—	—	—^{24}	—^{22}	—	—^{17}	—^{38}	65.1^{100}
B	Med	*Carduus acanthoides*[7]	—^{9}	—^{33}	—	—^{20}	—^{17}	—^{25}	—^{29}	46.4^{100}	—	—	19.6^{58}	—^{46}	—^{26}
T	Boreal	*Hordeum murinum*	—	—^{17}	—	—^{8}	—	—	—^{14}	—^{6}	—^{33}	—	—	33.6^{54}	28.6^{48}
B	SPont	*Cynoglossum officinale*	—	—	—	—	—	—	—	—^{7}	39.8^{22}	—	—	—	—
B	Eur	*Verbascum phlomoides*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
Diagnostic species for ass. ***Berteroetum incanae***															
H	SPont	*Berteroa incana*	—^{3}	—	—	—^{20}	—^{17}	—	—	—^{6}	81.3^{100}	—	—	—	—
H	Eur-Sib	*Cichorium intybus*	—^{24}	—^{33}	—	—^{53}	32.8^{100}	—	—^{71}	—^{24}	19.8^{78}	—^{13}	18.2^{75}	23.8^{85}	—^{13}
H	Eur-Sib	*Linaria vulgaris*	—^{15}	—^{17}	—	—^{13}	—^{33}	—^{25}	—^{29}	—^{12}	—^{11}	—^{13}	—^{8}	—	—
Diagnostic species for ass. ***Poo compressae-Tussilaginetum farfarae***															
H	Eur-As	*Tussilago farfara*	—	—	—	—	—^{17}	—	—	—	—^{11}	84.4^{100}	—^{8}	—	—
Diagnostic species for community of ***Achillea pannonica - Elytrigia repens***															
H	Pann-Bal	*Achillea pannonica*	—	—	—	—	—	—	—	—	—	—	—	100^{100}	—
Diagnostic species for alliance ***Dauco-Melilotion***															
H	Eur-subMed	*Rumex acetosella*	—^{6}	—	—	—	—^{17}	—	—	—	—^{11}	—	—	34.3^{31}	—
Diagnostic species for alliance ***Onopordion acanthii***															
T	subMed	*Xeranthemum annuum*	—^{9}	—	—^{17}	—	—	52.1^{50}	—	—^{6}	—	—	—	—	—

