ECOLOGIA BALKANICA

International Scientific Research Journal of Ecology

Volume 14, Issue 2
December 2022

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

UNIVERSITY OF PLOVDIV PUBLISHING HOUSE

International Standard Serial Number
Online ISSN 1313-9940; Print ISSN 1314-0213 (from 2009-2015)

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Published by the Union of Scientists in Bulgaria – Plovdiv and the University of Plovdiv Publishing house – twice a year. Language: English.

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- Scopus (SJR_{2021} = 0.137; $SiteScore_{2021}$ = 0.4; $SNIP_{2021}$ = 0.241)
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- Thomson Reuters Master Journal List - Zoological Record (Web Of Science)
- Thomson Reuters BIOSIS Reviews (Web Of Science)
- Thomson Reuters Biological Abstracts (Web Of Science)
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- EBSCO
- CAB International – CABI Abstracts
- Elsevier (GEOBASE)
- Ulrich's Web (ProQuest)
- Google Scholar Search Engine and many others.

Contact Publisher

Union of Scientists in Bulgaria – Plovdiv
6 Metropolit Paisii Str., 4000 Plovdiv, BULGARIA
E-mail: ecologia_balkanica@abv.bg

University of Plovdiv Publishing House
24 Tsar Assen Str., 4000 Plovdiv, BULGARIA

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Macromycetes Diversity of Sharr Mountains in Kosovo

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Abstract. Our study in the Sharr Mountains of Kosovo began in 2017 and included 20 surveyed sites. The predominant vegetation consists of beech and oak forests, but also a forest of *Pinus heldreichii*, the Balkan endemic pine species. A total of 244 macromycete species were recorded, of which 211 belong to the *Basidiomycota* phylum and 33 to the *Ascomycota* phylum. Newly recorded in Kosovo are 95 species. Several species, such as *Zeus olympuis, Antrodia ramentacea, Heterosphaeria patella, Kneiffiella abdita* and *Resupinatus striatulus*, are among the rarely recorded ones.

Key words: macrofungi, ecology, National Park Sharr, Former Yugoslavia.

Introduction

Kosovo is located in southeastern Europe, covering the central part of the Balkan Peninsula and has an area of 10,887 km². It borders Albania to the southwest, Montenegro to the northwest, Serbia to the northeast and North Macedonia to the south. The Sharr Mts. in Kosovo covers an area of 53,469 ha, starting in the eastern part of the state border of the Republic of Kosovo with the Republic of North Macedonia and continuing to the west and southwest along the state border, through natural grasslands and various natural habitats, respectively, passing through the highest peaks of the Sharr Mts. chain in Kosovo (e.g. Bistra 2604 m a.s.l., Rudoka 2658 m a.s.l.), up to the border point of the three countries, Kosovo, North Macedonia and Albania (Anonymous, 2012). From biogeographical point of view, the Sharr Mts. belongs to the biogeographical regions of the Mediterranean, Central, and Boreal Europe, as well as the mountainous biogeographical region of Central-Southern Europe (Stevanović et al., 1994). The Sharr Mts. has alpine and continental climate influenced by the mid-continental climate, which makes it one of the most important and weighty centers of biodiversity not only for Kosovo, but also for the entire Balkan Peninsula, in terms of taxonomic richness of plants and diversity of natural habitats (Micevski & Matevski, 1987; Stevanović et al., 2007; Millaku et al., 2013; Anonymous, 2014; Tomović et al., 2014; Berisha et al., 2020).

The mycobiota of Kosovo has been poor studied, but is currently being enriched, especially with regards to the diversity of macromycetes. An important study by Tortić and Sylejmani (1982) provides the first data on the diversity of macromycetes in Kosovo, including 32 species, mainly from Bosnian pine (*Pinus heldreichii* H. Christ.) forests in the Sharr Mts. (specifically in Mt. Oshlak). Further data on macromycete diversity (83 species) are reported by Veselaj (2000) from Gërmia Park. In 2017-2018, with the support of Rufford Foundation, a comprehensive and detailed survey of macromycete diversity in Kosovo was conducted as part of the project "Fungi of Kosovo": Preparation of a Fungal Checklist and a Preliminary Red List and a Proposal for Important Fungal Areas". With a survey in oak forests, including results on the diversity of the two main taxonomic divisions of fungi, *Ascomycota* and *Basidiomycota*, the knowledge of fungal diversity developed and contributed significantly to the Mycobiota of Kosovo (Ramshaj et al., 2021).

Given the unique importance of biodiversity and the rare bioclimatic combinations with landscape mosaics, the Sharr Mountains undoubtedly represent an exceptional natural mountain range for the study and documentation of macromycete biodiversity in these particular ecosystems. Although other biological groups of the Sharr Mountains have been well documented (Krasniqi, 1972; Rexhepi, 1976; 1982; 1997; 2007; Millaku et al., 2013; Tomter et al., 2013; Ibrahimi et al., 2019), macrofungal communities have been so far overlooked. Although this knowledge gap has been repeatedly highlighted by several NGOs and government agencies, there was an immediate need to conduct a survey and compile a macrofungal checklist for the Sharr Mts. On this basis, this study is the first attempt to define and describe the macrofungal communities in the Sharr Mts. and to establish the first checklist of macrofungal species.

Matrial and Methods

Study area. Field research in different regions of Sharr Mts. began in March 2017 and ended in November 2021 (with only one species recorded in January 2022). Fungal diversity was recorded at 22 sites in the Sharr Mts. of Kosovo, covering several districts. In the district of Prizren 10 sites were surveyed, four in Shtërpca, three in Dragash, two each in Kaçanik and Suharekë districts and one in Elez Han. The mentioned sites are shown on the map in Fig. 1 and in Table 1, including complete information about their coordinates, elevations, and habitat types.

Collection and identification of samples. During the field study, we took macroscopic images of the samples in their original state before removing them from the substrate. To do this, we used a camera such as the Canon EOS 1000D, the Sony alpha A58, the iPhone 6S, and more recently the iPhone 11, as well as recording GPS coordinates. Some fungi were immediately described in the field in fresh condition, and then examined in the Mycological Laboratory of Skopje (Institute of Biology of the Faculty of Natural Sciences and Mathematics), using a light microscope (LW Scientific i4, in magnification up to 1000×) for microscopic characteristics of the spores, such as size, shape and color, as well as other essential morphological elements. Melzer's reagent and KOH 5%, were employed for visualisation and certain chemical reactions. For identification of the collected fungi, identification keys were used, by the following sources: Eriksson et al. (1978), Jülich (1984), Breitenbach & Kränzlin (1986), Antonín et Noordeloos (1993), Noordeloos et al. (2001; 2005), Galli (2001; 2007), Knudsen & Vesterholt (2012). The nomenclatural database Index Fungorum (http://indexfungorum.org/) was used to select the most appropriate names for taxa. The species are arranged alphabetically in the list below and the studied specimens are deposited in the first author's own herbarium Ramshaj Qendrim Private Collection (RQPC). All the fungi samples were collected and prepared by Qëndrim Ramshaj, Katerina Rusevska and Slavica

Tofilovska. While the final taxonomic identification has been done by Mitko Karadalev.

Abbreviations: *Fagus* (beech forests), *P. hel*. (*Pinus heldreichii* forest), meadows, *Q. c.* (*Quercus cerris* forest), rip. (riparian vegetation), pastures (mountain pastures), *P. peuce, Ab.-Fag.* (mixed forest of *Pinus peuce, Abies alba* and *Fagus sylvatica*), *Q. f-c* (ass. *Quercetum frainetto-cerris*).

Results

During the study period in the Sharr Mountains' ecosystems, 244 species (151 genera) were identified from the collected samples, representing 77 families (18 families of *Ascomycota* phyllum and 59 families to the *Basidiomycota* phyllum). The majority of taxa belongs to the *Basidiomycota* – 125 genera (211 species), while 26 genera (33 species) belong to *Ascomycota*. Considering trophic groups, mycorrhizal species are 65 species, 169 saprotrophic species and 10 parasitic species were identified. In the Fig. 2 are presented four saprobic species of *Ascomycota*, six mycorrhizal species of *Basidiomycota* in the Fig. 3, in the Fig. 4 are three parasitic species of *Basidiomycota* and in the Fig. 5, two rare species (*Zeus olympius* - *Ascomycota* and *Resupinatus striatulus* - *Basidiomycota* phylum).

List of Species

The list includes a brief description of the ecology for each species, as well as reported localities assigned to specific districts and documents them with additional data such as: forest association, habitat or substrate characteristics, geographic coordinates, elevation, date of collection, names of collectors, and the voucher number of the specimen (currently in the first author´s personal collection). Asterisk is used to note the newly recorded fungal species on the Sharr Mts.

Table 1. The number of taxa recorded at each locality and the characteristics of sampling sites.

No.	Locality (General)	Place of collecting	No. of taxa	Latitude	Longitute	Altitude (m)	Forest Association
1(10)	Prizren	Prevalla	28	42.189250	20.979861	1300	*Fagus*
		Prevalla	30	42.171111	20.967778	1150	*Fagus*
		Prevalla (Mt. Oshlak)	35	42.184833	20.953375	1600	*P. hel.*
		Sredskë	24	42.169861	20.849167	700	rip.
		Kopana Voda	2	42.208611	20.833333	1560	*Fagus*
		Qadrak	4	42.164444	20.774722	1230	*Fagus*
		Novosellë	1	42.213000	20.785472	1125	*Fagus*
		Novosellë	1	42.209722	20.782778	1110	meadows
		Jabllanicë	5	42.166944	20.776944	1100	*Fagus*
		Jabllanicë	2	42.165722	20.762944	1280	*Fagus*
2 (4)	Shtërpcë	Shtërpcë	16	42.223611	21.050833	900	*Q.c.*
		Brezovicë	11	42.179969	21.029669	1687	*Fagus*
		Brod	31	42.265278	21.108611	700	*Fagus*
		Firajë	12	42.259444	21.086111	730	rip.
3 (3)	Dragash	Krushevcë	18	41.998333	20.615556	1150	*Fagus*
		Rrenc	7	42.086800	20.660972	1054	pastures
		Kuk	5	42.096750	20.713028	1100	meadows
		Mushtisht	22	42.263889	20.905278	1370	*P. peuce, Ab.-*

		(Gradanc)					*Fag.*
4 (2)	Suharekë	Dolloc	1	42.288611	20.916472	824	meadows
5 (2)	Kaçanik	Glloboçicë	56	42.153889	21.200406	900	*Q. f-c.*
		Vata - Dubravë	8	42.253333	21.172500	780	*Fagus*
6 (1)	Elez Han	Krivenik	29	42.123333	21.260278	700	*R. f-c.*

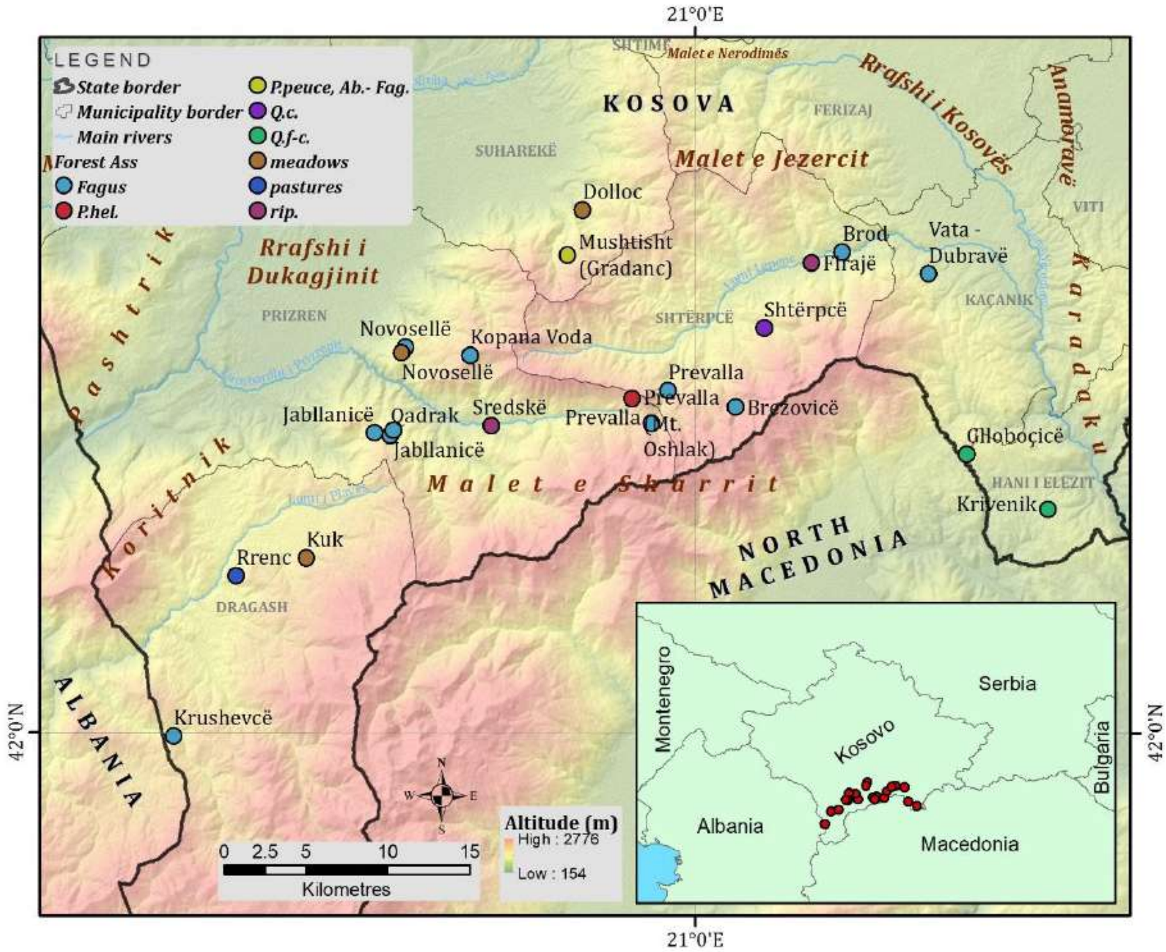

Fig. 1. Maps with localities of fungal species in the Sharr Mountains of Kosovo.

Ascomycota

**Ascocoryne sarcoides* (Jacq.) J.W. Groves & D.E. Wilson (*Gelatinodiscaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/124), beech forest, fallen branch and leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

Bertia moriformis (Tode) De Not. (*Bertiaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Calycina citrina* (Hedw.) Gray (*Pezizellaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK, MP, det. MK with GK. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/132), beech forest, on dry branch, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

**Cudoniella clavus* (Alb. & Schwein.) Dennis (*Tricladiaceae*)

Prizren: Prevalla, N42 N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, MK, MTh, KR et TJ, det. MK et KR.

**Diatrype disciformis* (Hoffm.) Fr. (*Diatrypaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/192), beech forest mixed with tree of *Pinus*, on dry branch of *Fagus sylvatica*, 10 Oct 2019, leg. QR, det. MK et QR. – Opojë: Kuk, N42°05'48.3", E20°42'46.9", 1100 m, RQPC (2021/459), *Populus* forest, on dry branch, 29 Apr 2021, QR et AM, det. MK et QR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

Diatrype stigma (Hoffm.) Fr. (*Diatrypaceae*)

Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on dry branch of *Fagus sylvatica*, 25 Jul 2021, leg. QR, det. MK, ST et QR. – Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Eutypella alnifraga* (Wahlenb.) Sacc. (*Diatrypaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Helvella lacunosa* Afzel. (*Helvellaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

**Heterosphaeria patella* (Tode) Grev. (*Heterosphaeriaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on herbaceous twigs, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Humaria hemisphaerica* (F.H. Wigg.) Fuckel (*Pyronemataceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/226), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. QR et MK.

**Hymenoscyphus syringicolor* Svrček (*Helotiaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on rotten leaf of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Hypomyces hyalinus (Schwein.) Tul. et C. Tul. (*Hypocreaceae*)

Kaçanik: Glloboçicë, 900 m, *Quercus pubescens* forest, on fruit body of *Amanita vaginata*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Hypomyces lateritius* (Fr.) Tul. (*Hypocreaceae*)

Elez Han:Krivenik, N42°07'24", E21°15'37", 700 m, *Pinus nigra* plantings, on fruit body of *Lactarius deliciosus*, 5 Jun 2018, MK, KR et TJ, det. MK et KR.

**Hypoxylon deustum* (Hoffm.) Grev. (*Hypoxylaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce*, *Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

**Hypoxylon fragiforme* (Pers.) J. Kickx f. (*Hypoxylaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on dry branch of *Fagus sylvatica*, 2 Jul 2021, leg. QR, det. MK, ST et QR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch of *Fagus sylvatica*, 24 Jul 2021, leg.

QR, det. MK ST et QR. – Dragash: Krushevc, N41°59'54", E20°36'56", 1150, beech forest, on dry branches of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Hypoxylon fuscum (Pers.) Fr. (*Hypoxylaceae*)

Prizren: Kopana Voda, N42°12'31", E20°49'60", 1560 m, RQPC (2020/457), beech forest, on dry branch of *Fagus sylvatica*, 16 Nov 2020, leg. QR, det. MK, ST et QR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Corylus avellana*, 9 Oct 2017, leg. MK GK et MP, MK et GK. – Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Hypoxylon rubiginosum* (Pers.) Fr. (*Hypoxylaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR. det. MK et KR.

**Jackrogersella multiformis* (Fr.) L. Wendt, Kuhnert & M. Stadler (*Hypoxylaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on dry branches of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR. det. MK et KR.

**Kretzschmaria deusta* (Hoffm.) P.M.D. Martin (*Xylariaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg. MK MTh, KR et TJ, det. MK et KR. – Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on trunk of *Carpinus betulus*, 31 May 2017, leg. MK, EM et KR, det. MK et KR.

**Mollisia cinerea* (Batsch) P. Karst. (*Mollisiaceae*)

Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on rotten wood of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

**Nemania serpens* (Pers.) Gray (*Xylariaceae*)

Prizren: Jabllanicë, N42°09'56.6", E20°45'46.6", 1280 m, RQPC (2020/455), beech forest, on dry branch of *Cornus mas*, 16 Nov 2020, leg. QR, det. MK et QR. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Peziza arvernensis Roze et Boud. (*Pezizaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil surface and decaying wood, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Rhytisma acerinum* (Pers.) Fr. (*Rhytismataceae*)

Opojë: Kuk, N42°05'48.3", E20°42'46.9", 1100 m, RQPC (2021/461), *Populus* forest, on leaves of *Acer pseudoplatanus*, 29 Apr 2021, leg. QR et AM, det. MK et QR.

Sarcoscypha coccinea (Gray) Boud. (*Sarcoscyphaceae*)

Shtërpcë, N42°13'25", E21°03'03", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on living tree of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Tapesia fusca* Fuckel (*Mollisiaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, rotten wood of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Trichoderma viride* Pers. (*Hypocreaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Ustulina deusta* (Hoffm.) Maire (*Xylariaceae*)

Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on cut trunk of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

**Verpa bohemica* (Krombh.) J. Schröt. (*Morchellaceae*)

Opojë: Kuk, N42°05'48.3", E20°42'46.9", 1100 m, RQPC (2021/462), *Populus* forest, on soil, under *Populus* sp., leaf litter, 29 Apr 2021, leg. QR et AM, det. MK et QR.

**Xanthoria parietina* (L.) Th. Fr. (*Teloschistaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on branch of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Xylaria carpophila* (Pers.) Fr. (*Xylariaceae*)

Prizren: Prevalla, N42°11'21.3", 20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Xylaria hypoxylon* (L.) Grev. (*Xylariaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, beech forest, on rotten wood, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on leaf litter and rotten wood of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren: Qadrak, N42°09'52.0", E20°46'29.0", 1230 m, RQPC (2020/454), beech forest, on soil, rotten wood, 20 Nov 2020, leg. QR, det. MK, ST et QR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Xylaria polymorpha* (Pers.) Grev. (*Xylariaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK, MP, det. MK et GK.

**Zeus olympius* Minter & Diam. (*Rhytismataceae*)

Prizren: Prevalla(Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on dry branches of living *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Basidiomycota

Agaricus campestris L. (*Agaricaceae*)

Prizren: Novosellë, N42°12'35.0", E20°46'58.0", 1110 m, meadow, on soil, leaf litter, 22 Nov 2020, leg. QR, det. MK, ST et QR. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on soil, leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Amanita caesarea (Scop.) Pers. (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita dryophila Consiglio et Contu (*Amanitaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita eliae Quél. (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita franchetii (Boud.) Fayod (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita lividopallescens (Gillet) Bigeard et H. Guill. (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Amanita muscaria* (L.) Lam. (*Amanitaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/115), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

Amanita pantherina (DC.) Krombh. (*Amanitaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–*

cerris, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita rubescens Pers. (*Amanitaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita subnudipes (Romagn.) Tulloss (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Amanita vaginata (Bull.) Lam. (*Amanitaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Amphinema byssoides* (Pers.) J. Erikss. (*Atheliaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus heldreichii*, 1 Jan 2018, leg. MK, KR et TJ, det. MK et KR.

**Antrodia ramentacea* (Berk. & Broome) Donk (*Fomitopsidaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on trunk of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Apioperdon pyriforme (Schaeff.) Vizzini (*Lycoperdaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg. MK MTh, KR et TJ, det. MK et KR.

Armillaria mellea (Vahl) P. Kumm. (*Physalacriaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on stump of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det MK et GK. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce*, *Abies alba* meixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

**Athelia neuhoffii* (Bres.) Donk (*Atheliaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on rotten wood, 21 Apr 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dry branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Auricularia auricula-judae (Bull.) Quél (*Auriculariaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dead tree of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Auricularia mesenterica (Dicks.) Pers. (*Auriculariaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), dead branch of *Salix alba*, 21 Apr 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dry dead tree of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Bjerkandera adusta (Willd.) P. Karst. (*Phanerochaetaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, KR et EM, det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK GK et MP, det. MK et GK. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, *Alnetum*, close to the river, on stump of *Fagus sylvatica*, 28 Apr 2018, leg MK. MTh, KR et TJ, det. MK et KR. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/135), beech forest, on dry branch of *Fagus sylvatica*, 10 Oct 2019, leg. QR, det. MK et QR. – Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on dry branch of *Fagus sylvatica*, 2 Jul 2021, leg. QR, det. MK, ST et QR.

Boletus aereus Bull. (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Boletus reticulatus Schaeff. (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Botryobasidium vagum* (Berk. & M.A. Curtis) D.P. Rogers (*Botryobasidiaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Bovista nigrescens* Pers. (*Lycoperdaceae*)

Prizren: Prevalla, N42°09'54.0", E20°57'09.0", 1948 m, RQPC (2019/140), mountain pasture, on soil, 6 Oct 2019, leg. et det. MK et KR.

Bovista plumbea Pers. (*Lycoperdaceae*)

Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on soil with dung of animals, 25 Jul 2021, leg. QR, det. QR, ST et MK. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Bovistella utriformis* (Bull.) Demoulin & Rebriev (*Lycoperdaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on trunk of *Fagus sylvatica*, 21 Mar 2017, leg. et det. MK. – Prizren: Prevalla, N42°09'54.0", E20°57'09.0", 1948 m, mountain pasture, on soil, 6 Oct 2019, leg. et det. MK et KR.

Byssomerulius corium (Pers.) Parmasto (*Irpicaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10"E, 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead branch of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer (*Psathyrellaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, meadow, on soil, 9 Oct 2017, leg. MK. GK et MP, det. MK et GK.

Cantharellus cibarius Fr. (*Hydnaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Cerioporus leptocephalus* (Jacq.) Zmitr. (*Polyporaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Cerioporus varius (Pers.) Zmitr. & Kovalenko (*Polyporaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK MTh, KR et TJ, det. MK et KR. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Cerrena unicolor (Bull.) Murrill (*Polyporaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on stump of *Betula pendula*, 31 May 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/157), beech forest, dry branch of *Fagus sylvatica*, 10 Oct 2019, leg. QR, det. MK et QR.

**Chlorophyllum rhacodes* (Vittad.) Vellinga (*Agaricaceae*)

Prizren, Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on soil, leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Clavulina rugosa* (Bull.) J. Schröt. (*Hydnaceae*)

Dragash, N42°04'28.9", E20°38'21.8", 960 m, *Pinus nigra* plantation, on soil, leaf litter, 6 Nov 2021, leg. QR, det. QR et MK.

Clitocybe nebularis (Batsch) P. Kumm. (Agaricales)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, llaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren: Qadrak, N42°09'52.0", E20°46'29.0", 1230 m, RQPC (2020/451), beech forest, on soil, leaf litter, 20 Nov 2020, leg. QR, det. MK, ST et QR. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/281), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

**Clitocybe odora* (Bull.) P. Kumm. (*Tricholomataceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/164), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

**Collybia cookei* (Bres.) J.D. Arnold (*Tricholomataceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on woody debris of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Coniophora arida* (Fr.) P. Karst. (*Coniophoraceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Coprinellus disseminatus* (Pers.) J.E. Lange (*Psathyrellaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on stump of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson (*Psathyrellaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on rotten wood of *Fagus sylvatica*, 21 Mar 2017, leg. et det. MK.

**Coriolopsis gallica* (Fr.) Ryvarden (*Polyporaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10"E, 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dry branches of *Clematis* sp., 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Cortinarius melanotus Kalchbr. (*Cortinariaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto-cerris*, on bare soil, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Craterellus undulatus* (Pers.) E. Campo & Papetti (*Hydnaceae*)

Prizren: Qadrak, N42°09'52.0", E20°46'29.0", 1230 m, RQPC 2020/452, beech forest, on soil, rotten wood, 20 Nov 2020, leg. QR, det. MK, ST et QR.

**Crepidotus mollis* (Schaeff.) Staude (*Crepidotaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Crustoderma corneum* (Bourdot & Galzin) Nakasone (*Sparassidaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Cyanoboletus pulverulentus (Opat.) Gelardi, Vizzini et Simonini (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Dacryobolus karstenii* (Bres.) Oberw. ex Parmasto (*Dacryobolaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus* *heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Daedalea quercina (L.) Pers (*Fomitopsidaceae*)

Prizren: Jabllanicë, N42°10'01.0", E20°46'37.0", 900 m, beech forest, on trunk of *Fagus sylvatica*, 21 Apr 2017, leg AS, det. MK. – Kaçanik : Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on stump of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Daedaleopsis confragosa* (Bolton) J. Schröt. (*Polyporaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on dry stem of *Salix caprea*, 21 Mar 2017, leg. MK, EM et KR. det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31",700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det MK et GK.

**Diplomitoporus flavescens* (Bres.) Domański (Polyporales)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Efibula tuberculata (P. Karst.) Zmitr. & Spirin (*Phanerochaetaceae*)

Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on dry branch of *Corylus avellana*, 25 Jul 2021, leg QR, det. MK, ST et QR.

**Entoloma sericeum* Quél. (*Entolomataceae*)

Suharekë: Dolloc, N42°17'19.0", E20°54'59.3", 824 m, meadow, on soil, 4 Jan 2022, leg. et det. QR.

Entoloma sinuatum (Bull. ex Pers.) P. Kumm. (*Entolomataceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Entoloma vernum* S. Lundell (*Entolomataceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on soil, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Exidia glandulosa (Bull.) Fr (*Auriculariaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Salix alba*, 9 Oct 2017, leg. MK, GK, MP, det. MK et GK. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil, leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Exidia saccharina* Fr. (*Auriculariaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Exidiopsis calcea* (Pers.) K. Wells (*Auriculariaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on dry branches of living *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Exidiopsis effusa* (Bref. ex Sacc.) Möller (*Auriculariaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018 leg. MK, MTh, KR et TJ, det. MK et KR.

**Flammula alnicola* (Fr.) P. Kumm. (*Strophariaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on soil, 9 Oct 2017, leg. MK. GK et MP, det. Mk et GK.

**Flammulina velutipes* (Curtis) Singer (*Physalacriaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on trunk of *Alnus glutinosa*, 9 Nov 2017, leg. MK, GK et MP, det. MK et GK.

Fomes fomentarius (L.) Fr. (*Polyporaceae*)

Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on trunk of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR. – Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on living tree of *Alnus glutinosa*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on living tree of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, on trunk of *Quercus cerris*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Fomitopsis pinicola (Sw.) P. Karst. (*Fomitopsidaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/203), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR. – Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on trunk of *Fagus sylvatica*, 2 Jul 2021, leg. QR, det. MK, ST et QR. – Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on dead trunk of *Fagus sylvatica*, 31 May 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on trunk of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch (*Hymenochaetaceae*)

Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on trunk of *Carpinus betulus*, 31 May 2017, leg. MK, EM et KR, det. MK et KR.

Galerina marginata (Batsch) Kühner (*Hymenogastraceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Ganoderma applanatum* (Pers.) Pat. (*Polyporaceae*)

Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on trunk of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

**Gloeocystidiellum luridum* (Bres.) Boidin (*Stereaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead branch of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Gloeoporus pannocinctus* (Romell) J. Erikss. (*Irpicaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on fallen branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Gymnopus dryophilus (Bull.) Murrill (*Omphalotaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil, rotten wood, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on soil, leaf litter, 5 Jun 2018, leg. MK, KR et TJ. det. MK et KR. – Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on soil, wood debris, 25 Jul 2021, leg. QR, det. MK, ST et QR.

Gymnopus fusipes (Bull.) Gray (*Omphalotaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", *Quercetum frainetto–cerris*, on stem of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Hapalopilus rutilans* (Pers.) Murrill Boletus (*Phanerochaetaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on stem and fallen branch of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, 900 m, *Quercetum frainetto–cerris*, on fallen branch of *Quercus cerris*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/221), beech forest, dry branch of *Fagus sylvatica*, 10 Oct 2019, leg. QR, det. MK et QR.

**Hebeloma sinapizans* (Paulet) Gillet. (*Strophariaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/224), beech forest, on soil, leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

Hemileccinum impolitum (Fr.) Šutara (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil, leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Heterobasidion annosum* (Fr.) Bref. (*Bondarzewiaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on root of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Hortiboletus engelii (Hlaváček) Biketova & Wasser (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on soil, leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Hygrophorus chrysodon* (Batsch) Fr. (*Hygrophoraceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, under *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren: Novosellë, N42°12'35.0", E20°46'58.0", 1125 m, beech forest, on soil, leaf litter, 22 Nov 2020, leg. QR, det. MK, ST et QR.

Hymenochaete cinnamomea (Pers.) Bres (*Hymenochaetaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, fallen branches of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Hymenochaete rubiginosa (Dicks.) Lév. (*Hymenochaetaceae*)

Prizren: Jabllanicë, N42°10'01.0", E20°46'37.0", 1100 m, beech forest, on dry branch of *Fagus sylvatica*, 21 Apr 2017, leg. AS, det. MK

Hymenopellis radicata (Relhan) R.H. Petersen (*Physalacriaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on soil, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Hyphoderma nemorale K.H. Larss. (*Hyphodermataceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on fallen branch of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Hyphoderma setigerum* (Fr.) Donk (*Hyphodermataceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead branch of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Hyphodermella corrugata (Fr.) J. Erikss. & Ryvarden (*Phanerochaetaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on fallen branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Hyphodontia quercina (Pers.) J. Erikss. (*Hyphodontiaceae*)

Shtërpcë: Brezovica, N42°13'11", E21°00'35", 920 m, *Alnetum*, close to the river, on fallen branch of *Carpinus orientalis*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Hypholoma fasciculare (Huds.) P. Kumm. (*Strophariaceae*)

Kaçanik: Glloboçicë. N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on stump of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Hypholoma lateritium (Schaeff.) P. Kumm. (*Strophariaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on stump of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Imperator rhodopurpureus (Smotl.) Assyov et al. (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Inocybe dulcamara (Pers.) P. Kumm. (*Inocybaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Inocybe geophylla* P. Kumm. (*Inocybaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, RQPC (2021/463), *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

**Inonotus hastifer* Pouzar (*Hymenochaetaceae*)

Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on trunk of *Fagus sylvatica*, 25 Jul 2021, leg. QR, det. MK. ST et QR.

**Kneiffiella abdita* Riebesehl & Langer (*Hyphodontiaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Kneiffia subalutacea* (P. Karst.) Bres. (*Hyphodontiaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Kuehneromyces mutabilis (Schaeff.) Singer & A.H. Sm. (*Strophariaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK, MP, det. MK et GK.

**Laccaria amethystina* Cooke (*Hydnangiaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, RQPC (2021/464), *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Laccaria laccata (Scop.) Cooke (*Hydnangiaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Lactarius azonites (Bull.) Fr. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Lactarius deliciosus (L.) Gray (*Russulaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, in leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Lactarius zonarius (Bull.) Fr. (*Russulaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Lactifluus piperatus (L.) Roussel (*Russulaceae*)

Shtërpcë: N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Lactifluus vellereus (Fr.) Kuntze (*Russulaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), in leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Lentinus arcularius (Batsch) Zmitr. (*Polyporaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead branch of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Lepiota castanea* Quél. (*Agaricaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), in leaf litter, 9 Oct 2017, MK, GK et MP, det. MK et GK.

**Leucocybe connata* (Schumach.) Vizzini, P. Alvarado, G. Moreno & Consiglio (*Lyophyllaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/292), beech forest, in leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

**Lycoperdon excipuliforme* (Scop.) Pers. (*Agaricaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, beech forest, in leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

Lycoperdon perlatum Pers. (*Agaricaceae*)

Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, beech forest, in leaf litter, 10 Oct 2019, leg. QR, det. MK et QR.

Lyomyces crustosus (Pers.) P. Karst. (*Schizoporaceae*)

Shtërpcë: Brezovica, N42°13'11", E21°00'35", 920 m, *Querco–Carpinetum orientalis*, on fallen branch of *Carpinus orientalis*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), wood debris, 9 Oct 2017, MK, GK et MP, det. MK et GK.

Marasmius oreades (Bolton) Fr. (*Marasmiaceae*)

Opojë: Kuk, N42°05'48.3", E20°42'46.9", 1100 m, meadow, on soil, leaf litter, 29 Apr 2021, leg. QR et AM, det. MK et QR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Melanoleuca brevipes* (Bull.) Pat. (*Tricholomataceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), in leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Melanoleuca graminicola* Kühner & Maire (Agaricales)

Opojë: Kuk, N42°05'48.3", E20°42'46.9", 1100 m, RQPC (2021/460), meadow, on soil, leaf litter, 29 Apr 2021, leg. QR et AM, det. MK et QR.

**Mucidula mucida* (Schrad.) Pat. (*Physalacriaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on rotten wood of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/319), beech forest, on dry branch of *Fagus sylvatica*, 10 Oct 2019, leg. QR, det. MK et QR.

**Mycena acicula* (Schaeff.) P. Kumm. (*Mycenaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, in leaf litter, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Mycena cyanorhiza* Quél. (*Mycenaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on stump of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Mycena galericulata (Scop.) Gray (*Mycenaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dead trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Prevalla, N42°10'16.0", E20°58'04", 1150 m, RQPC (2019/311), beech forest, on dry branch of *Fagus sylvatica*, wood debris, 10 Oct 2019, leg. QR, det. MK et QR.

**Mycena pseudocorticola* Kühner (*Mycenaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on bark of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Mycena renati*Quél. (*Mycenaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Mycena sanguinolenta* (Alb. & Schwein.) P. Kumm (*Mycenaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. - Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dry branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Mycoacia livida* (Pers.) Zmitr. (*Meruliaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Neoboletus erythropus (Pers.) C. Hahn (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Neoboletus xanthopus (Klofac & A. Urb.) Klofac & A. Urb. (*Boletaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Omphalotus olearius (DC.) Singer (*Omphalotaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on trunk cut of *Quercus cerris*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Oxyporus obducens* (Pers.) Donk (*Oxyporaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Panellus stipticus* (Bull.) P. Karst (*Mycenaceae*)

Prizren: Jabllanicë, N42°09'56.6", E20°45'46.6", 1300 m, beech forest, on stump of *Fagus sylvatica*, 21 Apr 2017, leg. AS, det. MK. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Panus neostrigosus Drechsler-Santos & Wartchow (*Panaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on wood and stump of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Paxillus rubicundulus* P.D. Orton (*Paxillaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), in leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Peniophora cinerea* (Pers.) Cooke (*Peniophoraceae*)

Prizren: Jabllanicë, N42°09'56.6", E20°45'46.6", 1280 m, beech forest, on dry branches of *Fagus sylvatica*, 16 Nov 2020, leg. QR, det. MK et QR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on dry branches of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dry branches of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on dry branches of *Corylus avellana*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Peniophora incarnata (Pers.) P. Karst. (*Peniophoraceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on fallen branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Peniophora lycii (Pers.) Höhn. & Litsch (*Peniophoraceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on fallen branch of living tree of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Peniophora pini* (Schleich. ex DC.) Boidin (*Peniophoraceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on dry branches of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Peniophora quercina (Pers.) Cooke (*Peniophoraceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, dry branches of living *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Peniophorella pratermissa* (P. Karst.) K.H. Larss. (*Rickenellaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Peniophorella pubera (Fr.) P. Karst (*Rickenellaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), dry branches of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, on fallen branch of *Quercus cerris*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Phanerochaete laevis* (Fr.) J. Erikss. & Ryvarden (*Phanerochaetaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on branch of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Phanerochaete sordida (P. Karst.) J. Erikss. & Ryvarden (*Phanerochaetaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on fallen branch and stump of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Phanerochaete velutina (DC.) P. Karst. (*Phanerochaetaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Phellinus igniarius* (L.) Quél. (*Hymenochaetaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on living tree of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Phellinus pomaceus* (Pers.) Maire (*Hymenochaetaceae*)

Prizren: Jabllanicë, N42°10'01.0", E20°46'37.0", 1100 m, beech forest, on living tree of *Prunus* sp., 21 Apr 2017, leg. AS, det. MK.

**Phlebiopsis gigantea* (Fr.) Jülich (*Phanerochaetaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Phloeomana hiemalis* (Osbeck) Redhead (*Porotheleaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on bark of living tree of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Pleurotus pulmonarius (Fr.) Quél. (*Pleurotaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Pluteus cervinus (Schaeff.) P. Kumm. (*Pluteaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on trunk of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on wood of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

**Podofomes mollis* (Sommerf.) Gorjón (*Polyporaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on dry branches of *Corylus avellana*, 28 Apr 2018, leg. MK, Mth, KR et TJ, det. MK et KR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

Porostereum spadiceum (Pers.) Hjortstam & Ryvarden (*Polyporaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on dry branch of *Fagus sylvatica*, 28 Apr 202018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys (*Strophariaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, meadow, on soil, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Pseudosperma rimosum (Bull.) Matheny & Esteve-Rav. (*Inocybaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, CFK (2021/465), *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

**Psilocybe coronilla* (Bull.) Noordel. (*Hymenogastraceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, meadow, on solil, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Pycnoporus cinnabarinus (Jacq.) P. Karst. (*Polyporaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, on fallen branch of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Prizren: Kopana Voda, N42°12'31", E20°49'60", 1560 m, CFK (2020/458), beech forest, on dry branch of *Fagus sylvatica*, 16 Nov 2020, leg. QR, det. MK, ST et QR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch and wood of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

Ramaria flava (Schaeff.) Quél. (*Gomphaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Resupinatus striatulus* (Pers.) Murrill (*Pleurotaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus heldreichii*, 1 Jun 2018, leg, MK,KR et TJ, det. MK et KR.

Rubroboletus rhodoxanthus (Krombh.) Kuan Zhao & Zhu L. Yang (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula adusta (Pers.) Fr. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula anthracina Romagn. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula aurea Pers. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula chloroides (Krombh.) Bres. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula cyanoxantha (Schaeff.) Fr. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Russula emetica* (Schaeff.) Pers. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula foetens Pers. (*Russulaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula fragilis Fr. (*Russulaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, CFK (2021/465), *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, in leaf litter, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Russula grata Britzelm. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula grisea Fr. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula heterophylla (Fr.) Fr. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula maculata Quél. (*Russulaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, in leaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula olivacea Pers. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula risigallina (Batsch) Sacc. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula squalida Peck (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula subfoetens W.G. Sm. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula vesca Fr. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula virescens (Schaeff.) Fr. (*Russulaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Russula zvarae Velen. (*Russulaceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Sarcomyxa serotina* (Pers.) V. Papp (*Mycenaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, CFK (2021/466), *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on cut trunk of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Schizophyllum commune Fr. (*Schizophyllaceae*)

Shtërpcë, N42°13'25", E21°03'03", 1100 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Alnus glutinosa*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.M. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Pinus peuce*, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Schizopora paradoxa (Schrad.) Donk (*Schizoporaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Shtërpcë, N42°13'25", E21°03'03", 900 m, *Alnetum*, close to the river, on stump of *Fagus sylvatica*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. - Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on branch of *Carpinus betulus*, 31 May 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR. – Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on dry branch of *Fagus sylvatica*, 25 Jul 2021, leg. QR, det. MK, ST et QR.

**Scopuloides hydnoides* (Cooke & Massee) Hjortstam & Ryvarden (*Meruliaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Scitinostroma alutum Lanq. (*Peniophoraceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on fallen branch of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Sistotrema brinkmannii* (Bres.) J. Erikss. (*Hydnaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Steccherinum fimbriatum (Pers.) J. Erikss. (*Steccherinaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. Mk, KR et TJ, det. MK et KR.

Steccherinum ochraceum (Pers. ex J.F. Gmel.) Gray (*Steccherinaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on dry branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen branch of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Stereum hirsutum (Willd.) Pers. (*Stereaceae*)

Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR. – Dragash: Rrenc, N42°05'12.48", E20°39'39.5", 1054 m, mountain pasture, on dry branch & wood of *Fagus sylvatica* fallen on ground, 25 Jul 2021, leg. QR, det. MK, ST et QR. – Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on fallen branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Shtërpcë: Brezovica, N42°13'11", E21°00'35", 920 m, *Alnetum*, close to the river, on trunk of *Carpinus orientalis*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Jabllanicë, N42°10'01.0", E 20°46'37.0", 1100 m, beech forest, on trunk of *Fagus sylvatica*, 21 Apr 2017, leg. AS, det. MK. – Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on trunk of *Cornus mas*, 31 May 2017, leg. MK, EM et KR, det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, 5 Jun 2018, on fallen branch of *Quercus frainetto*, leg. MK, KR et TJ, det. MK et KR.

Stereum rugosum Pers. (*Stereaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, MK, MTh, KR et TJ, det. MK et KR.

**Strobilurus tenacellus* (Pers.) Singer (*Physalacriaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on cone of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Suillellus luridus (Schaeff.) Murrill (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Suillellus mendax (Simonini & Vizzini) Vizzini, Simonini & Gelardi (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Suillellus queletii (Schulzer) Vizzini, Simonini & Gelardi (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Suillus granulatus (L.) Roussel (*Suillaceae*)

Prizren: Prevalla ((Mt. Oshlak)), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, in llaf litter, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, on soil, 1 Jun 2018, MK, KR et TJ, det. MK et KR.

**Szczepkamyces campestris* (Quél.) Zmitr. (*Polyporaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300m, beech forest, on dry branches of *Corylus avellana*, 28 Jan 2018, MK, MTh, KR et TJ, det. MK et KR.

**Tomentella bryophila* (Pers.) M.J. Larsen (*Thelephoraceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, woody debris & rotten wood of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Tomentella ferruginea* (Pers.) Pat. (*Tomentellaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, rotten wood of *Quercus frainetto*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Trametes gibbosa* (Pers.) Fr. (*Polyporaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on dry branch of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Prizren:

Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on stump of *Fagus sylvatica*, 28 Apr 2018, leg, MK, MTh, KR et TJ, det. MK et KR.

Trametes hirsuta (Wulfen) Lloyd (*Polyporaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Prizren: Jabllanicë, N42°10'01.0", E20°46'37.0", 1100 m, beech forest, on stump of *Fagus sylvatica*, 21 Apr 2017, leg. AS, det. MK. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on dry branch & stump of *Quercus cerris*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Trametes ochracea (Pers.) Gilb. & Ryvarden (*Polyporaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on trunk of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Trametes pubescens* (Schumach.) Pilat (*Polyporaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10"E, 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead trunk of *Alnus glutinosa*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on dead trunk of *Betula pendula*, 31 May 2017, leg. MK, EM et KR, det. MK et KR.

**Trametes suaveolens* (L.) Fr. (*Polyporaceae*)

Shtërpcë: Firajë, N42°15'34", E21°05'10", 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead trunk of *Alnus glutinosa*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

Trametes versicolor (L.) Lloyd (*Polyporaceae*)

Prizren: Jabllanicë, N42°10'01", E20°46'37", 1100 m, beech forest, on stump of *Fagus sylvatica*, 21 Apr 2017, leg. AS, det. MK. – Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on dry branches of *Corylus avellana*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on dry branch of *Quercus cerris*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on cut trunk of *Fagus sylvatica*, 13 Nov 2021, leg. QR, det. MK, ST et QR. – Shtërpc: Brezovicë, N42°10'47.89", E21°01'46.81", 1687 m, beech forest, on dry branch & wood of *Fagus sylvatica*, 24 Jul 2021, leg. QR, det. MK, ST et QR.