Diagnostic species for order *Onopordetalia*, order *Agropyretalia repentis* & class *Artemisietea vulgaris*															
H	Eur-Med	*Centaurea solstitialis*	—^{12}	—	—	—	—^{17}	—^{25}	—^{14}	—^{29}	—	—	—^{33}	59.5^{100}	—^{17}
H	Eur-As	*Marrubium vulgare*	—	—	—	—	—	—	—	—	—	—	—	53.9^{31}	—
H	subMed	*Geranium pyrenaicum*	—	—	—	—	—	48.5^{25}	—	—	—	—	—	—	—
T	SPont	*Torilis ucranica*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
B	Med	*Scolymus hispanicus*	—	—	—	—	—	—	—	—	—	—	39.5^{17}	—	—
H	subMed	*Verbascum densiflorum*	—	—	—	—^{7}	—	—	—	—	39.2^{22}	—	—	—	—
B	Eur-OT	*Dipsacus laciniatus*	—^{3}	—^{17}	—	—^{7}	37.9^{50}	—	—	12.7^{24}	—	—^{25}	—^{8}	—	—
H	subMed	*Salvia verticillata*	—	34.1^{33}	—^{17}	—^{13}	—	—	—	—^{12}	—	—	—	—	—
H	Eur-Sib	*Saponaria officinalis*	—	—	—	—	—	—	—^{14}	—	33.9^{22}	—	—	—	—
H	Eur-Sib	*Achillea millefolium*	—^{18}	—^{50}	—	30.8^{60}	—^{17}	—^{50}	—	—^{35}	—^{11}	—	—	—	—
Diagnostic species for class *Stellaretea mediae*															
T	Kos	*Chenopodium album*	—^{3}	—^{17}	—	—^{20}	—	—	—	—^{29}	—^{25}	—	—	56.6^{85}	—^{17}
T	Eur-As	*Euphorbia helioscopia*	—^{9}	—	—	—	—	—	—^{14}	—^{6}	—	—	—	46.7^{46}	—^{9}
H	Eur-As	*Sonchus arvensis*	—^{3}	—	—	—	—	—	—	—	—	—	39.5^{15}	—	—
T	Eur-Sib	*Silene noctiflora*	—	—	—	—	—	—	36.5^{17}	—	—	—	—	—	—
T	Eur-Med	*Cyanus segetum*	33.1^{12}	—	—	—	—	—	—	—	—	—	—	—	—
T	Eur-subMed	*Adonis aestivalis*	—^{6}	—	—	—	—	—	—	30.9^{18}	—	—	—	—	—^{4}
Diagnostic species for class *Sisymbrietea*															
T	Eur-Sib	*Crepis tectorum*	—	—	—	—	—	—	—	—	—	—	—	72^{54}	—
T	Eur-Med	*Artemisia annua*	—	—	—	—	—	—	—	—	—	—	—	66.5^{46}	—
T	subBoreal	*Erodium cicutarium*	—^{3}	—^{17}	—	—^{7}	—^{17}	—^{50}	—^{14}	—^{6}	—^{22}	—	—^{8}	53.7^{92}	—^{13}
T-B	Eur-As	*Descurainia sophia*	—	—	—	—	—	—	—	—	—	—	—	46.7^{31}	—^{9}
T	Kos	*Chenopodium strictum*	—	—	—	—	—	—	—	—	45.7^{22}	—	—	—	—
T-B	Eur-As	*Malva pusilla*	—	—	—	—	—	—	—	—	45.7^{23}	—	—	—	—
T-B	subMed	*Bromus squarrosus*	—	—	—	—	—	—	42.8^{29}	—	—^{11}	—	—	—	—
T-B	Eur-As	*Sisymbrium orientale*	—	—	—	—	—	—	—	—	37.6^{22}	—	—	—	—^{9}
Diagnostic species for class *Polygono-Poeteae annuae*															
T	Kos	*Ochlopoa annua*	—^{6}	—	—	—	—	—	—	—^{6}	—^{22}	—	—	83.7^{100}	—^{4}
T-B	Eur-As	*Herniaria glabra*	—	—	—	—	—	—	—	—	45.7^{22}	—	—	—	—
B-H	Kos	*Plantago major*	—^{3}	—	—	—^{7}	42.3^{50}	—	—	—^{12}	—^{11}	—	—	22.6^{31}	—
Diagnostic species for class *Epilobietea angustifolii*															
B	Eur-OT	*Dipsacus fullonum*	—^{3}	—	—	—	—	—	—	—	—	—	—	51.2^{31}	—
H	Eur-As	*Symphytum officinale*	—^{4}	—	—	—	—	—	—	—	—	—	—	51.2^{32}	—
T-B	Eur-As	*Conium maculatum*	—^{12}	—	—^{17}	—^{13}	—^{17}	—	—^{14}	—^{6}	—	—	—^{17}	—	40.3^{57}
H	Adv	*Fallopia* x *bohemica*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
T	Eur	*Veronica sublobata*	—	—	—	—	39.5^{18}	—	—	—	—	—	—	—	—
H	Eur	*Euphorbia polychroma*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
Diagnostic species for class *Papaveretea rhoeadis*															
T	Eur-As	*Alopecurus myosuroides*	—^{18}	—	—	—^{7}	—^{17}	52.9^{75}	—	13.9^{29}	—	—	—^{17}	—	—^{9}
T	Eur-Sib	*Papaver rhoeas*	—^{3}	—^{17}	—	—^{13}	—	—	47.8^{71}	—^{24}	—^{11}	—	—^{17}	—	—^{26}
T	Eur-As	*Lamium amplexicaule*	—^{3}	—	—	—	—	45.5^{25}	—	—	—	—	—	—	—
T	Eur-As	*Veronica polita*	—^{3}	—	—	—	—	45.5^{26}	—	—	—	—	—	—	—
T	Eur-Med	*Vicia tetrasperma*	—	—	39.5^{17}	—	—	—	—	—	—	—	—	—	—
T	Eur-Med	*Vicia pannonica*	—	—	—	—	—	—	—	—	—	—	35.7	—	—
T	Eur-Med	*Ranunculus arvensis*	—	—	—	—	—	—	—	33.1^{12}	—	—	—	—	—
T	Eur-Med	Cyanus segetum	33.1^{12}	—	—	—	—	—	—	—	—	—	—	—	—
Diagnostic species for class *Chenopodietea*															
T	subMed	*Cota altissima*	—^{6}	—	—	—	—	—	—^{14}	—^{6}	—^{11}	—	—^{8}	48.9^{54}	—^{4}
T	Eur-Med	*Vicia sativa agg.*	—^{15}	—	—^{17}	—	—	44.7^{50}	—	—^{6}	—	—	—^{17}	—	—
T	subMed	*Dasypyrum villosum*	—	—	—	—	—	—	—^{29}	—	—	—	43^{42}	—	—^{9}
B	subMed	*Echium italicum*	—	—	39.5^{17}	—	—	—	—	—	—	—	—	—	—
T	Med-Cas	*Vulpia ciliata*	—	—	—	—	—	—	—	—	—	—	39.5^{17}	—	—
T	Eur-As	*Vicia peregrina*	—	39.5^{17}	—	—	—	—	—	—	—	—	—	—	—
T	Eur-Med	*Alyssum simplex*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
H	Eur-As	*Rumex pulcher*	—	—	—	—	—	—	—	—	—	—	—	—	34.9^{13}
Diagnostic species for class *Digitario sanguinalis-Eragrostietea minoris*															

T	Adv	*Amaranthus deflexus*	—	—	—	—	—	—	—	—	—	—	—	91.4 ^{85}	—
H	subMed-As	*Sorghum halepense*	—	—	—	—	—	—	—	—	—	—	48.5 ^{25}	—	—
T	Eur-Sib	*Plantago arenaria*	—	—	—	—	—	—	—	—	45.7 ^{22}	—	—	—	—
H	Boreal	*Anisantha tectorum*	—	—	—	—	—	—	—	—	45.7 ^{23}	—	—	—	—
T	Boreal	*Dysphania botrys*	—	—	—	—	—	—	—	—	45.7 ^{24}	—	—	—	—
H	Kos	*Cynodon dactylon*	—	—	—	— ^{7}	— ^{17}	—	— ^{29}	— ^{18}	35 ^{56}	—	— ^{17}	— ^{15}	— ^{22}
T	Boreal	*Setaria viridis*	— ^{3}	—	—	—	— ^{17}	—	—	—	31.1 ^{22}	—	—	—	—
Diagnostic species for class *Festuco-Brometea*															
H	Boreal	*Erigeron acer*	—	—	—	—	—	—	—	—	—	—	—	66.5 ^{46}	—
H	Pont-Med	*Achillea coarctata*	—	—	—	—	—	—	—	—	65.2 ^{44}	—	—	—	—
H	subMed	*Cynoglottis barrelieri*	—	—	—	—	—	—	—	—	65.2 ^{45}	—	—	—	—
T	Eur-Med	*Trifolium striatum*	— ^{9}	—	—	—	—	56.8 ^{50}	—	— ^{12}	—	—	—	—	—
H	Pann-Bal	*Achillea crithmifolia*	—	—	—	—	—	—	—	—	56.2 ^{33}	—	—	—	—
H	Eur-Sib	*Artemisia campestris*	—	—	—	— ^{7}	—	—	—	—	50.6 ^{33}	—	—	—	—
H	subMed-As	*Bothriochloa ischaemum*	—	—	49.4 ^{33}	—	—	—	—	—	—	—	— ^{8}	—	—
B	Eur-Sib	*Chondrilla juncea*	— ^{3}	—	—	—	—	—	— ^{14}	—	46.6 ^{44}	—	— ^{17}	—	—
B	Eur-Med	*Inula britannica*	—	—	—	—	—	—	—	—	—	—	—	46.6 ^{23}	—
B	Pont-subMed	*Silene bupleuroides*	—	—	—	—	—	—	—	—	45.7 ^{22}	—	—	—	—
B	Eur-Med	*Lactuca viminea*	—	—	—	—	—	—	—	—	45.7 ^{22}	—	—	—	—
H	Eur-subMed	*Achillea collina*	— ^{3}	—	44 ^{33}	—	—	—	— ^{14}	—	—	—	—	—	—
H	SPont	*Potentilla argentea*	— ^{12}	—	—	— ^{27}	— ^{17}	— ^{25}	— ^{14}	—	—	—	— ^{8}	42.5 ^{62}	—
H	Eur-Med	*Inula oculus-christi*	—	—	39.5 ^{17}	—	—	—	—	—	—	—	—	—	—
H	subMed	*Anthericum liliago*	—	39.5 ^{17}	—	—	—	—	—	—	—	—	—	—	—
H	Eur-As	*Poa bulbosa*	—	—	—	—	—	—	—	—	37.9 ^{22}	—	— ^{8}	—	—
H	Pont-Med	*Trifolium leucanthum*	— ^{3}	—	—	—	—	—	—	—	—	—	35.9 ^{17}	—	—
B	CSEur	*Erysimum diffusum*	— ^{6}	—	—	—	—	—	—	—	34 ^{22}	—	— ^{8}	—	—
H	Eur-As	*Medicago falcata*	— ^{6}	— ^{17}	32.4 ^{33}	—	— ^{17}	—	—	—	—	—	—	— ^{8}	—
H	Eur-As	*Euphorbia seguieriana agg.*	—	—	32.8 ^{33}	— ^{7}	— ^{18}	—	—	— ^{12}	— ^{11}	—	—	—	—