**Trechispora farinacea* (Pers.) Liberta (*Hydnodontaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Trechispora mollusca* (Pers.) Liberta (*Hydnodontaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on stump of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Trichaptum fuscoviolaceum* (Ehrenb.) Ryvarden (Hymenochaetales)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on fallen tree of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Tricholoma sejunctum (Sowerby) Quél. (*Tricholomataceae*)

Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, among wood debris, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Tricholoma terreum (Schaeff.) P. Kumm. (*Tricholomataceae*)

Elez Han: Krivenik , N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Tubaria furfuracea* (Pers.) Gillet (*Tubariaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, in leaf litter, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Tubulicrinis glebulosus* (Fr.) Donk (*Hymenochaetaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldraichii* forest, rotten wood of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Tubulicrinis medius* (Bourdot & Galzin) Oberw. (*Hymenochaetaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldraichii* forest, rotten wood of *Pinus halepensisi*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Tubulicrinis subulatus* (Bourdot & Galzin) Donk (*Hymenochaetaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten wood of *Pinus heldreichii*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Typhula fistulosa* (Holmsk.) Olariaga (*Typhulaceae*)

Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

**Typhula sclerotioides* (Pers.) Fr (*Typhulaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR.

**Vitreoporus dichrous* (Fr.) Ginns (*Meruliaceae*)

Prizren: Prevalla, N42°10'16", E20°58'04", 1150 m, beech forest, on fallen branch of *Fagus sylvatica*, 28 Apr 2018, leg. MK, MTh, KR et TJ, det. MK et KR. – Shtërpcë: Firajë, N42°15'34", E21°05'10"E, 730 m, azonal vegetation (*Salix, Alnus, Populus*), on dead branch of *Salix alba*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR.

**Vuilleminia alni* Boidin, Lanq. & Gilles (*Vuilleminiaceae*)

Prizren: Sredskë, N42°10'11.5", E20°50'57", 700 m, *Alnetum* (close to the river Bistrica), on dry branches of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on dry branches of living *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Vuilleminia comedens (Nees) Maire (*Vuilleminiaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, beech forest, on stump of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det, MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, on dry branch of living *Quercus cerris*, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Vuilleminia cystidiata Parmasto (*Vuilleminiaceae*)

Kaçanik: Vata – Dubravë, N42°15'12", E21°10'21", 780 m, beech forest, on dry branches of living *Cornus mas*, 31 May 2017, leg. MK, EM et KR, det. MK et KR.

Xerocomellus chrysenteron (Bull.) Šutara (*Boletaceae*)

Prizren: Qadrak, N42°09'52.0", E20°46'29.0", 1230 m, CFK (2020/453), beech forest, on soil, leaf litter, 20 Nov 2020, leg. QR, det. MK, ST et QR. – Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR. – Elez Han: Krivenik, N42°07'24", E21°15'37", 700 m, *Quercetum frainetto–cerris*, among wood debris, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Xerocomellus subtomentosus(L.) Quél. (*Boletaceae*)

Kaçanik: Glloboçicë, N42°09'14", E21°12'1.46", 900 m, *Quercetum frainetto–cerris*, in leaf litter, 5 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Xeromphalina cauticinalis* (Fr.) Kühner & Maire (*Mycenaceae*)

Suharekë: Mushtisht (Gradanc), N42°15'50.0", E20°54'19.0", 1370 m, *Pinus peuce, Abies alba* mixed with *Fagus sylvatica*, on soil, leaf litter with mosses, 13 Nov 2021, leg. QR, det. MK, ST et QR.

Xylodon asper (Fr.) Hjortstam & Ryvarden (*Schizoporaceae*)

Prizren: Prevalla (Mt. Oshlak), N42°11'05.40", E20°57'12.15", 1600 m, *Pinus heldreichii* forest, on rotten tree of *Pinus halepensis*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

**Xylodon radula* (Fr.) Țura, Zmitr., Wasser & Spirin (*Schizoporaceae*)

Prizren: Prevalla, N42°11'21.3", E20°58'47.5", 1300 m, beech forest, on dry branches of *Corylus avellana*, 28 Apr2018, leg. MK, MTh, KR et TJ, det. MK et KR.

Xylodon raduloides Riebesehl & Langer (*Schizoporaceae*)

Shtërpcë, N42°13'25", E21°03'03", 900 m, *Quercus cerris* forest, on fallen branch of *Quercus cerris*, 1 Jun 2018, leg. MK, KR et TJ, det. MK et KR.

Xylodon sambuci (Pers.) Țura (*Schizoporaceae*)

Dragash: Krushevc, N41°59'54", E20°36'56", 1150 m, *Alnetum*, close to the river, on dry branch of *Fagus sylvatica*, 21 Mar 2017, leg. MK, EM et KR, det. MK et KR. – Shtërpcë: Brod, N42°15'55", E21°06'31", 700 m, beech forest, on fallen branch of *Alnus glutinosa*, 9 Oct 2017, leg. MK, GK et MP, det. MK et GK.

Discussion

As a result of field work and the microscopic examination under LM of the materials, collected from the studied areas in the Sharr Mountains in Kosovo, 244 species of macrofungi are reported. The majority of taxa belongs to *Basidiomycota* (211 species), while 33 species belongs to *Ascomycota*. As for novelties, 95 species were newly indetified in the Sharr Mts alone, while 166 species are new to Kosovo. From the collected material, the *Ascomycota* phylum is characterized by 29 species of saprophytes, three species of the genus *Xylaria* (*X. carpophila, X. hypoxylon, X. polymorpha*), three of the genus *Hypoxylon* (*H. fragiforme, H. fuscum, H. rubiginosum*), two of the genus *Diatrype* (*D. disciformis, D. stigma*) and other genera with one species. The saprophilous species from the phylum *Basidiomycota* include 132 species: with six species the genus *Mycena* (*M. acicula, M. cyanorhiza, M. galericulata, M. pseudocorticola, M. renati, M. sanguinolenta*) and the genus *Trametes* (*T. gibbosa, T. hirsuta , T. ochracea, T. pubescens, T. suaveolens, T. versicolor*), five species of the genus *Peniophora* (*P. cinerea, P. incarnata, P. lycii, P. pini, P. quercina*), four the genus *Xylodon* (*X. asper, X. radula, X. raduloides, X. sambuci*), with three species *Phanerochaete* (*Ph. laevis, Ph. sordida, Ph. velutina*), the genus *Tubulicrinis* (*T. glebulosus, T. medius, T. subulatus*), and the genus *Vuilleminia* (*V. alni, V. comedens, V. cystidiata*). The other genera that are not mentioned above with one or two species.

Mycorrhizal species (66 species) collected belong only to *Basidiomycota* phylum; 19 species of the genus *Russula* (*R. adusta, R. anthracina, R. aurea, R. chloroides, R. cyanoxantha, R. emetica, R. foetens, R. fragilis, R. grata, R. grisea, R. heterophylla, R. maculata, R. olivacea, R. risigallina, R. squalida, R. subfoetens, R. vesca, R. virescens, R. zvarae*); ten species are from the genus *Amanita* (*A. caesarea, A. dryophila, A. eliae, A. franchetii, A. lividopallescens , A. muscaria, A. pantherina, A. rubescens, A. subnudipes, A. vaginata*); three species of *Entoloma* (*E. sericeum, E. sinuatum, E. vernum*); genus *Lactarius* (*L. azonites, L. deliciosus, L. zonarius*); genus *Suillellus* (*S. luridus, S. mendax, S. queletii*). One or two species represent the remaining genera.

In the case of parasitic species of *Ascomycota* two species were recorded from the genus *Hypomyces* (*H. hyalinus, H. lateritius*), one from each *Hypoxylon* (*H. deustum*) and *Rhytisma* (*R. acerinum*), while the parasitic species of the phyllum *Basidiomycota* are *A. mellea, F. fomentarius, F. torulosa, G. applanatum, H. annosum* and *S. commune.*

Fig. 2. Saprobic species of *Ascomycota*: 1. *Helvella lacunosa*, 2. *Xylaria polymorpha*, 3. *Xylaria hypoxylon, Verpa bohemica*. Photo: Q. Ramshaj.

Fig. 3. Mycorrhizal species of *Basidiomycota*: 1. *Laccaria laccata,* 2. *Amanita pantherina,* 3. *Amanita muscaria,* 4.*Tricholoma terreum,* 5. *Lactarius deliciosus,* 6. *Russula cyanoxantha.* Photo: Q. Ramshaj.

Fig. 4. Parasitic species of *Basidiomycota*: 1. *Fomes fomentarius*, 2. *Schizophyllum commune,* 3. *Armillaria mellea.* Photo: Q. Ramshaj.

Noteworthy species are *Zeus olympius, Antrodia ramentacea, Heterosphaeria patella*, *Kneiffiella abdita* and *Resupinatus striatulus* which are reported as very rare. At an altitude of 1600 m, in Prevalla, the rare species *Zeus olympius* (Minter et al., 1987; Minter, 1996), was found on dead portions of twigs and small branches of Bosnian pine (*Pinus heldreichii* – an endemic Balkan pine), the only place known so far (Karadelev, 2018). According to Stoykov et al. (2014) the known distribution of this species encompasses Bulgaria (Pirin Mts., Mt. Slavyanka, Mt. Vitosha) and Greece (Mt. Olympus, Mt. Pindus). In Northern Macedonia, it was recorded by the Bulgarian mycologist Boris Asyov in September 2016, above Lake Ohrid (Galičica National Park) on dead leaves of *Ostrya carpinifolia* Scop., (Stoykov, 2020). In the Global Fungal Red List, the Current Assessment status for *Z. olympius* is "Under Assessment" and based on IUCN criteria the status for this species is provisionally listed as Critically Endangered on global scale. (Anonymous, 2011).

Antrodia ramentacea (Berk. & Broome) Donk, a species of bracket polypores fungi, wood-decomposing saprotrophic species (Bzdyk et al., 2019), mostly grows on wood of coniferous trees, like on branches of *Pinus nigra* (Doğan & Kurt, 2016), but sometimes also on hardwood, on dead attached and fallen *Salix* branches (Ainsworth, 2001). In Italy there is extensively, but rare distributed in Sicily, mainly collected in a mixed conifer wood of *Pinus halepensis, P. pinea* L., *Cupressus sempervirens* L. and *C. arizonica* Greene (Venturella et al., 2007). It is also listed a rare species in Finland, causing brown rot on dead wood of *Pinus sylvestris* (Niemela 1981). In Kosovo it was found at only one locality (Mt. Oshlak), on trunk of *Pinus heldreichii.*

Heterosphaeria patella (Tode) Grev., species from family *Helotiaceae*, as saprobes on dead or dried plant material (Leuchtmann, 1987), of herbaceous plants as we have found it on herbaceous twigs, beech forest from one locality (Prevalla). *H. patella* can be found on dead stalks of herbaceous plants of *Angelica sylvestris* or on dying stems of *Anthriscus cerefolium* (Hyde et al., 2020).

Kneiffiella abdita Riebesehl & Langer, a saprotrophic species, grows on dead wood of both coniferous and deciduous trees. The only registered location during this study is

Prevalla (rays of the Oshlaku Mountains) in the rich forest area of *Pinus heldreichii*, on fallen branches of *P. heldreichii*. According to Karadelev (2018) it is a very rare species in Kosovo. In the Czech Republic (Holec & Beran, 2006) it is included in the Red List of Macromycetes in the category CR (critically endangered species). Recorded as a rare species it is reported also in Slovakia (Tejklová & Zíbarová, 2020), known from Podunajská nížina Lowlands, on fallen trunks of *Populus* sp.; furthermore in higher elevations in dominated forests by *Fagus*: Vsetínské vrchy hills, near Karolinka, on stump and fallen trunk of *Fagus sylvatica* (Vampola & Vágner 1995) but in accordance with Ryvarden & Melo (2017) the species has preference for *Pinus* likewise.

Resupinatus striatulus (Pers.) Murrill, saprobic species, growing at the bottom of rotten wood mainly on coniferous trees, based on molecular and phylogenetic studies is included in the *Pleurotaceae* family (Thorn et al., 2000; Binder et al., 2005). In Kosovo it is known only one locality (Prevalla, Mt. Oshlak) - an area of Bosnian pine forest (*Pinus heldreichii*), and is evaluated as rare species (Karadelev, 2018), from rotten wood of *Pinus heldreichii*. In adjacent countries it has been recently recorded from Turkey (Akçay, 2021).

The research on fungi in Bosnian pine (*Pinus heldreichii* H. Christ.) is particularly interesting. Bosnian pine is a tertiary relict and endemic to the western Balkans and southern part of the Apennine peninsula. It is an Oro-Mediterranean species occurring at one locality in Sharr Mountains (Prevalla) at altitudes between 1600 and 1700 m. In these surveys, a total of 33 species were registered here, which is undoubtedly a small number of species. Most of the species such as *Amphinema byssoides, Exidiopsis calcea, Fomitopsis pinicola, Heterobasidion annosum, Peniophora pini, Peniophorella praetermissa, Suillus granulatus, Strobilurus tenacellus, Trichaptum fuscoviolaceum*, as well as the species of the genus *Tubulicrinis* (*T. glebulosus, T. medius, T. subulatus*) are common species commonly found in pine forests. In addition, some rare species such as *Antrodia ramentacea, Botryobasidium vagum, Kneiffiella abdita, Resupinatus striatulus* and *Zeus olympius* were collected, which are discussed in more detail above in the text. The ecological requirements of fungi in such forests surely have some particularities which should be studied in the future. The appearance of rare species promises that many interesting finds await future investigators. *P. heldreichii* forests are of key importance for nature conservation, protection against gravitational natural hazards, landscape conservation, and recreation.

Fig. 5. Rare species: 1. *Zeus olympius*, photo Tome Jovanovski, 2. *Resupinatus striatulus,* Photo: Mitko Karadelev.

Acknowledgements

For the implementation of this study is undoubtedly the great role and contribution of all those who helped in any way in the expansion of the mycobiota of Kosovo, especially for the field support, we would like to acknowledge: (in alphabetical order) Aldin Skenderi, Artan Mehmeti, Emri Murati, Gerhard Kost, Marija Pitoshevska, Mathias Theiss, Tome Jovanovski and Toni Mitrovski. Valbon Bytyqi is thanked for preparing the map of the surveyed areas.

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Received: 05.07.2022
Accepted: 20.09.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 31-38

Assessment and Maping Soil Erosion Risk in the Watershed of Sedelska River

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Abstract. The geographical features of Bulgaria such as steep slopes, rugged terrain, and unregulated forest activities, and easily susceptible to erosion soils are considered as main reasons for the erosion processes we observe. One of the most affected region from soil erosion in the country is the Struma River watershed. Sedelska River is one of its torrential tributaries, part of its middle reaches. On the territory of Sedelska River erosion control activities have been conducted in the past, but there are still signs of erosion in the watershed. The study aims to assess potential and actual soil erosion risk in forest territories and to determine the spatial distribution of them. The total assessment in the Sedelska River is "low to moderate" potential soil erosion risk and "very low to low" actual soil erosion risk. Part of the territories, mainly around the first grade tributaries are with "moderate to strong" risk of erosion, which showed the prerequisite for bank erosion in the watershed and the need for sustainable management practices.

Key words: soil erosion, risk assessment, Sedelska River.

Introduction

Natural hazards become more severe with each passing year. This situation brings a negative impact on all terrestrial ecosystems, where among the most affected are forests. According to many authors they are recognized as highly sensitive and vulnerable to changes in environmental conditions (Grabherr et al., 2000; Michelsen et al., 2011; Kozyr 2014; Takur et al., 2021). As in the definition of vulnerability meant that a variety of elements, including sensitivity and lack of capacity to cope and adapt (IPCC, 2014). Although in themselves they are vulnerable, the forest plays a key role in soil protection (Meléndez-Pastor et al., 2017).

Soils are a limited natural resources and considered the biggest threat to them is soil erosion. This environmental problem occures in all parts of the terrestrial world. It is a constant process, which can be very intensive if there is a combination of adverse factors. Another important thing about soil erosion is that we commonly are aware of the consequences only when it could be too late or too expensive to solve it (Spalevich et al., 2020). For that reason methods for assessing soil erosion risk are applied. In general, these erosion methods are mathematical descriptions of the relationships between the amount of eroded soil and erosion factors. The most famous model used is the Universal Soil Loss Equation (USLE) developed by Wishmeier & Smith (1978) and those derived from it. Nowadays models used the potential of Geographic Information Systems (GIS), which give us an opportunity to establish territories at risk and gives accurate information which could be easily used by decision-makers to mitigate negative consequences by applying appropriate silviculture and agriculture practices and if it is needed to conduct erosion control activities.

For Bulgaria, one of the most affected by soil erosion territories is the watershed of Struma River. In the watershed many factors influence, but the most significant are steep slopes (Martensson et al., 2001) and easily prone to erosion soils. In the past most of the forest territories in the watershed are cut down by locals to make places for pasture. This activity greatly worsened the condition of the forest and reduce its potential.

In some tributaries of the Struma River, in the upper part of its watershed a methodology for potential and actual soil erosion risk in forest territories is applied (Pavlova-Traykova & Marinov, 2018; Pavlova-Traykova, 2019) and the received results are comparable with the results received by applying other models for soil erosion risk assessment. In the middle part of Struma, where our object of investigation – Sedelska River is situated, this methodology is also chosen to be applied. Through its application we will also track the change in risk levels since the application of similar methodology 20 years ago (Marinov et al., 2002).

The purpose of the investigation is to assess potential and actual soil erosion risk in the forest territories in the Sedelska River watershed and to establish and mapped the territories according to the degrees of risk.

Material and Methods

The object of investigation is Sedelska River catchment area (Fig. 1), which is the right tributary of Struma River and its catchment area cover 50.2 km^2 of the eastern slopes of Maleshevska Mountain (Marinov, 2014). Despite erosion control activities like afforestation and limitation of pasture and unregulated activities in the forest, the current ecological situation in the watershed showed that about 30% of forest territories are under bad ecological conditions (Marinov, 2014). Most of these territories are in the belt up to 600 m. For the period 2071-2099, it is expected more than 50% of these territories to become under bad ecological conditions (Marinov, 2014).

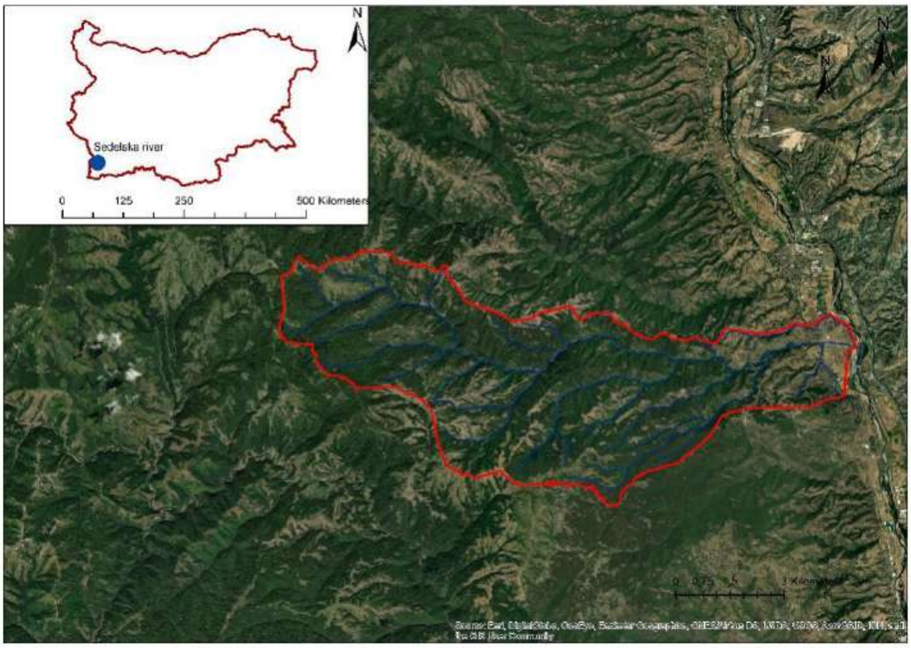

Fig. 1. Location of Sedelska River watershed.

In the present investigation, an assessment of potential and actual soil erosion risk was made only for forest territories, applying part of the "Methodology for preparing the national long term programme for protecting from erosion and flooding in the forest lands" (Marinov et al., 2009). An adapted version of methodology of MERA project is used (Stoev et al., 1997). The steps for assessment of potential and actual soil erosion risk have been precented in details in some other investigations (Pavlova-Traykova et al., 2017; Pavlova-Traykova & Marinov, 2018; Pavlova-Traykova, 2019). Some of the factors are directly taken from the Forest management plan of Strumyani. The subsection is the basic unit of area.

Precipitation influence on soil erosion development is assessed with rainfall erosivity index (R factor). All forest territory with altitude of 1000 m a.s.l. have annual index of erosion 1 (600 MJmm/hah), from 1000 to 1200 m a.s.l. – index 2 (601 – 1000 MJmm/hah), and over 1200 m a.s.l. are with rainfall index 3 (1001 – 2000 MJmm/hah) (Rousseva et al., 2010).

Soil index (*Is*) is obtained by multiplying the class according to the degree of erosion and class according to the type of erosion from forest management plans and are divided into three soil indexes– 1 (with value 1), 2 (with value 2 or 3) and 3 (with value 4 and 6). In case there are no data in forest management plans about bare area, about areas not suitable for forest barrens and areas without information for degree and type of erosion, soil index (*Is*) of 2 is accepted, and for gullies, landslides and sliding - the soil index is 3.

Topography factor is classified in four indexes: 1 – to 10^{o}, 2 - 11- 20^{o}, 3 - 21 – 30^{o} and 4 – above 30^{o}.

Potential soil erosion risk is determine by multiplication of *R* factor, slope index and soil index. Potential soil erosion index is 1 when the value is less then 4, 2- when the values are from 4 to 9 and 3- when the values are above 9.

For influence of vegetation cover a data from forest management plans is used. Vegetation index 1 have crops and forest with density above 0.6, these with density 0.3-0.6- index 2, and open stands, not suitable for forest area, barrens, gullies, landslides and landslips - index 3.

Actual soil erosion risk is determined by multiplication of potential soil erosion risk index and vegetation cover index in six grade scale - from very low (index 1) to strong (index 6). Assessment for the forest territories in the watershed is determined according to the value from sum of "moderate", "moderate to strong" and "strong" actual soil erosion risk grade.

Result and Disscusion

The significant part of the area of Sedelska River watershed are forest territories. From 50.2km^2, 41.8km^2, are forests. The characteristics of the watershed are presented in table 1. The length of main River current is 18 km. The territories on sunny and shady exposure are almost with the same area and the flat territories are only 0.64 km^2.

Table 2 are presented the main factors for soil erosion risk. It is obvious from the results, that the factor that influenced potential erosion most is the slope index. Most of the forest territories are on steep slopes with degrees 21-30°. This is a prerequisite for easy transportation of eroded particles from slopes to River bed as a result of this water quality will be reduced (Montanarella et al., 2016; Lal, 2017) and infrastructure disruptions may occur. Slope index is considered as main for the entire watershed of Struma, part of which is Sedelska and separately some of its tributaries (Martensson et al., 2001; Pavlova-Traykova et al., 2017; Pavlova-Traykova, 2019; Pavlova-Traykova & Marinov, 2021).

Table 1. Characteristics of Sedelska River watershed.

	Main charcteristics	Unit	Results
Sedelska River watershed	Area	km^2	50.2
	Length	km	18
	Average altitude	m	774
	Average slope	o	20
	Slope	km^2	
	< 10 ^{o}		0.9
	11–20 ^{o}		10.3
	21-30 ^{o}		32.94
	>30 ^{o}		6.06
	Slope exposure	km^2	
	Sunny (S, SE, SW, W)		23.27
	Shady (N, NW, NE, E)		26.29
	Flat		0.64

Table 2. Area distribution of main soil erosion risk factors.

R factor	Area, км^2	Soil index (Is)	Area, км^2	Slope index	Area, км^2
1	38.52	1	30.39	1	1.34
2	4.29	2	6.34	2	8.3
3		3	6.08	3	29.96
				4	3.21
Total area	42.81		42.81		42.81

The potential risk of erosion is presented in Table 3. The predominant degree for potential soil erosion is "low", but there is a presence of almost 40% of territories with a "moderate" degree. This distribution allows making a final assessment of "low to moderate" potential risk in forest territories. For the total area of the watershed risk in 2002, it is established that 64% of the territory is with "strong" potential soil erosion risk (Marinov et al., 2002). These results are mainly because non-forest territories are included and also in 2002 forest territories are considered for almost 64% and now these territories are about 86%. Normally in the eroded objects, the non-forest territories are with worse soil characteristics which led to this assessment. The great advantage of forest vegetation is expressed in the fact that, at the same time as limiting erosion, it supplies a large amount of organic matter, which supports soil formation processes on these eroded terrains (Pavlova-Traykova et al., 2018).

From the spatial distribution of forest area, it is well presented that the territories with "strong" risk are mainly around the tributaries from first grade in the part of the watershed near Drakata village. This distribution is an indicator of the presence of strong bank erosion, which actually is established on field. A significant part of the watershed is with "moderate" erosion. If not well thought out proper management, in situation of climate change these territories could pass into next level of risk.

For assessment of actual soil erosion risk, the influence of vegetation is considered. The table 4 are presented the distribution by vegetation index. Most of the forest are with full protection from soil erosion - index 1, but 32% are with moderate protection, and some part of the forest is with poor protection.

In 2002, the assessment for actual soil erosion risk is "moderate to low" (Marinov et al., 2002), now after calculation (Table 5), the total assessment of actual soil erosion risk is assessed as "low to very low". This is probably the positive influence of forest vegetation, which was found to have increased its area. In the current assessment, there is the presence of territories in all degrees of risk, but their area in the higher degrees is not significant. However, it is necessary to pay attention to the territories in the 4th, 5th, and 6th degrees to avoid the risk of worsening their condition and reaching irreversible processes.

The spatial distribution of actual soil erosion risk is presented in Fig. 3. Some of the territories with actual risk concur with these with potential risk. It seems that the forest area above the Drakata village is not only with the potential of "strong" erosion, but actual erosion is also to the highest degree.

Table 3. Potential soil erosion risk at forest territories.

Potential soil erosion risk		Distribution of forest territories	
Index	Degree	Area, км^2	Area,%
1	Low	24.97	58.3
2	Moderate	16.37	38.2
3	Strong	1.47	3.4
Total		42.81	100

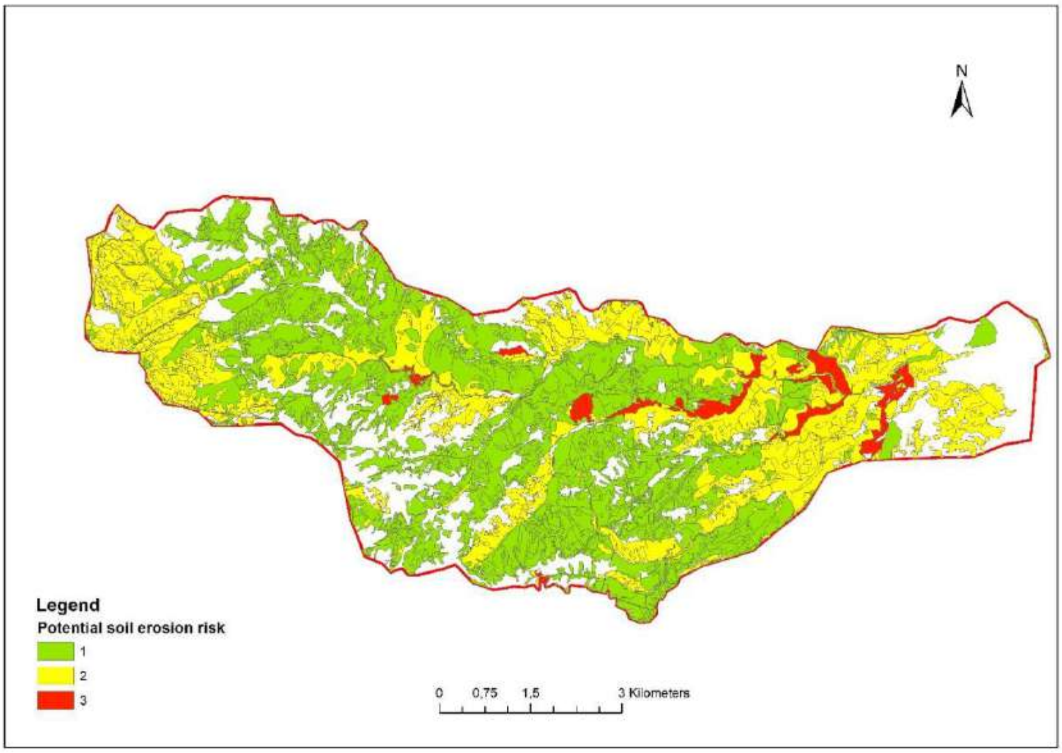

Fig. 2. Potential soil erosion risk at forest territories.

Table 4. Vegetation index.

Vegetation index	Area, км^2	Area,%
1	22.87	53.42
2	13.71	32.03
3	6.23	14.55
Total	42.81	100

Table 5. Actual soil erosion risk on forest territories.

Actual soil erosion risk		Distribution of forest territories	
Index	Degree	Area, км^2	Area,%
1	Very low	13.93	32.5
2	Low	16.34	38.2
3	Low to moderate	3.65	8.5
4	Moderate	4.95	11.6
5	Moderate to strong	3.84	9.0
6	Strong	0.11	0.2
Total		42.81	

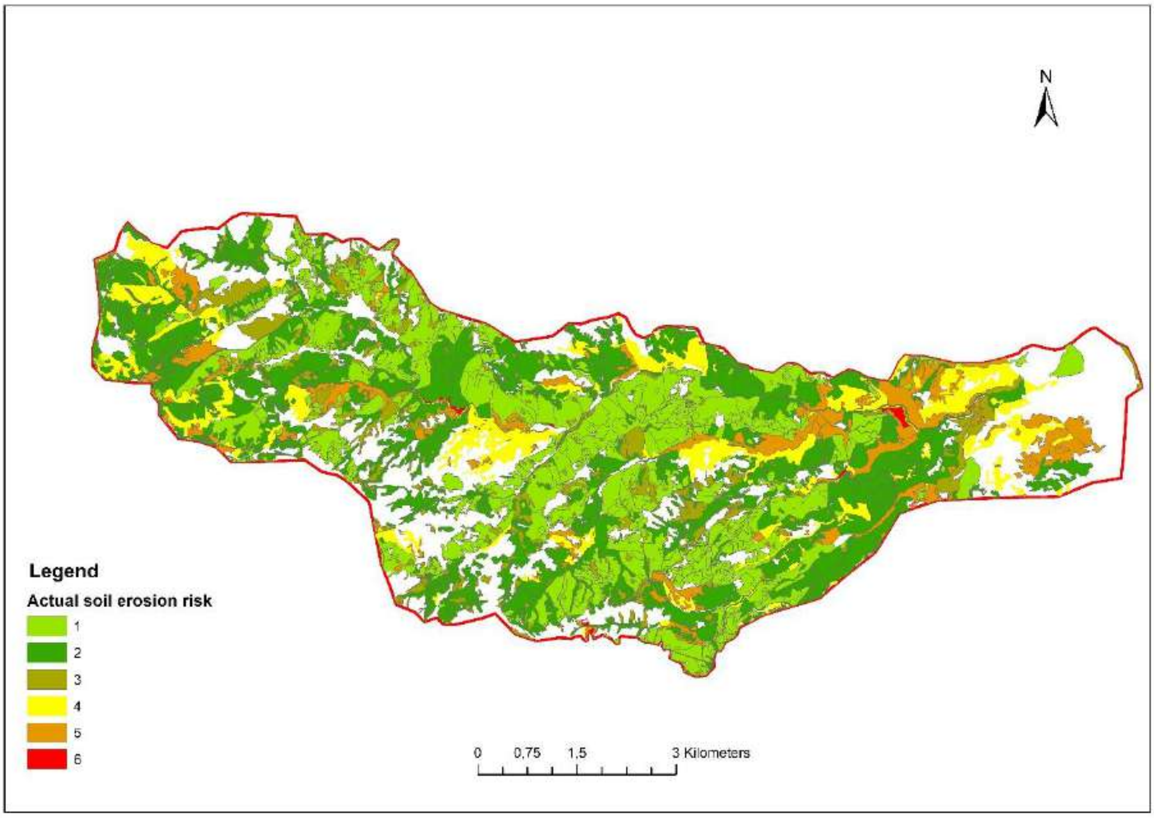

Fig. 3. Actual soil erosion risk at forest territories.

Conclusion

The main factor for erosion processes in the Sedelska River watershed is the topographical factor (steep terrain). The presence of territories assessed with "strong" risk indicates the need for additional erosion control activities. Attention should be paid to the coastal erosion and coastal stabilization activities should be undertaken.

In the case of improper management of the forest territories with risk of actual and potential erosion, they can easily pass into a stronger degree of risk.

Acknowledgments

The present study was conducted in relation to implementation of a project approved for financing on the basis of the Fund for Scientific Research Competition for financing fundamental scientific research of young scientists and postdoctoral students - 2021, with number КП-06-М56/5 with topic "Soil erosion assessment and efficiency of applied erosion control activities in Sedelska River, tributary of Struma River".

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Received: 13.07.2022
Accepted: 19.08.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 39-50

Impact of Fertilizing with Organic Composted Mixture on the Quality of Natural Grass Stands in the Semi-mountain Regions of Bulgaria

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Abstract. The impact of a composted mixture of *Pteridium aquilinum* L. with cattle manure on the composition and quality of natural grass stand, transitional type *Chrysopogon gryllus-Agrostis capillaris* in the conditions of the Central Balkan Mountain in Bulgaria was observed. Surface treatment with the composted mixture increased the share of useful legumes and grass fodder species. The percentage share of the components in the grass mass increased by 24.15 and 9.00%, respectively. Graphical regression models were developed to predict the amount of calcium, nitrogen, phosphorus and cellulose through the percentage of legumes and grasses in the treated grass stands, which in practice is significantly more economical and fast. Grass stands fertilized with 2000 kg/da composted mixture (with the highest share of legumes) had the highest content of crude protein (with 29.9%), crude fat (with 44.0%) and minerals (with 10.6%). The highest *in vitro* digestibility of the dry matter was observed in the variants treated with 1000 kg/da and 3000 kg/da composted mixture, where the excess in the values of the indicator was from 4.0 to 4.7% compared to the control. A high positive correlation was found between the indicators of dry matter yield with the content of crude protein (r = 0.84), minerals (r = 0.82), calcium (r = 0.96), phosphorus (r = 0.95), nitrogen (r = 0.84) and cellulose (r = 0.93) in the dry matter of the treated grass stands.

Key words: bio-fertilization, *Pteridium aquilinum*, quality and nutritional value of fodder.

Introduction

The biodiversity of natural grass stands is closely linked to the effective management and implementation of agrotechnical events affecting soil fertility and plant development dynamics (Parton, 1993; Mitev et al., 2013; Iliev et al., 2020; 2021). The use of biofertilizers as a substitute for chemicals is at the heart of sustainable agriculture and a good alternative for increasing soil fertility and microbiological diversity (Samadhiya et al. 2013; Yalew et al. 2020). The choice of new ecological products makes it possible to reduce costs (Churkova, 2021), improve productivity and grass composition. Feeding with some organic fertilizers reduces the degree of lignification and provides optimal conditions for obtaining environmentally friendly biomass with improved qualitative indicators (Bozhanska, 2019).

In this context, plant extracts of *Pteridium aquilinum* L. (bracken fern) used as organic products registered a high pesticidal and fungicidal effect (Mala et al., 2019). The biomass of *Pteridium aquilinum* L. is high in nitrogen and potassium, which makes it suitable for composting (Pitman & Webber, 2013). The processes related to the decomposition and preparation of the composted mixture allow to include inoculants to improve the moisture retention capacity and the quality of the peat material (Antonius et al., 2015).

Manure and urea are the main sources of N in the stage of composting and enrichment of the soil with the necessary nutrients. The high temperature in the fermentation process removes toxins from the leaf mass, which makes *Pteridium aquilinum* a potential organic substrate suitable for growing plants (Pitman & Webber, 2013).

The eagle fern is a perennial herbaceous plant, whose stems are modified mainly in underground rhizomes, which inhibits the development of useful grassland (Alexander et al., 2016; Dragicevic, 2019). The yield of dry leaf mass reaches 4-16 t/ha per year. One way to destroy this aggressive weed is through regulated annual mowing (Suazo et al., 2015).

Composting the aboveground mass of *Pteridium aquilinum* L. is used in modern organic agricuture as: herbicide product in some vegetable crops, biostimulator of seed germination of plants, antifungal agent and biofuel (Donnelly et al., 2002). It has also been found that the composition of minerals in the leaf mass of *Pteridium aquilinum* increases the growth and number of nodules in legumes of the genus *Trifolium* (Donnelly et al., 2006).

The aim of the present study was to determine the effect of a mixture of composted plant mass of *Pteridium aquilinum* L. with fresh cattle manure on the composition and qualitative indicators of a fodder from a natural grass stand (transitional type *Chrysopogon gryllus-Agrostis capillaris*) in the the Central Balkan Mountain region.

Material and Methods

The experiment was conducted at the Research Institute of Mountain Stockbreeding and Agriculture, Troyan (Department of Mountain Meadow Farming and Fodder Production) and covered a four-year study period (2016-2019).

The experiment was set up in 2016, on a natural grass stand (transitional type C*hrysopogon gryllus-Agrostis capillaris*), in the Makaravets area, at an altitude of 460 m, by the block method in 4 replications, with a plot size of 5 m^2. Fertilizing with a composted mixture (*Pteridium aquilinum* + fresh cattle manure) was applied annually, on the surface (manually by spraying) before the onset of active vegetation of grass species (in the period 20. February-10. March).

Experimental variants were:

1. Control (not fertilized with composted mixture);
2. Fertilizing with composted mixture at a rate of 1000 kg/da;
3. Fertilizing with composted mixture at a rate of 2000 kg/da;
4. Fertilizing with composted mixture at a rate of 3000 kg/da;
5. Fertilizing with composted mixture at a rate of 4000 kg/da;

The experimental areas were harvested in the phenophase of tasseling - onset of blossoming - ear formation (for grass species).

The composting mixture is prepared by the method of English nurseries and landscape contractors for growing coniferous and deciduous species (*Picea abies*, *Pinus sylvestris*, *Fraxinus excelsior* and *Betula pendula*) in forest nurseries (Pitmen, 1994).

Biomass of *Pteridium aquilinum* was mowed with a hand-held thumb mower in late spring-early summer (May-June) – Fig. 1. The cut fresh mass was cut with a combine twice with a particle size of 20 mm and 6-10 mm, respectively (in 48-50% of the fraction).

The prepared organic matter was placed on a wooden grill (composter made of 20 m^3 branches of *Populus nigra* and *Betula pendula*, sufficient for machine processing). Fresh cattle manure was added (ratio - 50:50) to balance and compensate for the lack of nitrogen in the eagle fern and neutralize some of the toxic alkaloid compounds. After 15 days (incubation period), the mixture was mixed with a power tiller with metal shovels, which continued for 120-140 days (every 20 days). The composted mixture was covered with silage polyethylene.

Fig. 1. Vegetable mass of *Pteridium aquilinum* and composted mixture.

During the first five weeks, the temperature (recorded on a scale of a soil platinum-resistant thermometer located in the center of the bowl) in the compost was 60-62°C, which accelerated the process of degradation of the carcinogen *ptaquiloide*. The critical minimum humidity of 70-75% and the temperature of 45-47°C were the most optimal conditions for the effective aerobic decomposition of the homogeneous mass.

The reaction in the composted heap was monitored with a mobile pH meter (model - ZD-06) (starting value of pH = 5.0-6.0 to pH = 8.0 in the final product).

The composted mixture (after six months) of eagle fern and manure acquired the character of a peat substrate (loose granular fraction) with dark brown to black color.

The following indicators were observed:

Dry matter yield (kg/da) - determined by mowing, weighing of grass in the different replications for each harvest plot followed by drying the samples to a constant weight at 105°C and recalculated for 1 da.

Botanical analysis of grass stand (%) was determined by weight by analysis of grass samples taken immediately before mowing. The percentage share of each species per year from the group of grasses and legumes, motley grasses (total) in both modes of use and their total ratio in the main botanical groups (grasses, legumes and motley grasses) was established.

The chemical composition of dry fodder was analyzed according to *Weende* analysis: Crude protein (CP, g kg^{-1}) according to *Keldahl* (according to BDS/ISO-5983); Crude fiber (CFr, kg 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; Nitrogen-free extractable substances (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 reagent - spectophotometer (*Agilent 8453 UV - visible Spectroscopy System*) that measure in the sphere of 425 ηm.

The fibrous structural elements in the plant cell were analyzed at a laboratory:

Neutral Detergent Fibers (NDF, g kg^{-1} DM); Acid detergent fiber (ADF, g kg^{-1} DM) and Acid detergent lignin (ADL, g kg^{-1} DM) by the Van Soest and Robertson (1979) detergent assay and in vitro dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides were empirically calculated: Hemicellulose (g kg^{-1} DM) = NDF-ADF and Cellulose (g kg^{-1} DM) = ADF-ADL. The lignification degree is expressed as the percentage of ADL and NDF.

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

Results and Discussion

Botanical composition of natural grass stand treated with a composted mixture of eagle fern and cattle manure

Fertilizing regime and soil composition affect species diversity in natural grass stands, control plant resistance and productivity (Královec et al., 2009; Pavlů et al., 2011; 2012).

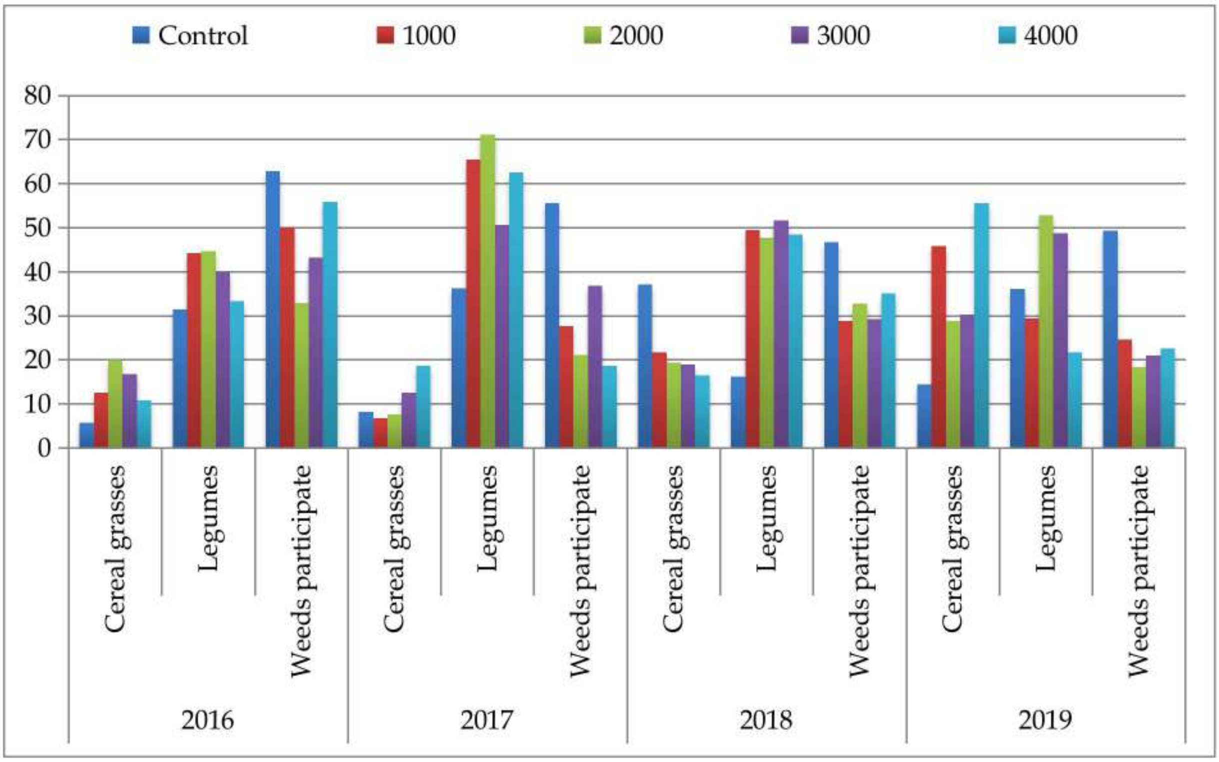

Fig. 2. Botanical composition of natural grass stand, transitional type (*Chrysopogon gryllus-Agrostis capillaris*) treated with composted mixture (%, by groups).

For the four-year test period, the highestshare of legumes and grasses was observed in the grass mass that was treated on the surface with 2000 and 4000 kg/da of composted mixture, respectively (Fig. 2). The excess of the species by groups is by 24.15% (for legumes) and 9.00% (for grasses) compared to the untreated control.

Yalew et al. (2020) report that compost fertilizer proven increases the legume species composition and their ratio in the volume of plant mass.

The results obtained during the study period show that natural grass stands with 2000 and 3000 kg/da of composted mixture imported have a higher presence of legumes than meadow grasses. The ratio of grasses:**legumes** is:

- 20.00:**44.70**% (in the variants with imported dose - 2000 kg/da composted mixture) and 10.80:**33.30** (in the variants with imported dose - 3000 kg/da composted mixture) - first experimental year (2016);
- 7.60:**71.20**% (in the variants with imported dose - 2000 kg/da composted mixture) and 12.60:**50.60**% (in the variants with imported dose - 3000 kg/da composted mixture) - second experimental year (2017);
- 19.40:**47.80**% (in the variants with imported dose - 2000 kg / da composted mixture) and 19.00:**51.70**% (in the variants with imported dose - 3000 kg/da composted mixture) - third experimental year (2017);
- 28.80:**52.80**% (in the variants with imported dose - 2000 kg / da composted mixture) and 30.30:**48.70**% (in the variants with imported dose - 3000 kg/da composted mixture) - fourth experimental year (2019);

During the first three experimental years, the percentage of fodder crops in the legume group in the variants treated with the lowest (1000 kg/da) and highest (4000 kg/da) dose of composted mixture was again higher than that of grasses. An exception is the fourth (last) year, when the share of grasses in the composition of the grass mass was higher by 16.3 to 33.8% compared to that of legumes.