T	Eur-As	*Medicago minima*	— ^{3}	—	—	—	—	—	31.3 ^{29}	—	—	—	37.6 ^{33}	—	—
H	Pont-Med	*Eryngium campestre*	— ^{18}	—	31.5 ^{50}	—	—	— ^{50}	— ^{14}	— ^{12}	— ^{11}	—	— ^{8}	— ^{9}	—
Diagnostic species for class *Molinio-Arrhenatheretea*															
H	Eur-As	*Mentha arvensis*	— ^{3}	—	—	—	—	—	—	—	—	—	—	75.2 ^{62}	—
H	Eur-As	*Alopecurus pratensis*	— ^{15}	—	—	— ^{5}	— ^{17}	—	—	— ^{6}	—	—	—	70.9 ^{85}	— ^{4}
H	Kos	*Poa pratensis*	— ^{18}	—	—	— ^{27}	— ^{17}	—	—	— ^{6}	—	—	—	65.6 ^{85}	—
B	subMed	*Crepis biennis*	— ^{3}	—	—	—	61.7 ^{50}	—	—	—	—	—	— ^{8}	—	—
H	Eur-As	*Veronica chamaedrys*	— ^{6}	—	—	—	—	—	—	—	—	—	—	48.7 ^{31}	—
H	Boreal	*Rumex crispus*	— ^{21}	— ^{50}	— ^{17}	— ^{20}	42.8 ^{83}	—	24.5 ^{57}	— ^{29}	—	—	— ^{8}	—	—
H	Eur-Med	*Lotus corniculatus*	—	—	— ^{17}	— ^{7}	38.2 ^{67}	— ^{25}	—	— ^{18}	—	25.3 ^{50}	—	16.5 ^{38}	—
T	Eur-Med	*Trifolium dubium*	— ^{3}	—	— ^{17}	—	— ^{17}	—	—	—	35.9 ^{33}	—	—	—	—
H	Eur-As	*Dactylis glomerata*	— ^{24}	— ^{33}	— ^{17}	19.8 ^{60}	34.7 ^{83}	—	— ^{43}	— ^{24}	— ^{33}	— ^{38}	— ^{8}	—	— ^{13}
H	Kos	*Prunella vulgaris*	33.1 ^{12}	—	—	—	—	—	—	—	—	—	—	—	—
Diagnostic species for class *Trifolio-Geranietea sanguinei*															
H	Eur-Sib	*Astragalus cicer*	—	39.5 ^{17}	—	—	—	—	—	—	—	—	—	—	—
H	Med-OT	*Silene coronaria*	—	—	—	35.3 ^{13}	—	—	—	—	—	—	—	—	—
Diagnostic species for class *Crataego-Prunetea*															
Ch-P	Eur-As	*Prunus cerasifera*	—	—	— ^{17}	— ^{7}	—	—	—	—	—	—	— ^{8}	42.3 ^{38}	—
P	subMed	*Pyrus communis* subsp. *pyraster*	—	—	—	—	—	—	—	—	—	—	39.5 ^{17}	—	—
Other species with Phi>30															
B-H	Bal-Anat	*Tripleurospermum tenuifolium*	—	—	—	—	—	—	—	—	—	—	—	100 ^{100}	—
T	Eur-As	*Holosteum umbellatum*	—	—	—	—	—	69.3 ^{50}	—	—	—	—	—	—	—
T-B	Eur-Med	*Alyssum alyssoides*	—	—	—	—	—	69.3 ^{50}	—	—	—	—	—	—	—
B	subMed	*Centaurea stoebe*	— ^{3}	—	—	— ^{7}	—	—	—	— ^{6}	57.8 ^{56}	—	—	—	—
T	Kos	*Polygonum arenastrum*	—	—	—	—	—	—	—	—	56.2 ^{33}	—	—	—	—