The data from the analysis show that the surface treatment with the composted mixture (plant mass of *Pteridium aquilinum* L. + beef manure) increases the share of useful legumes and cereal feed species and limits that of plants from the group of grasses, which improves productivity and quality of the formed biomass.

Basic chemical composition of natural grass stand treated with composted mixture

The formation of a vegetative mass with a high density of legume components is a major factor in increasing the concentration of crude protein in the dry matter.

The effect of the applied fertilization on the chemical composition of the natural grass cover (transitional type *Chrysopogon gryllus-Agrostis capillaris*) is presented in Table 1. With the highest dry matter content (900.2 g kg^{-1}), crude protein (160.2 g kg^{-1}), crude fats (26.5 g kg^{-1}) and minerals (71.1 g kg^{-1}) are grass fertilizers with 2000 kg/da composted mixture (the variant with the highest share of legumes). The excess in the values of the indicators compared to the untreated control was by 29.9% (for CP), 44.0% (for CF) и 10.6% (for Ash).

Table 1. Basic chemical composition (g kg^{-1}) of natural grassland (transitional type *Chrysopogon gryllus-Agrostis capillaris*) treated with composted mixture (average for the experimental period).

Variants	DM	CP	CF	CFr	Ash	NFE	Ca	P	N
Control	899.9	123.3	18.4	446.4	64.3	247.6	16.9	1.3	17.8
1000	898.0	154.5	17.6	390.2	67.3	268.4	56.9	2.3	22.2
2000	900.2	160.2	26.5	403.1	71.1	239.3	52.5	2.5	21.8
3000	899.9	157.8	25.4	430.0	69.3	217.4	50.2	2.5	20.4
4000	899.7	135.6	21.0	414.2	66.3	262.7	51.7	2.7	19.5
Mean±Sx	***899.5±0.5***	***141.3±4.4***	***21.7±2.0***	***416.8±8.4***	***67.7±1.1***	***252.0±7.8***	***45.6±1.4***	***2.3±0.1***	***20.3±0.6***
SD	***0.9***	***12.6***	***4.0***	***22.1***	***2.6***	***13.8***	***16.2***	***0.6***	***1.8***

The highest content of carbohydrates (268.4 g kg^{-1}), calcium (56.9 g kg^{-1}) and nitrogen (22.2 g kg^{-1}) was observed in the dry matter in the variants treated with 1000 kg/da composted mixture. The results obtained are consistent with those found by Nemera et al. (2018) namely that application of cattle manure in natural pasture exhibited higher ash due to the fact that cattle manure is rich in phosphorous, potassium, magnesium, and calcium.

The maximum dose of composted mixture (4000 kg/da) does not significantly affect the species diversity and quantity of legumes. The grass mass of the variant has the lowest concentration of crude protein (135.6 g kg^{-1}), mineral composition (66.3 g kg^{-1}) and nitrogen (19.5 g kg^{-1}), and the highest content of phosphorus (2.7 g kg^{-1}) in the dry matter compared to the other treated variants.

The grass stand treated with doses of 1000 and 3000 kg/da of composted mixture has the lowest (390.2 g kg^{-1}) and highest (430.0 g kg^{-1}) crude fiber content in the dry matter composition, respectively. In relative terms, the difference in the values of the indicator is from 3.3 to 10.2%.

On average for the test period, the compositions of the composted mixture have a positive effect on the mineral composition and the content of macronutrients (Ca, N and P) in the dry matter, the most significant being the excess in the concentration of the element calcium.

Fibrous structural components of the cell walls of natural grass stand treated with a composted mixture

The grass stands in the fertilizing variants have a lower content of neutral-detergent fibers, neutral-detergent lignin and hemicellulose (Table 2). The values of the indicators vary from 539.3 to 602.4 g kg^{-1} (for NDF), from 97.5 to 171.2 g kg^{-1} (for ADL) and from 173.6 to 199.5 g kg^{-1} (for Hemicellulose). The decrease in concentration compared to the average value of the quality parameters was from 1.3 to 11.6% (for NDF), from 6.9 to 47.0% (for ADL) and from 6.2 to 18.4% (for Hemicellulose).

Table 2. Fiber structural components of cell walls (g kg^{-1}) of a natural grass stand (transitional type *Chrysopogon gryllus-Agrostis capillaris*) treated with composted mixture (average for the experimental period).

Variants	NDF	ADF	ADL	Hemicellulose	Cellulose	IVDMD
Control	610.4	397.7	183.8	212.7	213.9	584.7
1000	544.3	361.8	99.0	182.5	262.8	612.2
2000	573.7	397.0	132.5	176.7	264.5	582.2
3000	539.3	365.7	97.5	173.6	268.2	608.2
4000	602.4	402.9	171.2	199.5	231.7	577.6
Mean±Sx	***574.0±14.6***	***385.0±10.5***	***136.8±17.4***	***189.0±5.8***	***248.2±8.4***	***593.0±8.8***
SD	***32.5***	***19.6***	***40.0***	***16.6***	***24.1***	***16.0***

According to Nemera (2016), the application of organic fertilizer did not significantly affect the NDF content of a natural pasture, but according to Delevatti et al. (2019) fertilizing has a significant effect on the fiber composition of the plant cell in natural grass stands.

The results of the treated variants show that the natural grass mass with imported 2000 and 4000 kg/da composted mixture has a higher concentration of neutral-detergent fibers, acid-detergent fibers and acid-detergent lignin.

The lignin fraction is a major factor influencing the digestibility of grass stand. The higher values of the indicator in the variants treated with 2000 and 4000 kg/da composted mixture also determine the lower *in vitro* digestibility of the dry matter (by 0.4-1.2%) compared to that of the grass stands with imported 1000 and 3000 kg/da composted mixture (although the variants fertilized with 2000 kg/da composted mixture registered the highest share of legumes in grass stands and the highest crude protein content, and fertilizers with a dose of 4000 kg/da composted mixture - the lowest concentration of crude protein). The values for *in vitro* digestibility of the dry matter in variants with 1000 kg/da (612.2 g kg^{-1}) and 3000 kg/da (608.2 g kg^{-1}) exceed the control by 4.0-4.7%.

The cellulose concentration in all fertilizer variants is higher than in the untreated control. The values of the indicator vary from 231.7 g kg^{-1} to 268.2 g kg^{-1}. The excess over the control is from 8.3 to 25.4%.

Compared with cellulose, the content of hemicellulose in the dry matter of the treated grass stands, as well as the degree of lignification indicate a downward trend. The fully digestible polioside (hemicellulose) values were lower by 6.2% (4000 kg/da) to 18.4% (3000 kg/da) compared to the untreated control (212.7 g kg^{-1}). With the highest lignification coefficient (28.40) are the grass stands treated with the maximum dose of composted mixture (4000 kg/da), whereas the lowest one was found in those treated with 3000 and 1000 kg/da (18.23-18.25) in the control variant - 29.17 (Fig. 3).

Correlation and regression dependences of dry matter yield with the botanical and qualitative composition of natural grassland (transitional type Chrysopogon gryllus-Agrostis capillaris) treated with a composted mixture

Table 3 shows the correlation dependences between some key indicators characterizing the productivity and quality of treated biomass.

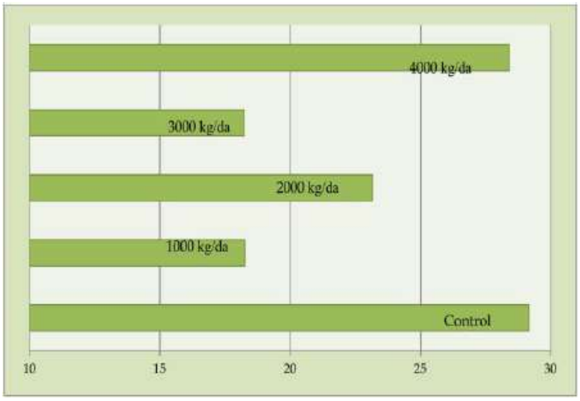

Fig. 3. Degree of lignification (coefficient) of natural grass stands (transitional type *Chrysopogon gryllus-Agrostis capillaris*) treated with composted mixture.

Table 3. Correlation and regression dependences of dry matter yield with the botanical and qualitative composition of a natural grass stand (transitional type *Chrysopogon gryllus-Agrostis capillaris*) treated with a composted mixture. *Legend:* *(P <0.05).

	Grasses	Legumes	Yield drymass	DM	CP	CF	CFr	Ash	NFE	Ca	P	N	NDF	ADF	ADL	Hemicell	Cellulose	IVDMD
Grasses	1																	
Legumes	0.25	1																
Yield dry mass	0.56	**0.94***	1															
DM	-0.35	-0.07	-0.13	1														
CP	0.27	**0.91***	0.84	-0.46	1													
CF	-0.10	0.67	0.58	0.66	0.30	1												
CFr	-0.52	-0.73	-0.77	0.60	-0.90	-0.01	1											
Ash	0.00	**0.94***	0.82	0.21	0.75	**0.85***	-0.48	1										
NFE	0.60	-0.04	0.14	-0.71	0.27	-0.66	-0.64	-0.34	1									
Ca	0.67	**0.86***	0.96	-0.37	0.86	0.36	-0.84	0.67	0.30	1								
P	**0.73***	**0.81***	0.95	-0.10	0.67	0.53	-0.64	0.67	0.13	0.94	1							
N	0.26	**0.91***	0.84	-0.45	1.00	0.31	-0.90	**0.76***	0.26	0.86	0.67	1						
NDF	-0.51	-0.87	-0.90	0.50	-0.93	-0.29	**0.85***	-0.68	-0.26	-0.96	-0.83	-0.93	1					
ADF	-0.51	-0.56	-0.61	**0.85***	-0.83	0.23	**0.92***	-0.27	-0.67	-0.77	-0.52	-0.83	**0.85***	1				
ADL	-0.74	-0.81	-0.94	0.38	-0.80	-0.32	**0.80***	-0.60	-0.32	-0.99	-0.95	-0.80	**0.94***	**0.75***	1			
Hemicellulose	-0.37	-0.92	-0.92	0.05	-0.77	-0.70	0.56	-0.87	0.19	-0.88	-0.89	-0.77	0.87	0.48	**0.86***	1		
Cellulose	**0.73***	**0.79***	0.93	-0.09	0.65	0.53	-0.61	0.66	0.10	0.93	1.00	0.65	-0.83	-0.51	-0.95	-0.90	1	
IVDMD	-0.07	0.28	0.17	-0.66	0.48	-0.14	-0.26	0.17	-0.06	0.34	0.13	0.47	-0.55	-0.56	-0.33	-0.39	0.16	1

A high positive correlation was found between dry matter yield and crude protein content (r = 0.84), minerals (r = 0.82), calcium (r = 0.96), phosphorus (r = 0.95), nitrogen (r = 0.84) and cellulose (r = 0.93). Correlation coefficients among dry matter yield with crude fiber content (r = -0.77), neutral-detergent fiber (r = -0.90), acid-detergent fiber (r = -0.61), acid-detergent lignin (r = -0.94) and hemicellulose (r = -0.92) were negative.

In the fertilization variants, the increased crude protein content in the dry matter composition strongly correlated with the amount of ash (r = 0.75), Ca (r = 0.86), P (r = 0.67) and N (r = 1.00). The values of the indicator are in strong negative dependence with the amount of crude fiber (r = -0.90) and the structural fiber components of the cell walls (r = from -0.77 to -0.93).

The high negative correlation dependence (r = -0.77 to -0.99) between the concentration of Ca, P and N with the fiber components of the cell walls (NDF, ADF and ADL) allows the amount of macronutrients to be determined with relatively high accuracy by the content of neutral-detergent fibers, acid-detergent fibers and acid-detergent lignin.

Theoretical regression lines and the regression equations between the weight percentage of legumes in the treated grass stands with the yield of dry matter, crude protein, ash and cellulose are presented in Fig. 4.

The equations by which it is possible to predict the amount of these indicators are respectively:

$$Y = 6.6091x + 42.333\ (R^2 = 0.8827);$$
$$Y = 1.2575x + 85.87\ (R^2 = 0.8195);$$
$$Y = 0.2736x + 55.58\ (R^2 = 0.8928);$$
$$Y = 5.0689x + 55.809\ (R^2 = 0.621).$$

The correlation coefficients between the quantitative share of legumes in the composition of the grass stand and the content of calcium, nitrogen and phosphorus have a high absolute value (r = 0.81-0.91), which corresponds to a strong empirical linear dependence (Fig. 5).

The equations by which it is possible to predict the amount of macronutrients in the composition of the dry matter are:

$$Y = 1.5473x - 22.63\ (R^2 = 0.7467) \text{ - for Ca;}$$
$$Y = 1.1812x + 12.349\ (R^2 = 0.8277) \text{ - for N;}$$
$$Y = 0.0494x + 0.0744\ (R^2 = 0.6491) \text{ – for P.}$$

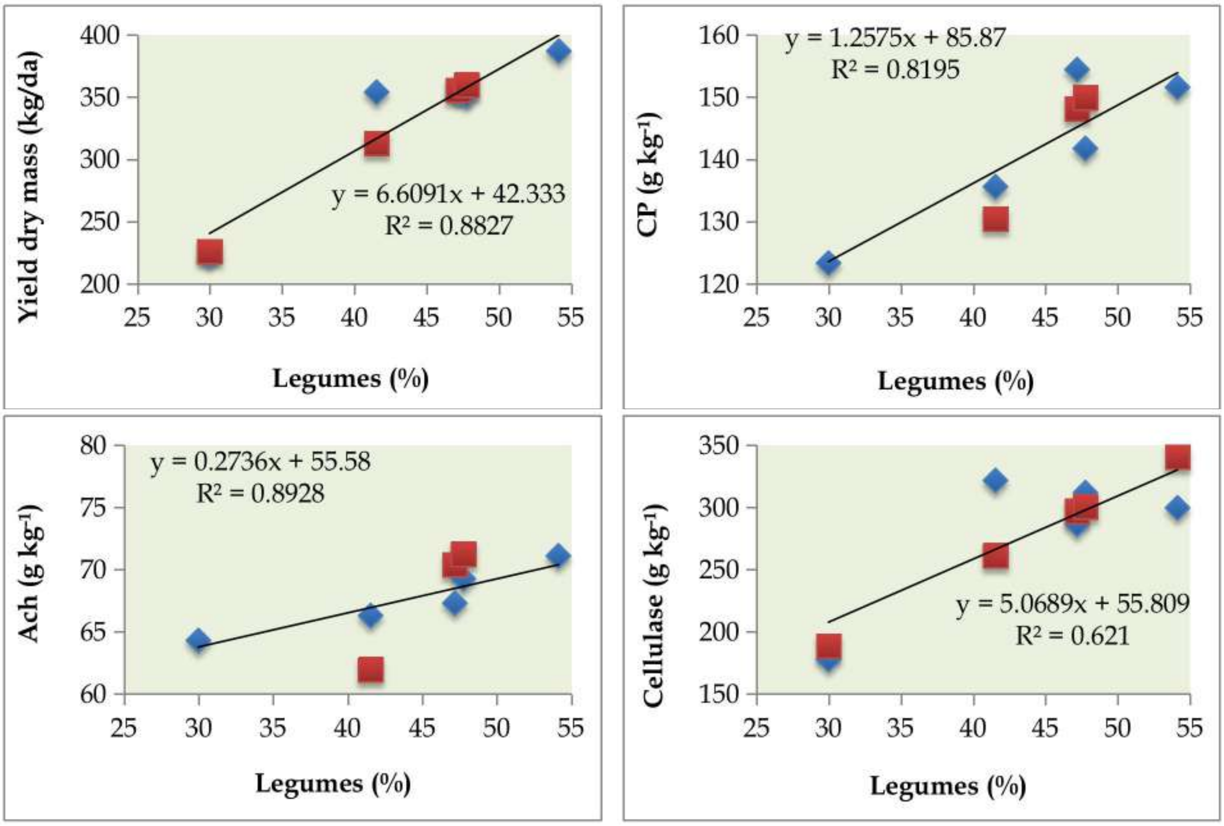

Fig. 4. Regression dependence between the percentage of legumes in grass stand with dry matter yield and the content of crude protein, ash and cellulose.

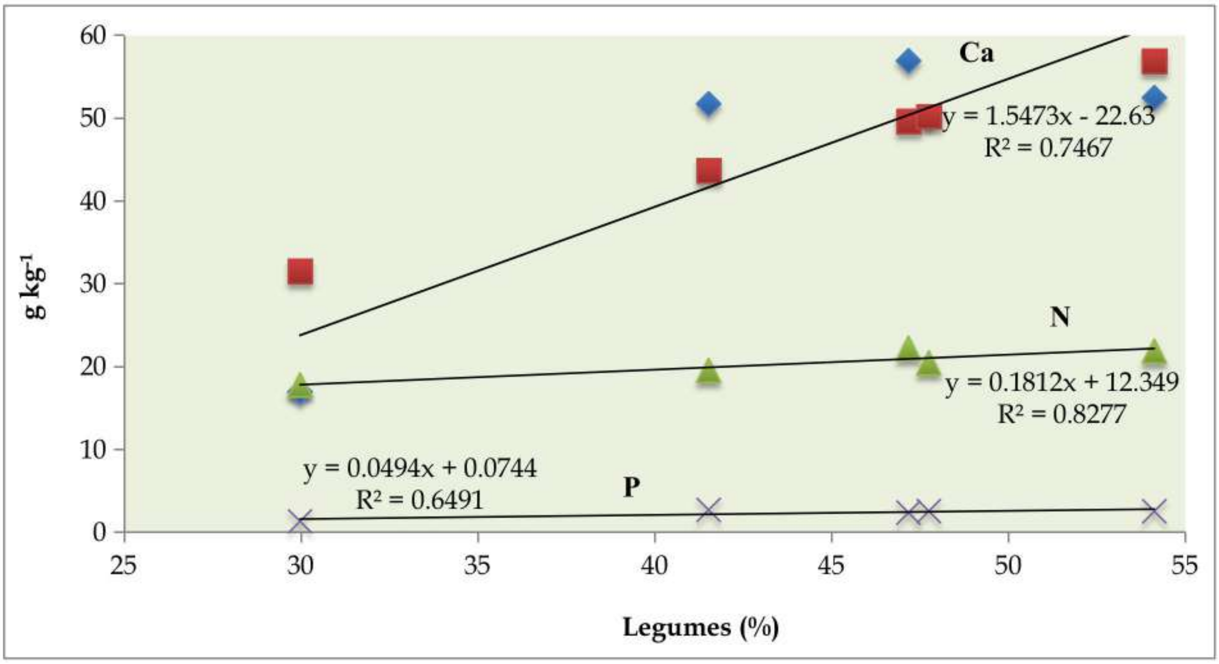

Fig. 5. Regression dependence between the percentage of legumes in grass stand with the content of nitrogen, phosphorus and calcium in the dry matter.

The weight percentage of meadow grasses had the greatest impact on the change in the average values of the element phosphorus and polyoside - cellulose (Fig. 6). The results of the analysis show a high positive correlation (r = 0.73) between the indicators.

The established graphical regression models reveal a good possibility for their approximate determination by the percentage share of grasses in the grassland:

$Y = 0.1207x – 0.2108$ ($R^2 = 0.5326$) - for P;

$Y = 12.667x - 20.683$ ($R^2 = 0.5335$) - for Cellulose.

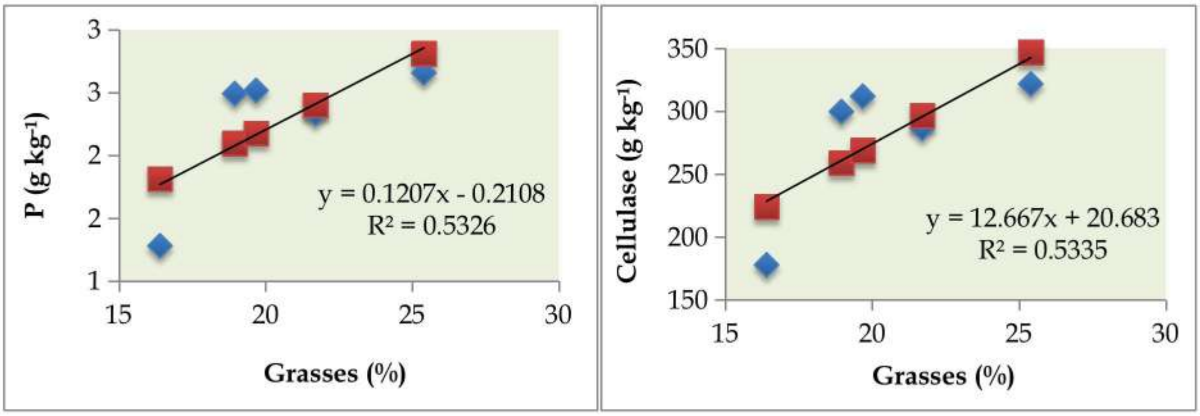

Fig. 6. Regression dependence between the percentage of legumes in grass stand with the content of phosphorus and cellulose in the dry matter.

From the point of view of practice, it will be significantly more economical, quick and easy to determine the estimated amount of calcium, nitrogen, phosphorus and cellulose by the botanical composition (legumes and cereals) of the stand.

Conclusions

The applied surface fertilizing increased the percentage share of useful legumes and grasses in the composition of the grass stand by 24.15 and 9.00%, respectively. The weight percentage of meadow grasses had the greatest impact on the change in the average values of calcium, nitrogen, phosphorus and cellulose in the dry matter of the treated grasses. From the point of view of practice, the developed graphical regression models based on the percentage share of legume and grass components in the amount of the obtained biomass is significantly more economical and fast method for predicting the amount of studied macronutrients and cellulose.

Variants with an imported dose of 2000 kg/da composted mixture registered the highest content of crude protein (160.2 g kg^{-1}), crude fat (26.5 g kg^{-1}) and minerals (71.1 g kg^{-1}). The excess in the values of the indicators compared to the untreated control was respectively 29.9% (for CP), 44.0% (for CF) and 10.6% (for Ash).

The grass stands in the fertilizing variants had a lower content of neutral-detergent fibers (by 1.3 to 11.6%), neutral-detergent lignin (from 6.9 to 47.0%) and hemicellulose (from 6.2 to 18.4%) compared to the untreated control.

The values for *in vitro* digestibility of the dry matter in variants with 1000 kg/da (612.2 g kg^{-1}) and 3000 kg/da (608.2 g kg^{-1}) exceeded the control by 4.0-4.7%.

A high positive correlation was found between dry matter yield and crude protein content (r = 0.84), minerals (r = 0.82), calcium (r = 0.96), phosphorus (r = 0.95), nitrogen (r = 0.84) and cellulose (r = 0.93).

In the fertilizing variants, the increased crude protein content in the dry matter composition strongly correlated with the amount of ash (r = 0.75), Ca (r = 0.86), P (r = 0.67) and N (r = 1.00). The values of the indicator are strongly negatively correlated with the amount of crude fiber (r = -0.90) and the structural fiber components of the cell walls (r = from -0.77 to -0.93).

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Received: 04.07.2022
Accepted: 05.10.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 51-62

Analysis of the Microbiological Characteristics of Soils from the Territory of Vitosha Nature Park, According to the Differences in the Environmental Conditions

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Abstract. An analysis of the microbiological status of 13 soil profiles from the territory of Vitosha Nature Park was carried out. Eight of the studied soils were defined as *Dystric* Cambisols (Brown Forest soils), and the other five as Umbrisols (Mountain Meadow soils). In order to assess the biogenicity and the prevailing processes of transformation of soil organic matter, the total microbial number was determined, as well as the percentage distribution of the main microbiological groups (spore-forming bacteria, non-spore-forming bacteria, actinomycetes and micromycetes) of the *A* horizon of the studied soils. The highest microbial abundance was observed in the altitude range from 1000 m a.s.l. up to 1600 m a.s.l. In higher altitudes there was a decrease in the total microbial number. At an altitude of over 2000 meters, soil microorganisms are strongly suppressed. According to the data received in the process of the study it has been found that at such altitudes, higher levels of organic carbon and total nitrogen do not result in greater microbial abundance. Altitude dynamics affects the values of the total microbial number, but no relationship has been established with the distribution of microbial groups. The results show a higher microbial abundance in the tested areas under deciduous vegetation compared to the ones under coniferous and grass vegetation. It was found that the lower pH values cause a greater abundance of the micromycete group.

Key words: microorganisms, total microbial number, forest soil, vegetation cover, altitude.

Introduction

Vitosha Mts. is among the few Bulgarian mountains that are subjected to immediate anthropogenic pressure, especially in summer, when there is a mountain-valley circulation, typical of the northern slopes of the mountain, where slope winds carry polluted air masses from the nearby capital – Sofia (Kadinov, 2021a). However, according to a study by Kadinov (2019), the presence of fine dust particles has a background status, with less than 1% due to transport from Sofia and does not indicate exceedances of particulate matter (PM) deposition rates on the soil. This is confirmed by the seasonal dynamics of the PM, whose maximum is in the summer, while in the capital it is in the winter (Kadinov, 2021b).

This is a prerequisite for analysis of the territory of Vitosha Nature Park as an environmentally friendly ecosystem.

Soil microorganisms are an essential part of the forest food network. They play a major role in the transformation of soil organic matter and the preparation of organic component transformation for inclusion in the biogeochemical cycle. A large number of studies are focusing on the important role of microorganisms as predictors of environmental change (Baldrian et al., 2012; Trivedi et al., 2013; Jansson & Hofmockel, 2020). Due to their sensitivity and adaptability, microorganisms can serve as an indicator of soil health in forest areas and from there they can provide timely information on the state and dynamic changes occurring in forest ecosystems as a result of global climate change. It is the rapid response to changes in the environment that allows microorganisms to be used as an indicator of changes in soil status (Kennedy et al., 1995; Pankhurst et al., 1995).

The abundance and distribution of microbial groups are important indicators of soil health and soil quality (Kennedy et al, 1995; Kibblewhite et al., 2008; Sharma et al., 2011).

The presence of high biogenicity of forest soils implies the processes of transformation of organic matter at a rate that provides the necessary nutrients for plants and maintains a regular cycle of substances.

Soil microorganisms are a key element of forest soils. They are involved in the transformation of plant materials into humus (Vanmechelen et al., 1997).

A number of studies have examined the influence of soil parameters on the abundance, activity and dynamics in the distribution of soil microorganisms. The main components influencing the microbial soil biota are considered to be the organic matter content, the content of total nitrogen and organic carbon and the acidity of the soil habitat (Fierer & Jackson, 2006; Lauber et al., 2009; Romanowicz et al., 2010).

Shen et al. (2013) point out that bacterial diversity and community composition are influenced by soil pH. The pH(H_2O) in the range of 5 and 7 was found to be optimal for microbiological activity and nutrient availability (Vammechelen et al., 1997). Although pH is an important factor in the development of microorganisms, Cho et al. (2016) and Lauber et al. (2009) established that it is not fundamental to soil biogenicity. Vegetation, and in particular the combination of tree species, influences soil microbiological activity (Algusto et al., 2015; Snajdr et al., 2013). The microbial diversity and percentage distribution of the main microbiological groups are influenced by the different dominant plant species (Prescott & Grayston, 2013).

Margestin et al. (2008) found that microbial activity decreases with increasing altitude and with decreasing temperature in soils of alpine and subalpine zones. Opposite data were also obtained in the study of Siles et al. (2016), in which an increase in abundance with an increase in altitude was reported in the study zones due to higher amounts of organic carbon, total nitrogen and other nutrients. The same study states that there are no stable trends in the composition and distribution of microorganisms with increasing altitude. In a large number of studies (Fritze et al., 2000; Taylor et al., 2002; Eilers et al., 2012), it has been stated that microbial abundance is highest in the *A* horizon. In order to have comparability of the results in the analysis of different soil units, the present study focuses on the biogenicity reported in *A* horizon.

Heterogeneity in forest soils determine various parameters for the development of soil microbial biota. The main aim of the present study is to analyze some of the microbiological parameters of forest soils from the territory of Vitosha Nature Park and their interrelations with soil parameters and the altitude.

Materials and Methods

The subject of the study is soils from the territory of Vitosha Nature Park. The studied territory covers almost the entire territory of Vitosha Mountain. According to Sabev & Stanev (1963) the territory of the Park falls into two climatic regions - the temperate continental climatic subregion - Climatic region of the hills and mountainous parts of Western Central Bulgaria and Mountain climatic region. Frequent short-term precipitations are observed under the influence of intramass convective clouds. As a result, summer precipitation significantly exceeds winter ones (Koleva-Lizama, 2018). The duration of the period with air temperature above 10 °C varies from 93 to 144 days, and the temperature sum above 10 °C is between 1130 °C and 2030 °C (Koleva-Lizama, 2018).

Vitosha Nature Park is characterized by the presence of a vertical band in relation to the vegetation cover. According to Velchev (2002) on the territory of the Park were expressed all plant belts except the alpine. Vitosha Nature Park is characterized by a rich and diverse flora. Of the higher seed plants, 1500 species have been identified, which represents half of the flora of Bulgaria (Tsavkov & Dimova, 2001). Research by Stefanov (1939) shows that among the deciduous species in Vitosha mountain pure beech (*Fagus sylvatica* L.) forests are with the highest prevalence. The current area of beech forests is 40.5% of the territory (Management Plan of Vitosha Nature Park, 2014). According to the data of the Management Plan of Vitosha Nature Park, 2014 the coniferous tree species occupy 34.7% of the afforested area, and with the largest share is *Pinus sylvestris* - 18.4%.

According to Ninov (2002) the territory of Vitosha mountain falls into 2 soil provinces. The first is Vitosha-Srednogorian mountain province, which is dominated by *Dystric – Eutric* Cambisols and Umbrisols, and the second - Mediterranean soil region, High Vitosha-Rila-Pirin-Rhodope province. Cambisols and Umbrisols are widespread in it.

The most widespread are the *Dystric – Eutric* Cambisols, which occupy the range between 600 m - 800 m above sea level as the lower limit and 1800 m above sea level as the upper limit (Petkov et al., 1983).

Soil samples were taken in November 2021, to analyze the peak development of microbial communities at the end of the autumn season. Microbiological samples were collected using sterile instruments. The collected soil samples were placed in sterile paper bags according to all sterility guidelines. Thirteen soil profiles were surveyed. Soil material for analysis was collected from *A* horizon. Laboratory analyzes were performed to determine the main soil characteristics, as follows - soil acidity (pH(H_2O)) - ISO 10390; the organic carbon content - modified method of Turin (Filcheva & Tsadilas, 2002), and the Kjeldahl digestion (ISO 11261) method was carried out to determine total nitrogen content.

Microbiological studies of the collected soil samples were performed immediately after their collection. Basic microbiological parameters related to soil biogenicity were analyzed - total microbial number, determination of percentage distribution of different microbial groups (spore forming bacteria, non-spore forming bacteria, actinomycetes and micromycetes). Koch's method was used to determine the total microbial number. The method includes successive dilutions and subsequent inoculation on appropriate elective agar medium (Davis et al., 2005). For the determination of spore-forming bacteria, the soil extract was previously pasteurized. Nutrient agar was used for the cultivation of spore-forming bacteria and non-spore-forming bacteria. Actinomycete isolation agar was used to isolate actinomycetes. Capek Dox agar is used for the isolation of micromycetes (Parks & Roland, 1997). The count was expressed as colony forming units per gram dry soil (CFU/g dry soil) under logarithm (lg). Software was used to perform the statistical data processing - StatSoft Statistica 12 under significance thresholds 95%.

Results

Thirteen soil profiles were studied and analyzed. The morphological characteristics of the soil profiles are shown in Table №1. The soils from tested areas (TAs) 1-8 are determined as *Dystric* Cambisols and the soils form tested areas 9-13 as Umbrisols, according to the WRBSR (2006,2007).

The considered soil profiles are set at an altitude of 1032 m to 2261 m in order to cover the variety of altitude gradient of the Vitosha Nature Park (Fig. 1).

The soils of all tested areas are acidic, which is due to the leaching of the bases from the profile. The more acidic reaction of the soil is due to the increased amount of precipitation and the decrease in temperatures with increasing altitude. This trend is clearly expressed when considering the reported acidity of soils in the range 1500-1800 m altitude compared to soils in the range 1000-1500 m altitude. This acidification is also due to the fact that at higher altitudes mainly coniferous vegetation predominates, which determines the course of acidification processes in soils.

Table 1. Main characteristics of soil profiles.

TA	**Soil Unit**	**Location**	**Altitude (m)**	**Exposure**	**Vegetation**	**Soil horizons**	**pH**	**Humus (%)**	**Org. C g.kg^{-1}**	**Total N g.kg-1**	**C:N**
1.	Dystric Cambisols	42°38'29.73"N 23°13'4.56"E	1032	N	*F. sylvatica*	A	5.90	3.08	18.20	1.28	14
2.	Dystric Cambisols	42°38'17.00"N 23°13'9.27"E	1057	W	*Q. petraea* *F. sylvatica*	A	5.50	3.20	18.72	1.49	13
3.	Dystric Cambisols	42° 27' 16'' N, 23° 13' 14'' E	1151	S	*Q. petraea* *Q. cerris* *Q. frainetto*	A	5.40	3.30	19.50	1.33	15
4.	Dystric Cambisols	42° 29' 53'' N, 23° 12' 51'' E.	1163	S	*C. betulus* *C. monogyna* *C. avellana*	A	5.90	5.83	33.80	3.83	9
5.	Dystric Cambisols	42° 31' 11'' N, 23° 21' 9'' E	1171	S	*Q.s petraea* *P. nigra* *Q. cerris*	A	5.30	7.60	44.10	2.42	18
6.	Dystric Cambisols	42°36'28.37"N 23°17'50.53"E	1289	N W	*F. sylvatica*	A	5.30	4.46	25.90	2.00	13
7.	Dystric Cambisols	42°35'50.79"N 23°14'12.26"E	1565	N W	*P. abies*	A	4.30	9.39	54.50	4.74	12
8.	Dystric Cambisols	42°35'20.81"N 23°18'37.25"E	1598	SE	*P. sylvestris*	A	5.00	10.56	61.40	5.26	12
9.	Umbrisols	42°35'17.79"N 23°14'47.25"E	1788	SE	*J. communis*	A	4.60	6.78	38.17	5.85	7
10.	Umbrisols	42°35'25.36"N 23°17'24.96"E	1793	E	*P. peuce*	A	4.70	6.98	39.98	5.93	7
11.	Umbrisols	42°35'26.48"N 23°17'24.99"E	1793	E	*F. valida*	A	4.60	7.18	41.04	5.60	7
12.	Umbrisols	42°35'14.17"N 23°17'19.88"E	1845	SE	*P. mugo*	A	5.00	12.01	69.8	5.31	13
13.	Umbrisols	42°33'49.28"N 23°16'48.22"E	2261	S	*N. stricta, Vaccinium sp, Juniperus sp.*	A	4.50	20.02	117.4	9.4	12

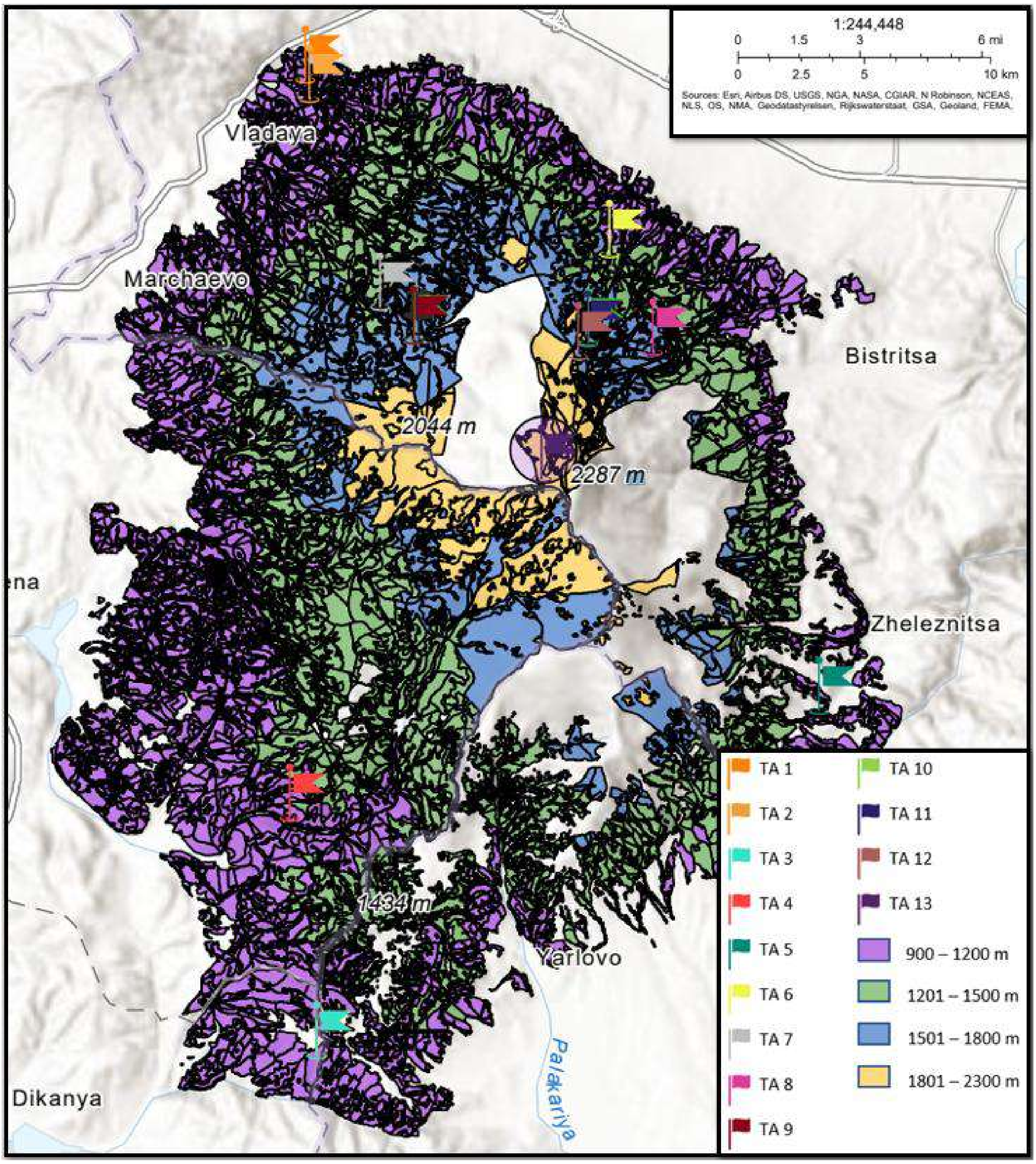

Fig. 1. Location of tested areas (TAs) on the territory of Vitosha Nature Park.

Correlation analysis was performed at 95% significance thresholds to assess the relationship between elevation increase and changes in humus percentage. The reported correlation coefficient is 0.83. As a result of this relationship, there is a direct proportionality between changes in altitude and organic carbon content (r = 0.82). Soils located at higher altitudes contain more organic carbon. The obtained data on the content of organic carbon in the studied soils show that it varies from low to very high (according to the Vanmechelen scale, 1997).

The strongest correlation was found between the increase in altitude and the change in total nitrogen content. The correlation is positive, in which the content of total nitrogen increases with increasing altitude (r = 0.96). The nitrogen content was assessed as average in TA1,2,3,5 and 6, as high in TA4 and TA7 and as very high in TA 8,9,10,11,12,13 according to the scale of Vanmechelen (1997). The organic carbon/total nitrogen ratios are in the range of 7-18. They are rated as very low to value 16 and as low to value 18 on the Vanmechelen scale. The results obtained show that the decomposition prosses are in an advanced stage.

Soil biogenicity is a dynamic indicator that is related to the parameters of the soil. The dynamics in the number and percentage distribution of soil microorganisms is an indicator of the activity of the transformation of soil organic matter. The results for the total microbial number and the distribution of the microbial community reflect the biogenicity of the studied soils. The results of the analyzed microbiological indicators are shown in Table 2.

Table 2. Total microbial number and number of main microbial groups in lg CFU/g dry soil.

TA	Total microbial number	Spore forming bactiria	Non-spore forming bacteria	Actynomicetes	Micromycetes
1.	6.32 ± 0.15	5.58 ± 0.16	5.72 ± 0.19	4.88 ± 0.23	5.13 ± 0.37
2.	6.36 ± 0.15	5.62 ± 0.16	5.85 ± 0.19	4.62 ± 0.23	5.09 ± 0.37
3.	6.40 ± 0.15	5.96 ± 0.16	6.11 ± 0.19	5.00 ± 0.23	5.26 ± 0.37
4.	6.41 ± 0.15	5.88 ± 0.16	6.18 ± 0.19	5.10 ± 0.23	5.28 ± 0.37
5.	6.32 ± 0.15	5.88 ± 0.16	6.00 ± 0.19	5.17 ± 0.23	5.27 ± 0.37
6.	6.31 ± 0.15	5.90 ± 0.16	6.00 ± 0.19	5.06 ± 0.23	5.08 ± 0.37
7.	6.30 ± 0.15	5.84 ± 0.16	5.90 ± 0.19	5.49 ± 0.23	5.30 ± 0.37
8.	6.26 ± 0.15	5.73 ± 0.16	6.04 ± 0.19	4.93 ± 0.23	4.88 ± 0.37
9.	6.10 ± 0.15	5.71 ± 0.16	5.74 ± 0.19	5.26 ± 0.23	4.43 ± 0.37
10.	6.11 ± 0.15	5.51 ± 0.16	5.65 ± 0.19	5.15 ± 0.23	5.59 ± 0.37
11.	6.18 ± 0.15	5.69 ± 0.16	5.76 ± 0.19	5.42 ± 0.23	5.21 ± 0.37
12.	6.00 ± 0.15	5.54 ± 0.16	5.69 ± 0.19	5.15 ± 0.23	4.30 ± 0.37
13.	5.96 ± 0.15	5.51 ± 0.16	5.59 ± 0.19	5.17 ± 0.23	4.72 ± 0.37

The total microbial number of microorganisms in the tested accumulative horizons –varied from 5.96 lg CFU/g dry soil to 6.41 lg CFU/g dry soil.

The tested area (TA) 4 of Dystric Cambisols at an altitude of 1163 m, under mixed deciduous vegetation, stands out with the highest biogenicity. The tested area with the lowest total microbial count is at the highest altitude (TA 13). The obtained results show a greater microbial abundance under deciduous or mixed vegetation, compared to conifers and grasslands. This is a result of both greater diversity and reduced competition in soils under deciduous forests, and of the reduced temperature and reduced activity of microbial communities at higher altitudes, where coniferous vegetation predominates.

Statistical processing of the results was performed, and a correlation between the microbiological indicators and the environmental parameters was found.

Statistical evaluation of the results shows the presence of a high statistical coefficient between the biogenicity of the studied soils and the increase in altitude at 95% significance thresholds. The correlation is negative. The high correlation coefficient (r = - 0.92) determines the strong influence of altitude on the amount of soil microflora (Fig. 1). As a redistributor of the other factors of the relief, the changes in the altitude lead to changes not only in the temperature and water regime, but also to changes in the vegetation cover. The obtained results clearly show that at altitudes above 1600 meters, the soil microbial abundance is reduced, and above 2000 meters, the soil microbial biota is strongly suppressed (Fig. 2).

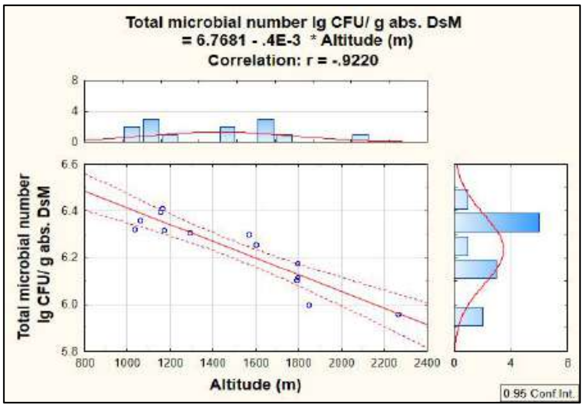

Fig. 2. Statistical relationship between total microbial number and altitude.

A correlation was made between the pH values and the reported biogenicity of the tested areas (Fig. 3). The correlation coefficient is relatively low (0.67), which shows a weak relation between pH values and differences in the microbial abundance of the studied soil profiles. The data obtained correspond to other studies in which it was found that soil acidity is not a major factor in its biogenicity (Cho et al., 2016; Lauber et al., 2009).

Although Khatoon et al. (2017) established that organic carbon is a source of nutrients and plays a vital role in maintaining the activity of soil microflora, its content and impact on soil microbial biota should be considered comprehensively together with other factors of the environment.

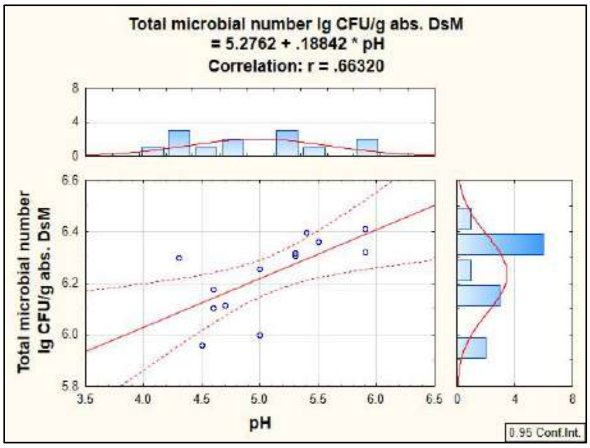

Fig. 3. Statistical relationship between total microbial number and pH.

The results of the correlation analysis show that there is an inverse relationship between the content of organic carbon and the abundance of soil microorganisms in the studied soil profiles (Fig. 4). This negative correlation is related to the positive correlation between the increase in altitude and the increase in the content of organic carbon. The obtained relationships show that although the carbon content increases with increasing altitude, the microbial biota decreases, especially above 1800 m. This decrease in the total microbial number in the high parts of Vitosha Nature Park is associated with an increase in the suppressive effect of environmental factors - mainly the temperature decrease.

When looking for a correlation between the total biogenicity of the studied soil profiles, relative to the content of total nitrogen, we have found once again an inverse relationship (Fig. 5). Although the total nitrogen content increases with increasing altitude, this does not lead to an increase in microbial abundance. Again, the strong influence of the height gradient on the microbial abundance of forest soils stands out.

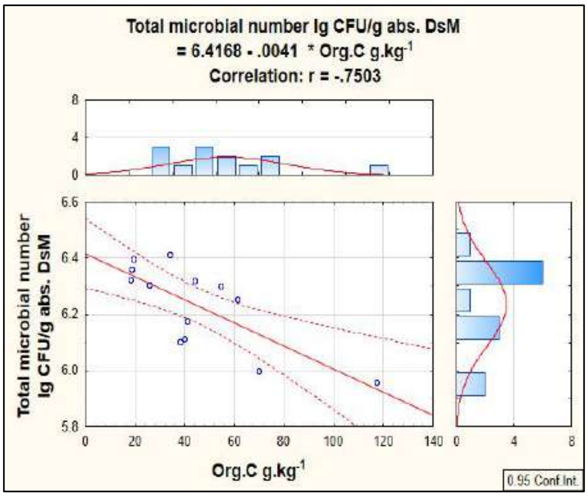

Fig. 4. Statistical relationship between total microbial number and organic carbon content.

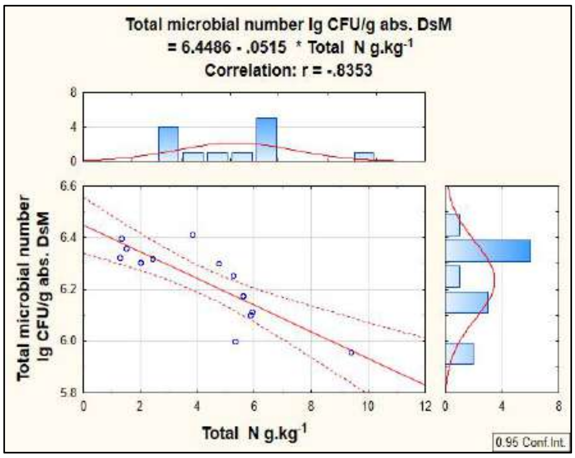

Fig. 5. Statistical relationship between total microbial number and total nitrogen.

In the present study, altitude stands out as one of the main factors related to the total microbial number of the studied soils.

Fig. 6 shows the composition of the microbial communities by groups as a percentage of the total microflora for the individual TAs., including acidity.

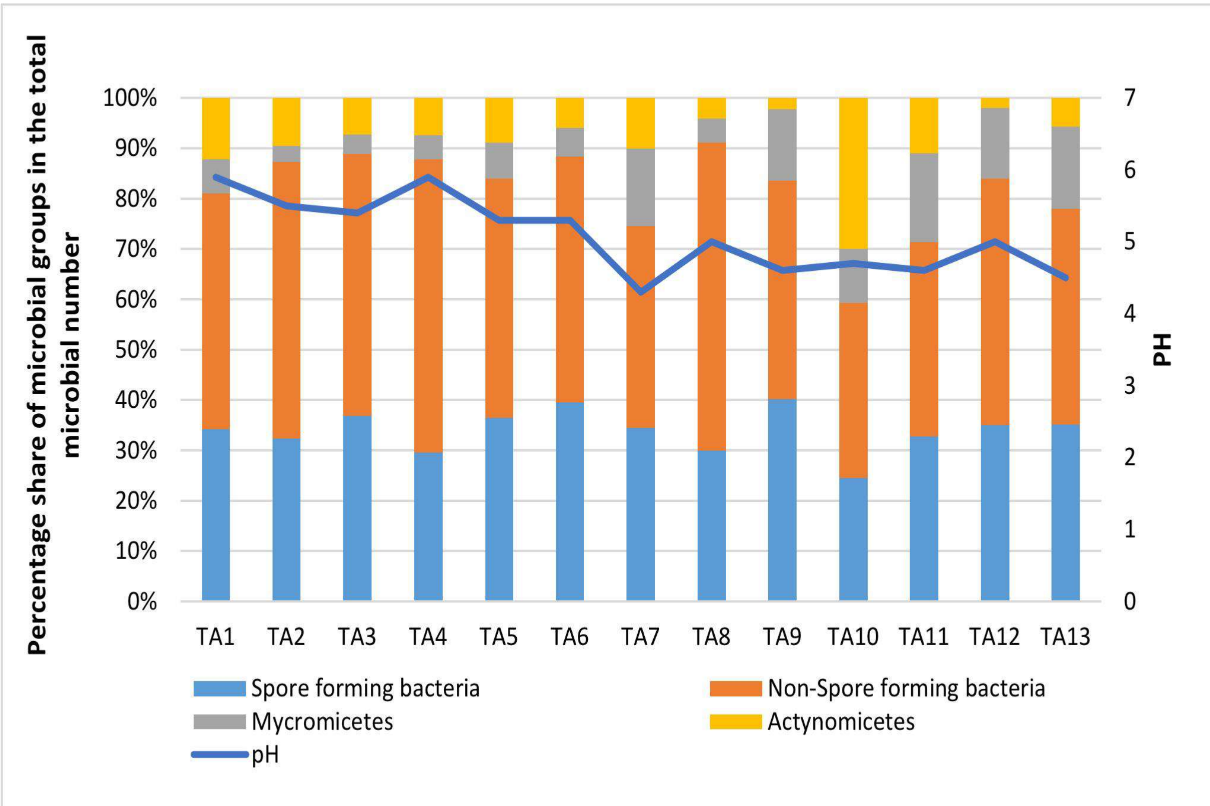

Fig. 6. Percentage share of microbial groups in the total microbial number, (%).

The predominance of a specific group of microorganisms in the soil shows the predominance of certain processes in the transformation of soil organic matter. Non-spore-forming bacteria dominate in all studied tested areas, followed by spore-forming ones. This distribution of microbial groups is an indication of the predominance of the processes of transformation of more easily degradable soil organic matter.

There is no established relationship between the distribution of the main microbial groups and a change in the content of organic carbon, total nitrogen or change in altitude. However, in terms of acidity, there is an increased percentage of micromycetes involved as part of the total microflora in soils with a pH below 5 (TA 7,9,10,11,12,13).

Discussion

One of the main tasks of the present study was to look for relationships between changes in the parameters of the studied soil profiles, changes in altitude and changes in microbial abundance and the relative participation of basic microbial groups in forest soil microbial community.

The study of soil microorganisms is a dynamic process. Their adaptability to the environment, together with their ability to react faster than all other participants in forest ecosystems, makes them an extremely interesting object of analysis. Numerous studies are being conducted around the world to find lasting links between soil microorganisms and the environment. Studies have been carried out to determine soil acidity as a driving factor for microbial abundance. (Lauber et al., 2009; Shen et al., 2013). Conducted are studies that prove the priority impact of the content of organic carbon (Khatoon et al., 2017) and total nitrogen (Egamberdieva, 2011) and others who point out that altitude should be considered as a fundamental factor for the development of soil microflora in forest areas, due to its major role in the distribution of other environmental factors (Margestin et al., 2008; Siles et al., 2016). All these studies demonstrate the need for a comprehensive and in-depth approach involving long-term research.

In the present study, we aimed to cover the maximum altitude range of the Park area, which would cover the diversity of soil microbial habitats. We studied the availability of a statistical relationship between soil parameters, changes in altitude and microbial abundance, including the dynamics of the distribution of basic microbial groups. The present study shows a significant negative correlation between microbial abundance and altitude increase. The results of our study show the main role that altitude plays in the abundance of microorganisms from the soils of Vitosha nature park.

Conclusions

We concluded that altitude is the environmental factor most strongly related to total microbial number. It was found that the greatest microbial abundance, occurs at an altitude of 1000 to 1600 m. The content of organic carbon and total nitrogen, although important for the development of soil microflora are strongly influenced by altitude and its role in redistribution of temperature, humidity and the predominant vegetation cover. Soil acidity is not associated with changes in microbial abundance, but an increased amount of micromycetes is found in more acidic soils, regardless of their altitude. Greater microbial abundance was found in the sample areas under deciduous vegetation compared to coniferous and grassy vegetation.

The present study, as well as all its results, can serve as a basis for conducting a multi-year analysis by seasons of the surveyed areas. This would make it possible to carry out a more in-depth analysis and to look for sustainable relationships over a long-term period of time, covering various environmental factors, potentially affecting soil microbial biota.

Acknowledgements

The current study was performed in the framework of "Study of interrelations in the system "soil - soil microorganisms - tree composition" in forest stands on the territory of „Vitosha" Nature Park." – KP-06-N56/7" funded by Research Fund of Ministry of Education and Science in Bulgaria.

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Received: 27.04.2022
Accepted: 16.10.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 63-74

Monitoring of Heavy Metals Migration into Edaphic Horizons of Coal Mine Dumps

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Abstract. Mining of minerals is accompanied by direct irreversible changes in the biosphere. An analysis of the latest researches on the technogenic hazard of rock dumps at coal mines, as well as specifics of their reclamation and phytomelioration in a global context, necessiates the study of the ecological state and natural phytomelioration of the Nadiya mine waste heap in the Lviv-Volyn coal basin (Ukraine). This scientific work presents the results of research on heavy metals migration into the genetic horizons of the rock dump, as well as their influence on forming the phytomeliorative cover on its surface. It has been revealed that the studied site of the No.4 rock dump in the 0-15 cm horizon, which is located from the eastern side near the foot, is the most contaminated with heavy metals. The indicators of heavy metal content at the background site, which is located within a radius of 3 km from the rock dump, are the lowest in the 0-15 cm horizon. The uneven distribution of heavy metals in the genetic horizons of the rock dump substrate is caused by frequent landslides, changing rock acidity, heterogeneity of natural overgrowth, and existing combustion sources. In the event of precipitation, toxic compounds, heavy metals and other harmful substances are washed out from the rock dump surface, and in our case, these substances also enter the natural water body.

Key words: rock dump, coal mine, ecological hazard, natural overgrowth, technogenic reservoir, heavy metals.

Introduction

The Lviv-Volyn coal basin functioning has led to numerous negative changes in flora and fauna, atmosphere, hydrosphere, biosphere, and also had a significant impact on the life and health of people of Ukraine and Poland, on the border with which it is located (Bosak et al., 2020). The basin includes three mining districts – Chervonohrad, Novovolynsk and Southwest districts, mining in which began in the 60s of the twentieth century.

In the global context, a lot of scientific papers have been devoted to the environmental and technogenic hazard of rock dumps. Among such works, it is worth noting the scientific research of Polish scientists (Abramowicz et al., 2021; Nadudvari et al., 2021). In particular, changes in organic and mineral substances caused by thermal effects have been revealed in coal waste. Irregular fractures and fissures appear inside and at the edges of the organic substance particles, which oxidize, volatilize and plasticize. Mineral phases have undergone oxidation, dehydration, structure restoration and recrystallization (Nadudvari et al., 2021). The vegetation area distribution due to changes in soil thermals has been studied for three periods within the selected transect, on which three types of surfaces with different soil thermals and spontaneous combustion directions have been distinguished. The temperature ranges from +9.9°C to +139°C at a depth of 20 cm and at the same time from +3.1°C to +69.0°C on the surface. The total organic carbon content in all samples ranges from 1.7% to 7.6% and at the same time from 3.1% to 4.5% in places of active fire. The total nitrogen concentration ranges from 0.023% to 0.29%. The soil (pH) reaction ranges from 5.8 to 8.0 (in H_2O). The vegetation area variability in time and space indicates the directions of displacement of the fire spots. The analysis has shown that underground temperature has a significant impact on the distribution and species composition of plants growing on coal dumps (Abramowicz et al., 2021).

Studies conducted at coal mines in North Africa (Onifade & Genc, 2018) have revealed that coal has a higher reactivity to spontaneous combustion than shale. Both materials show that an increase in the content of carbon, moisture, hydrogen, volatile substances, nitrogen and a decrease in the ash content can add to the tendency to spontaneous combustion. An approximate and final analysis of the tested samples indicates that these properties can be used as a tool to measure the tendency to spontaneous combustion.

Further research on petrographic analysis and detailed geotechnical study of coal and coal shale has been conducted to assess their impact on predicting and minimizing spontaneous heating events (Wu et al., 2019; Petlovanyi et al., 2020; Vo et al., 2022). In addition to the impact of rock dumps, significant damage to the environment is caused by oil pollution of soils resulting from emergency situations (Karabyn et al., 2019; Popovych et al., 2019).

The distribution of heavy metals depending on the places of burning rock dumps and the influence of these phenomena on natural phytoremediation processes within the limits of the Lviv-Volyn coal basin has not been fully investigated. The development of vegetation on waste dumps of coal mines depends on the temperature of the substrate, microclimate, climate, radiation background and other landscape-transforming factors. We did not find a combination of this kind of research in the process of analyzing scientific sources. For the most part, researchers limit themselves to the analysis of one direction of research on rock dumps of coal mines.

Materials and Methods

The geographical location of the Lviv-Volyn coal basin corresponds to the zone of Male Polissia, the climate of which is influenced by the air masses of the Atlantic. Since the Chervonohrad Mining District was the first to be mined, the largest rock volume is concentrated on its waste heaps. Nadiya mine, with a waste heap volume of 2869.4 thousand m^3, is one of the largest mines in the district (Fig. 1).

To determine the technogenic-ecological situation and take substrate samples for composition analysis in stationary laboratories, 7 sites are selected in the zone of the Nadiya mine rock dump influence for rock sampling from depths of 0-15 cm and 0-20 cm. In addition, water samples are taken from the centre of a technogenic reservoir, formed as a result of anthropogenic activities, which accumulates effluents from the dump. Coordinates of the rock dump location are 50.296540, 24.271369.

The edaphotopes from rock dumps are sampled according to the standard (DSTU ISO 10381-1:2004). The analysis of the micronutrient content in the selected rock substrates is performed using the ICP-MS device in the chemical laboratory of the Freiberg University of Mining and Technology (Saxony, Germany). The method for performing analyses is microwave induced combustion with subsequent measurement by the method of inductive-plasma mass spectrometry. The maximum permissible concentrations (MPC) of heavy metal content in the rock are compared with the data given in the works (Miedviediev & Laktionova, 1998; Kuraeva et al., 2012).

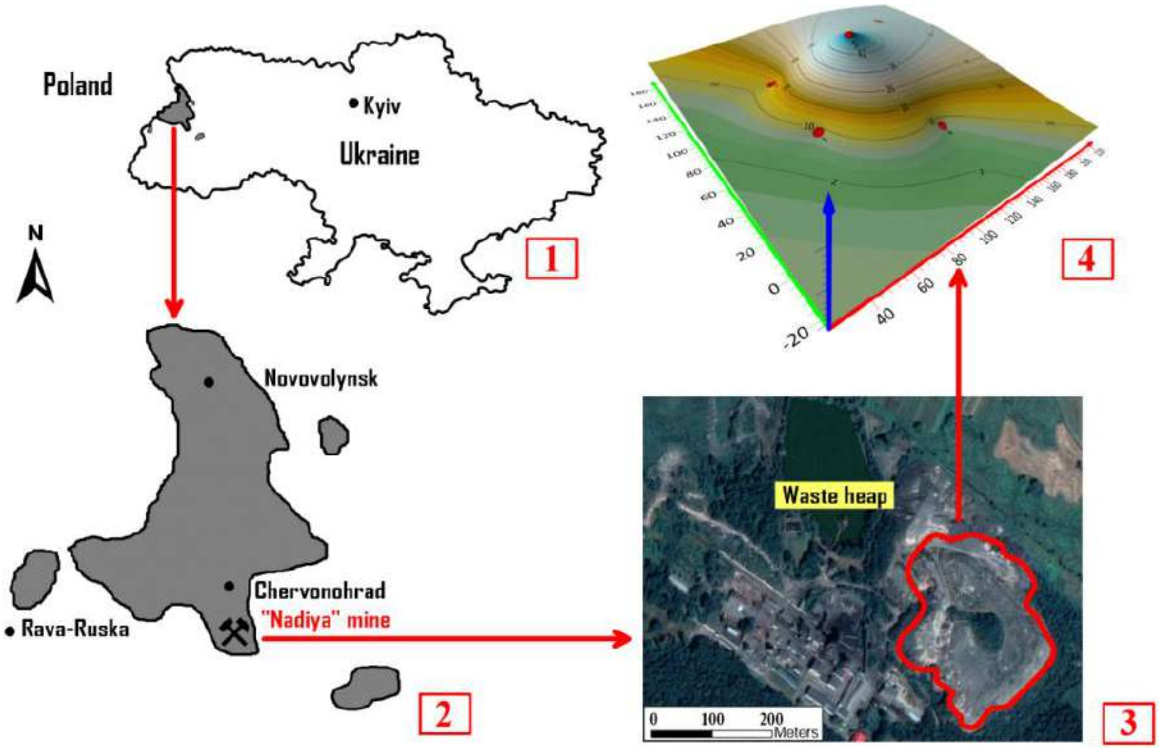


Fig. 1. Layout of the studied object: 1 – a map of Ukraine with the designation of the Lviv-Volyn coal basin; 2 – the Lviv-Volyn coal basin scheme with the designation of the Nadiya mine; 3 – the Nadiya mine map with the designation of a rock dump, made using GoogleMaps; 4 – 3-D rock dump model.

Results and Discussion

Analysis of the gross content of heavy metals Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb in the 0-15 cm and 0-20 cm horizons of the Nadiya mine rock dump in the Chervonohrad Mining District indicates that their values do not exceed the maximum permissible concentrations for soils. The only value exceeding the maximum permissible concentrations is set for Cu (studied site No.4) near the rock dump foot from the east. However, the indicators of heavy metal content in comparison with the background values (studied site No.6) exceed for individual elements by dozens of times.

The highest Mn content (894.6 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Mn content (19 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump. In the 0-20 cm horizon, the highest Mn content (259.6 mg/kg) is observed at the studied site No.2 on the western side of the rock dump slope. The lowest Mn content in the 0-20 cm horizon (25.6 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Mn (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 2.

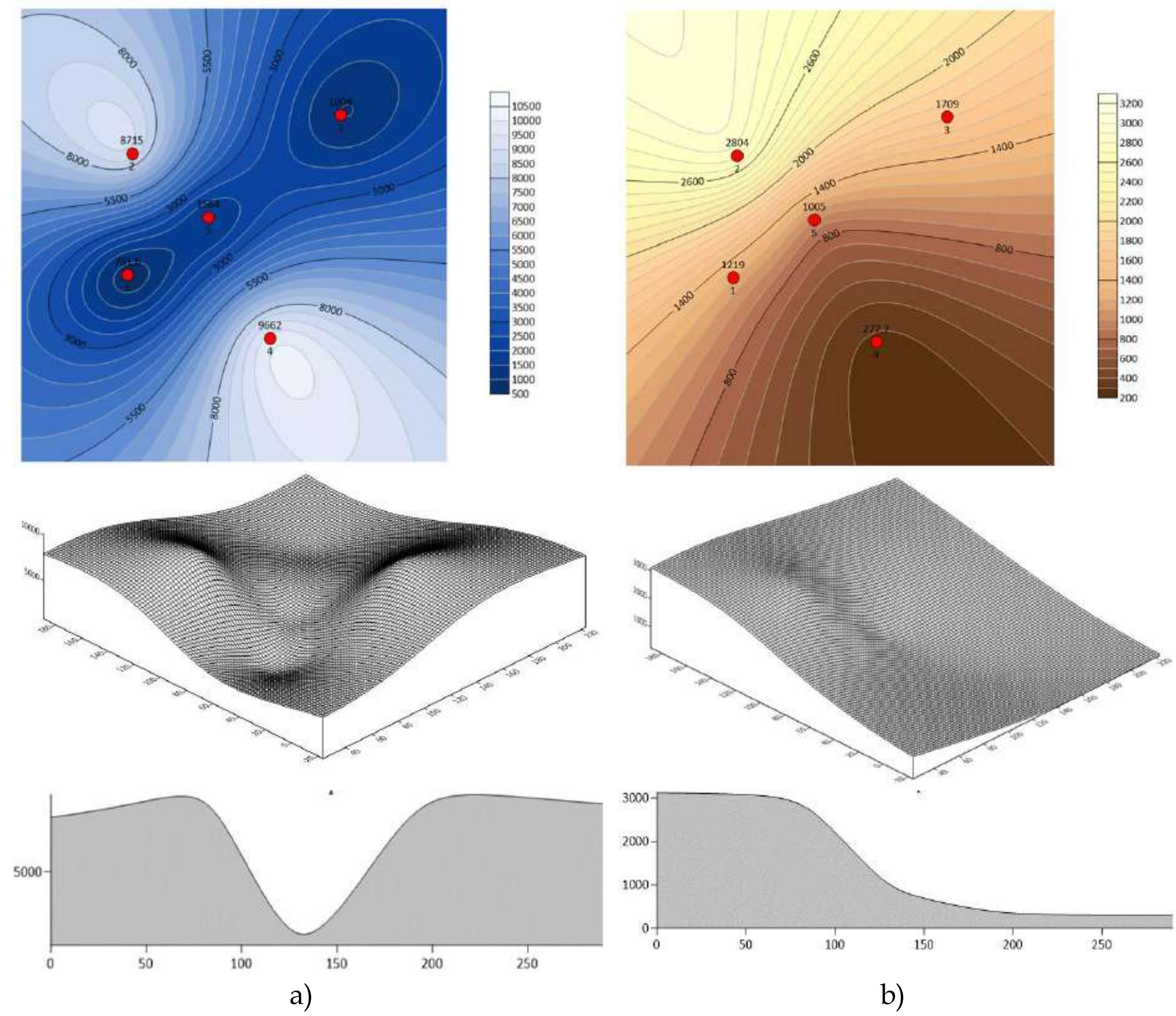
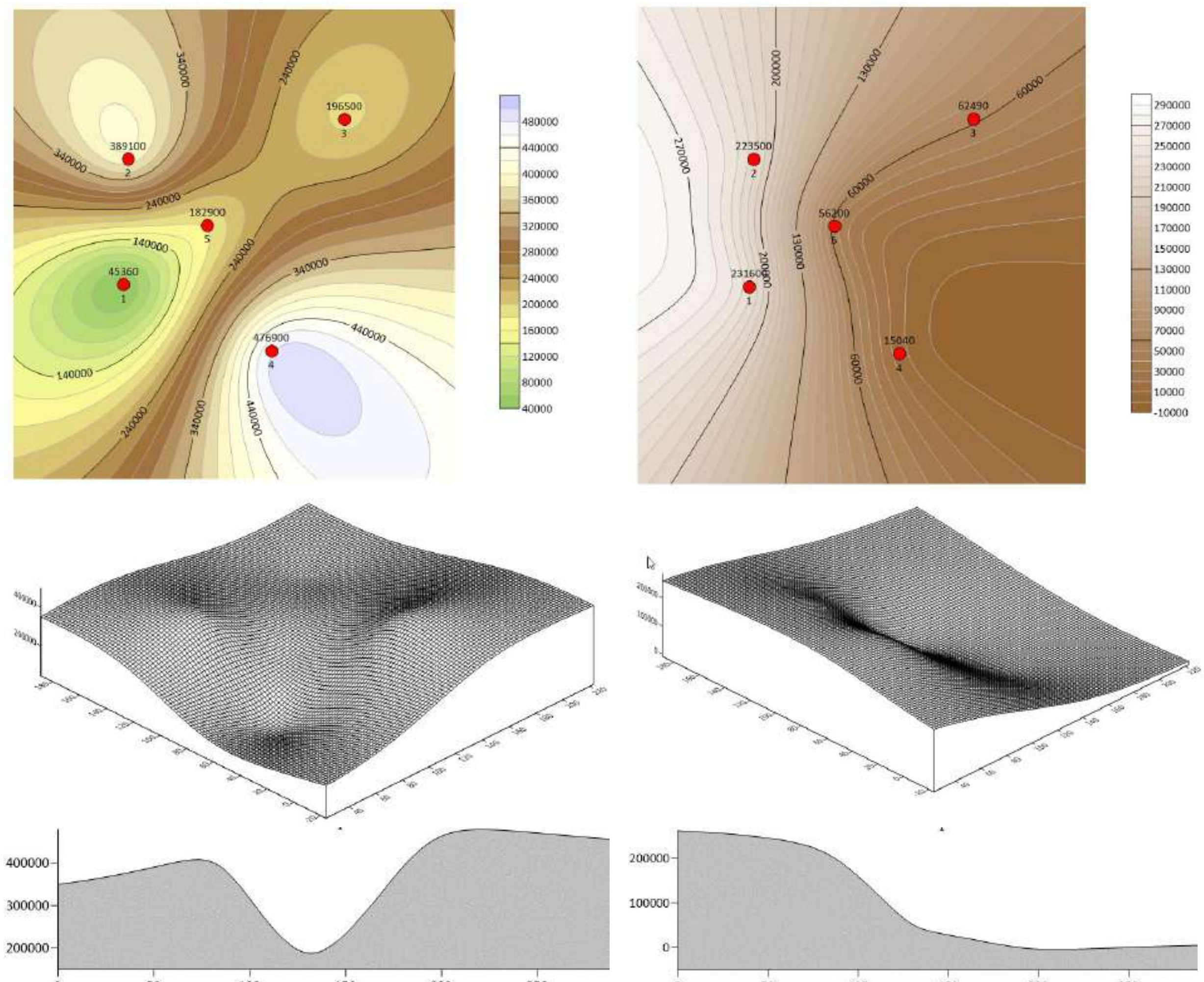


Fig. 2. Distribution of Mn (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.

The highest Fe content (44157.4 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Fe content (1128.7 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump.

In the 0-20 cm horizon, the highest Fe content (21444.4 mg/kg) is observed at the studied site No.1 on the southern side near the rock dump foot. The lowest Fe content in the 0-20 cm horizon (1392.5 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Fe (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 3.

The highest Co content (23.8 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Co content (0.3 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump.



a) b)

Fig. 3. Distribution of Fe (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.

In the 0-20 cm horizon, the highest Co content (9.2 mg/kg) is observed at the studied site No.2 on the western side of the rock dump slope. The lowest Co content in the 0-20 cm horizon (0.4 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Co (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 4.

The highest Ni content (67.6 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Ni content (10.9 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump.

In the 0-20 cm horizon, the highest Ni content (36.1 mg/kg) is observed at the studied site No.6 (area with a background value). The lowest Ni content in the 0-20 cm horizon (11.8 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Ni (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 5.

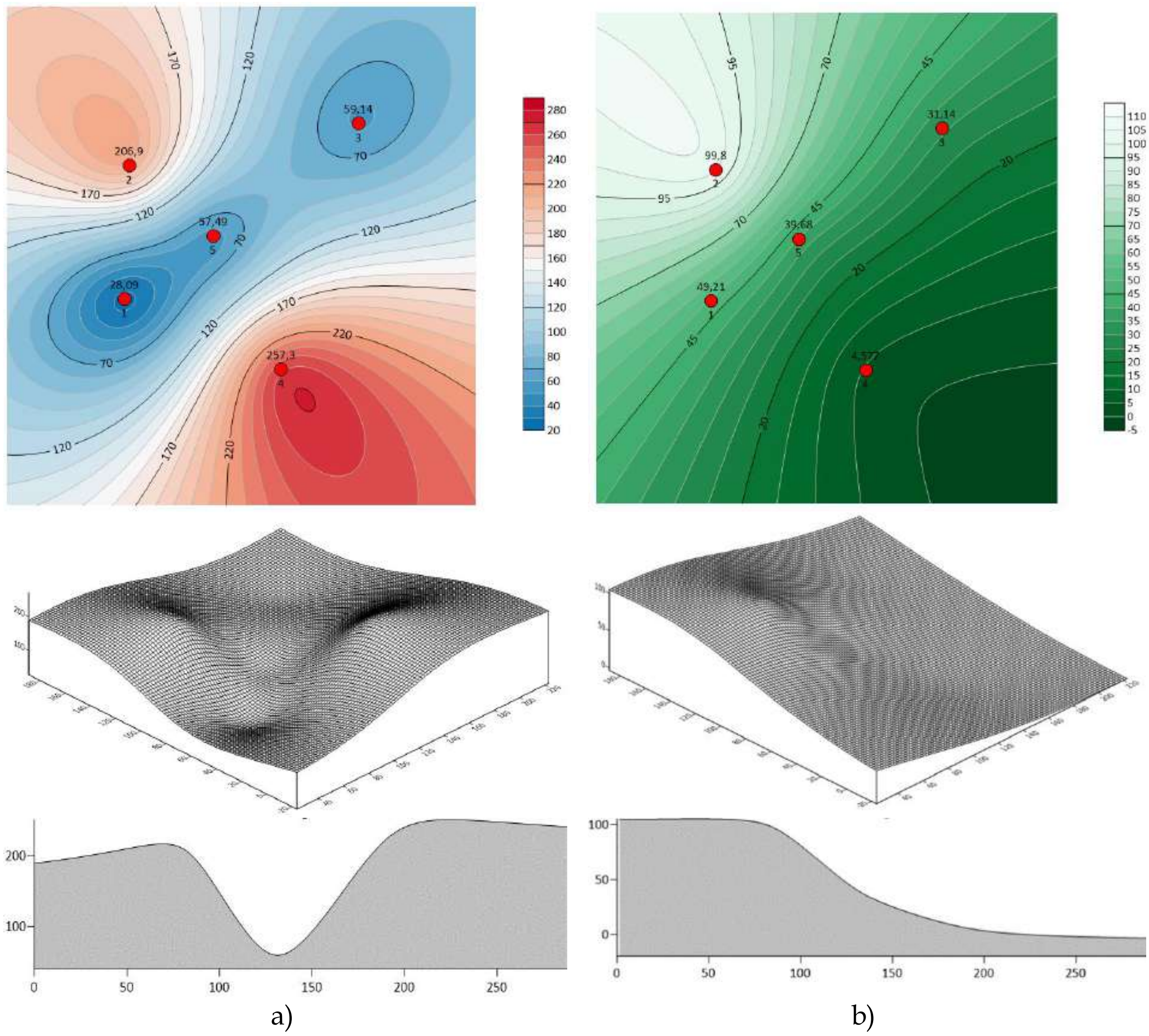
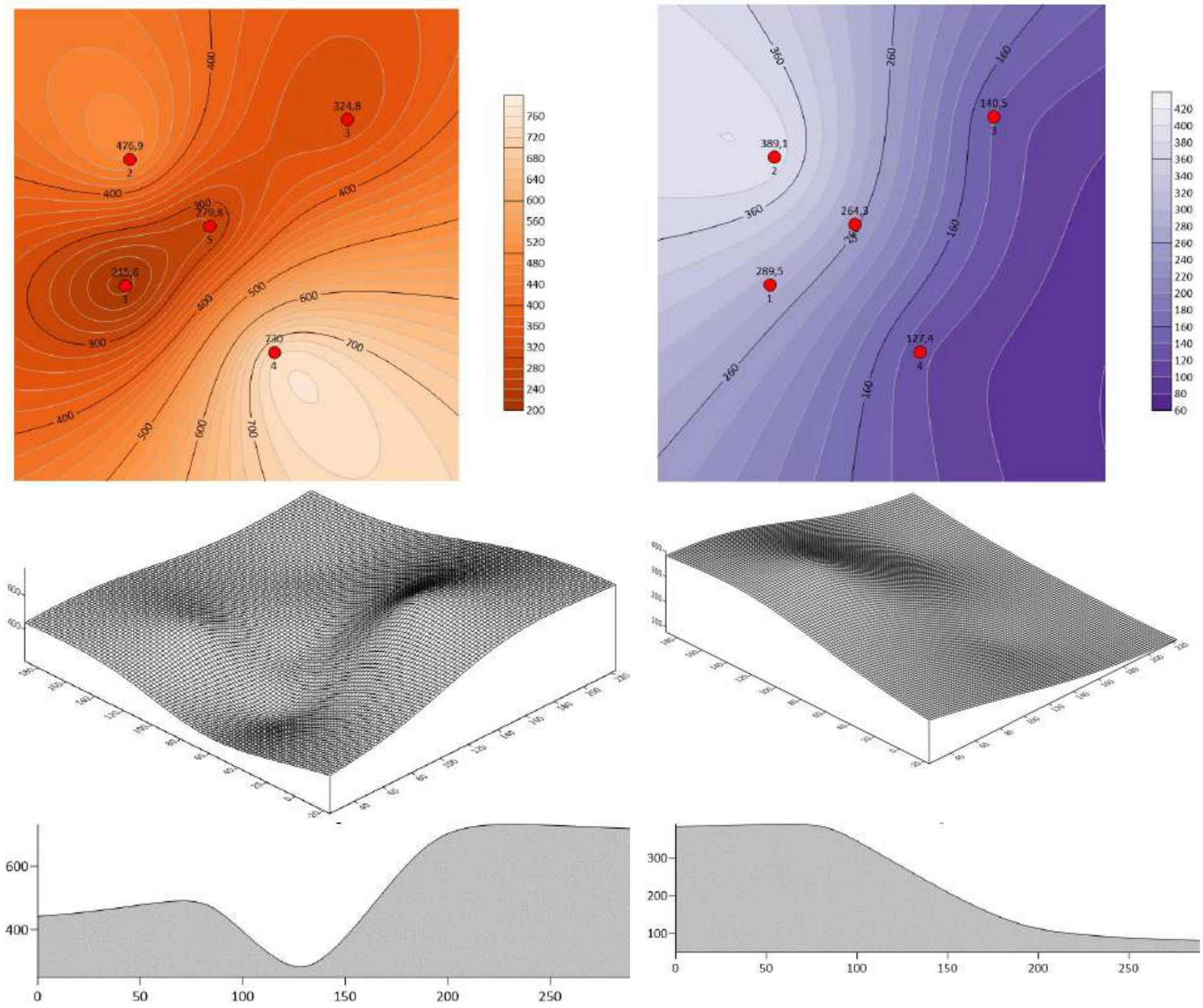


Fig. 4. Distribution of Co (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm

The highest Cu content (55.7 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Cu content (1.3 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump.

In the 0-20 cm horizon, the highest Cu content (34.6 mg/kg) is observed at the studied site No.6 (area with a background value). The lowest Cu content in the 0-20 cm horizon (1.8 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Cu (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 6.

The highest Zn content (67.9 mg/kg) is observed at the studied site No.4 in the 0-15 cm horizon on the eastern side near the rock dump foot. The lowest Zn content (2.4 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump.



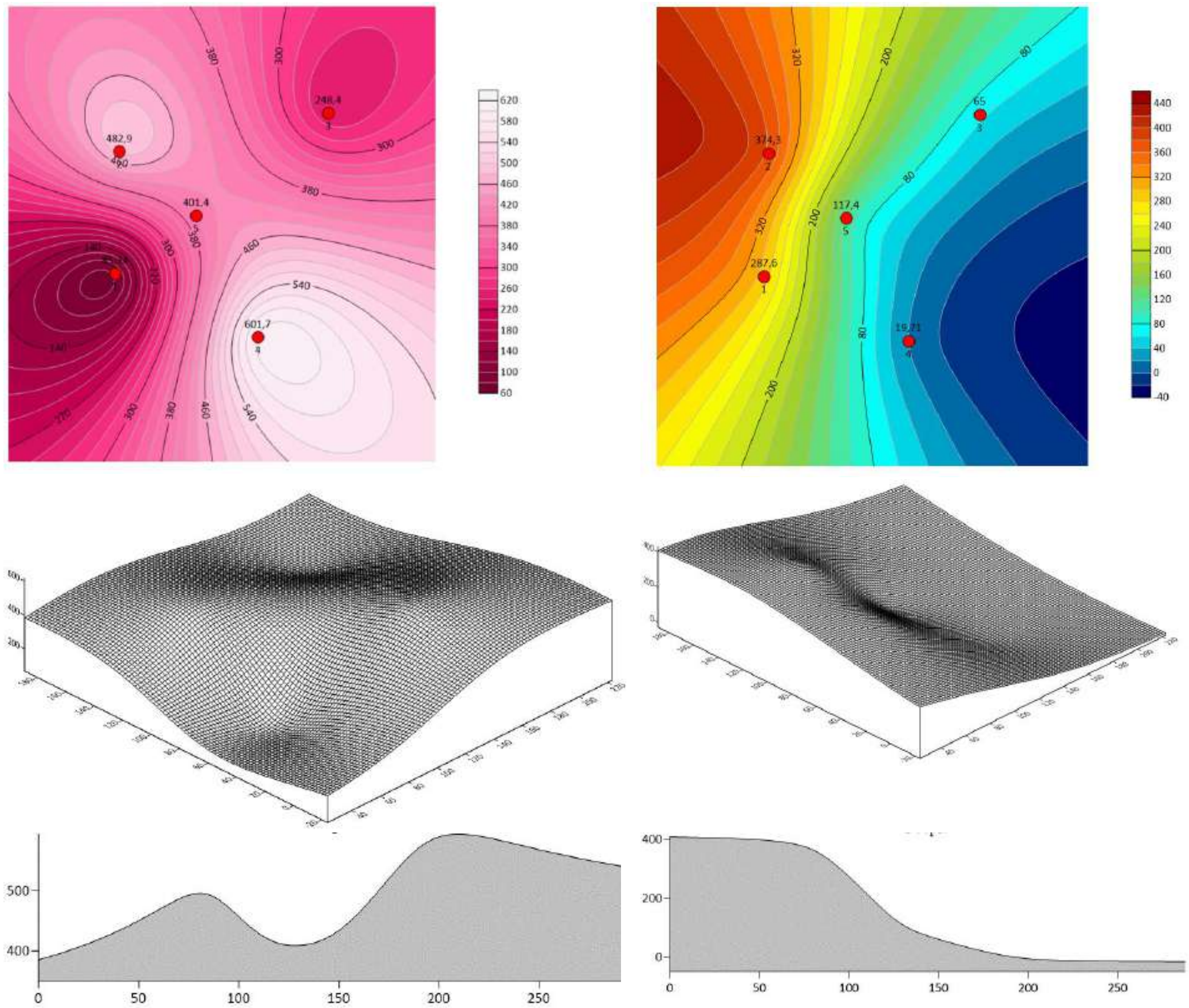
a) b)

Fig. 5. Distribution of Ni (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.

In the 0-20 cm horizon, the highest Zn content (32.3 mg/kg) is observed at the studied site No.2 on the western side of the rock dump slope. The lowest Zn content in the 0-20 cm horizon (6.7 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot. Modeling of Zn (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 7.

The highest Cd content (0.3 mg/kg) is observed at the studied site No.6 (background site) in the 0-20 cm horizon. The lowest Cd content in the 0-20 cm horizon (0.15 mg/kg) is observed at the studied site No.4 on the eastern side near the rock dump foot.

The lowest Cd content (0.14 mg/kg) is observed at the studied site No.6 in the 0-15 cm horizon, which is the background and is located 3 km south of the rock dump. The highest Cd content (0.26 mg/kg) in the 0-15 cm horizon is observed at the studied site No.5 on the rock dump top. Modeling of Cd (mg/kg) distribution in the edaphic horizons of the Nadiya mine rock dump in the 0-15 cm and 0-20 cm horizons is given in Fig. 8.



a) b)

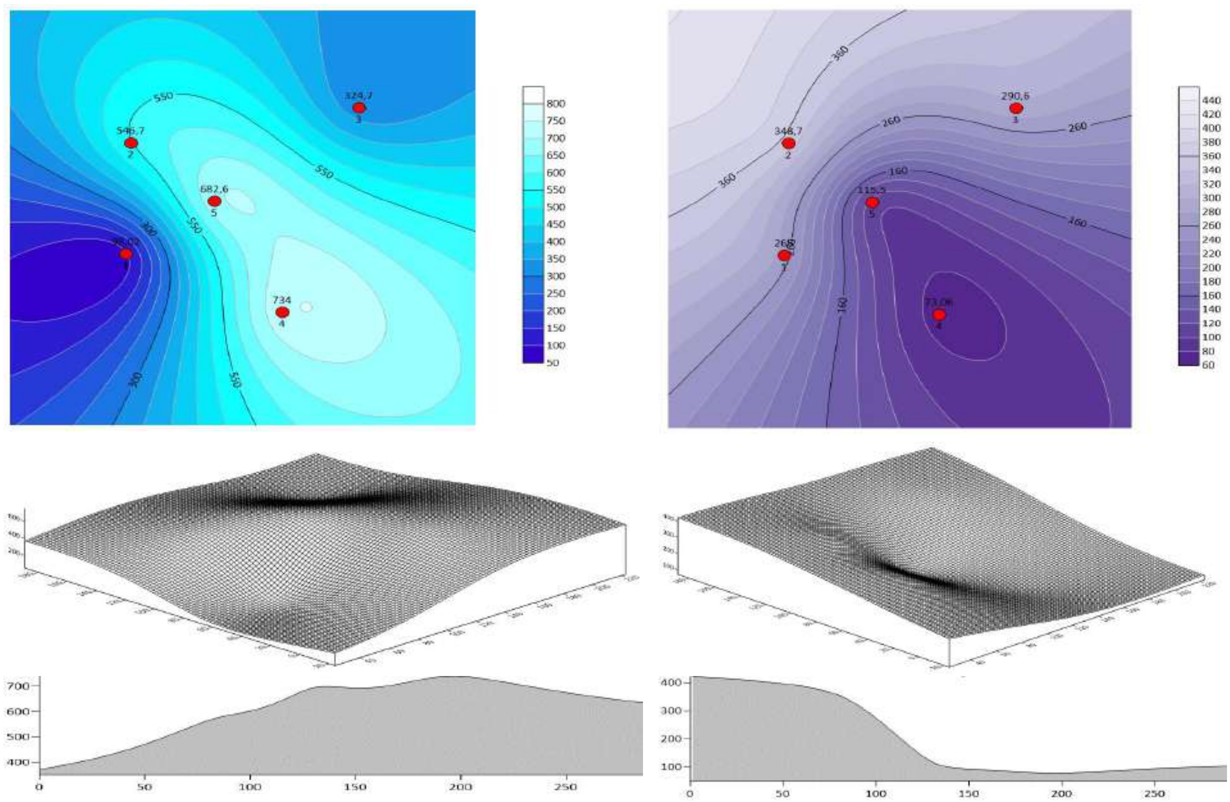
Fig. 6. Distribution of Cu (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.

The uneven distribution of heavy metals in the genetic horizons of the rock dump substrate is caused by frequent landslides, changing rock acidity, heterogeneity of natural overgrowth, and existing combustion sources. In the event of precipitation, toxic compounds, heavy metals and other harmful substances are washed out from the rock dump surface, and in our case, these substances also enter a natural water body.

To study the pollution of the reservoir, located on the north-western side of the Nadiya mine rock dump foot, a water sample has been taken and the physical-chemical properties have been tested. The obtained data are compared with the maximum permissible concentrations. It has been determined that the maximum permissible concentrations exceed NH_4 and are 2.1 mg/dm^3. In general, the water is characterized by a high degree of salinity, since chlorides and sulphates reach values of 47.9 mg/dm^3 and 120 mg/dm^3, respectively. Physical-chemical properties of water in the studied pond are presented in Table 3.