H	Eur-Sib	*Cota tinctoria*	—	—	—	—	—	—	—	—	—^{11}	—^{13}	—	52.2^{46}	—
H	Pont-Med	*Bituminaria bituminosa*	—	—	—	—	—	—	—	—	—	—	48.5^{25}	—	—
T	Eur-As	*Persicaria hydropiper*	—	—	—	—	—	—	—	—	—	48.5^{25}	—	—	—
Ch	Carp-Bal	*Syringa vulgaris*	—	—	—	—	—	48.5^{25}	—	—	—	—	—	—	—
T	Pont-Med	*Petrorhagia prolifera*	—^{3}	—	—	—	—	—	47^{43}	—	—^{11}	—	—	—^{15}	—
H	Eur-Med	*Scrophularia canina*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
T	Eur-Med	*Senecio leucanthemifolius subsp. vernalis*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
T	Eur-Med-CAs	*Draba verna*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
H	Eur-Med	*Verbascum longifolium*	—	—	—	—	39.5^{17}	—	—	—	—	—	—	—	—
P	Eur	*Fagus sylvatica*	—	—	—	—	39.5^{18}	—	—	—	—	—	—	—	—
P	Adv	*Robinia pseudoacacia*	—	—	—	—^{7}	—	—	—	—	39.2^{22}	—	—	—	—
H	Pont	*Festuca valesiaca*	—	—	—	—	—	39^{25}	—	—	—	—	—	—	—
H	subBoreal	*Lythrum salicaria*	—	—	—	—	—	—	36.5^{17}	—	—	—	—	—	—
H	Eur	*Anchusa hybrida*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
H	Eur-Med	*Rumex sanguineus*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
H	Eur-Med	*Scleranthus perennis*	—	—	—	—	—	—	36.5^{15}	—	—	—	—	—	—
H	Eur-Sib	*Vincetoxicum hirundinaria*	—	—	—	—	—	—	36.5^{14}	—	—	—	—	—	—
T-B	Med	*Cirsium ligulare*	—^{3}	—	—	—^{13}	—	—	—	—	—	35.9^{25}	—	—	—
T	Eur-Med	*Vicia hirsuta*	—^{12}	—	34.7^{50}	—^{7}	—	—^{25}	—^{29}	—^{12}	—	—	—	—^{17}	—
T-B	subMed	*Vicia grandiflora*	—^{6}	33.7^{50}	—^{17}	—^{20}	—	—^{25}	—^{14}	—^{12}	—	—	—	—	—^{13}

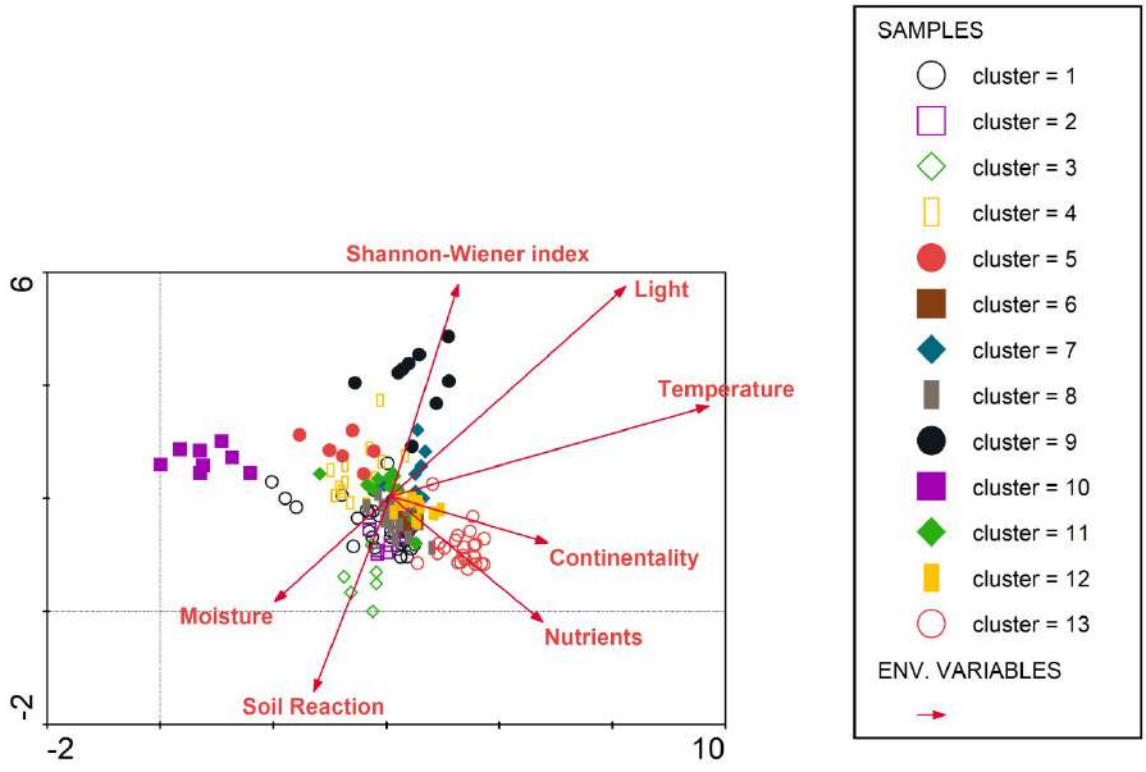

Fig. 2. Ordination diagram of the analyzed data set along the first two axis.

Discussion

Despite the wide distribution of the communities of the *Artemisietea vulgaris* class in the country, studies, focusing on this vegetation are restricted to the western and the central parts of Southern Bulgaria that fall within the temperate and transitional climate zones. The current study focused on 10 associations that are widely distributed. Their ecology and floristic composition are similar to those of the associations that are distributed in the Southeastern and the Central Europe e.g. Romania (Coldea, 2012), the Czech Republic (Láníková, 2009), Slovakia (Jarolímek et al., 1997), Austria (Mucina, 1993), etc.). The phytocoenoses of the *Artemisietea vulgaris* class are located in termophylic and sunny habitats and different stages of anthropogenic pressure, stabilization of substrates and species' composition diversity may be observed. The low-growing vegetation of *Tussilago farfara* is presented by *Poo compressae–Tussilaginetum farfarae.* The association includes early successional communities, typical of habitats with recently disturbed soils (Láníková, 2009). On the contrary *Onopordion acanthii* represents an ancient ruderal vegetation, which has probably been common since the Neolithic age and consists of many archeophytes. The species' composition of the *Potentillo argenteae-Artemisietum absinthii* and *Carduo acanthoidis-Onopordetum acanthi* in Bulgaria includes more perennial species than the phytocoenoses in the Central Europe (Láníková, 2009). On the other hand the alliance *Convolvulo arvensis-Elytrigion repentis* includes semi-natural and ruderal communities, whose species composition includes many typical species for neighbouring grassland vegetation types of classes *Festuco-Brometea*, *Molinio-Arrhenatheretea* and *Trifolio-Geranietea*.