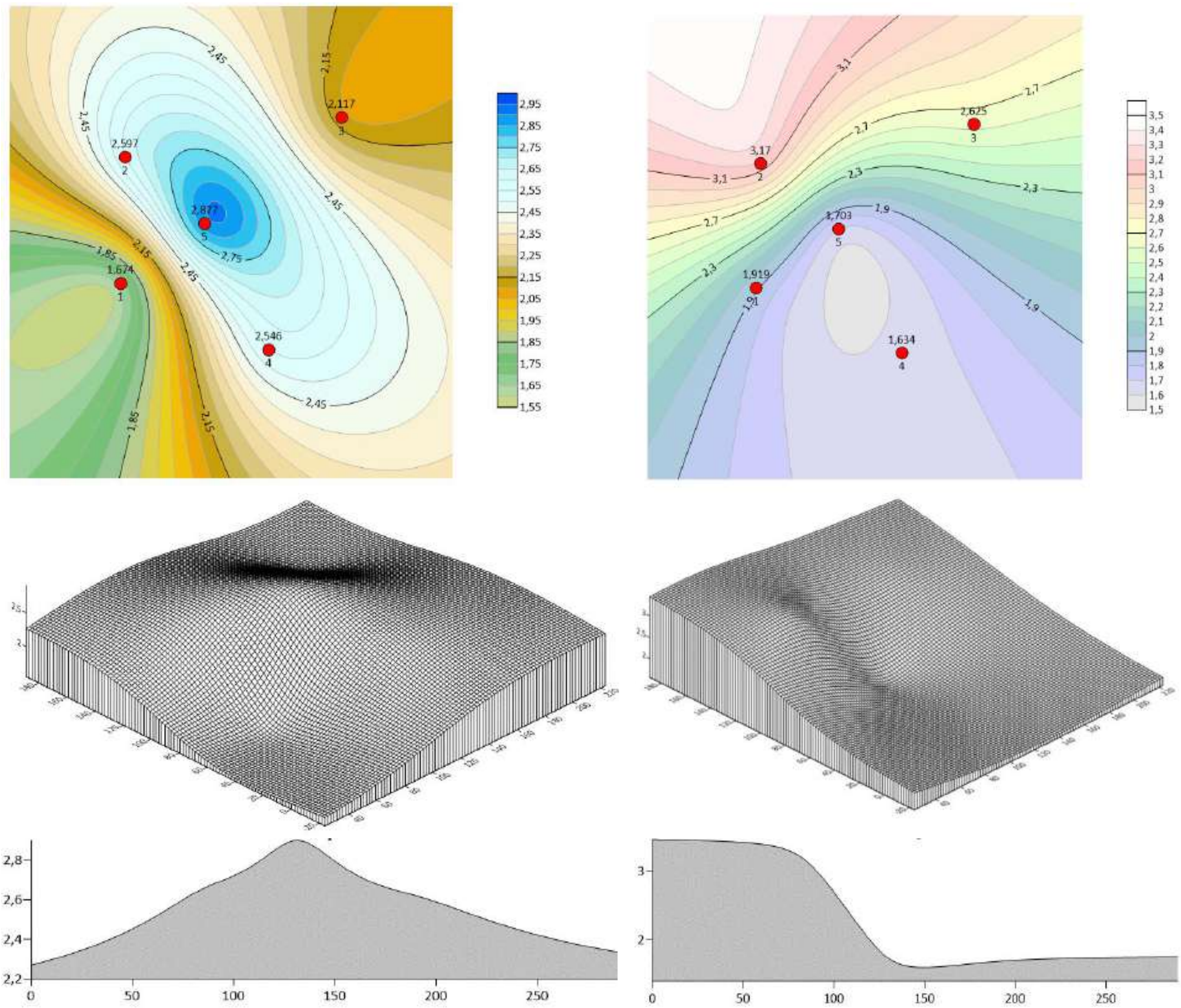
Table 3. Results of studying the physical-chemical parameters of the reservoir in the zone of the Nadiya mine rock dump influence.

Seq. No.	Indicator name	Result	MPC
1.	Odor threshold at 20 °C, points	0	to 2
2.	Transparency, cm	25	more than 20
3.	Hydrogen indicator (pH)	7.8	6.5-8.5
4.	Suspended substances, mg/dm3	25.1	not normal
5.	Total dry matter, mg/dm^3	109.7	to 1000
6.	Total hardness, $mg\text{-}eq/dm^3$	1.0	to 7.0
7.	Carbonate hardness, $mg\text{-}eq/dm^3$	0.9	to 6.5
8.	Hydrocarbons (HCO_3^-), mg/dm^3	54.6	to 300
9.	Chlorides (Cl^-), mg/dm^3	47.9	to 250
10.	Sulphates (SO_4^{2-}), mg/dm^3	120	to 500
11.	Nitrites (NO_2^-), mg/dm^3	0.74	to 3.3
12.	Nitrates (NO_3^-), mg/dm^3	5.6	to 45
13.	Phosphates (PO_4^{3-}), mg/dm^3	0	not normal
14.	Calcium (Ca^{2+}), mg/dm^3	17.3	not normal
15.	Magnesium (Mg^{2+}), mg/dm^3	5.0	to 80
16.	Total Ferrum (Fe_{tot}), mg/dm^3	0.2	to 0.3
17.	Salt ammonium (NH_4^+), mg/dm^3	2.1	to 2.0
18.	Sum of $Na^+ + K^+$, mg/dm^3	25.6	to 300



a) b)

Fig. 7. Distribution of Zn (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.



a) b)

Fig. 8. Distribution of Cd (mg/kg) in the edaphic horizons of the Nadiya mine rock dump: a) 0-15 cm; b) 0-20 cm.

Thus, in order to reduce the reservoir salinity in the zone of the rock dump influence, it is recommended to set the geochemical barriers near the foot, as well as to plant salt-resistant species and violents in the coastal zone. Since wastewater from the rock dump enters the studied reservoir almost without restriction.

Conclusions

Rock dumps of coal mines entail a significant technogenic burden on the environment due to landscape-transforming, chemical, and physical factors. The physical-chemical, ecological and phytocenotic properties of the Nadiya mine rock dump of the Chervonohrad Mining District, which belongs to the Lviv-Volyn Coal Basin (Ukraine), have been studied in the paper.

The study of the gross content of heavy metals Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb in the 0-15 cm and 0-20 cm horizons of the rock dump indicates that their values do not exceed the maximum permissible concentrations set for soils by state standards of Ukraine. The value of Cu (studied site No.4) near the rock dump foot from the east has been determined as exceeding the maximum permissible concentrations. However, the indicators of heavy metal content in comparison with the background values (studied site No.6) exceed for individual elements by dozens of times. It has been revealed that the studied site No.4 in the 0-15 cm horizon, which is located on the eastern side near the rock dump foot, is the most contaminated with heavy metals. The indicators of heavy metal content at the background site, which is located within a radius of 3 km from the rock dump, are the lowest in the 0-15 cm horizon.

In the event of precipitation, toxic compounds, heavy metals and other harmful substances are washed out from the rock dump surface and enter the natural water body. Thus, in order to reduce the reservoir salinity in the zone of the Nadiya mine rock dump influence, it is recommended to set the geochemical barriers near the foot, as well as to plant salt-resistant species and violents in the coastal zone.

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Received: 26.06.2022
Accepted: 22.10.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 75-84

New records and impact of tachinid parasitoids of Thaumetopoea pityocampa (Lepidoptera: Notodontidae) in Bulgaria

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Abstract. The impact of larval and pupal parasitoids of *Thaumetopoea pityocampa* was studied in laboratory conditions, during the period February-October 2021. The material, 138 hibernating caterpillars of summer form and 415 larvae of winter form intercepted during processions, was collected from two localities – Dobrostan Vill. and Gotse Delchev, respectively. In the land of Kovil Vill., which is occupied by the winter form, the winter nests of *T. pityocampa* were checked for infection by parasitoids. In this study, all isolated parasitoids belong to the family Tachinidae. As a result, 3 species – *Phryxe vulgaris* (Fallén, 1810), *Compsilura concinnata* (Meigen, 1824) and *Bothria frontosa* (Meigen, 1824) were found to parasitized *T. pityocampa*. A relatively high parasitism rate of *T. pityocampa* winter form by tachinids was observed – 21%, of which *P. vulgaris* parasitized 15.2% of the sample. Conversely, the parasitism rate of *T. pityocampa* summer form is low – 5%, in which *C. concinnata* parasitized 1.4% from the sample. Two puparia of *B. frontosa* were collected from larval winter nests of *T. pityocampa* in the region of Kovil Vill. This is the first record of the Notodontidae family as a host of *Bothria frontosa*. A complete up-to-date list of trophic connections of *T. pityocampa* with tachinid parasitoids, reported in Bulgarian and international publications is provided, as well as their impact on pest populations.

Key words: pine processionary moth, Tachinidae, parasitism rate, host record.

Introduction

Thaumetopoea pityocampa (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is a forest insect, which caterpillars cause severe defoliation of pine and cedar plantations throughout the Mediterranean region, including Southwestern Europe and Balkan Peninsula (Hódar et al., 2003; Kanat et al., 2005; Jacquet et al., 2012; Roques et al., 2015; Mirchev et al., 2017). A part of the northern border of its range passes Bulgaria (Roques et al., 2015). Pine processionary moth (hereafter abbreviated as PPM) is present in over 20 countries with Atlantic and Mediterranean climate (Battisti et al., 2015), with the exception of Bulgaria, where Continental climate predominates (Sabev & Stanev, 1959). Globally, a worldwide vertical and horizontal expansion of PPM range to the north and to higher altitudes is being reported (Battisti et al., 2006), also in Bulgaria (Mirchev et al., 2017; Zaemdzhikova et al., 2018). This gradual increase of PPM attacks in recent decades has a negative impact on the economic, social and environmental functions of the pine forests (Arnaldo et al., 2010; Aimi et al., 2006; Carus, 2010). In this regard, natural enemies play an important role in controlling the population density of pests, and they also have a great impact suppressing incipient pests' outbreaks in various ecosystems (Žikić et al., 2017). The natural enemies, such as parasitoids, predators, insectivorous birds and pathogens might have a role in controlling processionary moth species (Battisti et al., 2015).

In the Mediterranean area, 18 parasitoids and approximately 14 predators have been reported as natural regulators of *T. pityocampa* (Battisti et al., 2015). A list of all known entomophagous of *T. pityocampa* in Bulgaria is given in Table 1. In the country, 13 primary parasitoids (six chalcidoid wasps, one ichneumonid, *Trichogramma* sp., five tachinid species) and 5 predators have been identified. Among them, the regulatory role of egg parasites has been well studied (Tsankov et al., 1996 a, b; Mirchev et al., 2021). Given the recent increase in the frequency of PPM outbreaks in pine forests of Central Bulgaria reported by Zaemdzhikova et al. (2018), as well as the real potential for increasing their frequency in the future, it is important to know the role of other enemies on pest populations in the country. In this context, the aim of the present work is to establish the species composition and impact of tachinid parasitoids of *T. pityocampa* in Bulgaria. To achieve this proposal, our own observations were supplemented with all known country data, as well as these from international publications.

Table 1. List of primary parasitoids and predators of *Thaumetopoea pityocampa* in Bulgaria.

Stage	Parasitoids	Source
Eggs	*Ooencyrtus pityocampae* (Mercet, 1921) (Hymenoptera: Encyrtidae)	Mirchev et al. (1998)
	Anastatus bifasciatus (Geoffroy, 1785) (Hymenoptera: Eupelmidae)	
	Eupelmus vesicularis (Retzius, 1783) (Hymenoptera: Eupelmidae)	
	Baryscapus servadeii (Domenichini, 1965) (Hymenoptera: Eulophidae)	
	Eupelmus vladimiri Fusu, 2017 (Hymenoptera: Eupelmidae)	Boyadzhiev et al. (2020)
	Pediobius bruchicida (Rondani, 1872) (Hymenoptera: Eulophidae)	
	Trichogramma embryophagum (Hartig, 1838) (Hymenoptera: Trichogrammatidae)	Mirchev et al. (1998)
Larvae/ Pupae	*Heteropelma megarthrum* (Ratzeburg, 1848) (Hymenoptera: Ichneumonidae)	Zankov (1960)
	Compsilura concinnata (Meigen, 1824) (Diptera: Tachinidae)	Russkoff (1929-1930)
	Exorista fasciata (Fallén, 1820) (Diptera: Tachinidae)	Georgiev et al. (2022)
Larvae	*Exorista segregata* (Rondani, 1859) (Diptera: Tachinidae)	Russkoff (1929-1930)
Pupae	*Phryxe vulgaris* (Fallén, 1810) (Diptera: Tachinidae)	Hubenov (1983)
Larvae	*Phorocera grandis* (Rondani, 1859) (Diptera: Tachinidae)	
	Predators	
Eggs	*Ephippiger ephippiger* (Fiebig, 1784) (Orthoptera: Tettigoniidae)	Mirchev et al. (2019a)
	Pterolepis germanica (Herrich-Schäffer, 1840) (Orthoptera: Tettigoniidae)	
	Dermestes lardarius Linnaeus, 1758 (Coleoptera: Dermestidae)	Zankov (1960)
	Formica rufa Linnaeus, 1761 (Hymenoptera: Formicidae)	
Larvae	*Oecanthus pellucens* (Scopoli, 1763) (Orthoptera: Gryllidae)	Zaemdzhikova & Doychev (2020)

Material and Methods

The biological material, a total of 553 *T. pityocampa* larvae, was collected during the period February-April 2021 in two localities of *Pinus nigra* J.F.Arnold – Dobrostan Vill. (Rhodope Mts.) and Gotse Delchev (Southern Pirin Mt.). In Dobrostan the PPM summer form occurs (Mirchev et al., 2019b) and the winter one is widespread in the region of Gotse Delchev (Zaemdzhikova 2020). From the first locality, 138 overwintering larvae were dug out from the soil, and from the second one – 415 larvae were intercepted at the beginning of processions. In the laboratory of Entomology at Forest Research Institute (Bulgarian Academy of Sciences), the collected larvae were placed in plastic containers with ventilated holes (40x30x20 cm). A layer of 5 cm sterilized sand was added to each box, simulating the soil environment required for burying of the caterpillars. After larvae built cocoons, they were removed from the boxes and stored in dark at room temperature until their pupation. The cocoons were checked once a week for pupae present. All cocoons and pupae were isolated in separate containers. All samples (larvae, prepupae, pupae) were checked 3 times a week for the emergence of adults or puparia of parasitoids. In addition to the collected samples, in the vicinity of Kovil Vill. (Rhodope Mts.), where the PPM winter form occurs, the nests of caterpillars have been directly observed for infestation by parasitoids (Fig. 1). Twenty PPM winter nests were checked.

The parasitism rate was calculated as the number of insects in a sample from which parasitoids had emerged divided by the total number of insects, including these parasitoids, which were not yet identified (conserved as puparia).

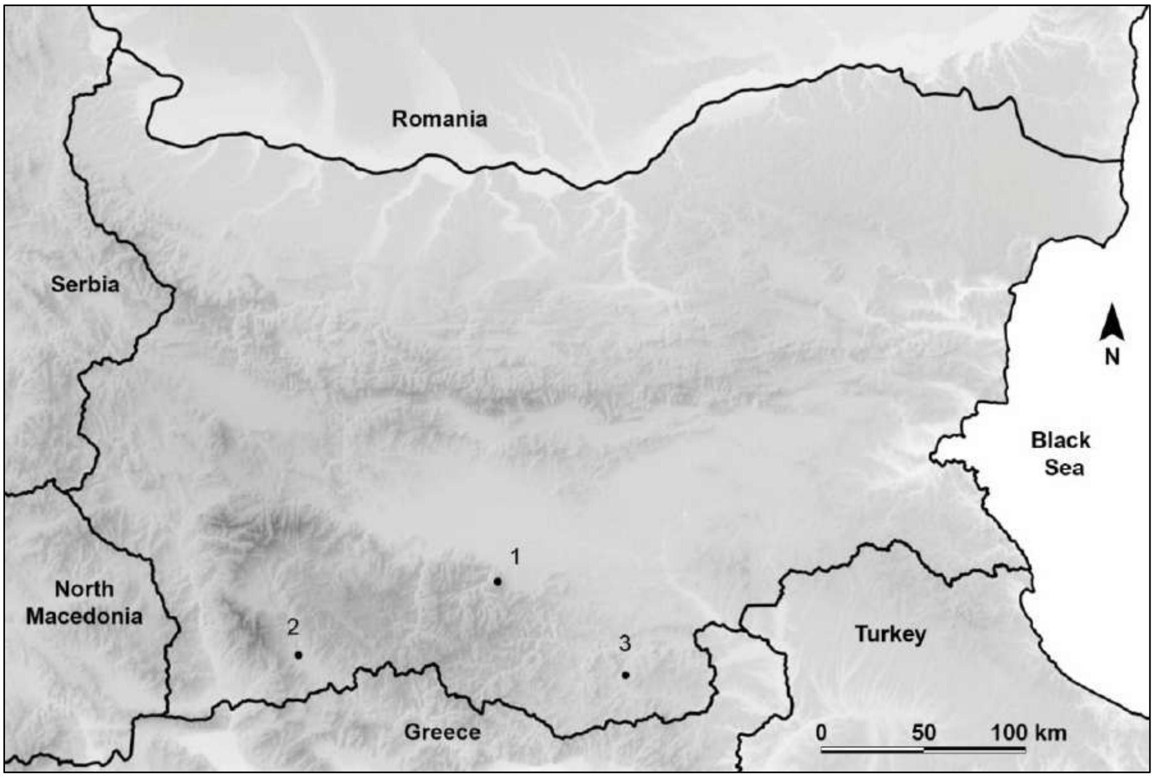

Fig. 1. New localities of *Compsilura concinnata* (1), *Phryxe vulgaris* (2) and *Bothria frontosa* (3) in Bulgaria.

Results

General. In the present study, all isolated parasitoids from *T. pityocampa* belong to the subfamily Exoristinae (Diptera: Tachinidae). In the result, three species were established by the host – *Phryxe vulgaris* (Fallén, 1810), *Compsilura concinnata* (Meigen, 1824) and *Bothria frontosa* (Meigen, 1824). For one species, *B. frontosa* – a host of Notodontidae family has been reported for the first time.

Compsilura concinnata

Material examined: Western Rhodope Mts., land of Dobrostan Vill. (41°54'10''N/24°55'07''E), 1100 m a. s. l., date of coll. 12.III.2021, 2 ♀♀, emerged 4.IV.2021 (det. Z. Hubenov). The adults were reared from prepupae of PPM summer form.

Distribution: Holarctic-Paleotropical-Australian. *In Bulgaria* – common: Western Danubian Plain, Popovo-Provadiya district, Loudogorie-Dobroudzha district, Western and Middle Predbalkan, Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Podbalkan Basins; Thracian Lowland, Belasitsa Mts., Boboshevo-Simitli Valley, Kroupnik-Sandanski-Petrich Valley, Rila Mts., Pirin Mts., Slavyanka Mt., Western Rhodope Mts., Eastern Rhodope Mts., Northern Black Sea Coast (Hubenov, 2021).

Parasitoid-host list: many lepidopteran species, most of the reported hosts are species of the Notodontidae family (Tschorsinig, 2017). *Trophic connections in Bulgaria: Calyptra thalictri* (Borkhausen, 1790), *Lymantria dispar* (Linnaeus, 1758), *Euproctis chrysorrhoea* (Linnaeus, 1758), *Hyphantria cunea* (Drury, 1773), *Leucoma salicis* (Linnaeus, 1758) (Lepidoptera: Erebidae); *Eriogaster lanestris* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae); *Archips xylosteana* (Linnaeus, 1758) (Lepidoptera: Tortricidae); *Orthosia cruda* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae); *Malacosoma neustria* (Linnaeus, 1758) (Lepidoptera: Lasiocampidae) (Hubenov, 1985); *Subacronicta megacephala* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae) (Hubenov, 1985; 2001); *Thaumetopoea solitaria* (Freyer, 1838), *T. processionea* (Linnaeus, 1758) (Hubenov, 1985) and *T. pityocampa* (Lepidoptera: Notodontidae) (Russkoff, 1929-1930; Georgiev et al., 2022).

Phryxe vulgaris

Material examined: Pirin Mts., region of Gotse Delchev (41°35'17''N/23°44'24''E), 554 m a. s. l., date of coll. 30.IV.2021, 32 ♀♀, 31 ♂♂, emerged 15-19.IX.2021 (det. Z. Hubenov). All specimens were reared from pupae of PPM winter form.

Distribution: Holarctic. *In Bulgaria:* Loudogorie-Dobroudzha district, Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Lozenska Planina Mt., Thracian Lowland, Belasitsa Mts., Kroupnik-Sandanski-Petrich Valley, Rila Mts., Pirin Mts., Slavyanka Mt., Rhodope Mts., Northern Black Sea Coast (Hubenov, 2021).

Parasitoid-host list: Many hosts from the Coleoptera, Hymenoptera and Lepidoptera orders. Only two hosts are known of the family Notodontidae – *Thaumetopoea processionea* and *T. pityocampa* (Tschorsinig, 2017). *Trophic connections in Bulgaria: Sphinx pinastri* Linnaeus, 1758 (Lepidoptera: Sphingidae); *T. pityocampa* (Lepidoptera: Notodontidae) (Hubenov, 1983; Georgiev et al., 2022); *Lycia hirtaria* (Clerck, 1759), *Erannis defoliaria* (Clerck, 1759) (Lepidoptera: Geometridae) (Hubenov, 1985).

Bothria frontosa

Material examined: Eastern Rhodope Mts., in the vicinity of the Kovil Vill. (41°30'17''N/25°39'41''E), 500 m a. s. l., date of coll. 27.II.2021, two puparia were observed in the winter nest of PPM winter form (they were reared in laboratory), 1 ♀, emerged 04.IV.2021 (det. Z. Hubenov).

Distribution: Transpalaearctic, Holoeurosiberian. *In Bulgaria* – Western Stara Planina (Balkan) Mts., Sofia Basin, Vitosha Mt., Thracian Lowland; Pirin Mts. (Hubenov 2021).

Parasitoid-host list: Until now, only two hosts are known – *Mesogona acetosellae* (Denis & Schiffermüller, 1775) and *Noctua comes* Hübner, 1813 (Lepidoptera: Noctuidae) (Tschorsinig, 2017). *Trophic connections in Bulgaria:* No records.

Parasitism rate. Significant differences in the parasitism rate of *T. pityocampa* were observed in the localities (Table 2). In the region of Gotse Delchev, where the winter form is widespread, the rate of parasitism is high – 21%, while in Dobrostan, where summer form occurs, it is only – 5%. The parasitism rate in both localities is 12% of an average.

Relatively high parasitism rate of *T. pityocampa* by *Phryxe vulgaris* has been found, which is the main result of this study. It parasitized 15.2% of the larvae of PPM winter form, while the other tachinid species – *Compsilura concinnata* parasitized 1.4% of the larvae of PPM summer form. All adults of *P. vulgaris* emerged (in laboratory condition) in the middle of September, while *C. concinnata* and *B. frontosa* – at the beginning of April.

Table 2. Parasitism rate of *Thaumetopoea pityocampa* by tachinid species.

Locality	Phenological form	Collected sample, number	Parasitism		Emerged adults of identified parasitoids				
					Species	Reared from (host stage)	Emerged	Parasitism	
			N	%				N	%
Dobrostan Vill.	summer	138 (overwintering larvae)	7	5	*Compsilura concinnata*	prepupae	4.IV.2021	2	1.4
Gotse Delchev	winter	415 (larvae in procession)	87	21	*Phryxe vulgaris*	pupae	15-19.IX.2021	63	15.2

Discussion

Trophic connections recorded by us. Three trophic connections of *Thaumetopoea pityocampa* with tachinid parasitoids were found, as follows – *Compsilura concinnata*, *Phryxe vulgaris* and *Bothria frontosa.* Of these, *B. frontosa* is a new parasitoid record for *T. pityocampa.* The other two tachinids are known to parasitize on *T. pityocampa* in the country. The first report for *C. concinnata* is by Russkoff (1929-1930) and *P. vulgaris* by Hubenov (1983). From our findings and literature sources, a complete up-to-date list of trophic connections of tachinid parasitoids with *T. pityocampa* is given in Table 3.

Trophic connections evidenced in Bulgaria. Six tachinid species have been associated with *T. pityocampa* in the country so far. For three parasitoids (*Bothria frontosa*, *Exorista fasciata* and *Phryxe vulgaris*) this host is known only for the country, and for two of them – *B. frontosa* and *E. fasciata* (Georgiev et al., 2022) the host record is new of the family Notodontidae (Tschorsinig, 2017).

World-wide trophic connections. Until now, 14 parasitoid-host relationships are known. Five of them are considered questionable – *Townsendiellomyia nidicola* (Townsend, 1908), *Pelatachina tibialis* (Fallén, 1810), *Siphona cristata* (Fabricius, 1805), *Cyrtophloeba ruricola* (Meigen, 1824) and *Zenillia* sp. The remaining nine parasitoid-host associations were confirmed in different studies (Tschorsinig, 2017).

Unobserved trophic connections in Bulgaria. Among the known 14 trophic connections, 8 remain unknown for the country, of which 5 are questionable (Tschorsinig, 2017). The remaining three are unconfirmed at this time. Among them, *Phryxe caudata* (Rondani, 1859) is absent for the list of Bulgarian dipterans (Hubenov, 2021). The other two tachinids – *Carcelia iliaca* (Ratzeburg, 1840) and *Pales pavida* (Meigen, 1824) are present in the country (Hubenov, 2021), but they have not been associated with *T. pityocampa*.

Parasitism rate. In this study, a relatively high mortality of PPM winter form larvae caused by tachinid parasitoids in the region of Gotse Delchev was found, where *Phryxe vulgaris* parasitized 15.2% from the larvae in the sample. According to Georgiev et al. (2022) the mortality of PPM larvae, caused by *P. vulgaris* in the region in Eastern Rhodopes (Kandilka) and Struma Valley (town of Sandanski) is 2.5 and 2.4%, respectively. This is 6 times lower than the observed parasitism rate in this study.

Surprisingly but so far, this cosmopolitan species has been reported as a parasitoid of the pine processionary moth only in the country (Tschorsinig, 2017). *P. vulgaris* is extremely polyphagous on different families of Lepidoptera with relatively large caterpillars (Tschorsnig & Herting 1994). Probably it parasitized the larvae of the PPM in the winter nest or during their moving to pupation in the soil, similarly to *Phryxe caudata* (Buxton, 1990; Zamoum et al., 2017).

Relatively low parasitism rate of the larvae sample of PPM summer form has been found – 5%. In this case – *Compsilura concinnata* parasitized 1.4% of the collected overwintering PPM larvae. For Bulgaria, the known parasitization level of PPM by this tachinid fly ranged between 0.5% (Fotinovo Vill.) and 5.3% and (Dobrostan Vill.) (Rhodope Mts.) (Georgiev et al., 2022). Overall the observed country's maximum mortality is lower than known maximum parasitism rate reported by Battisti et al. (2015) – 7%. *C. concinnata* is polyphagous generalist species, attacking over 100 species in the Palearctic region (Herting, 1960). Most of the hosts are lepidopterous larvae (Tschorsnig, 2017). Three to four generations of *C. concinnata* occur per year, with the larvae overwintering in host larvae or pupae (Culver, 1919). In this case, the fly probably attacked the host larvae on trees or on the soil during their autumn migration, then it overwintered in larval stage inside the host prepupae. Only two host records for *Bothria frontosa* dating back to the middle of the last century have been found in the literature (Tschorsnig, 2017). For the first time in this study data on a host of *B. frontosa* of the Notodontidae family are provided, which is the third host record for the tachinid parasitoid.

Table 3. List of known tachinid parasitoids of developmental stages of *Thaumetopoea pityocampa*. *The data recorded by:* [R] Russkoff, 1929-1930; [H] – Hubenov, 1983; [G] – Georgiev et al., 2022; [ZD] – current study (Zaemdzhikova & Doychev, 2020); [T] – Tschorsnig, 2017; [B] – Battisti et al., 2015. *Legend:* * - first record for Bulgaria; (Q) – the parasitoids-host connection is questionable.

Stage	Species	New connect-ion found by us	Confirm-ed known connections in this study	Total of connect-ions evidenced in Bulgaria	Total world-wide trophic connections evidenced - number, author, country	Known connect-ions not observed in Bulgaria	Connect-ion known only for Bulgaria	First record of the Noto-dontidae family	Period of activity (month)	Max. morta-lity (%)
Larva	*Phorocera grandis*			1 [H*]	numerous [T]				IV [H]	unknown
Pupa	*Phryxe vulgaris*		1 [H]	1 [H*,G,ZD]	3 [H,G,ZD] Bulgaria		1		XIII – XI [G]; IX [ZD]	15.2 [ZD]
Larva	*Phryxe caudata*				numerous [T]	1			IX–X and II–III–IV [B]	10 [B]
Larva	*Compsilura concinnata*		1 [R]	1 [R*,G]	numerous [T]				IV-V [B]; III [G]	7 [B]
Larva	*Bothria frontosa*	1		1 [ZD*]	1 [ZD] Bulgaria		1	1 [ZD]	IV [ZD]	unknown
Larva / Pupae	*Exorista fasciata*			1 [G*]	1 [G] Bulgaria		1	1 [G]	III–IV [G]	2.2 [G]
Larva	*Exorista segregata*			1 [R*]	numerous [T]				IV [B]	5 [B]
Not found	*Carcelia iliaca*				1 [T] Southern France	1				
	Townsendiellomyia nidicola				1 [T] only for Italy (Q)	1 (Q)				
	Zenillia sp.				1 [T] only for Portugal (Q)	1 (Q)				
	Pales pavida				1 [T] only for	1				

					Corsica					
	Pelatachina tibialis				1^{T} only for Italy (Q)	1(Q)				
	Siphona cristata				1^{T} only for Austria (Q)	1(Q)				
Larva	*Cyrtophloeba ruricola*				1^{T} only for Morocco (Q)	1(Q)				
Total result		1	2	6	14	8 (5)	3	2		

Acknowledgements. We are grateful to Prof. Dr Zdravko Hubenov (National Museum of Natural History, Bulgarian Academy of Sciences) for the identification of tachinid species. This work has been carried out in the framework of the National Science Program "Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № D01-279/03.12.2021).

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Received: 16.09.2022
Accepted: 20.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 85-92

Sex, Age and Blood Group Structure of Myopia and Astigmatism in South Bulgaria Population

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Abstract. Myopia and astigmatism are common visual abnormalities in human populations worldwide. The studies in Bulgaria concerning the population structure in context of these defects and thair relations with sex, age, etc. metrics are limited. Reasonably the present study aims to analyze peculiarities in the manifestation of myopia and astigmatism by characterizing the sex, age and AB0 blood group structure of a reproductive population in southern Bulgaria. The study includes 817 individuals of both sexes, aged between 18 and 59 years. They voluntarily have filled out a questionnaire, giving diseases information (myopia and astigmatism), after they had been diagnosed by a physician. AB0-blood group affiliation has been determined in laboratory conditions by a routine technique. Based on the obtained results, it is concluded that female sex and age over 44 years are risk factors for manifestation of the studied refractive abnormalities, and blood groups A and 0 hide a greater risk for their joint manifestation.

Key words: myopia, astigmatism, sex, age, AB0 blood groups.

Introduction

Multifactorial diseases are an object of increased interest because of their complex etiology. Their manifestation depends on genetic and environmental factors such as sex, age, body composition, different social factors and habits (Nikolova & Petrov, 1982; 1986; Petrov et al., 1987; Nikolova, 1994; Baird et al., 2010; Nikolova & Boyadjiev, 2011; Tedja et al., 2019; Ivanova et al. 2021).

Refractive errors are, widespread throughout the world. Myopia has a frequency varying between 1% and 10% in different human populations. Data indicate that pathological myopia annually causes blindness at an incidence of 1 to 5‰ (Asakuma et al., 2012; Pan et al., 2013). Astigmatism is another common visual defect, usually reported at birth, and a marker for predisposition to other refractive errors (Mackey et al., 2011).

Different studies, provide information on the heritability pattern of astigmatism and pointed out its wider prevalence in specific ethnic groups (Harvey et al. 2010; Fan et al., 2011; McKean-Cowdin et al., 2011; Mackey et al., 2011; Lopes et al., 2013) and also variations by age and sex (Gudmundsdottir et al. 2000; Shih et al. 2004; Hashemi et al. 2005).

In Bulgaria, studies on the structural characterization of refractive errors are limited (Ivanova et al., 2018) and this motivates the conduct of the present study, which aims to analyze peculiarities in the manifestation of myopia and astigmatism by characterizing the sex, age and AB0 blood group structure of reproductive population in southern Bulgaria.

Material and Methods

A total of 817 individuals (30.2% men and 69.8% women), aged between 18 and 59 years and divided into two age categories – up to and over 44 years – have been included in the present study. It has conducted within a five-year period, from 2017 to 2021. The individuals included in the study are all of Bulgarian ethnic origin, from settlements in the territory of the Plovdiv Region, Southern Bulgaria. The participants voluntarily and after obtained informed consent, have filled out a questionnaire, designed according to the objectives of the study, in which they indicated whether they suffered from certain multifactorial diseases, including myopia and astigmatism. Only cases diagnosed by a specialist physician are included in the analysis.

AB0 blood grouping has been determined in the laboratory conditions using a routine technique of Moss or has been directly accepted after an official document provided by a clinical laboratory. The software package IBM SPSS Statistics, version 22.0 has used for statistical analysis of the reported results.

Results

Sex. The results regarding distribution of myopia and astigmatism, according to sex are presented in Table 1. As could be seen, 30.6% of female and 22.7% of male have myopia, and 24.7% of female and 16.5% of male – astigmatism. The established differences, regarding both diseases are statistically significant ($p = 0.030$, $\chi^2 = 4.700$ and $p = 0.014$, $\chi^2 = 6.038$ respectively).

Table 2 presents results, regarding simultaneous reporting of both visual abnormalities, depending on sex. It becomes clear, that such a joint manifestation is presented in a high percentage of the studied individuals – 33.3% for men, and twice higher for women – 66.7%. The established difference is distinct and statistically significant ($p < 0.001$, $\chi^2 = 14.344$).

Table 1. Myopia and astigmatism – distribution by sex ($p \leq 0.03$). *Legend:* N – Number of individuals, % - percentage; (+) presence of disease; (-) absence of disease.

Sex	N %	Myopia			Astigmatism		
		+	**-**	**Total**	**+**	**-**	**Total**
Male	N	50	170	220	37	187	224
	%	22.7	77.3	100.0	16.5	83.5	100.0
Female	N	159	361	520	130	397	527
	%	30.6	69.4	100.0	24.7	75.3	100.0
Total	N	209	531	740	167	584	751
	%	28.2	71.8	100.0	22.2	77.8	100.0

Table 2. Myopia and astigmatism – simultaneous manifestation in dependence to sex ($p < 0.001$). *Legend:* N – Number of individuals, % - percentage; (+) presence of disease; (-) absence of disease.

Myopia and astigmatism		Sex	
		Male	Female
-	N	78	284
	%	21.5	78.5
+	N	167	334
	%	33.3	66.7

Age. The distribution of participants who suffer from myopia and astigmatism, according to sex and age, shows distinct differences. The results indicate that among the age group up to 44 years, both myopia and astigmatism occur with greater frequency in women – 28.2% and 24%, respectively, than in men – 20.7% and 14.6%, respectively. For both diseases, the reported differences are statistically significant: $p = 0.050$, $\chi^2 = 3.827$ for myopia and $p = 0.008$, $\chi^2 = 7.121$ for astigmatism – Table 3.

A comparison of older participants of both sexes, also demonstrate that myopia manifests in a higher percentage for females (43.8%), compared to males (33.3%). It is important to note that, despite the lack of a categorical statistical significance, the proportion of females with myopia is approximately 10% higher than that for males ($p = 0.290$, $\chi^2 = 1.118$).

The results of the present study show that in the age category over 44, astigmatism manifests with a similar frequency within representatives of both sexes – 28.4% among women and 28.1% among men ($p = 0.977$, $\chi^2 = 0.001$) – Table 3.

Blood group affiliation. Table 4 presents data on the distribution of study participants, depending on their AB0 blood group type.

Myopia occurs more often in persons with blood groups B (31.3%) and AB (31.1%), and least often - in those with blood group 0 (27.7%). Astigmatism occurs with a similar frequency - 20.3% for blood group 0 to 24.8% – for blood group A. The established differences between the compared categories are not statistically significant ($p = 0.475$, $\chi^2 = 0.511$ for myopia and $p = 0.712$, $\chi^2 = 0.136$ for astigmatism).

The results of the present study show that the two visual abnormalities occur together in part of the studied individuals, from all four blood groups ($p = 0.358$, $\chi^2 = 0.846$). The highest number is found for the participants with myopia and astigmatism, who belong to blood groups A (36.3%) and 0 (32%) – Table 5.

Table 3. Myopia and astigmatism - age structure in the representatives of both sexes ($p \leq 0.05$). *Legend:* N – Number of individuals, % - percentage; (+) presence of disease; (-) absence of disease.

Age groups	Sex	Number	Myopia		Astigmatism	
			+	-	+	-
Up to 44	Male	N	38	146	28	164
		%	20.7	79.3	14.6	85.4
	Famale	N	124	316	107	339
		%	28.2	71.8	24.0	76.0
	Total	N	162	462	135	503
		%	26.0	74.0	21.2	78.8
Over 44	Male	N	12	24	9	23
		%	33.3	66.7	28.1	71.9
	Famale	N	35	45	23	58
		%	43.8	56.3	28.4	71.6
	Total	N	47	69	32	81
		%	40.5	59.5	28.3	71.7

Table 4. Myopia and astigmatism – distribution depending on AB0 blood group affiliation (p > 0.4). *Legend:* N – Number of individuals, % - percentage; (+) presence of disease; (-) absence of disease.

AB0 blood group	Number %	Myopia			Astigmatism		
		+	-	Total	+	-	Total
0	N	56	146	202	42	165	207
	%	27.7	72.3	100.0	20.3	79.7	100.0
A	N	74	172	246	63	191	254
	%	30.1	69.9	100.0	24.8	75.2	100.0
B	N	40	88	128	29	100	129
	%	31.3	68.8	100.0	22.5	77.5	100.0
AB	N	28	62	90	21	73	94
	%	31.1	68.9	100.0	22.3	77.7	100.0
Total	N	198	468	666	155	529	684
	%	29.7	70.3	100.0	22.7	77.3	100.0

Table 5. Myopia and astigmatism – simultaneous manifestation in dependence to AB0 blood group affiliation (p > 0.3). *Legend:* N – Number of individuals, % - percentage; (+) joint manifestation of diseases; (-) absence of disease.

Myopia and astigmatism		AB0 Blood group			
		0	A	B	AB
-	N	79	113	58	40
	%	27.2	39.0	20.0	13.8
+	N	126	143	72	53
	%	32.0	36.3	18.3	13.5
Total	N	205	256	130	93
	%	30.0	37.4	19.0	13.6

Discussion

Visual abnormalities are defined under the general name of "refractive errors" and often appear together. People who have astigmatism are prone to myopia or hyperopia (Sanil et al., 2018), which is also proven from the results of the present study.

Literature data show that in East Asia (China, Japan, Republic of Korea and Singapore) myopia has a higher occurrence – approximately 50% among the population, compared to Australia, Europe, North and South America, where its prevalence is relatively rarer (Holden et al., 2016). It is also reported a frequency of 36.4% of myopia among 8-years old children in Taiwan and China in 2016, while its prevalence among 6-year-old children in Netherlands was only 2.4% for 2018 (Hsu et al., 2016; Tideman et al., 2018).

According to Sanil et al. (2018), the frequency of the disease in a similar age range (35.5 – 37.0 years) is approximately the same for both sexes, which is not confirmed by the results from our study, showing a predominant occurrence for the female sex. A series of studies have demonstrated that myopia is most common at school age and its prevalence decreases with increasing age (Leung et al., 2012; Sanfilippo et al., 2015; Natung et al., 2017; Gomez-Salazar et al., 2017). In our study, the data indicate that the frequency of this abnormality is lower in the age category up to 44 years and significantly higher in the age group over 44 years, a trend, valid for both sexes (Table 3).

Regarding astigmatism, the literature reports that its prevalence increases significantly with age (Wong et. al., 2000; Saw et. al., 2002), being most significant in adults over 40 years (Saw et al., 2002; Bourne et al., 2004; Hashemi et al., 2005). Such a tendency, very clearly manifested in men, can be confirmed by the data of our study – Table 3. Pontikos et al. (2019) find negative associations of astigmatism with earlier age and female sex. The results of other researchers (Gudmundsdottir, 2000; Leung, 2012; Gomez-Salazar, 2017) demonstrate the association of the astigmatism with the male sex and the increasing age. In our study, a higher frequency of astigmatism is found among women aged up to 44 years and a similar frequency of occurrence of the anomaly among representatives of both sexes in the age category over 44 years (Table 3).

Some authors report that astigmatism usually occurs together with myopia and hyperopia at a later age (Farbrother et al., 2004). According to Sanil et al. (2018), its prevalence is approximately equal in men and women at an age of about 35 years, increases toward the age of 65 – 69 years, and then, above 70 years there is a plateau, with a large percentage of people with astigmatism (64%) who also have myopia. The results of our study confirm that the frequency of the abnormality increases with age and that there is a significant percentage of individuals suffering from both refractive errors.

Regarding the blood group affiliation of individuals suffering from myopia and astigmatism no significant differences were found and the simultaneous manifestation of the two refractive anomalies is more common in the blood groups A and 0. According to other authors, blood group B predominates among patients with myopia (Garg and Pahwa, 1965; Seth and Chahal, 2004; Gupta and Nishi, 2013; Sonal et al., 2020). We found no information regarding astigmatism and any blood group affiliation reported.

Conclusions

Based on the results of the present study, we could conclude that: 1) the female sex and the age over 44 years are risk factors for myopia and astigmatism; 2) the blood groups A and 0 hold a greater risk for their joint expression.

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Received: 24.07.2022
Accepted: 20.11.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 93-111

Accumulation of Heavy Metals and Metabolites in Taraxacum officinale (L.) Weber ex F.H.Wigg. on Polymetallic Contaminated Area

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Abstract. Technogenic terrains are one of the most unfavorable terrains for occupying of plant species. However, there are species that are distributed in such places and their reaction to such conditions is interesting. Some of them can be used for remediation of such terrains. The purpose of this study is to establish relationships among the heavy metals in the roots and aboveground part of T. officinale on the one hand and the metabolites synthesized under conditions of polymetallic contamination in Osogovo Mine, West Bulgaria. The metabolites were identified by GC/MS. There were determined 19 soil characteristics, content of 15 elements in aboveground and in the root system of *Taraxacum officinale*, as well as 20 metabolites. The concentrations of the studied elements in the aboveground part were significantly higher than the ones in the root system. There were found 40 significant correlations among the studied soil indicators and the studied metabolites in the roots. The number of statistically significant correlations (106) among the studied soil indicators and the studied metabolites in the aboveground parts were determined. There are also statistically significant correlations (38) among the studied chemical elements in the roots, the metabolites in the roots, and the metabolites in the aboveground part as well as 32 statistically significant correlations among the studied elements in the aboveground part of *T. officinale* and the studied metabolites in the aboveground and underground parts.

Key words: technogenic soils, heavy metals, *Taraxacum officinale*, metabolites.

Introduction

Terrain and soil resources formed or affected by mining activities are characterised by degrading properties. Depending on the mined resources, the newly formed and/or affected soils display different and specific characteristics. Sustainable management of post-mining areas is strongly linked to having good knowledge of soil and plant resources, and of the changes in the synthesis of important metabolites caused by unfavourable soil characteristics.

As a result of ore mineral extraction and processing, post-mining areas form specific habitats (Donov et al., 1978), and the formed tailings ponds, especially after mining and processing activities have ceased, can be a significant point source of pollution. Due to the high levels of heavy metals and metalloids, these sites can pose a serious environmental problem, but also a significant threat to people's health due to the degree of persistence and accumulation (Conesa et al., 2006; Martínez-Carlos et al., 2022). The environmental consequences of the presence of heavy metals and metalloids in tailings ponds can also be serious for the surrounding areas which have not been affected by ore mining and processing (García-Lorenzo et al., 2015; Tsolova et al., 2016; Khademi et al., 2018; Martínez-Carlos et al., 2022).

The substrates formed can be characterized by a high content of the sand fraction and a low content of the clay fraction, high particle density and bulk density which result in degraded hydro-physical properties (Donov et al., 1978; Martínez-Pagán et al., 2011; Chrastný et al., 2011). The availability of plant nutrients (N, P, K) and Soil Organic Matter (SOM) is usually low. Soil acidity can be extremely high and can vary considerably (Hossner & Hons, 1992; Martínez-Pagán et al., 2011). Tailings ponds formed as a result of ore mining activities can have very high, even toxic levels of heavy metals and metalloids, which can significantly affect the formation of phytocoenosis (Fernandez et al., 2017; Kovačević et al., 2020).

Many authors have studied the stages of vegetation succession on such terrains, for example Charzyński et al. (2013) present a scheme for the replacement of plant communities of technogenic substrates in parallel with soil development. Denisenko et al. (1996) investigate primary successions that occur in areas disturbed by mining and open pit mining. Titova & Vershinina (2014) comment on the changes in the floristic structure of the phytocoenosis of mechanically disturbed soil in the conditions of natural ecosystem and against the background of partial biological reclamation. The possibility for self-restoration of the phytocoenosis after technogenic impact has been assessed and the importance of reclamation for the regenerative capacity of the natural ecosystem has been established. The main component of the newly formed phytocoenosis after the disturbance is the weed-ruderal vegetation. The greatest development of weeds of the family Asteraceae is observed - more than 44%. Partial recultivation (liming) contributes to increasing the total number of plants, intensive development of cereals and the emergence of legumes.

The macro- and microelements play an extremely important role in normal plant development (Gorbanov et al., 2005). Different plant species need certain species-specific levels of macro- and micro-elements for their normal development, and both deficiencies and high concentrations of them can have an inhibiting effect on plant development (Gorbanov et al., 2005; Verbruggen et al., 2009). According to a number of authors, the optimal levels for plant development are not only species-specific but also genotype-dependent (Yang et al., 2006; Zalesny & Bauer, 2007). Elements such as Co, Cu, Fe, Mn, Mo, Ni and Zn are essential elements required for the normal growth and photosynthesis, respiration, and metabolic processes, whereas elements such as As, Cd, Hg, Pb are not considered to be essential elements (Gorbanov et al., 2005; Rascio & Navari-Izzo 2011; Martínez-Carlos et al., 2022. A number of angiosperm species can be classified as hyperaccumulators of elements such as As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, Zn (Rascio & Navari-Izzo, 2011). Plant species accumulate different heavy metals and metalloids in different concentrations in different plant organs (Gorbanov et al., 2005).

Heavy metals can affect the synthesis of metabolites and metabolic processes to varying degrees (Lajayer et al., 2017; Zheljazkov et al., 2006). A number of authors report that stress from heavy metal contamination can increase the content of some metabolites (Tirillini et al., 2006; Lajayer, et al., 2016), while other authors report a reduction of some metabolites under heavy metal stress (Murch et al., 2003). On the other hand, differences in the synthesis of secondary metabolites can be observed in different populations of the same species, which may be due to abiotic factors (Doncheva et. al., 2014; Semerdjieva et. al., 2020). One of the species found in areas subject to anthropogenic pressure associated to heavy metal contamination is *Taraxacum officinale* (L.) Weber ex FHWigg (Maleci et al., 2013; Fazekašová et al., 2015), which has been the subject of extensive research related to the synthesized metabolites (Hook, 1994; Huber et al., 2015).

The aim of this study was to determine the influence of heavy metals in soil, roots and aerial parts of *T. officinale* and the content of metabolites in response to stress caused by polymetallic pollution. The formulated hypothesis, which we tested is that the stress caused by heavy metals in the soil lead to a change in the content of metabolites in plants.

Materials and Methods

The object of study were technogenic soils (Technosols (IUSS, 2014) formed in a tailings pond created as a result of ore mining and lead-zinc ore processing in Osogovo Mine complex, situated near the village of Gyueshevo, Kyustendil district, West Bulgaria, 950 m above sea level. They were found in the Middle forest vegetation zone (700-1200 m above sea level) of the Thracian forest vegetation area (Zahariev, 1979).

The taxonomy of the plant species is presented according to Delipavlov et al. (2003). The quantitative participation of species in phytocoenoses has been assessed by the 7 degree abundant scales of abundance and coverage (Braun-Blanquet, 1964). Five sample plots, 2x2 m have been set.

Five soil samples and plant (roots and aboveground parts) materials were collected in 2021 during peak flowering (May) of *T. officinale* and by means of applying a systematic sampling method (Petersen & Calvin, 1996), where the studied soils were taken from a depth of 0-20 cm, as close to the root system of *T. officinale* as possible. Soil sampling was done in accordance with the recommendations of Donov et al. (1974), and with the procedures described in ISO 10381-1: 2002. Preliminary sample preparation was performed according to ISO 11464: 2006.

Soil samples were analyzed for: pH (H_2O) - ISO 10390:2005; specific Electrical Conductivity EC ($\mu S.cm^{-1}$) was determined according to ISO 11265:1994; $CaCO_3$ was determined using the volumetric method in accordance with ISO 10693:1995; Total Kjeldahl Nitrogen TKN, $mg.kg^{-1}$ - according to FAO.2021; plant available P_2O_5, $mg.100g^{-1}$ and K_2O, $mg.100g^{-1}$ were determined using an Acetate-Lactate extraction solution (Ivanov, 1984); Soil Organic Matter – SOM, % was determined according to Donov et al. (1984); Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn, $mg.kg^{-1}$ were determined by extraction with NH_4NO_3 and subsequent determination by ICP-OES (ISO 19730:2008; Borge, 1997; Schöning & Brümmer, 2008); the results were converted to absolute dry weight in accordance with ISO 11465:1993.

Plant samples were cleaned to remove deposits, dried and ground with an agate mill, and homogenized in a metal-free homogenizer (Kowalenko, 1984). Procedures set out in ISO 10381-1:2002 were applied; microwave digestion system (M6 PreeKem) was used in accordance with Method 3052 (USEPA, 1996) for digestion of samples and subsequent determination by ICP-OES (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn, $mg.kg^{-1}$); Total Kjeldahl Nitrogen was determined in accordance with the AOAC method 2001.11; the results were converted to absolute dry weight in accordance with Rautio et al. (2010).

Preparation of extracts and GC/MS analysis: crude extracts were prepared from 100 mg powdered plant material macerated with 1 mL methanol in 2 mL Eppendorf tubes. Fifty µL of 3,5 dichloro-4-hidroxybenzoic acid (1 mg/mL) were placed in the beginning of the extraction procedure as internal standard. After 24 h of extraction at room temperature aliquot of 500 µL of each simple was transferred to glass vial and was dried. 100 µL pyridine and 100 µL of N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) were added to the dried samples and heated at 70 °C for 2 h. After cooling, 300 mL of chloroform were added and the samples were analyzed by GC/MS. The GC-MS spectra were recorded on a Thermo Scientific Focus GC coupled with Thermo Scientific DSQ mass detector operating in EI mode at 70 eV. A DB-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The conditions of the analysis were described by Berkov et al. (2021). The GC-MS spectra of the compounds in the extracts were deconvoluted by AMDIS 2.64 software (NIST, National Institute of Standardization and Technology, Gaithersburg, MD) before their comparison with those of standard compounds and NIST spectra library. The response ratios were calculated for each metabolite relative to the internal standard using the calculated areas for both components.

Descriptive statistics and t-test was applied for the purposes of data analysis using Excel in Mac. Pearson's product-moment correlation and LSD Post-Hoc (SPSS 26 for Mac) were used to measure the associations that exist among the soil characteristics, elements and metabolites in the plants studied. The selected level of significance was α=0.05.

Results and Discussion

Most of the plant species that are distributed in the territory are pioneering and represent a stage of primary succession of vegetation (Table 1).

Table 1. Floristic composition of the tailings pond of the Osogovo Mine.

Plant species	Cover/Abundance of species	Family	Biological type
Tussilago farfara L.	1	Asteraceae	perennial
Taraxacum officinale (L.) Weber ex F.H.Wigg.	2	Asteraceae	perennial
Senecio vulgaris L.	1	Asteraceae	annual
Centaurea stoebe L.	1	Asteraceae	perennial
Cirsium arvense L. (Scop.)	1	Asteraceae	perennial
Hieracium pilosella L.	1	Asteraceae	perennial
Echium vulgare L.	2	Boraginaceae	biennial
Equisetum palustre L.	1	Equisetaceae	perennial
Euphorbia cyparissias L.	1	Euphorbiaceae	perennial
Medicago lupulina L.	1	Fabaceae	annual
Pinus sylvestris L.	1	Pinaceae	tree
Festuca valesiaca Schleich. ex Gaudin	1	Poaceae	perennial
Poa pratensis L.	1	Poaceae	perennial
Clematis recta L.	1	Ranunculaceae	perennial
Populus nigra L.	2	Salicaceae	tree

Fifteen plant species have been identified, of which 2 are trees (*Pinus sylvestris* L. and *Populus nigra* L.), 1 biennial herbaceous, 2 annual herbaceous and 10 perennial herbaceous plants. *Pinus sylvestris* is represented by single plants up to 40 cm high, and from *Populus nigra* single plants are 1.5 m high. Prevailing species are of Asteraceae (40% of all species) and Poaceae (13%) families.

Taraxacum officinale was chosen for sampling for chemical analysis because it has higher average cover and occurrence than other species in research site.

The studied soils (Table 2) according to Penkov's classification (1996) are characterised by moderately alkaline reaction in water, low carbonate content, low SOM levels, very low TKN levels, low reserve of plant available K_2O (Nikolova et al., 2014), and very low reserve of plant available P_2O_5 (Nikolova et al., 2014). The studied elements in the soil (Al, Ba, Ca, Cd, Cu, Fe, Mg, Mn, Na, Ni, Pb, Zn) are arranged in descending order by mean values as follows: Ca>Mg>Mn>Zn>Na>Fe>Pb>Al>Ba>Cu>Cd>Ni. Although there were significant variations, SOM, Ca, K_2O, $CaCO_3$, EC, TKN had the lowest levels of variation (under 20%), whereas Pb, Al, Cu, Cd, Ni, Zn, Fe had the highest levels of variation (over 50%). SOM's lowest level of variation is most likely due to the nature of formation and accumulation of soil organic matter and is related to SOM being considered a stable soil indicator (Bogdanov, 2018). Essential and non-essential elements are present in the soil in different forms (Doichinova et al., 2013), which determines their availability to plants. A number of soil indicators such as pH, SOM, soil texture can affect the availability of essential and non-essential elements to plants. Heavy metal immobilization by forming organo-mineral complexes has been discussed by various authors (Doichinova et al., 2013; Gorbanov et al., 2005).

The element with the most significant difference between its content in the root system and in the aboveground part was ~~is~~ nickel, which is the element with the lowest concentration in the studied soils (Table 3). Significantly greater variations in the concentrations of most elements in the root system were found, while only N, Ca, Cu, Na and Ni were characterized by a greater variation in the aboveground part.

Table 2. Arithmetic means and coefficients of variation of studied soil indicators.

	mean	CV, %
$pH(H_2O)$	8,33	1,28
$CaCO_3$, %	1,5	16,79
EC, $\mu S.cm^{-1}$	156,92	17,77
K_2O, $mg.100g^{-1}$	12,08	12,84
P_2O_5, $mg.100g^{-1}$	0,74	33,26
TKN, $mg.kg^{-1}$	162,9	18,49
SOM, %	0,56	10,56
Al, $mg.kg^{-1}$	1,34	59,02
Ba, $mg.kg^{-1}$	1,11	20,13
Ca, $mg.kg^{-1}$	1037,49	11,47
Cd, $mg.kg^{-1}$	0,09	62,63
Cu, $mg.kg^{-1}$	0,68	59,62
Fe, $mg.kg^{-1}$	2,3	75,99
Mg, $mg.kg^{-1}$	33,68	36,36
Mn, $mg.kg^{-1}$	10,72	41,03
Na, $mg.kg^{-1}$	2,64	33,67
Ni, $mg.kg^{-1}$	0,005	67,82
Pb, $mg.kg^{-1}$	1,49	55,51
Zn, $mg.kg^{-1}$	9,96	69,23

CV – coefficient of variation

The elements were accumulated in the root system of *T. officinale* in the following order (arranged by mean values): Ca>N>K>Fe>Al>Mg>Mn>P>Zn>Na>Pb>Cu>Ni>Ba>Cd. The accumulation of the elements in the aboveground part of the plant was in the following order (arranged by mean values): K>Ca>N>Fe>Al>Mg>Mn>P>Zn>Pb>Na>Ni>Cu>Ba>Cd. Significant differences were found between the content of K and Ca in the roots and in the leaves, where the higher values of K and Ca in the aboveground part compared to the roots have been discussed by other authors (Hook et al., 1993). Various studies (Kabata-Pendias & Dudka, 1991; Krolak, 2003; Lisiak-Zielińska et al., 2020) have reported higher concentrations of the elements Mn, Ni, Pb, Zn, Fe in the leaves compared to their levels in the roots, which has also been confirmed by the data obtained from this study. In a study, Krolak (2003) reported higher concentrations of Cd and Pb in the leaves compared to the concentrations of Cd and Pb in the roots, but these data have not been confirmed by the present study. Although the accumulation of toxic elements in the roots and their immobilization is considered a good strategy for dealing with toxic concentrations of elements (Bini et al., 2012) *T. officinale* does not accumulate toxic elements in larger quantities in the roots, and successfully translocates essential and non-essential elements from the roots to the aboveground part.

Twenty one metabolites were identified in the aboveground part and root system of *T. officinale* including phenolic, organic and fatty acids, mono- and disaccharides, triterpene and triterpene acids, sugars alcohols (Table 4). The content of the most metabolites, except malic acid, fructose 2, myo-inositol, sucrose, β-amyrin, β-sitosterol and triterpene 2, were higher in the aboveground part than in the root system.

Table 3. Arithmetic means and coefficients of variation of the studied elements in the roots and aboveground part of *T. officinale*. Legend: CV – coefficient of variation; The values in **Bold** indicate statistically significant difference at p≤0,05.

	Roots		Aboveground part	
	Mean	CV, %	mean	CV, %
N, %	0,96	13,5	1,18	32,82
P, mg.kg^{-1}	**498,1**	26,99	**822**	10,44
K, mg.kg^{-1}	**8452**	13,43	**15846**	8,85
Al, mg.kg^{-1}	**3808,8**	18,33	**6452**	11,73
Ba, mg.kg^{-1}	**26,69**	18,88	**39,34**	13,38
Ca, mg.kg^{-1}	11333	17,51	12468	17,96
Cd, mg.kg^{-1}	2,78	24,5	2,97	17,92
Cu, mg.kg^{-1}	45,09	16,38	54,56	16,69
Fe, mg.kg^{-1}	**6486**	18,14	**11295**	12,17
Mg, mg.kg^{-1}	**1766**	12,67	**2787**	11,4
Mn, mg.kg^{-1}	**1582**	20,73	**2682**	13,25
Na, mg.kg^{-1}	274,4	10,51	277,8	19,87
Ni, mg.kg^{-1}	**32,85**	26,93	**67,46**	35,51
Pb, mg.kg^{-1}	**202,41**	19,32	**341,51**	16,29
Zn, mg.kg^{-1}	389,92	23,8	458,54	19,69

Table 4. Arithmetic means and coefficients of variation of the studied metabolites in the roots and aboveground part of *T. officinale*. Legend: CV – coefficient of variation; The values in **Bold** indicate statistically significant difference at p≤0,05.

Metabolites in the roots			Metabolites in the aboveground part		
	mean	CV, %		mean	CV, %
Protocatechuic acid	0,43	80,87	Protocatechuic acid	2,06	97,35
Quinic acid	21,56	76,74	Quinic acid	68,55	142,59
Caffeic acid	26,78	77,18	Caffeic acid	560,59	204,19

Chlorogenic acid	12,08	195,18	Chlorogenic acid	37,58	178,67
Malic acid	365,15	41,14	Malic acid	204,72	126,11
Meso-erythritrol	8,39	95,15	Meso-erythritrol	15,39	59,2
Octanoic acid	594,39	88,03	Octanoic acid	717,14	137,88
Fructose 1	556,53	78,85	Fructose 1	718,51	114,88
Fructose 2	1924,63	63,89	Fructose 2	73,77	101,82
Fructose 3	44,12	138,77	Fructose 3	210,12	134,05
Glucose	97,99	173	Glucose	550,56	176,68
Myo-Inositol	**626,23**	**75,75**	**Myo-Inositol**	**1779,8**	**69,24**
Sucrose	2471,99	61,9	Sucrose	1778,71	109
β-Amyrin	77,88	44,3	β-Amyrin	61,13	115,63
Tririterpe acid	188,11	51,58	Tririterpe acid	119,86	142,25
Succinic acid	11,94	79,86	Glyceric acid	105,64	54,67
Palmitic acid	44,91	82,78	Palmitic acid	301,6	131,28
β-Sitosterol	80,09	55,59	β-Sitosterol	44,6	102,32
Triterpene 1	62,41	56,08	Triterpene 1	96,63	123,33
Triterpene 2	187,25	60,54	Triterpene 2	126,98	125

There were found 40 significant correlations among the studied soil indicators and the studied metabolites in the roots (Table 5). Twenty of the studied metabolites (in the roots) correlated with 13 out of 18 soil indicators. The following considerable numbers of significant correlations have been established – 9 for protocatechuic acid, 6 for palmitic acid, triterpene 1, triterpene 2, 5 for sucrose. Much fewer significant correlations have been found for the other metabolites. The significant correlations between fructose 1 - Na and glucose – Ca are negative, whereas all other significant correlations are positive. As regards the studied chemical elements, there were no significant correlations only for plant available P and K, and Mg, as well as for $CaCO_3$, EC and SOM. Ba and Fe had 6 significant correlations each, lead – 5; TKN, Al, Cd have 4 each, and Zn – 3. A large number of statistically significant correlations (106) among the studied soil indicators and the studied metabolites in the aboveground parts, all of which are positive (Table 6) were determined. All metabolites showed statistically significant correlations with the studied soil parameters. Three to eight were the significant correlations with quinic acid and fructose, 7 significant correlations were observed with octanoic acid, myo-inositol, sucrose, β-amyrin, palmitic acid. Six correlations were observed with chlorogenic acid, malic acid, tririterpe acid, triterpene 1; five significant correlations were observed with protocatechuic acid, caffeic acid, glucose and triterpene 2. The other metabolites had fewer significant correlations with the studied soil parameters. Of the studied soil parameters, only pH (H_2O), $CaCO_3$ K_2O, P_2O_5, TKN, Ca and Mg didn't show any significant correlations. Copper correlated with 17 metabolites, lead and zinc with 15 metabolites each, aluminum and barium with 14 metabolites each, and cadmium with 8. The other elements and EC had fewer correlations.

A number of studies (Hook et al., 1993; Rai et al., 2004; Pandey & Tripathi, 2011) discuss the relationships between the concentrations of essential and non-essential elements in the growth medium and the synthesis of metabolites in different plants. The significant correlations were found among a number of elements (Cd, Cu, Fe, Mn, Mg, Ni, Pb, Zn) and the metabolites synthesized in the organs of *T. officinale*. They confirmed the results obtained by other studies, which showed that those elements affected the synthesis of metabolites (Lajayer et al., 2017). Despite the important role of essential nutrients (N, P, K) in the synthesis of metabolites and in the regulation of the metabolic regime (Gorbanov et al., 2005), the present study did not find any significant correlations among P_2O_5, K_2O, TKN (with the exception of TKN which correlated with the metabolites in the roots) and the metabolites in the roots and the aboveground part.

Table 5. Table of the Pearson correlation coefficients among the pH (H_2O), EC, SOM and the studied chemical elements in the soil and the metabolites in the root system of *T. officinale*. Legend: * Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	Ca, mg.kg^{-1}	Ba, mg.kg^{-1}	Al, mg.kg^{-1}	SOM, %	TKN, mg.kg^{-1}	P_2O_5 mg.100^{-1}	K_2O mg.100^{-1}	EC, µS.cm^{-1}	$CaCO_3$, %	pH (H_2O)
Protocatechuic acid	,61	,993**	,933*	,27	,882*	-,49	-,03	,02	-,31	-,01
Quinic acid	,37	-,02	-,23	-,58	-,03	,56	,32	-,28	,34	,22
Caffeic acid	,73	,62	,62	,51	,61	,16	,61	,15	-,84	,73
Chloro-genic acid	,41	,27	,26	,69	,47	,44	,60	,45	-,85	,880*
Succinic acid	,29	,59	,56	,85	,83	-,01	,15	,61	-,69	,50
Malic acid	,63	,84	,71	-,27	,63	-,39	-,07	-,32	,14	-,22
Meso-erythritrol	,45	-,03	-,15	,11	,14	,84	,73	,16	-,42	,879*
Octanoic acid	,65	,64	,61	,59	,70	,14	,52	,27	-,81	,69
Fructose 1	-,24	-,44	-,65	,00	-,04	,84	,12	,44	,24	,41
Fructose 2	,74	,57	,51	,40	,61	,31	,63	,15	-,72	,76
Fructose 3	,39	,29	,29	,73	,48	,39	,57	,47	-,87	,86
Glucose	-,944*	-,39	-,43	,43	,06	-,10	-,78	,75	,41	-,40
Myo-Inositol	,58	,83	,69	,37	,912*	-,07	,13	,25	-,35	,30
Sucrose	,52	,926*	,79	,09	,87	-,41	-,15	,02	-,02	-,13
β-Amyrin	,53	,883*	,78	,47	,955*	-,21	,06	,29	-,40	,24
Tririterpe acid	-,17	-,47	-,66	-,65	-,37	,56	-,02	-,16	,69	-,07
Palmitic acid	,47	,905*	,918*	,59	,87	-,50	-,01	,23	-,55	,11
β-Sitosterol	-,85	-,23	-,29	,56	,27	-,10	-,75	,86	,32	-,31
Triterpene 1	,54	,960**	,883*	,40	,943*	-,41	-,04	,18	-,34	,06
Triterpene 2	,62	,980**	,889*	,17	,87	-,45	-,05	-,02	-,20	-,05

Table 6. Table of the Pearson correlation coefficients among the pH (H_2O), EC, SOM and the studied chemical elements in the soil and the metabolites in the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

K_2O mg.100^{-1}	EC, µS.cm^{-1}	$CaCO_3$, %	pH (H_2O)		Zn, mg.kg^{-1}	Pb, mg.kg^{-1}	Ni, mg.kg^{-1}	Na, mg.kg^{-1}	Mn, mg.kg^{-1}	Mg, mg.kg^{-1}	Fe, mg.kg^{-1}	Cu, mg.kg^{-1}	Cd, mg.kg^{-1}
-,091	-,494	-,123	-,386	Protocatechuic acid	,915*	,970**	,896*	,49	,88	,70	,988**	,908*	,905*
-,137	-,251	-,141	-,288	Quinic acid	-,15	-,14	-,36	-,29	-,43	-,34	-,10	-,20	-,11
-,287	-,266	,07	-,448	Caffeic acid	,33	,55	,59	,23	,64	,00	,73	,49	,30
-,224	-,217	,015	-,351	Chlorogenic acid	-,03	,19	,26	-,15	,36	-,25	,44	,10	-,01
-,123	-,392	-,089	-,354	Malic acid	,41	,55	,58	-,04	,67	,28	,75	,42	,46
-,142	,951*	-,364	,377	Meso-erythritol	,81	,80	,61	,44	,53	,60	,74	,75	,80
-,157	-,168	-,089	-,254	Octanoic acid	-,34	-,16	-,23	-,41	-,19	-,61	,06	-,27	-,31
-,039	-,529	-,18	-,356	Fructose 1	,36	,57	,59	,15	,65	,07	,76	,48	,36
,164	-,649	-,329	-,224	Fructose 2	-,56	-,55	-,70	-,898*	-,67	-,53	-,38	-,69	-,43
-,139	-,06	-,154	-,173	Fructose 3	,27	,47	,45	,09	,49	-,08	,67	,38	,26
-,287	-,303	,118	-,468	Glucose	,00	,22	,30	-,13	,40	-,22	,46	,12	,01
,177	-,39	-,447	-,048	Myo-Inositol	-,18	-,34	-,34	-,63	-,29	,23	-,32	-,42	-,04
-,083	-,334	-,16	-,28	Sucrose	,66	,75	,63	,10	,64	,42	,88	,62	,70
-,228	-,351	-,014	-,443	β-Amyrin	,881*	,885*	,73	,32	,68	,71	,887*	,80	,907*
-,252	-,336	,044	-,454	Tririterpe acid	,74	,83	,74	,18	,76	,53	,939*	,71	,77
,319	,077	-,481	,393	Glyceric acid	-,46	-,55	-,75	-,56	-,81	-,42	-,56	-,59	-,38
-,227	-,352	-,044	-,444	Palmitic acid	,82	,908*	,930*	,46	,958*	,66	,967**	,85	,81
-,244	-,408	-,004	-,496	β-Sitosterol	-,06	-,19	-,22	-,65	-,15	,31	-,13	-,31	,10
-,22	-,387	-,001	-,454	Triterpene 1	,87	,928*	,85	,35	,85	,67	,984**	,84	,880*
-,303	-,298	,08	-,487	Triterpene 2	,907*	,947*	,84	,45	,80	,69	,955*	,88	,906*

Zn, $mg.kg^{-1}$	Pb, $mg.kg^{-1}$	Ni, $mg.kg^{-1}$	Na, $mg.kg^{-1}$	Mn, $mg.kg^{-1}$	Mg, $mg.kg^{-1}$	Fe, $mg.kg^{-1}$	Cu, $mg.kg^{-1}$	Cd, $mg.kg^{-1}$	Ca, $mg.kg^{-1}$	Ba, $mg.kg^{-1}$	Al, $mg.kg^{-1}$	SOM, %	TKN, $mg.kg^{-1}$	P_2O_5 $mg.100^{-1}$
,882*	,878	,880*	,905*	,81	,715	,729	,952*	,777	,569	,828	,896*	-,14	,41	-,808
,976**	,982**	,925*	,72	,87	,798	,898*	,984**	,923*	,583	,964**	,956*	,032	,691	-,722
,977**	,933*	,837	,655	,764	,852	,818	,932*	,943*	,474	,913*	,875	-,084	,654	-,757
,974**	,950*	,841	,605	,774	,819	,865	,930*	,948*	,528	,946*	,888*	-,039	,72	-,678
,946*	,941*	,895*	,804	,825	,768	,821	,975**	,871	,593	,913*	,927*	-,096	,564	-,758
-,225	-,174	-,098	-,614	,031	-,058	,031	-,302	-,103	-,474	-,164	-,175	,905*	,347	,262
,972**	,974**	,877	,597	,822	,793	,916*	,945*	,947*	,573	,977**	,921*	,045	,77	-,637
,832	,84	,872	,945*	,807	,662	,687	,932*	,711	,572	,782	,878*	-,136	,338	-,803
,517	,561	,695	,984**	,649	,355	,404	,716	,35	,512	,481	,673	-,17	-,027	-,675
,961**	,978**	,882*	,527	,842	,78	,952*	,928*	,950*	,563	,989**	,924*	,152	,841	-,584
,962**	,911*	,802	,647	,722	,836	,788	,912*	,927*	,477	,893*	,847	-,144	,624	-,74
,827	,910*	,949*	,879*	,913*	,568	,844	,963**	,715	,739	,886*	,964**	,068	,51	-,628
,954*	,967**	,923*	,78	,863	,758	,871	,988**	,884*	,624	,946*	,955*	-,023	,625	-,723
,962**	,932*	,881*	,776	,81	,827	,799	,963**	,898*	,504	,895*	,906*	-,088	,569	-,812
,967**	,928*	,85	,725	,774	,832	,798	,946*	,914*	,499	,899*	,884*	-,117	,595	-,783
,644	,778	,692	,264	,693	,335	,893*	,682	,645	,746	,856	,756	,297	,823	-,057
,956*	,929*	,894*	,801	,827	,827	,793	,968**	,886*	,492	,884*	,912*	-,065	,551	-,835
,929*	,891*	,865	,831	,793	,815	,734	,947*	,85	,463	,837	,879*	-,121	,474	-,862
,951*	,919*	,868	,792	,794	,813	,778	,957*	,881*	,509	,880*	,894*	-,123	,536	-,814
,973**	,923*	,838	,691	,763	,859	,793	,934*	,930*	,454	,893*	,871	-,103	,612	-,795

There were statistically significant correlations (38) among the studied chemical elements in the roots, the metabolites in the roots, and the metabolites in the aboveground part, which have been presented in Table 7. Only one statistically significant correlation was positive and all the rest were negative. There were found 21 statistically significant correlations among the studied elements in the roots and the identified metabolites in the roots. Mallic acid had ten significant correlations with the studied elements in the roots, and only one significant correlation with the studied chemical elements (with sodium) in the aboveground part.A large number of significant correlations were observed among sucrose in the roots and the studied elements in the roots (5), where, as in the case with malic acid, sucrose also had only one significant correlation with the studied elements and it was with Na.

A number of the identified metabolites in the aboveground part (quinic acid, caffeic acid, chlorogenic acid, meso-Erythritrol, octanoic acid, glucose, triterpene acid) correlated significantly with the studied elements in the roots, but on the other hand these same metabolites (in the roots) did not correlate with the studied elements in the roots.

Table 7. Table of the Pearson correlation coefficients among the studied chemical elements in the root system and the metabolites in the root system and the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). ± the compound has been found only in the roots. No statistically significant correlations were found among the studied elements in the roots and Protocatechuic acid, Fructose 1, Fructose 2, Fructose 3, Palmitic acid, β-Sitosterol, Triterpene 1, due to which they have not been presented in the table. The correlations marked in brown are among the studied elements in the roots and the metabolites in the roots. The correlations marked in green are among the studied elements in the roots and the metabolites in the aboveground part. Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	Malic acid		Succinic acid ±	Chlorogenic acid		Caffeic acid		Quinic acid	
N, %	,249	-,250	-,407	-,02	-,393	,082	-,255	,124	-,614
P, mg.kg-1	-,745	-,925*	,132	-,829	,413	-,832	,043	-,724	-,472
K, mg.kg-1	-,736	-,799	,211	-,8	,595	-,836	,253	-,704	-,220
Al, mg.kg-1	-,411	-,755	-,616	-,625	-,428	-,528	-,572	-,525	-,515
Ba, mg.kg-1	-,402	-,729	-,662	-,615	-,469	-,516	-,601	-,522	-,471
Ca, mg.kg-1	-,763	-,980**	-,248	-,899*	,058	-,861	-,278	-,801	-,457
Cd, mg.kg-1	-,613	-,907*	-,225	-,706	-,184	-,637	-,503	-,636	-,699
Cu, mg.kg-1	-,684	-,924*	-,035	-,737	,020	-,694	-,350	-,671	-,668
Fe, mg.kg-1	-,647	-,949*	-,289	-,777	-,151	-,71	-,462	-,689	-,65
Mg, mg.kg-1	-,806	-,950*	-,061	-,909*	,295	-,904*	-,068	-,813	-,357
Mn, mg.kg-1	-,586	-,934*	-,247	-,744	-,091	-,678	-,375	-,632	-,691
Na, mg.kg-1	-,884*	-,982**	-,165	-,922*	,052	-,898*	-,367	-,881*	-,397
Ni, mg.kg-1	-,664	-,696	,479	-,594	,544	-,632	,148	-,56	-,404
Pb, mg.kg-1	-,545	-,902*	-,098	-,665	-,044	-,603	-,343	-,562	-,783
Zn, mg.kg-1	-,815	-,974**	-,268	-,876	-,105	-,83	-,492	-,831	-,483

Triterpene 2		Triterpe acid		β-Amyrin		Sucrose		Myo-Inositol		Glucose		Octanoic acid		Meso-erythritol	
,149	-,252	,174	-,508	,252	-,459	,198	-,375	,361	-,573	,072	-,218	-,05	-,333	-,41	-,658
-,820	-,700	-,812	-,263	-,759	-,414	-,715	-,779	-,475	-,457	-,865	,31	-,774	,045	,654	,18
-,841	-,599	-,822	-,151	-,781	-,287	-,691	-,682	-,427	-,284	-,864	,071	-,728	,236	,588	,477
-,466	-,807	-,455	-,135	-,392	-,875	-,466	-,867	-,306	-,939*	-,529	,162	-,661	-,628	-,109	-,493
-,455	-,808	-,443	-,089	-,383	-,894*	-,461	-,861	-,311	-,951*	-,514	,139	-,657	-,66	-,162	-,496
-,829	-,889*	-,82	-,121	-,764	-,723	-,77	-,952*	-,551	-,761	-,878	,294	-,883*	-,303	,377	-,047
-,596	-,779	-,612	-,234	-,562	-,66	-,634	-,805	-,534	-,755	-,661	,671	-,716	-,476	,483	-,442
-,667	-,732	-,685	-,249	-,64	-,528	-,683	-,758	-,574	-,615	-,725	,71	-,725	-,306	,667	-,27
-,666	-,846	-,671	-,216	-,614	-,734	-,669	-,891*	-,518	-,815	-,731	,517	-,783	-,461	,394	-,361
-,887*	-,812	-,875	-,115	-,825	-,569	-,79	-,880*	-,548	-,586	-,925*	,227	-,868	-,088	,498	,193
-,631	-,807	-,63	-,308	-,563	-,699	-,605	-,878	-,418	-,789	-,702	,419	-,742	-,385	,366	-,348
-,880*	-,879*	-,891*	,02	-,86	-,652	-,884*	-,884*	-,758	-,679	-,914*	,591	-,911*	-,344	,593	-,024
-,649	-,388	-,668	-,227	-,65	-,021	-,608	-,406	-,509	-,064	-,674	,623	-,53	,225	,965**	,244
-,562	-,711	-,57	-,393	-,507	-,57	-,554	-,773	-,403	-,682	-,636	,576	-,658	-,323	,521	-,400
-,800	-,895*	-,814	-,014	-,778	-,725	-,83	-,897*	-,723	-,77	-,844	,633	-,881*	-,471	,509	-,204

There were 32 statistically significant correlations among the studied elements in the aboveground part of *T. officinale* and the studied metabolites in the aboveground and underground part (Table 8). All 22 significant correlations among the studied elements in the aboveground part and the metabolites in the roots were positive. All ten correlations between the studied elements and the metabolites in the aboveground part were negative. The only metabolites found both in the roots and in the aboveground part that correlated with some of the studied chemical elements were fructose 1, fructose 2 and β-sitosterol. No significant correlations were found between the studied elements in the aboveground part and malic acid (both in the roots and in the aboveground part), although malic acid (in the roots) correlated in many of the cases with the studied elements in the roots.

The toxic levels of essential and non-essential elements can cause oxidative stress and a number of other changes in physiology, including different localization of metal ions (in roots and leaves), accumulation and storage as non-toxic forms, formation of complexes with organic acids or peptides, etc., and unlock various mechanisms in the plant organism for adaptation to stress (Clijsters et al., 1999; Bretzel et al., 2013), which can result in a change in the amount of secondary metabolites (Lajayer et al., 2017). The increase or decrease in the content of various metabolites in plants subjected to metal stress has been discussed by other authors (Misra & Sharma, 1991;

Zheljazkov & Nielsen, 1996). Our data confirmed the increase in secondary metabolites caused by heavy metal stress reported by other authors (Lajayer et al., 2017), but there was also a decrease in the synthesis of secondary metabolites, which has been discussed by other authors (Murch et al., 2003). The significant correlations among Cu, Pb, Zn and the secondary metabolites in the leaves, and the lower concentrations of these same metals in the roots compared to those in the leaves confirmed data obtained from a study conducted by other authors on the impact of heavy metals on metabolites in *T. officinale* (Bretzel et al., 2013).

Table 8. Table of the Pearson correlation coefficients among the studied chemical elements in the aboveground part and the metabolites in the root system and the aboveground part of *T. officinale*. Legend: * Correlation is significant at the 0,05 level (2-tailed). ** Correlation is significant at the 0,01 level (2-tailed). No statistically significant correlations were found among the studied elements in the aboveground part and Quinic acid, Succinic acid, Malic acid, Meso-erythritol, Sucrose, β-Amyrin, Triterpene 1, Triterpene 2, Glyceric acid, due to which they have not been presented in the table. The correlations marked in brown are among the studied elements in the aboveground part and the metabolites in the roots. The correlations marked in green are among the studied elements in the aboveground part and the metabolites in the aboveground part. Due to the large amount of data, the correlation coefficients have been presented without 0 before the decimal point.

	Fructose 2	Fructose 1		Octanoic acid		Chlorogenic acid		Caffeic acid		Protocatechuic acid	
N, %	-,176	-,969**	,920*	-,714	-,305	-,706	,014	-,746	-,348	-,941*	-,65
P, mg.kg^{-1}	-,058	-,894*	,879*	-,497	-,111	-,505	,158	-,558	-,203	-,843	-,386
K, mg.kg^{-1}	-,292	-,862	,859	-,686	-,477	-,651	-,21	-,674	-,488	-,835	-,699
Al, mg.kg^{-1}	,995**	,107	,165	,383	,977**	,282	,919*	,173	,979**	,121	,605
Ba, mg.kg^{-1}	,986**	,197	,052	,403	,978**	,302	,903*	,201	,994**	,201	,614
Ca, mg.kg^{-1}	,051	-,644	,902*	-,263	-,176	-,239	-,067	-,301	-,195	-,575	-,264
Cd, mg.kg^{-1}	-,307	-,684	,628	-,317	-,286	-,298	-,108	-,313	-,404	-,622	-,277
Cu, mg.kg^{-1}	,321	-,54	,697	,018	,325	-,02	,422	-,103	,208	-,461	,178
Fe, mg.kg^{-1}	,404	-,509	,703	,064	,396	,02	,477	-,071	,286	-,428	,233
Mg, mg.kg^{-1}	,024	-,693	,913*	-,204	-,128	-,187	-,011	-,25	-,197	-,607	-,171
Mn, mg.kg^{-1}	,362	-,356	,526	,195	,409	,153	,438	,076	,286	-,275	,352
Na, mg.kg^{-1}	-,298	-,62	,525	-,276	-,234	-,264	-,063	-,275	-,359	-,565	-,222
Ni, mg.kg^{-1}	,519	-,772	,859	-,388	,431	-,457	,69	-,563	,388	-,746	-,161
Pb, mg.kg^{-1}	,577	-,203	,392	,314	,647	,247	,642	,157	,539	-,136	,513
Zn, mg.kg^{-1}	-,334	-,679	,628	-,316	-,32	-,292	-,147	-,305	-,436	-,616	-,288

β-Sitosterol		Palmitic acid		Tririterpe acid		Myo-Inositol		Glucose		Fructose 3		
-,891*	,44	-,879*	-,678	-,799	,733	-,943*	-,265	-,722	,474	-,671	-,022	-,942*
-,755	,706	-,718	-,362	-,635	,523	-,825	-,008	-,553	,671	-,422	,136	-,986**
-,796	,222	-,804	-,828	-,712	,895*	-,903*	-,371	-,632	,297	-,681	-,256	-,822
,052	-,292	,132	,65	,147	-,295	,437	,807	,145	-,483	,468	,907*	-,005
,116	-,378	,189	,671	,188	-,372	,518	,769	,172	-,557	,478	,895*	,12
-,526	,077	-,501	-,518	-,362	,935*	-,613	,092	-,254	,062	-,247	-,123	-,793
-,485	,907*	-,462	-,251	-,389	,383	-,716	-,049	-,311	,885*	-,261	-,111	-,845
-,36	,696	-,286	,197	-,197	,212	-,377	,509	-,115	,547	,12	,411	-,808
-,337	,627	-,258	,24	-,165	,208	-,323	,575	-,083	,466	,168	,464	-,786
-,511	,429	-,472	-,353	-,333	,794	-,652	,191	-,219	,377	-,16	-,053	-,918*
-,17	,705	-,092	,389	-,016	,035	-,193	,612	,055	,535	,297	,438	-,662
-,427	,962**	-,402	-,146	-,348	,231	-,645	-,031	-,283	,931*	-,214	-,057	-,774
-,751	,254	-,686	-,097	-,624	,319	-,516	,298	-,57	,151	-,284	,659	-,799
-,065	,552	,022	,593	,076	-,175	,025	,75	,124	,351	,426	,647	-,489
-,477	,897*	-,456	-,276	-,381	,412	-,723	-,066	-,3	,880*	-,265	-,151	-,842

Conclusions

Significant concentrations of available heavy metals have been found in technogenic soils on which phytocoenoses with the participation of *T. officinale* have been formed. There was a greater accumulation of elements (P, K, Al, Ba, Fe, Mg, Mn, Ni, Pb) in the aboveground part compared to the roots, which has confirmed previous data reported by other authors on the greater accumulation of heavy metals in the aboveground part. There were significantly more (38) correlations between the investigated chemical elements in the roots, metabolites in the roots and metabolites in the aerial part, compared to the correlations (32) between the investigated elements in the aerial part of *T. officinale* and the investigated metabolites in the aerial and underground parts. It was established that the investigated elements can influence (positively and/or negatively) the content of metabolites in the different parts of the plants. Also, affecting the content of a given metabolite in one plant part does not necessarily mean that the same metabolite will be affected in the same way in other plant parts. The obtained results confirm the formulated hypothesis - that the stress caused by heavy metals in the soil causes a change in the content of metabolites in plants.

Acknowledgments. The studies were funded by the project: Migration of heavy metals in the soil-plant system, SIS UF, № 1142/5.04.2021.

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Received: 01.09.2022
Accepted: 22.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 113-121

Effect of the Urban Heat Island in Plovdiv City (Bulgaria) on the Species Composition and Distribution of the Dragonflies (Insecta: Odonata)

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Abstract. The current paper researches the impact of the urban heat island effect on the species composition and distribution of dragonflies (Insecta: Odonata) along the Maritsa River in the city of Plovdiv, Bulgaria. The study was conducted on imaginal and larval forms of the species, and the studied area was divided into 3 sub-areas (urban, suburban and rural) according to the proximity to the city center. Along the urban gradient from the rural to the urban zone, an increase in air, water and soil temperature by ≈1-2ºC was observed. Differences were found also in the dissolved oxygen in the water, which had the highest values in the rural area (10.70 mg/l) and decreased towards the urban area, where it was 9.03 mg/l. Four dragonfly species were confirmed for the study area, and 2 new species were recorded. The most species were found in the urban zone, probably due to the higher temperatures compared to the other two areas, while at the same time, no larvae were found there, due to the lower amount of dissolved oxygen in the water. The current paper gives a better understanding of the impact that the urban heat island effect has on dragonflies in cities and aims to contribute for timely measures and decisions for the management of wetlands around urban areas.

Key words: temperature, dissolved oxygen, Odonata, dragonflies, Plovdiv City, urban area.

Introduction

One of the biggest problems of the XXI century are climate anomalies, which lead to major changes in wetland regimes and changes in environmental conditions and affect all organisms in these ecosystems. Indeed, wetlands are most vulnerable in and around cities due to the presence of the "heat island effect". Urban heat islands are formed when the natural landscape is gradually replaced by asphalt, concrete and gravel used to build roads, buildings and other structures that retain heat, and this causes the temperature in cities to rise several degrees compared to the surroundings them (Oke, 1982).

Dragonflies (Odonata) are good indicators in aquatic ecosystems (Kalkman et al., 2008), since they are closely related to habitat quality, and their life cycle is directly affected by climate change. The changes in the climate are also clearly noticeable in the city of Plovdiv (Bulgaria), and Maritsa River is extremely important water basin for the dragonflies in the area. Proper management of green spaces and especially wetlands in cities would ensure stability in urban systems (Lovejoy & Hannah, 2005). A better understanding of the impact that the urban heat island effect has on dragonflies in cities will contribute to timely measures and decisions for the management of wetlands around urban areas, which determines the relevance of the present work, which aims to investigate the impact of the urban heat island effect on the species composition and distribution of dragonflies (Insecta: Odonata) along the Maritsa River in the city of Plovdiv.

Material and Methods

Study area. The study period was from May to August 2022, when the dragonflies are most active. The studied area was divided into 3 sub-areas (Fig. 1.) according to the proximity to the city center. The first site is located directly in the central part of the city of Plovdiv and covers the territory from the "Vasil Aprilov" bridge to the point of confluence of the Parvenetska River with Maritsa River (Site 1 - urban zone). The second site covers the point of confluence of Parvenetska River to the bridge at the 6th kilometer west of the city (Site 2 - suburban zone) and the third site is the farthest from the urban part and covers the bridge at the 6th kilometer to a small dam before the village of Orizari (Site 3 - rural zone).

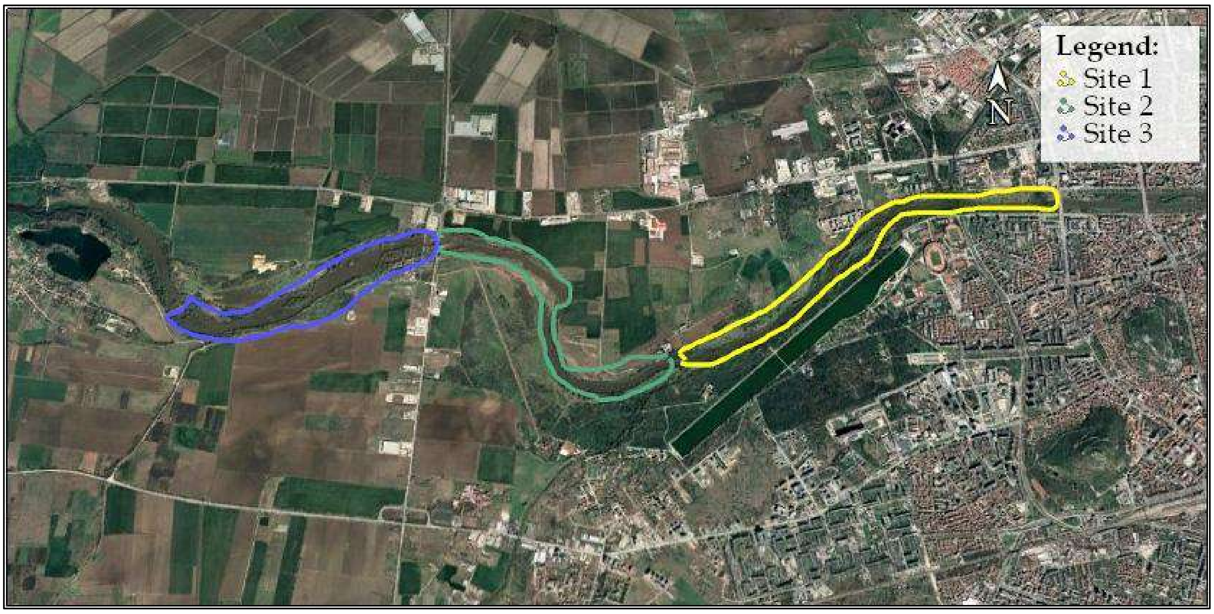

Fig. 1. Map of the study area with the boundaries of the studied sites.

Field surveys

The study was conducted on imaginal and larval forms of the order Odonata. The species composition of the imaginal individuals was established by means of transects (Gregory et al., 2004) at the north bank of Maritsa River. Where possible, the individuals were captured via entomological net, identified in the field and immediately released afterwards. Otherwise, a photographic technique with macro mode (Sony Alpha 58) was used for detailed photography of the registered species. The determination by photographic material was carried out later in laboratory conditions.

Larval material was collected using underwater live traps (Bock et al., 2009). Traps were placed at pre-selected points with similar characteristics in the three studied sites for comparability in data analysis. Traps were set monthly during the study period. After placing the traps, they are checked every 24 hours.

For the determination of the registered dragonflies (imago and larvae), we used specialized field guides by Beshovski (1994), Marinov (2000) and Smallshire & Swash

(2020), paying attention to the most taxonomically important features: size and shape of the eyes, shape and coloration of the prothorax, shape and coloration of legs, wing venation, coloration and shape of abdominal segments and copulatory apparatus of male and female specimens. For each captured larval individual, the determination was made by morphological marks with the help of a monocular magnifying glass. Valid species names are according to the "World Odonata Checklist" (Paulson et al., 2022).

At the points where the traps are placed, several abiotic factors (water, air and soil temperature, pH and dissolved oxygen in the water) are measured. The dissolved oxygen was measured using oximeter "Oxi 3205"; for measuring pH - an electronic pH meter "Lovibond SensoDirect 150", and for measuring the temperature of air, water and soil, a mercury thermometer was used, with accuracy to 0.1°C.