Studies of the class in Bulgaria need to continue. There are some other associations, belonging to the *Artemisietea vulgaris* class that we expect to be found in the country: *Carduetum hamulosi* F. Diaconescu 1978, *Artemisietum scopariae* Borza et Lupşa 1963, *Dauco-Cephalarietum transsylvanicae* M. Coroi et A.-M. Coroi 1998, *Bromo japonico-Aristolochietum* Ubrizsy 1967, *Dauco-Salvietum verticillatae* Soran 1962, *Centauretum calcitrapae* Mititelu ex Mititelu et Barabas 1975, which are well-known from Romania (Sanda et al., 2008; Coldea, 2012). The communities of *Cephalaria transsylvanica*, *Bromus japonica*, *Aristolochia clematitis*, *Salvia verticillata* and *Daucus carota* should also be studied. They are located within the vegetation stripes in agricultural areas. They also occur as a successional phase (in the second or third year) of the development of the ruderal vegetation in abandoned agricultural lands. These phytocoenoses are widely distributed but poorly studied.

The ruderal vegetation in the sub-Mediterranean zone of Bulgaria forms transitional phytocoenoses of the classes of *Artemisietea vulgaris* and *Stellarietea mediae* s.l. that are also poorly researched. The presence of other phytocoenoses from the order *Carthametalia lanati* S. Brullo in S. Brullo et Marcenò 1985 is also possible. The future regional analyses of this vegetation class should incorporate data of regional and national databases (Balkan Vegetation Database, Romanian Grassland Database, etc.), as well as the information from the European Vegetation Archive (Chytrý et al. 2016). It is expected that this is the right way to uncover the full syntaxonomical diversity of the class on Balkan peninsula and South-East Europe.

Conclusions

The current study evaluated the syntaxonomical diverisity of the *Artemisietea vulgaris* class on the territoriy of Bulgaria. One class (*Artemisietea vulgaris*), two orders (*Agropyretalia intermedio-repentis, Onopordetalia acanthii*), three alliances (*Convolvulo arvensis-Elytrigion repentis, Dauco-Melilotion* and *Onopordion acanthii*), ten associations (*Convolvulo arvensis-Elytrigietum repentis, Falcario vulgaris-Elytrigietum repentis, Convolvulo arvensis-Brometum inermis, Cardarietum drabae, Tanaceto vulgaris-Artemisietum vulgaris, Melilotetum albo-officinalis, Berteroetum incanae, Poo compressae-Tussilaginetum farfarae, Potentillo argenteae-Artemisietum absinthii* and *Carduo acanthoidis-Onopordetum acanthi*) and 1 plant community (*Achillea pannonica-Elytrigia repens*) were found. One order, 2 alliances and 9 associations are found for the first time in Bulgaria. The studied associations are well floristically and ecologically separated. Only the association *Poo compressae–Tussilaginetum farfarae* is found in steep, eroded and unstable habitats. All the other syntaxa are predominantly distributed on flat or slightly inclined terrains. The stands of the alliance *Dauco-Melilotion* represent the initial stages of the succession of antropogenic habitats and has a higher species diversity. On the other hand the associations of the alliances *Convolvulo arvensis-Elytrigion repentis* and *Onopordion acanthii* represent more stable antropogenic vegetation types. The soils are rich of nutrients and the soils moisture is higher because of the stronger shade effect and the higher litter accumulation, which leads to a lower species richness. The species composition and the ecology of the established syntaxa are similar to same communities from other parts of Europe.

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The following types of manuscripts are accepted: *short research notes* (up to 4 pages), *research articles* (4 to 10 pages) and *review papers* (10 to 20 pages). *Short research notes* are shorter submissions of a preliminary nature or those including new records or observed phenomenon, etc. *Research articles* should present significant original research in the various fields of ecology, mentioned above. *Review papers* should deal with topics of general interest or of contemporary importance, being synthetic rather than comprehensive in emphasis. Authors of review papers should consult with the Editor before submission. The Editor may also invite review articles concerning recent developments in particular areas of interest. The Editor reserves the right to decide if a manuscript should be treated as a short note or research article. In general, studies that are purely descriptive, mathematical, documentary, and/or natural history will not be considered for publication.

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