The data of the measured abiotic factors from the three zones were processed with descriptive statistics and compared using the Kruskal-Wallis test for independent samples, since the data did not have a normal distribution. In order to establish similarity between the three investigated areas (urban, suburban and rural), a cluster analysis was performed based on the quantitative data (number) (unweighted per-group average, Bray-Curtis similarity index), and for the distribution of the relationships of the species in the three areas, again, on the basis of the quantitative data, a corresponding analysis was made. Statistical package "PAST" v.4.11 was used for the statistical processing of the data. (Hammer et al., 2001).

Results and Discussion

Analysis of abiotic factors in the studied areas

The values of air, water and soil temperature, dissolved oxygen and water reaction (pH) in the studied parts of the Maritsa River in the city of Plovdiv during the whole period of study are presented in Fig. 2.

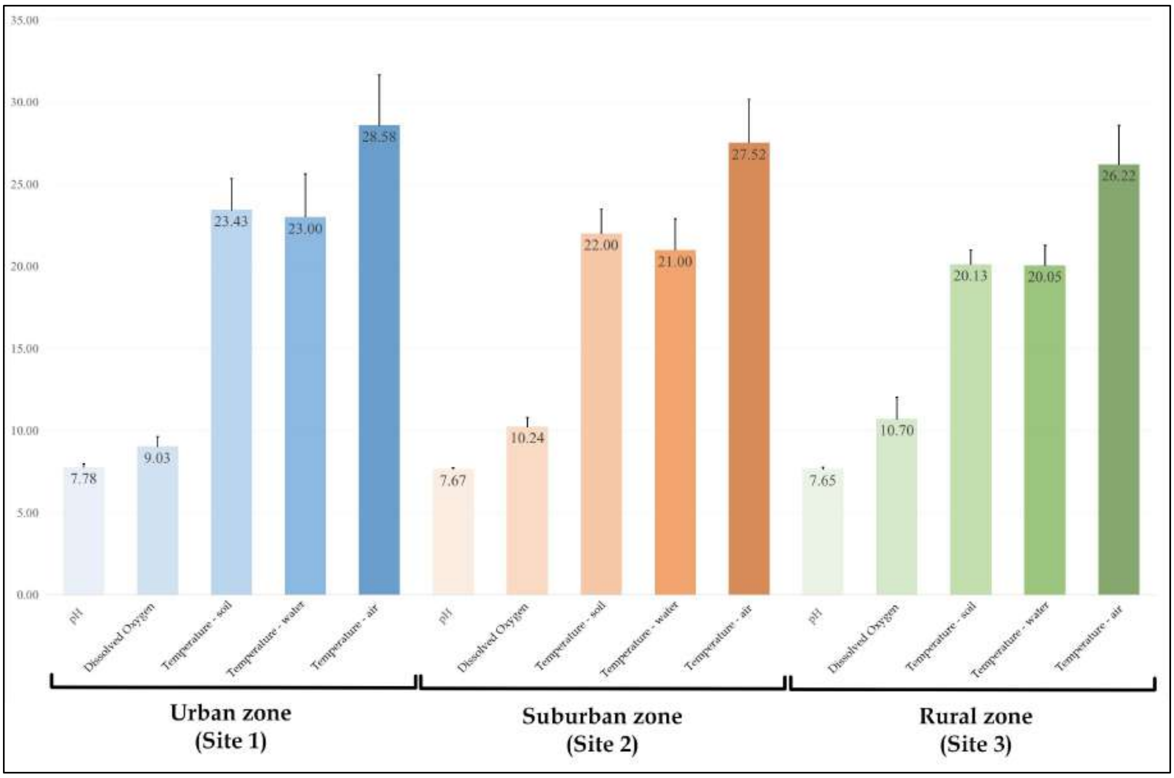

Fig. 2. Abiotic factors (mean values and standard deviations), measured along the urban gradient along the Maritsa River in the city of Plovdiv (total for 2022).

As it can be seen from the figure - along the urban gradient from the rural to the urban zone, an increase in air, water and soil temperature by ≈1-2ºC is observed. The Kruskal-Wallis test based on the pooled data for the whole period shows statistically significant differences when comparing the air temperature of the three zones (H=9.803, p=0.00742), as well as the water temperature (H=21.43, p=0.0189) and the soil temperature (H=40.75, p=0.046). In the studied area for the period May-August 2022, we can talk about the presence of a "urban heat island effect" along the urban gradient along the Maritsa River in the city of Plovdiv.

Statistically significant differences were also registered regarding the dissolved oxygen in the water (H=23.71, p=0.00000854), which has the highest values in the rural area (10.70 mg/l) and decreases towards the urban area, where it is 9.03 mg/l . The only indicator that did not show statistically significant differences when comparing the three zones is pH (H=2.213, p=0.33), which ranges between 7.65-7.78 (Fig. 2).

Species composition and distribution

Up to date, a total of 24 species from 8 families have been reported for the studied areas, which, out of a total of 71 species in Bulgaria, makes 33.8% of the species composition in the country (Gainzarain, 2017): *Aeshna affinis, Aeshna mixta, Anax ephippiger, Anax parthenope, Calopteryx splendens, Crocothemis erythraea, Coenagrion puella, Gomphus vulgatissimus, Ischnura elegans, Ischnura pumilio, Lestes barbarous, Lestes dryas, Libellula fulva, Ophiogomphus cecilia, Onychogomphus forcipatus, Ophiogomphus serpentinus, Orthetrum albistylum, Orthetrum brunneum, Platycnemis pennipes, Sympetrum meridionale, Stylurus flavipes, Sympetrum depressiusculum, Sympecma fusca, Sympetrum fonscolombii* (Klapalek, 1894; Petkov, 1914; 1921; Urbanski, 1947; Angelov, 1960; Beshovski, 1964; Rusev, 1966; Dumont, 1977; Uzunov et al., 1981; Marinov, 2001).

From the 24 species reported in the literature, only 4 species were confirmed in the field studies. Another 2 species are new to the studied area: *Calopteryx virgo* (Linnaeus, 1758) and *Orthetrum cancellatum* (Linnaeus, 1758) - Fig. 3-4.

Fig. 3. *Orthetrum cancellatum.*

Fig. 4. *Calopteryx virgo.*

Calopteryx virgo was found in the urban zone among coastal vegetation. A male was observed among a group of *Calopteryx splendens* and *Platycnemis pennipes. C. virgo* is a rheophilic species and inhabits more often the upper reaches of rivers at altitudes above 400 m (Marinov, 2000). *Orthetrum cancellatum* was found again in the central urban area with 2 males. The species is limnophilous but is found in water bodies throughout the country (Marinov, 2000).

The most numerous is *Calopteryx splendens*. Both single specimens and groups of about 20 or more individuals have been found in the three studied sites. Such groups have been observed near the banks of the river with the presence of coastal and submerged aquatic vegetation.

This indicates that macrophyte habitat cover is an important factor for dragonfly populations (Pereira et al., 2019). To avoid overheating, some species may seek shady locations (May, 1976; Mazzacano et al., 2014). Junior et al. (2015) observed high species richness of Anisoptera in habitats that were ecologically characterized between built-up and well-preserved areas, while Zygoptera showed specificity for preserved habitats. They have greater richness in protected areas than in degraded habitats (de Carvalho et al., 2013).

In larval stage, only 3 species were found from the suburban and rural zones: *Calopteryx splendens* (Harris, 1782), *Platycnemis pennipes* (Pallas, 1771) and *Libellula fulva* Muller, 1764, as well as one individual determined only to the family level (Libellulidae).

Table 1 presents the species composition and numbers of the recorded species and their distribution in the three sites (rural, suburban and rural).

Table 1. Distribution and abundance (N) of Odonata (imago et larvae) in the three zones along the urban gradient along the Maritsa River in the city of Plovdiv.

Site 1 (urban zone)				**Site 2 (suburban zone)**				**Site 3 (rural zone)**			
Species	N	Males	Females	Species	N	Males	Females	Species	N	Males	Females
Larvae											
				Platycnemis pennipes	5			*Calopterix splendens*	8		
				Calopterix splendens	11			Libellulidae	1		
								Libellula fulva	1		
Imago											
Platycnemis pennipes	2		2	*Libellula fulva*	1	1		*Calopterix splendens*	27	23	4
Calopterix splendens	7	4	3	*Calopterix splendens*	32	3	29	*Orthetrum albistylum*	1	1	
Calopterix virgo	1	1									
Libellula fulva	3	2	1								
Orthetrum cancellatum	2	2									

It can be seen from the table that no larvae were detected in the urban zone, which may be due to the relatively lower content of dissolved oxygen in the water. Larvae are entirely aquatic organisms that directly depend on oxygen levels, dissolved in the water (Beshovski, 1994). Most of the individuals found are from the suborder Zygoptera, whose larvae possess gill plates. These species prefer waters with higher dissolved oxygen content. Dragonfly larvae are affected by many physical and biological factors, some of which may be limiting to their distribution (Vannote et al., 1980; Power, 2006). Although there are many observations in this area, almost no experimental tests have been conducted (Leipelt, 2005). The most numerous species is *Calopteryx splendens*. Willigalla & Fartmann (2012) indicate that species richness increases along the urban gradient from the city center to the outskirts of the city.

Fig. 5 presents a dendrogram built on the basis of cluster analysis of the quantitative data (abundance) of Odonata species in the three studied areas. The dendrogram shows that the urban zone separates into a single cluster with only about 30% similarity to the other two zones, which form a second cluster with about 85% similarity between them. As already mentioned the urban zone is where the majority of species were found, some of which are not found in the other two areas. Dragonflies belong to the so-called warm-adapted animals (Marinov, 2000), accordingly, the high species diversity can probably be attributed to the higher average air temperature (Fig. 2). There is also evidence that species of the suborder Zygoptera are more sensitive to dynamics in environmental factors (Perez & Bautista, 2020; Sganzerla et al., 2021) and as it can be seen from Table. 1 in the urban zone, no large aggregations of species of the suborder Zygoptera were found. Another factor that may affect the distribution of dragonflies is the food resource. Higher temperatures may contribute to higher densities of other insects that represent a food base attracting dragonflies. Fig. 6. presents a graph from the correspondence analysis, which shows the distribution of species (based on the quantitative data) in relation to the three zones.

Some researchers believe that urbanization is having an adverse effect on dragonfly populations. One study shows that species richness increases from urban to rural areas (Willigalla & Fartmann, 2012). One of the reasons, according to the authors, is that in the center of the cities the number of water basins is much smaller and insignificant. The result of the studies conducted by Perez & Bautista (2020) and Sganzerla et al. (2021), proving that representatives of the suborder Zygoptera are more sensitive to changes in environmental factors compared to species of the suborder Anisoptera. Ambient relative humidity and temperature can affect the abundance of certain species in their populations, and vegetation in riparian habitats is critical for increasing dragonfly populations in highly urbanized areas (Chrislene et al., 2022).

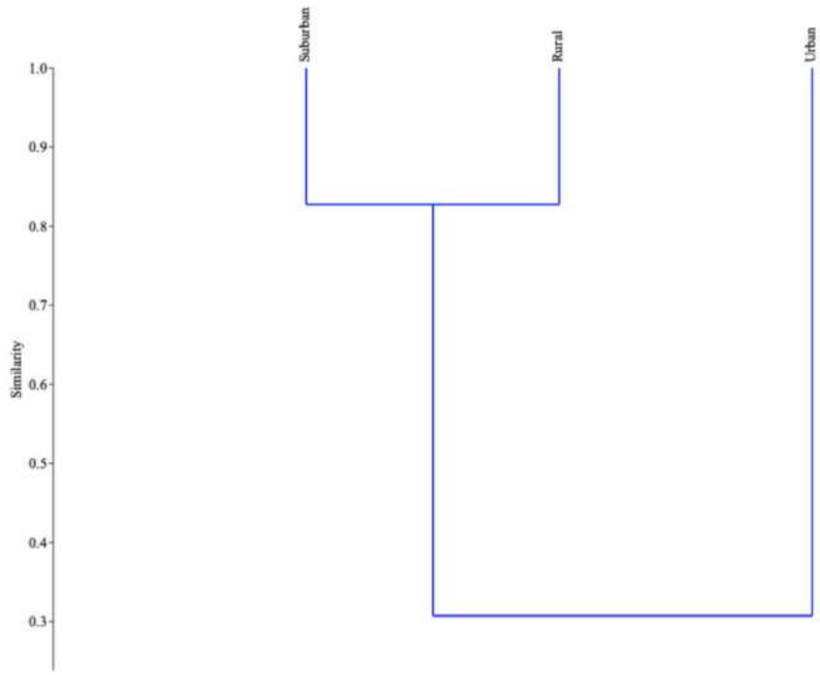

Fig. 5. Dendrogram (unweighted per-group average, Bray-Curtis similarity index) showing the similarity of dragonflies (based on quantitative data) in the three studied areas (urban, suburban and rural zones).

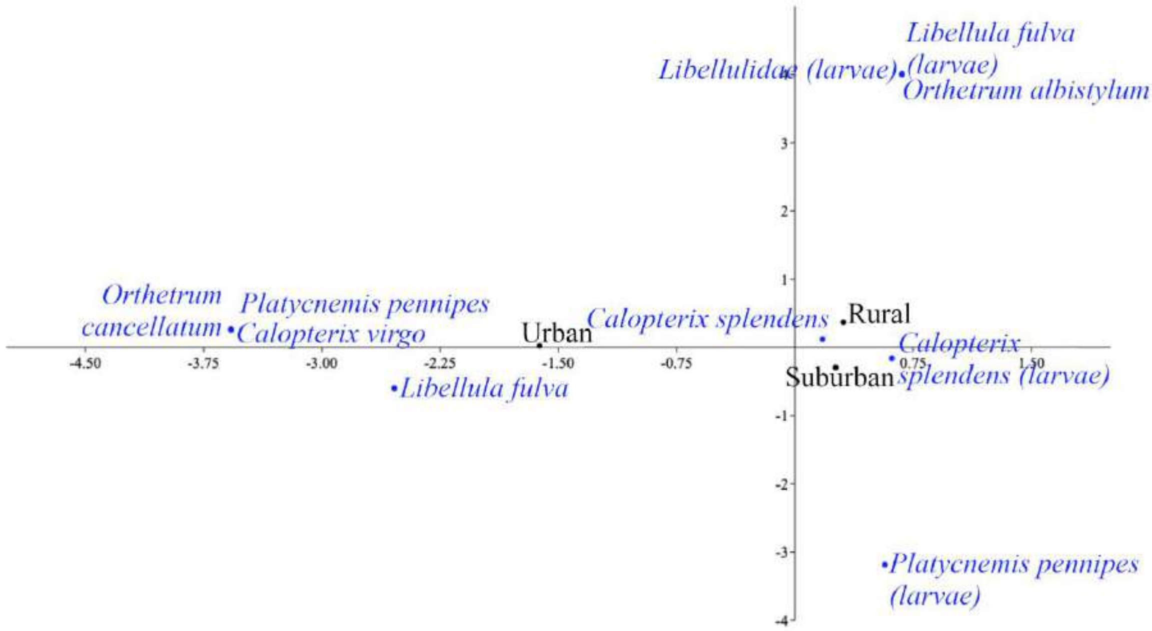

Fig. 6. Correspondence analysis showing the relationship of dragonfly species (based on quantitative data) in the three studied areas (urban, suburban and rural zones).

Another study conducted in Germany (Goertzen & Suhling, 2018) focused on examining the species richness of dragonflies in agricultural areas and in urban environments. The results show that the urban environment is characterized by the presence of more opportunistic and thermophilic species. The authors concluded that urban landscapes may have more diverse habitats and support greater species richness of dragonflies than agricultural areas. Another survey conducted in the Braunschweig area (northern Germany) from 1980 to 2009 recorded 51 species of dragonflies (Suhling et al., 2009). This research supports the proposition that cities have the potential to support a high species richness of dragonflies.

Conclusions

As a result of the research carried out and presented, we can summarize the following conclusions:

1. In the studied area during the sampling period (May-August 2022), we can talk about the presence of a "urban heat island effect" along the urban gradient along the Maritsa River in the city of Plovdiv. Air, water and soil temperatures rise by approximately 1-2ºC from the periphery to the city center.

2. A total of 4 species were confirmed and 2 new species of dragonflies were recorded for the study area (*Calopteryx virgo* and *Orthetrum cancellatum*), which increases the total number of Odonata species for the study are to 26.

3. The most species were found in the urban zone, which was probably due to the higher temperatures compared to the other two zones, while at the same time, no Odonata larvae were found in this area, due to the lower amount of dissolved oxygen in the water.

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Received: 01.10.2022
Accepted: 23.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 123-141

Histochemical and Biochemical Changes in Common Carp (Cyprinus carpio Linnaeus, 1785) Liver after Cypermethrin and Chlorpyrifos Exposure

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Abstract. Nowadays pollution of aquatic ecosystems with pesticides causes acute and chronic poisoning of fish, leading to serious damage to vital organs, such as the liver. Common carp (*Cyprinus carpio* Linnaeus, 1758) is a popular edible fish favored for culture due to its rapid growth, hardiness and reproduction in confined waters. The purpose of the present study was to investigate the negative effects of cypermethrin (CYP) and chlorpyrifos (CPF), based on their maximum allowable concentrations (Directive 2013/39/EU) on histochemical and biochemical biomarkers in the liver of common carp. The histochemical analysis included Periodic acid-Shiff stainig (PAS reaction) and Sudan Black B staining, while in the biochemical study different hepatic enzyme activities such as lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). The negative effects of the tested pesticides on fish were expressed with liver changes in the amount of glycogen and lipids, and enzyme changes of LDH, ASAT and ALAT, caused by the acute and chronic exposure to cypermethrin and chlorpyrifos under laboratory conditions. The results from such experimental set ups could be used in the legislation of protection water bodies from contamination, in areas near intensive application of plant protection products and also in implementing the Directive 2013/39/EU and Water Frame Directive by using multi-biomarker approaches.

Key words: histochemistry, biochemistry, fish, liver, biomarkers, enzymes, pollution, cypermethrin, chlorpyrifos.

Introduction

Today, more than 1400 different pesticides are used in the environment worldwide, mostly in agriculture. Over the past 50 years, the use of these substances has greatly increased the quantity and improved the quality of food for the growing population of the world (Manuel et al., 2008). A pesticide is a substance or mixture of substances designed to kill, repel or reduce damage from various pests (Eldridge, 2008). Activities, such as agriculture, fishing, forestry, construction, mining, urban development and soil pollution occurring in or near the watershed of a reservoir can lead to disturbance of water quality and fish health (Mustapha, 2009). Pollution of aquatic ecosystems with pesticides causes acute and chronic poisoning of fish, leading to serious damage to vital organs, such as the liver (Omitoyin et al., 2006, Velmurugan et al., 2007). In addition, water pollution, directly or indirectly, can lead to fish kills or increased concentrations of toxic chemicals in edible fish tissue, which could have a negative effect on the health of people consuming such fish (Adedeji & Okocha, 2012).

Common carp (*Cyprinus carpio*) belongs to the order *Cypriniformes* and the family *Cyprinidae*, which is considered to be the largest family of freshwater fish. It inhabits freshwater environments, especially lakes, rivers, and rarely saltwater areas (Barus et al., 2001). Common carp is widespread in almost all countries of the world, but it is very popular in Asia and some European countries (Weber & Brown, 2011; Kloskowski, 2011). Common carp is also a popular edible fish favored for culture due to its rapid growth, hardiness and reproduction in confined waters (Singh, 2014).

Chlorpyrifos (O-O-diethyl-O-(3, 5, 6 trichloro-2-pyridyl)-phosphorothioate) is an organophosphorus insecticide, which is used to control a wide range of pests, such as worms, cockroaches, grubs, fleas, termites, ants and lice. It is applied to crops including cotton, nuts, vegetables and ornamentals (Caceres et al., 2007). The pesticide is a moderately toxic broad-spectrum lipophilic insecticide (Mugni et al., 2016) and was developed in the 1960s to replace other persistent pesticides (Kumar et al., 2017).

Cypermethrin ([cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate) is a synthetic pyrethroid widely used in agriculture, household and animal husbandry mainly (Valles & Koehler, 1997). Cypermethrin contains chlorine atoms in a vinyl side chain of the compound. The presence of halogens contributes to greater insecticidal activity and high stability, as well as providing better residual activity against insects. Additionally, the presence of halogens leads to a higher potential for negative environmental effects (Bradbury & Coats, 1989).

The combined use of a set of complementary biomarkers that can both signal exposure to pollutants and quantify their impact on living organisms allows for a more comprehensive and integrative assessment of the biochemical and cellular effects induced by environmental pollutants (Linde-Arias et al., 2008; Cazenave et al., 2009).

The fish liver is a key organ that controls many functions and plays an important role in fish physiology, both in anabolism and catabolism (Bruslé & Anadon, 1996). The activity levels of plasma enzymes (alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH)] and metabolites (cortisol, glucose, cholesterol, total protein, creatinine, blood urea nitrogen and etc.)) are often used as sensitive indicators of the harmful effects of pesticideson vital fish tissues (Canli et al., 2018; Firat & Tutus, 2020). Toxic compounds interfere with key enzymatic processes that are associated with the animal's normal physiology. This includes carbohydrate, protein and lipid metabolism. In this regards, biochemical changes occur before the appearance of tissue pathological symptoms (Ksheerasagar et al., 2011).

Lipids can also be synthesized by the liver (endogenous lipid metabolism). Fish store lipids in various organs, including mesenteric membranes, muscles and liver. Hepatic lipid accumulation is induced by biochemical mechanisms, such as decreased hepatic lipid export, increased hepatic uptake of circulating fatty acids, decreased hepatic beta-oxidation, and increased hepatic fatty acid synthesis (Sheridan, 1988). Abnormal lipid accumulation in the liver has been suggested to be among the most common pathological hepatic responses to chemical exposure (Cave et al., 2011), however it is not that commonly studied in the aquatic toxicology research compared to other biological approaches (enzymes, histopathology, behavior, etc.).

Material and Methods

Certified, juvenile fish were purchased from the Institute of Fisheries and Aquaculture, Plovdiv, where they are bred and raised under strictly controlled conditions. The experimental individuals were of the same size-age group (average length 10.1 ± 0.4 (sd) cm; average weight 11.15 ± 0.6 (sd) g), without external pathological changes. The individuals were transported using polyethylene containers filled with water from the Institute of Fisheries and Aquaculture, Plovdiv, in which oxygen tablets were added. After transportation, the fish were separated into 100 L glass aquaria with pre-dechlorinated water to acclimatize for 2 weeks. During this period, survival and behavior of the fish were also tracked. In addition, during the acclimation period, the fish were fed with specialized carp pellets (CarpCo Excellent Koi Grower, Helmond, The Netherlands) and a twelve-hour light period (12:12) was provided. The fish were not fed for 2 days before the start of the experiment.

The tested concentrations of toxicants for the short-term and long-term laboratory experiment were provided and prepared by expert chemists. The concentrations were prepared according to Bulgarian legislation (Directive 2013/39/EU), representing 100%, 50%, 30 % of MAC-EQS (0.1 μg/L - CPF; 0.0006 μg/L - CYP) for each test chemical. The reason for choosing lower concentrations than MAC-EQS was that in real conditions biota are usually subjected to the chronic action of various toxicants that are presented in rather low concentrations. Moreover, the concentrations according MPC-EQS were applied as the experiment was conducted once. The pesticides were dissolved in methanol and an aquarium with clean, tap, dechlorinated water was used as a control.

The test fish were randomly divided into 15 individuals in the different concentrations of the pesticides, including a control group (no added chemicals) and were treated under static conditions for 96 hours and 30 days (acute, short-term and chronic, long-term exposure) with nominal and ecologically relevant concentrations of CPF and CYP (Yancheva et al., 2019). During the experiment, the fish were observed for macroscopic and behavioral changes (APHA, 2005). A methanol control group was not used because it was considered that its significantly low concentration would have no observed effects. The test was conducted in a static environment with no water changes for 96 hours and 30 days (Modesto & Martinez, 2010 a,b; Santos & Martinez, 2012; Filho et al., 2017; Sánchez et al., 2018). We also applied this model in our previous study on the subject (Georgieva et al., 2021; Yancheva et al., 2022).

The physico-chemical parameters of water: pH , temperature, °C, electrical conductivity, µS/cm, dissolved oxygen, mg/L were measured at the 96th hour and the 30th day using a combined instrument (MultiLine® Multi 3510 IDS, WTW-Xylem Analytics, Weilheim, Germany) and published in our previous research (Georgieva et al., 2021; Yancheva et al., 2022).

The liver samples from the individuals were processed using a Leica CM 1520 freezing microtome (Leica Microsystems, Wetzlar, Germany). 5 µm thick cryosections were prepared from the material. They were stained for lipids by Sudan Black B staining according to Daddy's (1896) method and PAS-reaction (Schiff-Iodic acid) according to McManus (1948) for polysaccharides (liver glycogen). The histochemical changes in the liver of experimental individuals were presented semiquantitatively according to our modified scale of Mishra & Mohanty (2008), as follows: (–) – negative reaction of histochemical staining; (+/–) – very weak positive histochemical reaction; (+) – weak positive histochemical reaction; (++) – moderate positive reaction to histochemical staining; (+++) – strong positive histochemical reaction in the hepatocytes.

The biochemical study of liver enzymes - LDH and the aminotransferases ASAT and ALAT, was carried out at the Technology Center at the Faculty of Biology, Plovdiv University "Paisii Hilendarski". The liver samples from both exposures were thawed on ice and homogenized with 50 mM, 300 mM NaCl (pH=7.4). The homogenates were centrifuged at 4°C for 15 min at 9000 rpm using a refrigeration centrifuge (MPW, Poland). After centrifugation, the supernatant was separated into new Eppendorf tubes and frozen at -80°C to measure the activity of the above enzymes. Lactate dehydronese activity of the liver extracts was determined using commercially available enzymatic kit (LDH FL DGKC, Cat. LD F245 CH, Chema Diagnostica, Italy). Alanine aminotransferase activity of the liver extracts was determined using commercially available enzymatic kit (GPT/ALT FL IFCC, Cat. LD F400 CH, Chema Diagnostica, Italy). Aspartate aminotransferase activity of the liver extracts was determined using commercially available enzymatic kit (GOT/AST FL IFCC, Cat. GO F400 CH, Chema Diagnostica, Italy). All the reactions of LDH, ALAT and ASAT were prepared according to the instructions of the manufacturer of the kits and. The decrease of the light absorbance of the reactions at 340 nm, due to the oxidation of NADH to NAD+ during the reactions was determined spectrophotometrically The enzyme activity was measured with a spectrophotometer (Beckman Coulter DU 80 model, Inc., Brea, CA, USA) at 25°C.

The amount of total protein in the tested samples was determined spectrophotometrically at 595 nm according to the method of Bradford (1976) and the protein content was calculated against a standard curve of bovine serum albumin. The obtained values of LDH, ALAT and ASAT and the protein content of the samples were used to calculate the specific enzyme activity (U/mg protein) of the enzymes of interest.

All the analyses of LDH, ALAT, ASAT and protein content were performed in triplicate and the obtained average values of the measurements were used to calculate the acticities of the corresponding enzymes including ±SD.

Results and Discussion

The PAS-reaction is a staining method used to detect polysaccharides, such as glycogen in tissues, and the staining is mainly purple-magenta in color (Ngokere et al., 2016). It is mainly used in medicine, but can be applied along with other combined biomarkers in aquatic toxicology to study the negative effect of various toxicants for better results. The PAS-reaction works on the basis of the oxidation by the periodic acid (or its salts) of 1,2-glycol groups contained in these compounds to aldehydes, which are detected with the help of Schiff's reagent. The staining with Sudan Black B detects the presence of fatty degeneration in the cells, with the cytoplasmic lipids stained dark gray or black.

As a result of the histochemical analysis by applying the PAS-reaction, we observed an increase in the amount of glycogen in the hepatocytes of the experimental individuals. We found an increase in glycogen according to the proposed scale both in CPF exposure and in all tested CYP concentrations. With a similar degree of expression, we observed an accumulation of glycogen in the cytoplasm of hepatocytes in after 30 days in both exposures (Table 1; Fig. 1, 3).

When monitoring lipid inclusions in the hepatocytes, we observed an increase in the degree of Sudan Black B staining. In the short-term exposure, the degree of staining was similar for both toxicants. When following the changes that occurred after the long-term exposure, we found an increase in the degree of lipid inclusions, again with a similar degree of expression for both test toxicants. The obtained results confirm the observed fatty degeneration with increased lipid inclusions in hepatocytes through the histopathological analysis, as well as its degree of expression (Table 2; Fig. 2, 4).

Table 1. *Degree of PAS-reaction in the liver of common carp.*

Exposure time	Control	0.03 µg/L CPF	0.05 µg/L CPF	0.01 µg/L CPF	0.0002 µg/L CYP	0.0003 µg/L CYP	0.0006 µg/L CYP
96th hour	+/-	+/-	+	+	+/-	+	++
30th day	+/-	+/-	+	+	+/-	+	++

Table 2. *Degree of Sudan Black B staining in carp (Cyprinus carpio) liver.*

Exposure time	Control	0.03 µg/L CPF	0.05 µg/L CPF	0.01 µg/L CPF	0.0002 µg/L CYP	0.0003 µg/L CYP	0.0006 µg/L CYP
96th hour	+	+	+	++	+	++	++
30th day	+	+	+	++	+	++	++

Similarly, to us, Shrivastava (2007) considered that changes in the amount of glycogen in the liver indicate changes in carbohydrate metabolism under the influence of various pesticides. Changes associated with an increase in the amount of glycogen in hepatocytes compared to the control group of fish may be due to changes in the amount of pyruvate, and this may affect the processes of glycogenesis, glycogenolysis and gluconeogenesis.

According to Yogesh & Venkateshwarlu (2022), significant changes in the levels of glucose, glycogen, total protein and free amino acids, as well as changes in the activities of the enzymes lactate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, protease, aspartate aminotransferase and alanine aminotransferase were observed in common carp tissues and the changes developed progressively with exposure time. In addition, changes in the carbohydrate metabolic enzymes indicate that pesticides cause metabolic disturbances and change aerobic metabolism to anaerobiosis, while the changes in protein metabolic enzymes explain that pesticides may interact with peptide sequences in the fish directly or indirectly.

An increase in the lipid inclusions in the cytoplasm of hepatocytes is due to fatty degeneration in the liver cells, which was also found in the histopathological examination of the liver (Georgieva et al., 2021; Yancheva et al., 2022). Increased synthesis of fatty acids leads to increased synthesis of triglycerides and hyperlipidemia associated with fatty infiltration in hepatocytes. In agreement with Ayoola (2008), we believe that the changes related to the alterations in the amount of glycogen and lipids in the liver of the experimental individuals can generally be due to a change in the processes of glycolysis, which depends on the administered concentrations of the toxicant, the duration of action or its chemical nature.

Lactate dehydrogenase (LDH, ES 1.1.1.27), which is an important biomarker enzyme, is contained in the cytoplasm of cells and is involved in maintaining the balance in carbohydrate metabolism. Any change in the metabolism of organisms as a result of toxic stress has a negative effect on its activity (Mommsen, 2000). In general, however, less is known about carbohydrate metabolism in the liver of fish. It has been studied mainly in their muscles, and the studies in the available literature, in which it is considered under the action of heavy metals and various organic toxicants on liver, are considerably less (Norton et al., 2000; Almeida et al., 2002; Cooper et al., 2002; Napierska & Podolska, 2008; Oliva et al., 2012).

The results from the analysis showed a decrease in LDH activity at the 96-hour exposure, and we observed a stronger inhibition at the CYP exposure (Fig. 5). Similar to the short-term test, at the 30-day exposure we found inhibition of the specific enzyme activity, observing a similar trend comparing the two toxicants (Fig. 6).

LDH is a tetrameric glycolytic enzyme that has been used as a potential marker of tissue damage (Diamantino et al., 2001). Viarengo (1985) proved that the action of various poisons can lead to a change in the activity of LDH by binding them to some functional groups. This enzyme is involved in the carbohydrate metabolism, but also serves as an indicator of chemical pollution of the natural environment. The study of

LDH activity can provide useful information about the cellular metabolism in organisms that are subjected to the action of different toxicants (Monteiro et al., 2006). We agree with Das et al. (2004 a,b) and Yousafzai & Shakoori (2011) that alterations in the LDH activity signal changes in cellular energy metabolism as a result of water pollution. For example, the increased activity of LDH can serve as an enzyme biomarker for the diagnosis of various disorders in tissues of many vertebrate organisms, which is often the result of oxygen depletion after the exposure to contaminants. This forces the organisms to start using energy produced through anaerobic processes. On the other hand, a decrease in the LDH activity can serve as a biomarker for increased glycolysis as a result of chemically induced stress in the body. A decrease in the activity of LDH as a result of chemically induced stress and an increased amount of free radicals leads to intensive processes of gluconeogenesis and glycogen accumulation, which is also proven by the histochemical analysis using the PAS reaction in our study. Along with the processes of gluconeogenesis, an enhanced process of glycolysis probably takes place, which in turn could lead to the accumulation of pyruvate, hence of Acetyl CoA and, as a result, to hyperlipidemia, which is again proven by the histochemical analysis with Sudan Black B in the current experiment. The stronger inhibition of the enzyme after the 30-day exposure we could also connect with the higher degree of the observed fatty degeneration through the histological and histochemical analysis, which again shows intense processes of glycolysis and hyperlipidemia.

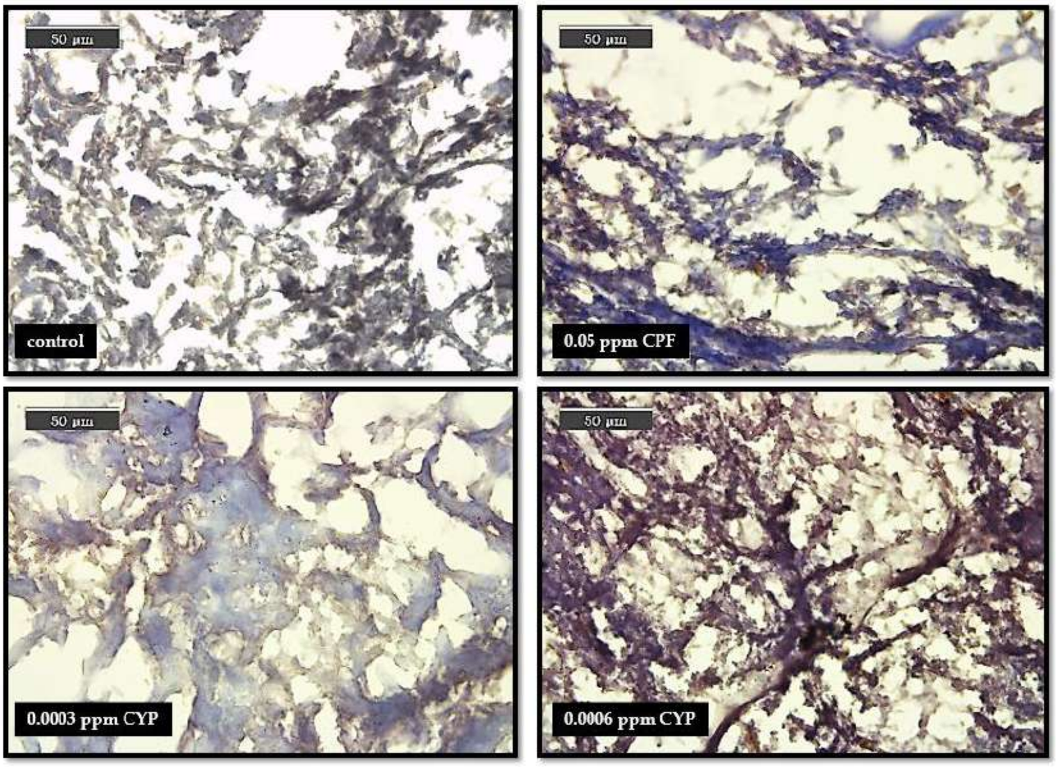

Fig. 1. Intensity of PAS-reaction in carp liver after 96-hour exposure.

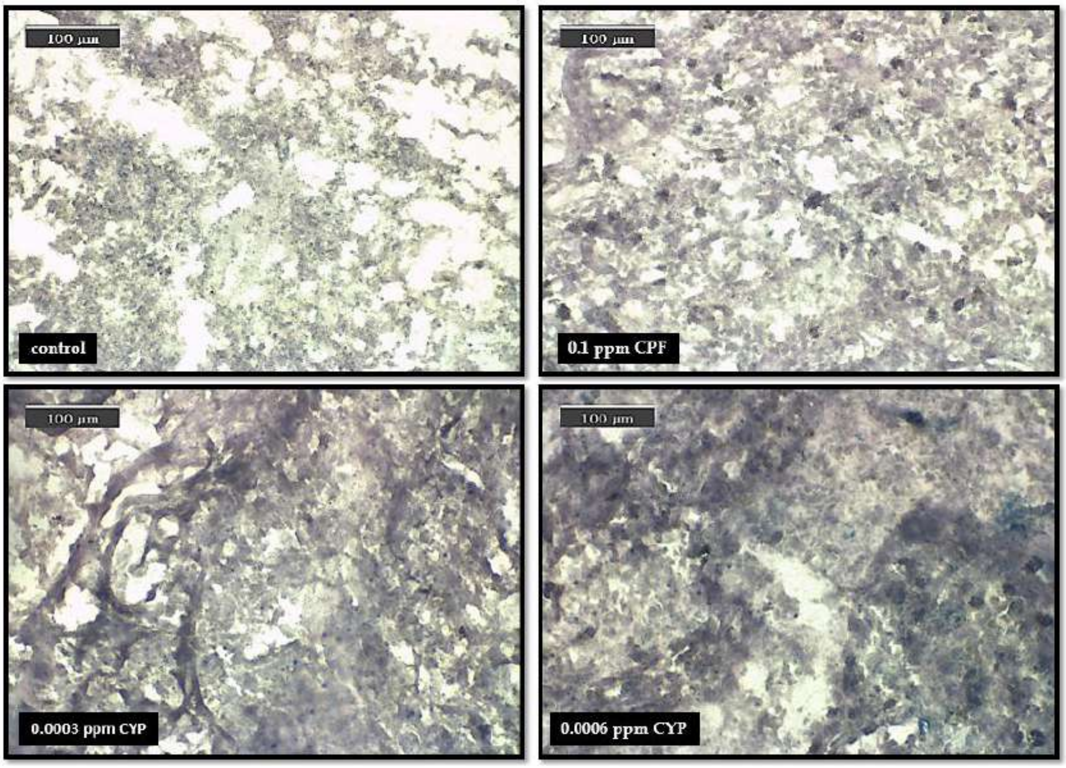

Fig. 2. Intensity of Sudan Black B staining in carp liver after 96-hour exposure.

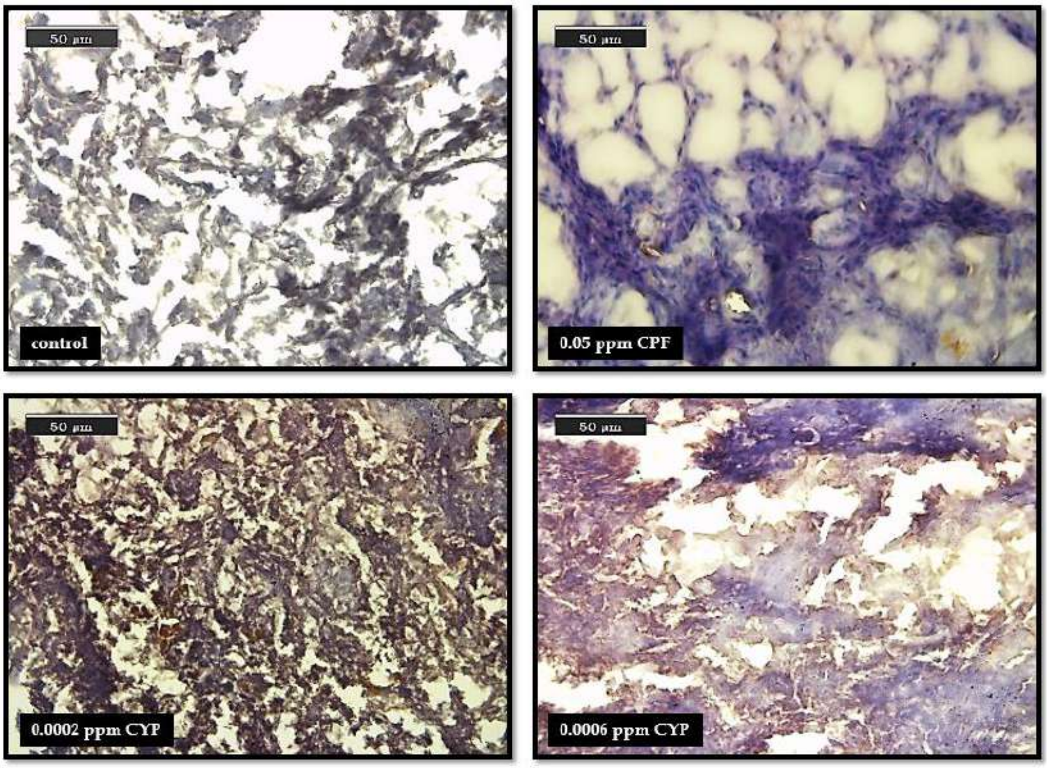

Fig. 3. Intensity of PAS-reaction in carp liver after 30-day exposure.

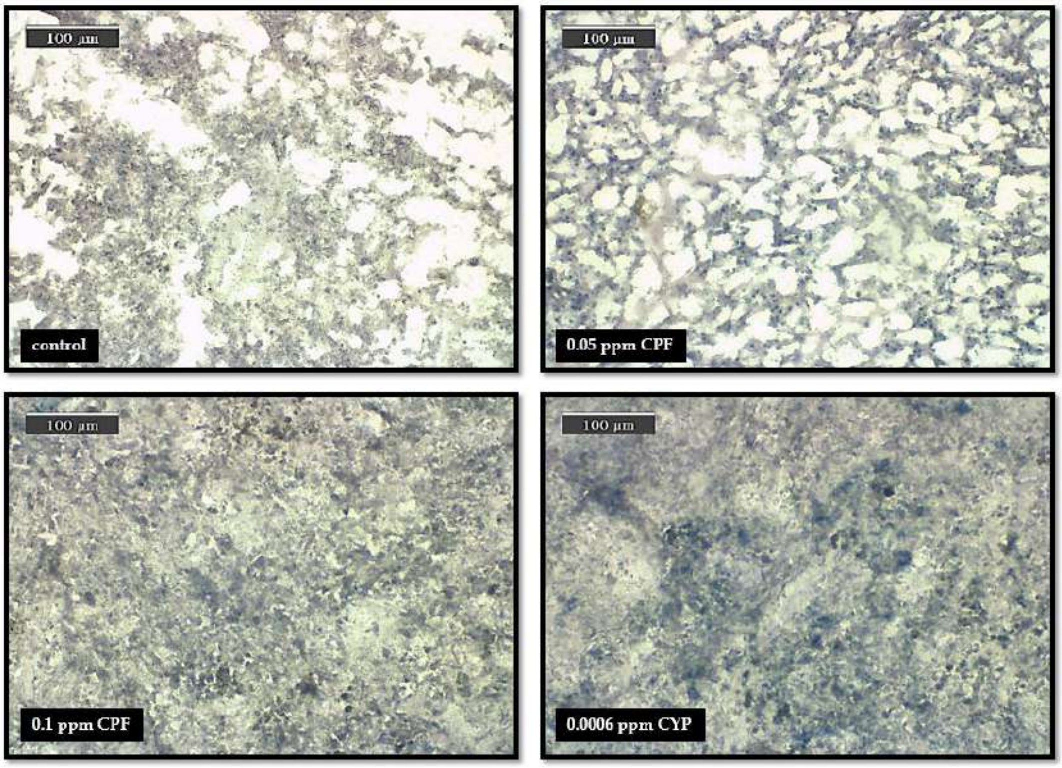

Fig. 4. Intensity of Sudan Black B staining in carp liver after 30-day exposure.

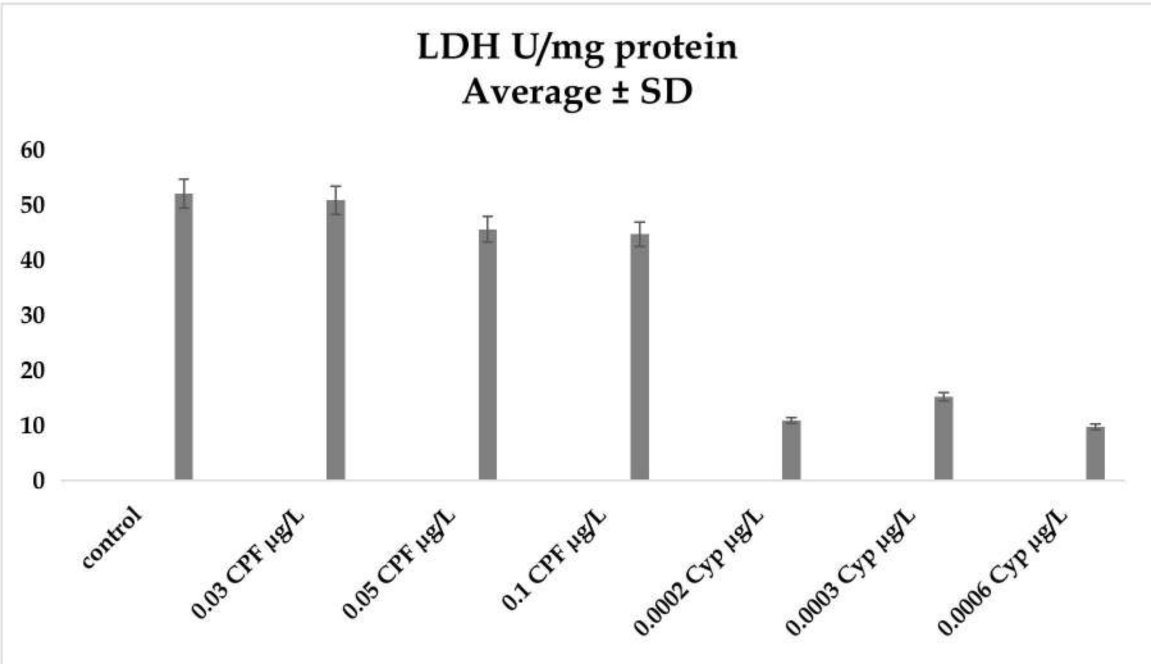

Fig. 5. Changes in the specific enzyme activity of LDH after 96-hour exposure to CPF and CYP.

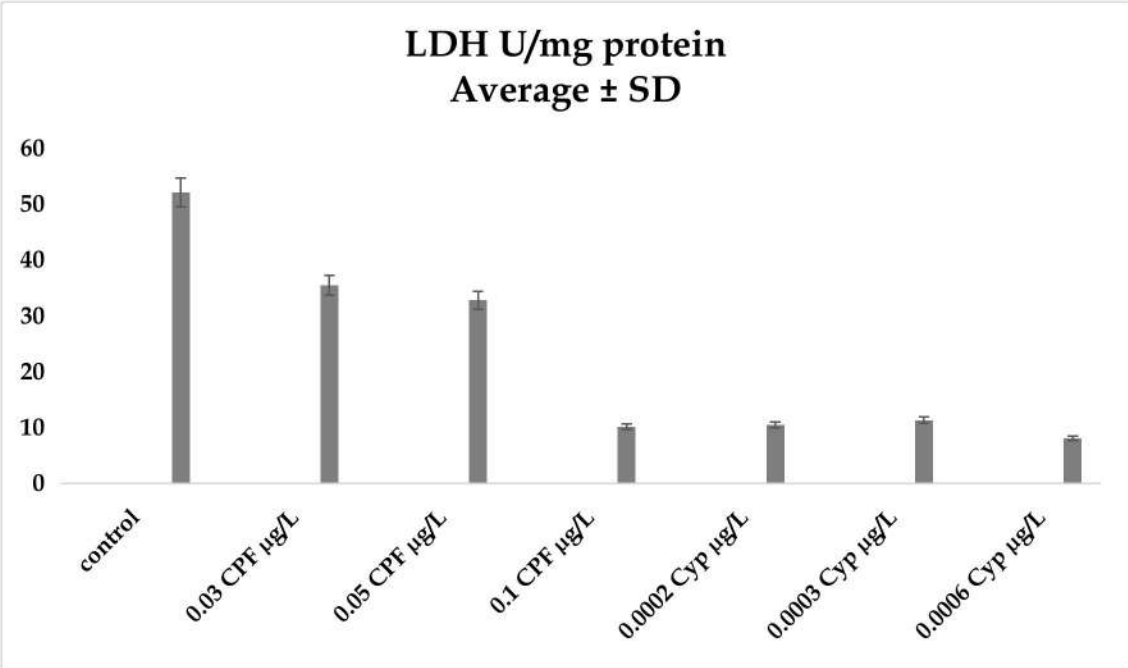

Fig. 6. Changes in the specific enzyme activity of LDH after 30-day exposure to CPF and CYP.

In addition, Rao (2006) found in his experiments an increase in the activity of LDH in the fish brain and gills, and a decrease in the activity of LDH in the liver under the influence of various pesticides, which clearly indicates different damage to the studied tissue.

Furthermore, Abhijith et al. (2016) found a decrease in the LDH activity in the gills and liver of *Catla catla* under the influence of methyl parathion (MP). According to the authors, the results show a higher degree of glycolysis under pesticide stress. According to Abhijith et al. (2016) MP can inhibit the aerobic and anaerobic metabolism of fish, leading to a decrease in the LDH activity, which was confirmed with the results obtained in the present study. Tripathi & Shasmal (2011) found reduction of the LDH activity in the gill, liver, brain and muscle of *Heteropneustes fossilis* exposed to CPF, which may be due to binding of pesticides or their metabolites to the enzyme molecule.

According to El-Shehawi et al. (2007) in ecotoxicological studies, the increase or decrease of ALAT and ASAT levels in the blood and other examined organs and tissues of fish can be used as a successful indicator of water pollution. ASAT is also considered a key enzyme for nitrogen metabolism and energy mobilization in invertebrates, is often used as a biochemical indicator of stress (Shobha et al., 2001). According to El-Shehawi et al. (2007) aminotransferases are widely used to diagnose the damage that toxins do to the liver in fish and some other organs, such as gills and muscles. In addition, transaminases (aminotransferases), have been widely used as sensitive markers of possible tissue damage, particularly liver toxicity, for many years in aquatic toxicology (Ramaiah, 2007).

The results on the changes in the aminotransferase activity showed inhibition of the specific enzyme activity in both the short-term and long-term exposure (Fig. 7, Fig. 8, Fig. 9, Fig. 10). We observed a stronger decrease in the specific activity after the 30-day exposure (Fig. 8, Fig. 10).

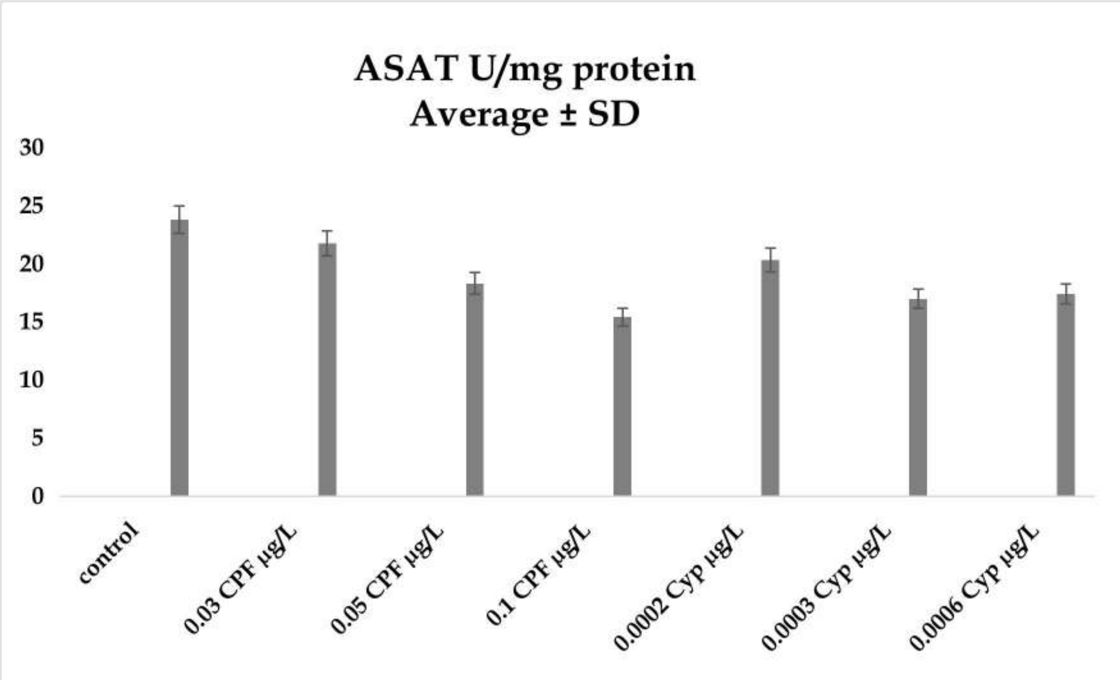

Fig. 7. Changes in the specific enzyme activity of ASAT after 96-hour exposure to CPF and CYP.

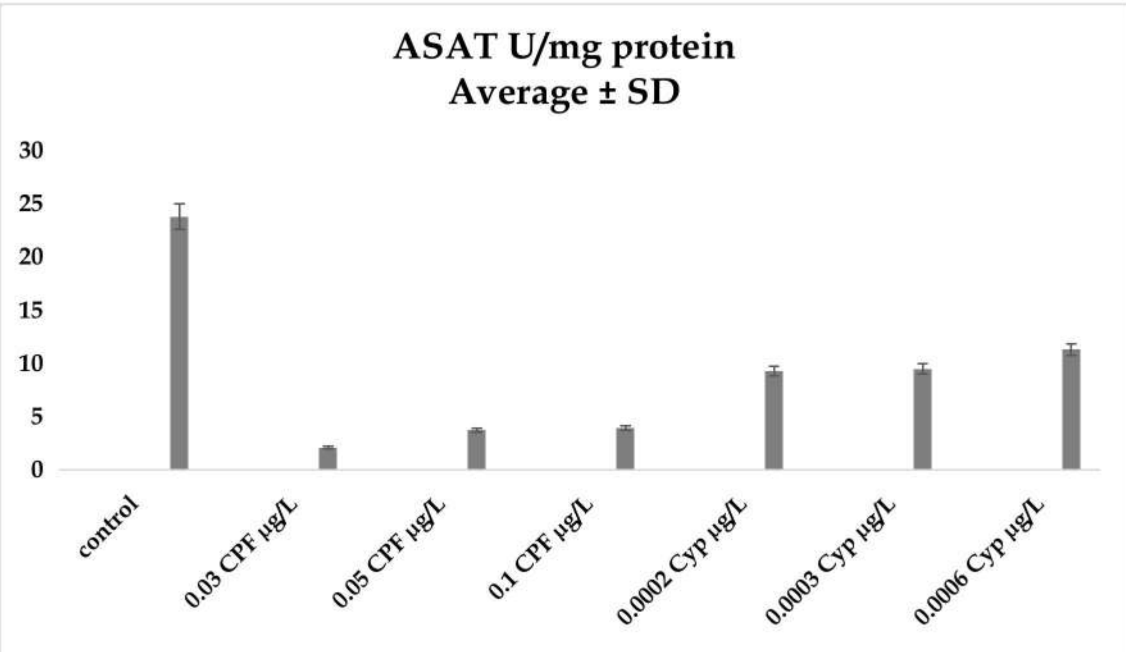

Fig. 8. Changes in the specific enzyme activity of ASAT after 30-day exposure to CPF and CYP.

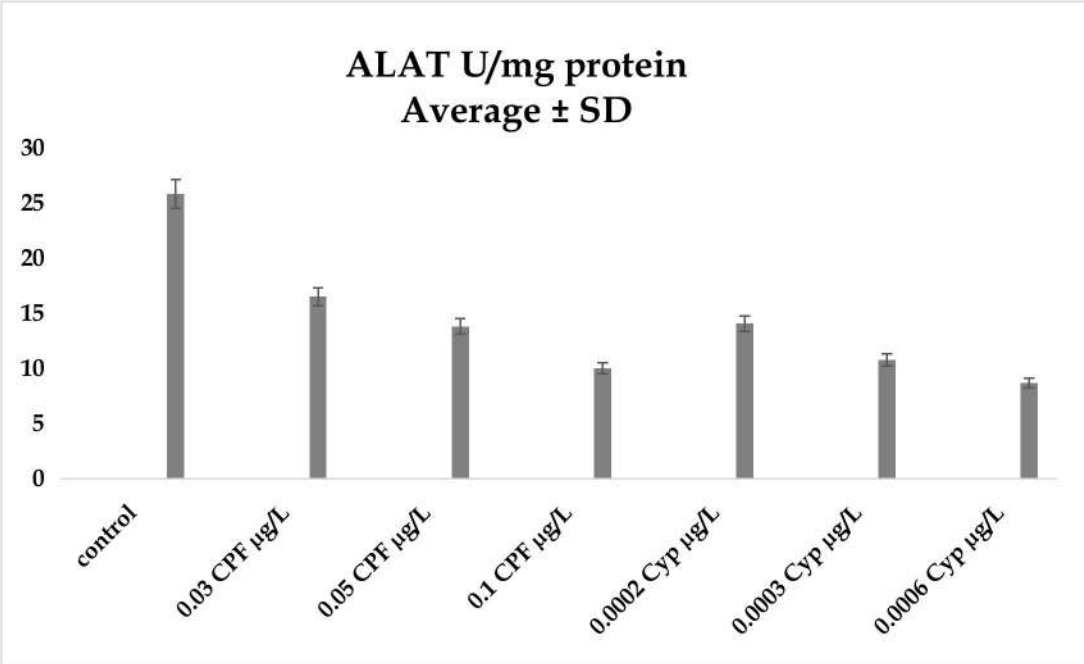

Fig. 9. Changes in the specific enzyme activity of ALAT after 96-hour exposure to CPF and CYP.

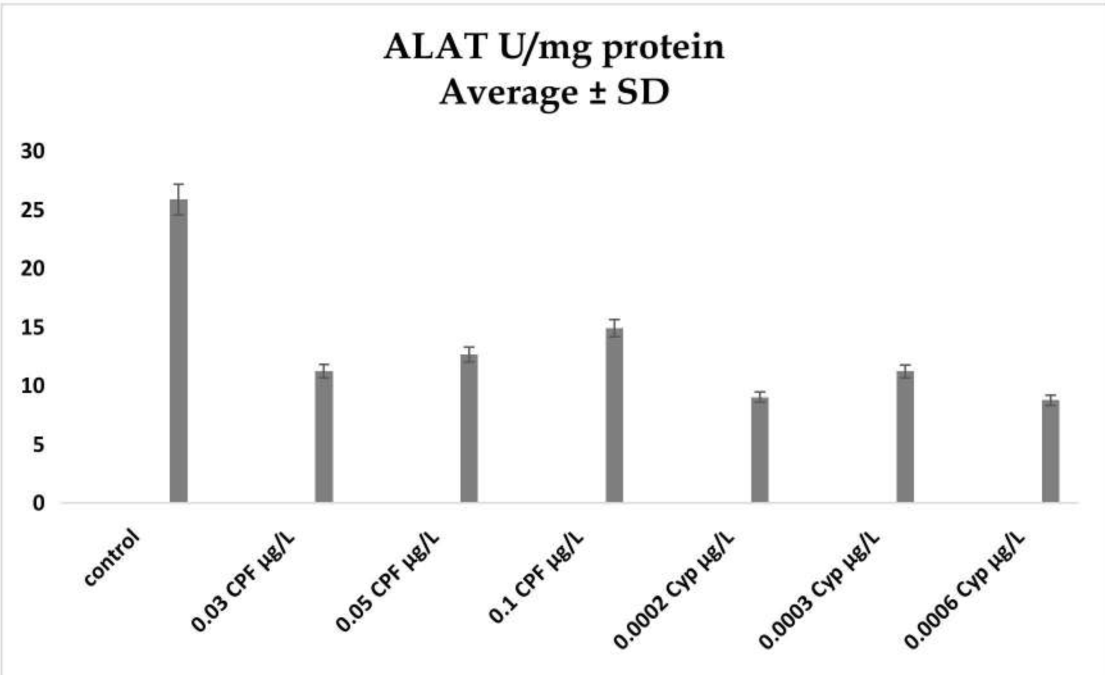

Fig. 10. Changes in the specific enzyme activity of ALAT after 30-day exposure to CPF and CYP.

A number of authors have followed the activity of aminotransferases in various organs of carp fish under the influence of different poisons. For example, Karan et al. (1998) measured a substantial increase in the ALAT activity, Velmurugan et al. (2008)

found an increase in the activity of aminotransferases in gills, brain and muscles of fish, Zikić et al. (2001) and Heydarnejad et al. (2013) indicated that the activity of aminotransferases increased in blood serum. Guo et al. (2002) reported an increase in the aminotransferase activity in liver under the influence of ytterbium.

Moreover, Al-Ghanim (2014) found an increase in ALAT, ASAT and GDH activity in all organs of fish exposed to cypermethrin. This suggests the active trans-deamination of amino acids to incorporate keto acids into the Krebs cycle to release the energy needed for new protein synthesis (Sivaramakrishna & Radhakrishnaiah, 1998). The elevation of these enzymes generally indicates amino acid utilization, while the elevation of transaminases suggests a strong efflux of metabolites under cypermethrin stress, as stress conditions generally induce an increase in the transamination pathway (Awasthi et al., 1984). Involvement of alternative pathways, such as aminotransferase reactions, is also possible due to inhibition of oxidative enzymes, such as isocitrate dehydrogenase and cytochrome C oxidase. Changes in the aminotransferase activity are often reflected in nitrogen metabolism and interdependent biochemical reactions. Elevated aminotransferase levels may be due to tissue damage after intoxication (Raju & Ramna, 1985). Amino acids appear to be mobilized to undergo transamination to 2-keto acids for use in the production of energy-rich compounds (Shobha et al., 2001)

Rao (2006) reported that the reduction of aminotransferase activity in the liver of *Oreochromis mossambicus* under the influence of organophosphorus pesticides may be due to liver damage. We agree with Abhijith et al. (2016) that the detoxification mechanism may not be efficient enough to prevent the toxicity and effect of the toxicant in the body. According to Tripathi & Shasmal (2011), a decrease in the specific activity of metabolic enzymes upon exposure to organophosphorus and pyrethroid pesticides indicates the direct impact of these toxicants on the enzyme activity. Neelima et al. (2013) also reported a decrease in the ALAT and ASAT activity when treating fish with cypermethrin.

The established decrease in the enzymatic activity of LDH, ASAT and ALAT after the long-term exposure with the test pesticides compared to short-term exposure could be also associated with the increased necrosis in the hepatocytes (Georgieva et al., 2021; Yancheva et al., 2022), which in turn indicates degenerative changes in the hepatic cells.

Conclusion

In sum, all concentrations of the tested pesticides cypermethrin and chlopyrifos in the short-term and long-term exposure negatively affected the histochemical and biochemical values in liver of common carp. The negative effects were expressed with liver changes in the amount of glycogen and lipids, and enzyme alterations in the specific activity of LDH, ASAT and ALAT. We found that these alterations affect the processes of glycogenesis, glycogenolysis, gluconeogenesis, carbohydrate and nitrogen metabolism. The results from such experimental set ups could be used in the legislation of protection water bodies from contamination in areas near intensive application of plant protection products and also in implementing the Water Frame Directive by using multi-biomarker approaches, which are not that common, but easy and reliable to apply, such as histochemistry.

Acknowledgments. The study is conducted with the financial help of project FP 21 BF 008 and project "Vita Plus" financed by Science Research Fund at the University of Plovdiv Paisii Hilendarski under the "Green Technologies" project line. The national program "Young scientists and postdoscs" funded by the Ministry of Education in Bulgaria is also highly appreciated.

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Received: 01.11.2022
Accepted: 22.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 143-159

Acute Histopathological Changes in Common carp (Cyprinus carpio Linnaeus, 1785) Gills: Pirimiphos-methyl, 2, 4 - Dichlorophenoxyacetic Acid and Propamocarb Hydrochloride Effects

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Abstract. A number of characteristics make fish excellent experimental models in toxicological research, especially for the contamination of aquatic systems. The main aim of the present study was to investigate the negative effects of different classes of pesticides (insecticide, herbicide and fungicide), based on their LC_{50} on the gills histological architecture of common carp (*Cyprinus carpio* Linnaeus, 1758). The effects of the tested pesticides on fish gills were expressed with histopathological alterations, such as proliferative, degenerative and changes in the circulatory system. Based on our results, the test insecticide showed higher toxicity with more severe irreversible necrotic changes in common carp gills compared to the herbicide and fungicide exposure. The identified histopathological changes in the fish gills can be successfully applied as reliable biomarkers for monitoring the degree of negative effects on the organisms due to the pesticide toxicity. The results from such experiments could be applied in the legislation in order to protect the water bodies from pesticide contamination, in areas with intensive application of plant protection products used in agricultural practices.

Key words: histology, fish, gills, biomarkers, pollution, pirimiphos-methyl, 2, 4 - dichlorophenoxyacetic acid, propamocarb hydrochloride.

Introduction

Pesticides play a significant role not only in the agriculture industry, because they increase the production of food, but also improve human health as they reduce the rate of vector-borne diseases (Blindauer et al., 1999). Pesticides, such as insecticides, herbicides and fungicides have been a part of practices in agriculture throughout the world for many years. Therefore, a great number of synthetic organic pollutants are daily released into many types of wastewaters, enter into natural water channels and accumulate in the aquatic environment (Agarwal et al., 2016; Bartczak et al., 2016; Goscianska et al., 2017). Unfortunately, the indiscriminate use of these compounds for improving production may have different impacts on non-target organisms, and especially aquatic animals. The World Health Organization (WHO, 1992) reported that around 3 million cases of pesticide poisoning occur annually, which is resulting in 220.000 deaths worldwide. Some of these chemicals are mutagenic (Garaj-Vrhovac & Zeljezic, 2000; Kumar et al., 2009; Nwani et al., 2010), related to development of cancers (Leiss & Savitz, 1995) or may cause developmental deficits (Arbuckel & Sever, 1998).

According to Qian & Lin (2015) organophosphorus compounds (OPs) have been widely used due to their low persistence under natural conditions and high effectiveness for pest control. In addition, OPs have been applied not only in agricultural industry, but also suburban and urban settings (Costa, 2018). The mode of action of OPs results inhibiting the enzyme acetylcholinesterase (AChE), which is responsible for conduction of nerve impulses during the process of neurotransmission, plays a vital role in the neurons development and formation of the network in the central nervous system (Boelsterli, 2007; Paraoanu & Layer, 2008; Arun Kumar & Jawahar, 2013). Also, OPs may act as an endocrine disruptors and producers of genotoxic, immunotoxic effects and oxidative stress in aquatic animals (Yonar & Sakin, 2011; Chang et al., 2013; Lavarias et al., 2013). According to Biswas et al. (2019) pesticide residues can be detected for long periods after their application due to the decreased biodegradation in the environment. Furthermore, they could be absorbed by fish, which leads to negative influence on their health and meat properties, and this will eventually have a negative effect on humans as well (Naiel et al., 2020).

Pirimiphos-methyl is a rapid-acting organophosphorus pesticide, which is frequently used in prevention and control of beetles, snout beetles and moths during the storage of the grains (Mhadhbi & Beiras, 2012). The use of these chemicals in water bodies in many developing countries could cause reduction of fisheries (Ayoola, 2008).

Among numerous herbicides, 2,4-dichlorophenoxyacetic acid is the most popular element of plant protection products. It is often applied in agriculture practices due to its low cost and good selectivity (Liu et al., 2016). 2,4-dichlorophenoxyacetic acid was developed during World War II (Suzuki, 2017) and was introduced on the market in the 1940s (Islam et al., 2018). The herbicide has high toxicity to broadleaf plants. It is used around houses, gardens, on golf courses, ball fields, parks, in agriculture and forestry (Garabrant & Philbert, 2002). Studies have described post-2,4-D exposure effects in different organisms, such as endocrine disruption (Guerrero-Estevez & Lopez-Lopez, 2016), reproductive disorders (Pattanasupong et al., 2004), genotoxicity (Lajmanovich et al., 2015) and carcinogenic effects (Loomis et al., 2015). In humans, 2,4-D has been related to the development of Parkinson's disease (Tanner et al., 2009) and autism (Roman, 2007). Herbicides cause damage to important vital organs and reduce the survival, growth and the reproduction of aquatic organisms (Rahman et al., 2002).

Propamocarb hydrochloride [propyl 3-(dimethylamino)propylcarbamate hydrochloride, is a systemic carbamated fungicide with protective action against phyco-mycetous diseases (*Phythium, Phytophthora spp.*) (Fernandez-Alba et al., 2001). According to Pieroh et al. (1978) propamocarb was introduced into European markets in 1978 for control of oomycetes in decorative crops and some vegetables.

The freshwater environment includes different groups of organisms like fish, amphibians, invertebrates, plants and microorganisms. Pesticides can have direct effects on them, as a result of the physiological action of the substance, and indirect effects, which include the ecological interactions between species (Preston, 2002).

Common carp (*Cyprinus carpio*) belongs to the order Cypriniformes and the family Cyprinidae, which is considered to be the largest family of freshwater fish. It usualy inhabits ponds, lakes and rivers, and also rarely inhabits brackish-water environments (Barus et al., 2001). The fish has excellent commercial value and may be a good model to study the responses to various environmental contaminants (Vinodhini & Narayanan, 2009). According to Xing et al. (2012) common carp is a widely distributed bottom-dwelling fish, with feeding habits, which expose them to many different types of environmental toxicants. Furthermore, they are easily captured and are often used for human consumption. All these features make common carp a very good indexical organism for the study of contamination on aquatic systems.

Fish gills are comparatively vulnerable to toxic stress due to their continual contact with contaminated water and large surface area. They are the first and the major site of uptake of waterborne toxicants and toxic impacts (Vigliano et al., 2006).

Histopathological alterations in fish gills have been frequently used as bioindicators in evaluation of the health of fish exposed to different pollutants, both in the laboratory and field studies. Therefore, histopathological changes can be used as tools for monitoring the effects of multiple contaminants, and are also a reflection of the whole ecosystem's health status. (Drishya et al., 2016; Olaniyi, 2020).

The present study aims to assess and compare for the first time the negative effects of different concentrations of pirimiphos-methyl, 2,4-dichlorophenoxyacetic acid and propamocarb hydrochloride**,** based on their LC_{50} on the gills histological architecture in the bioindicator species common carp (*Cyprinus carpio* Linnaeus, 1758), after acute (96h) laboratory exposure.

Material and Methods

Test species

Common carp selected as a bioindicator species due to its excellent commercial value and an excellent model to study the responses to various environmental contaminations (Vinodhini & Narayanan, 2009). According to Xing et al. (2012) common carp is a widely distributed bottom-dwelling fish, with feeding habits that expose them to many different types of environmental contaminants.

Test chemicals

In the present experiment, all pesticides were purchased as a commercial products used in the agricultural practice. The decreasing pesticide concentrations were prepared as a dilution of LC_{50} of each test toxicant. The applied concentrations were as follows: 10 µg/L and 60 µg/L pirimiphos-methyl; 50 µg/L and 100 µg/L 2,4-dichlorophenoxyacetic acid; 40 µg/L and 80 µg/L propamocarb hydrochloride.

Acute experimental exposure

Carp juveniles with normal morphology and no visible alterations, were provided by the Institute of Fisheries and Aquaculture (Plovdiv, Bulgaria). After transportation, fish were placed in a 100-L glass tank and they were not fed for 2 days before the experiment according to De Moura et al. (2017). After the acclimatization period (two weeks), fish were divided into eight groups (n = 15), including a control group with no added toxicant. Acute exposure of 96 hours in static conditions was carried out according to Modesto et al. (2010). The experimental setup was performed only once and as a pilot study in order to identify the histopathological effects of different concentrations of the tested pesticides. The basic physicochemical characteristics of the water, such as conductivity, dissolved oxygen, pH, and temperature, were measured once a day with a multi-parameter portable meter (MultiLine® Multi 3510 IDS, WTW-Xylem Analytics, Weilheim, Germany) according to APHA (2005). The basic physicochemical properties of the water stayed relatively constant during the experiment, without any significant changes between the control and the tested aquaria, therefore they will not be further discussed.

Histopathological analysis

Fish dissection was held carefully following the guidelines of Directive 2010/63/EU, regarding the protection of animals used for scientific purposes. For each pesticide concentration, the gills from 15 fish were fixed in 10% neutral buffered formaldehyde, washed in tap water and dehydrated in increasing ethanol concentrations, cleared with xylene and then infiltrated with liquid paraffin with a melting point of 54–56°C. Histological sections of 5 µm were prepared by using a rotary microtome (Leica RM 2125 RTS, Leica Microsystems, Wetzlar, Germany) and then stained with hematoxylin-eosin (H&E) following the method of Gautier (2011). Gills histological sections were examined with a light microscope (Leica DM 2000 LED, Leica Microsystems, Wetzlar, Germany) connected to a microscope camera (Leica DM 2000 LED, Leica Microsystems, Wetzlar, Germany). The histopathological alterations were classified using the semi-quantitative method according to Bernet et al. (1999), which we slightly modified. The severity of each histopathological alteration in the fish gills was specified according to the five-degree (0–5) severity gradation scale proposed by Saraiva et al. (2015). Lastly, organ index values (IO) were calculated using classes based on the scoring system introduced by Zimmerli et al. (2007).

Results

The results showed normal morphology of the gill histological structure in the control group. We observed primary lamellae, which were closely spaced and arranged in rows. The secondary lamellae were observed across the filaments, and they were covered by a single-layer epithelium. In the circulatory system in each lamella, we observed two main blood vessels: an afferent one, which extends from the gill arch to the tip of the filament, and an efferent blood vessel, which returns the blood to the gill arch. According to the five-degree (0–5) severity gradation scale, the control gill sections were determined as 0, despite the fact that in some individuals, we found lamellar lifting, which occupied less than 10% of the section surface. The normal histological structure of the common carp gills is shown in Table 1 and Fig. 1 A.

After the acute exposure with pirimiphos-methyl we observed histopathological alterations in the epithelial tissue of the gills, as well as in the circulatory system. The degree of expression of each of the observed changes is presented in Table 1. The histopathological lesions were scored according to Bernet et al. (1999). The changes were observed in both, the primary and secondary lamellae.

In regard to the first group of histopathological alterations classified according to Bernet et al. (1999), we found vasodilation of the blood vessels in the filament, which was expressed in a mild degree in both tested concentrations of pirimiphos-methyl (Table 1, Fig. 1 B,F). In contrast to the observed changes in the primary lamellae, in the secondary lamellae we found increasing of the degree of expression at the 60 µg/L pirimiphos-methyl (Table 1). In addition, we also found aneurisms of the secondary lamellae only at concentration of 60 µg/L pirimiphos-methyl (Fig. 1F).

Table 1. Histopathological lesions in common carp gills after acute (96-h) exposure to pirimiphos-methyl. Severity of the histopathological alterations, according to Saraiva et al. (2015) gradation scale: 0 – none; 1 – very mild alterations; 2 – mild alterations; 3 – moderate/pronounced alterations; 4 – severe alterations; 5 – very severe alterations.

Reaction pattern	Functional unit of the tissue	Alteration	Importance factor	Score value – concentrations of pirimiphos-methyl (µg/L)		
				Control	10	60
Changes in the circulatory system	*Filament*	Vasodilation	$W_{GC1} = 1$	0	2	2
	Secondary lamellae	Vasodilation	$W_{GC2} = 2$	0	3	4
	Secondary lamellae	Aneurysms	$W_{GC3} = 2$	0	0	2
Index for changes in the circulatory system				**I_{GC} =0**	**I_{GC} =8**	**I_{GC} =14**
Degenerative changes	*Gill epithelium (filament)*	Necrosis	$W_{GR1} = 3$	0	0	1
	Gill epithelium (secondary lamellae)	Necrosis	$W_{GR2} = 3$	0	1	1
Index for degenerative changes				**I_{GR} = 0**	**I_{GR}= 3**	**I_{GR}=6**
Proliferative changes	*Gill epithelium (filament)*	Edema	$W_{GP1} = 1$	0	1	1
		Proliferation of stratified epithelium	$W_{GP2} = 2$	0	3	2
		Proliferation of glandular cells	$W_{GP3} = 1$	0	1	0
	Gill epithelium (secondary lamellae)	Fusion	$W_{GP4} = 3$	0	3	3
		Lamellar lifting	$W_{GP5} = 1$	0	1	2
		Proliferation of stratified epithelium	$W_{GP6} = 2$	0	2	2
Index for proliferative changes				**I_{GP}=0**	**I_{GP}=20**	**I_{GP}=20**
Index for organ I_G				**I_G=0**	**I_G=31**	**I_G=40**

The degenerative changes in the filament were expressed in a very mild degree of necrosis of the epithelial tissue only at the highest concentration. Likewise, necrotic lesions were also observed in the secondary lamellae of the gills in a very mild degree (Table 1; Fig. 1D). The proliferative changes were detected in both, the filament and secondary lamellae. Edema was expressed in a very mild degree at both concentrations. Moreover, the proliferation of the stratified epithelium of the filament was observed in moderate degree at 10 µg/L pirimiphos-methyl and in mild degree at 60 µg/L pirimiphos-methyl. The proliferation of glandular cell in the filament was found only at the highest insecticide concentration in a very mild degree of expression. As a more severe proliferative change - fusion was detected at both tested concentrations in a moderate degree (Fig. 1 C). In regard to the proliferative alterations, we observed also lamellar lifting and proliferation of the stratified epithelium of the gill tissue. Lamellar lifting was expressed in a very mild degree at the lowest concentration and in a mild degree at 60 µg/L pirimiphos-methyl. Furthermore, proliferation of the stratified epithelium of the secondary lamellae was expressed in a mild degree (Table 1). Comparing the indices of histopathological changes in the circulatory system (I_{GC}), for the lower pirimiphos-methyl concentration of 10 µg/L I_{GC} was 8, while at the concentration of 60 µg/L pirimiphos-methyl I_{GC} was found to be 14. This indicates that the higher concentration of pirimiphos-methyl has a greater effect on the degree of expression of the changes in the circulatory system, which proves that it has a more severe effect on the structure of the organ too (Table 1). Moreover, we observed vasodilatation mainly at the secondary lamellae. Likewise, to the changes in the circulatory system, the indices of degenerative changes (I_{GR}) showed a tendency to increase the degree of expression. According to Table 1, at the lowest tested concentration I_{GR} was 3, while at the highest concentration we observed I_{GR} 6. The indices for proliferative changes (I_{GP}) after pirimiphos-methyl were 20 for both tested concentrations, but we found different degrees of expression of the proliferative lesions (Table 1). Based on the obtained results and the scheme proposed by Zimmerli et al. (2007) we calculated that gill index (I_G) falls into the Class IV (index 31-40) for both concentrations of pirimiphos-methyl. Furthermore, we observed more severe degenerative alterations and changes in the circulatory system at the concentration of 60 µg/L pirimiphos-methyl.

The observed histopathological alterations under the action of 2,4-dichlorophenoxyacetic acid were found to be more severe than those observed in pirimiphos-methyl exposure (Table 2).

After 2,4-dichlorophenoxyacetic acid treatment, alterations of sinus vasodilation were detected in the filament in a mild degree at the concentration of 50 µg/L and in a moderate degree at the concentration of 100 µg/L. In addition to the changes observed in the primary lamellae, similar degree of vasodilation of secondary lamellae was found at both experimental concentrations. The observed degrees in both, filament and secondary lamellae, were increased with the increasing concentration of the herbicide (Table 2, Fig. 2 B,E).

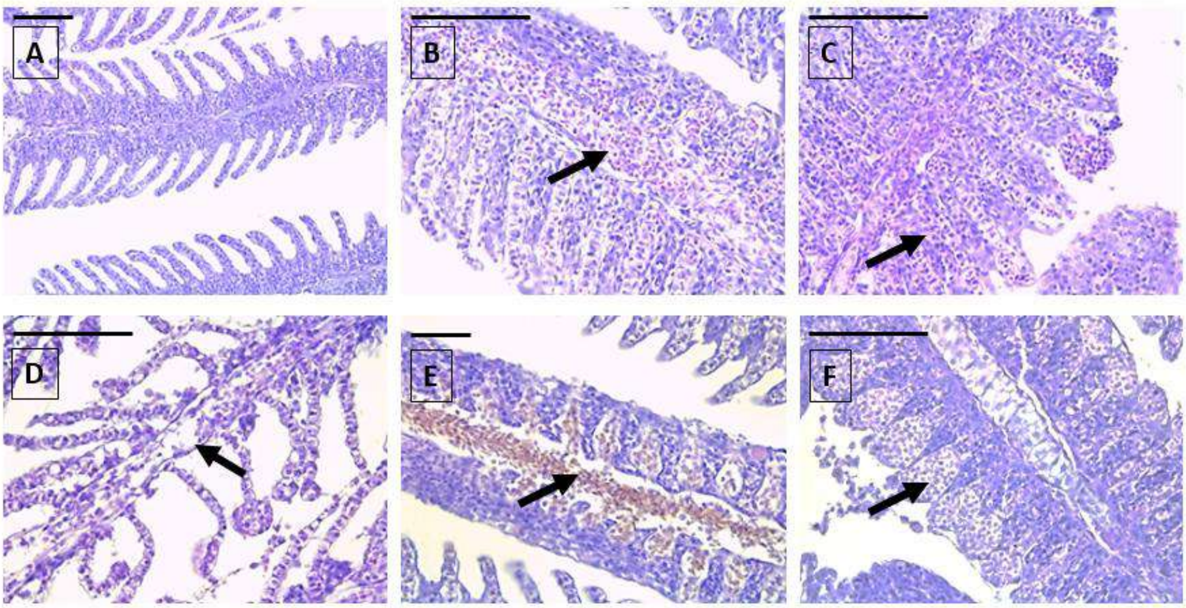

Fig. 1. Histopathological alterations in carp gills after acute exposure with pirimiphos-methyl exposure (H&E): A - control group, x200; B – vasodilatation in the filament at 10 µg/L pirimiphos-methyl, x 400; C – fusion of the secondary lamellae at 10 µg/L pirimiphos-methyl, x 400; D – degenerative changes (necrosis) at 60 µg/L pirimiphos-methyl, x400; E – vasodilatation in the filament at 60 µg/L pirimiphos-methyl, x200; F – aneurisms of the secondary lamellae 60 µg/L pirimiphos-methyl, x400.

Table 2. Histopathological lesions in the gills of common carp after 96-h exposure to 2,4-dichlorophenoxyacetic acid. Severity of the histopathological alterations, according to Saraiva et al. (2015) gradation scale: 0 – none; 1 – very mild alterations; 2 – mild alterations; 3 – moderate/pronounced alterations; 4 – severe alterations; 5 – very severe alterations.

Reaction pattern	Functional unit of the tissue	Alteration	Importance factor	Score value – concentrations of 2,4-Dichlorophenoxyacetic acid (μg/L)		
				Control	50	100
Changes in the circulatory system	*Filament*	Vasodilation	$W_{GC1} = 1$	0	2	3
	Secondary lamellae	Vasodilation	$W_{GC2} = 2$	0	2	3
	Secondary lamellae	Aneurysms	$W_{GC3} = 2$	0	0	0
Index for changes in the circulatory system				**$I_{GC} = 0$**	**$I_{GC} = 6$**	**$I_{GC} = 9$**
Degenerative changes	*Gill epithelium (filament)*	Necrosis	$W_{GR1} = 3$	0	1	0
	Gill epithelium (secondary lamellae)	Necrosis	$W_{GR2} = 3$	0	0	1
Index for degenerative changes				**$I_{GR} = 0$**	**$I_{GR} = 3$**	**$I_{GR} = 3$**
Proliferative changes	*Gill epithelium (filament)*	Edema	$W_{GP1} = 1$	0	2	2
		Proliferation of stratified epithelium	$W_{GP2} = 2$	0	3	4
		Proliferation of glandular cells	$W_{GP3} = 1$	0	1	0
		Fusion	$W_{GP4} = 3$	0	3	3
	Gill epithelium (secondary lamellae)	Lamellar lifting	$W_{GP5} = 1$	0	2	3
		Proliferation of stratified epithelium	$W_{GP6} = 2$	0	2	2
Index for proliferative changes				**$I_{GP} = 0$**	**$I_{GP} = 26$**	**$I_{GP} = 30$**
Index for organ I_G				**$I_G = 0$**	**$I_G = 35$**	**$I_G = 41$**

The degenerative changes in the histological structure of the gills were expressed in a very mild degree of necrosis of the epithelial tissue. In the filament, necrosis was found only at the concentration of 50 μg/L, while at the concentration of 100 μg/L necrosis was found only in the secondary lamellae (Table 2, Fig. 2C). The proliferative changes were detected in both, the filament and secondary lamellae of the carp gills. Edema was expressed in a mild degree at the both concentrations. Furthermore, the proliferation of the stratified epithelium of the filament was observed in a moderate to severe degree and showed an increase with increasing concentrations of the pesticide. The proliferation of glandular cell in the filament showed very mild degree of expression, with a decreased degree at the increased concentration of the herbicide. Fusion was detected at both concentrations in a moderate degree (Fig. 2D). The proliferative alterations found in the secondary lamellae were expressed in lamellar lifting and proliferation of the stratified epithelium of the tissue. Lamellar lifting was observed in a mild to moderate extend. Meanwhile, proliferation of the stratified epithelium of the secondary lamellae was expressed in a mild degree of expression at both tested concentrations (Table 2, Fig. 2F). Comparing the indices of histopathological changes in the circulatory system (I_{GC}), for the lower concentration I_{GC} was 6, while for the higher concentration I_{GC} was 9. This indicates that the higher concentration of 2,4-dichlorophenoxyacetic acid has a greater effect on the degree of expression of the changes in the circulatory system of the organ, which proves that it has a more severe effect on the structure of the organ as well (Table 2). Concerning the indices for degenerative changes (I_{GR}), the calculated values were similar (I_{GR} 3) at both tested concentrations (Table 2). Unlike the indices of degenerative changes, those for proliferative changes (I_{GP}) after the 2,4-dichlorophenoxyacetic acid exposure increased to the increasing pesticide concentration. For the concentration of 50 µg/L exposure I_{GP} was 35 and for the 100 µg/L tested concentration I_{GP} was 41 (Table 2). According to the scheme proposed by Zimmerli et al. (2007) and our results after 2, 4-dichlorophenoxyacetic acid exposure, the calculated gill index falls into the Class IV (index 31-40) for the lower concentration of the pesticide, which means the structure of the tissue is with pronounced histopathological alterations. The higher tested concentration falls into the Class V (index > 41), which showed that the structure of the tissue is affected by severe histopathological alterations. The indices of both experimental concentrations of the tested herbicide confirm the toxicity of 2,4-dichlorophenoxyacetic acid.

After the exposure with propamocarb hydrochloride we observed histopathological alterations in the epithelial tissue of the gills and in the circulatory system. The degree of expression of each of the histopathological changes is presented in Table 3. Moreover, the histopathological lesions were scored according to Bernet et al. (1999) in three groups – lesions in the circulatory system of the organ, degenerative and proliferative changes (Table 3). The changes were observed in both, the primary and secondary lamellae of the fish similarly to the other two tested pesticides.

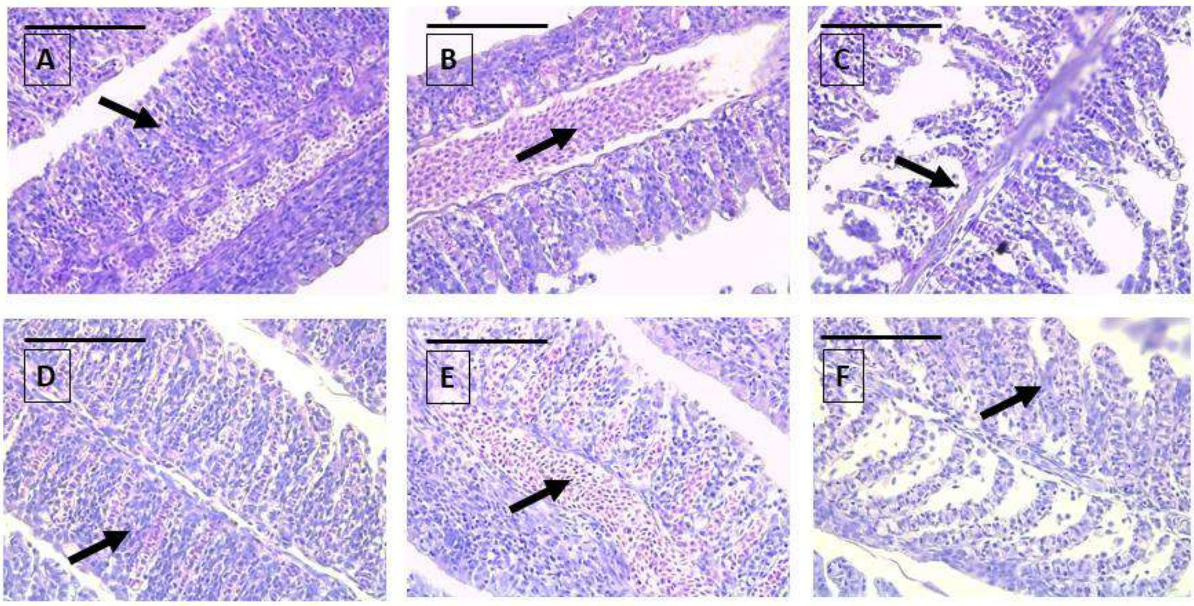

Fig. 2. Histopathological lesions in common carp gills after 2,4-Dichlorophenoxyacetic exposure (H&E), x400: A – proliferation of stratified epithelium in the filament after exposure with 50 µg/L 2,4-Dichlorophenoxyacetic acid; B – vasodilation of the blood vessel in the filament after exposure with 50 µg/L 2,4-Dichlorophenoxyacetic acid; C – degenerative changes of the stratified epithelium in the filament after exposure with 50 µg/L 2,4-dichlorophenoxyacetic acid; D – fusion of the secondary lamellae after exposure with 100 µg/L 2,4-dichlorophenoxyacetic acid; E - vasodilation of the blood vessel in the filament after exposure with 100 µg/L 2,4-dichlorophenoxyacetic acid; F - proliferation of stratified epithelium in the filament after exposure with 100 µg/L 2,4-dichlorophenoxyacetic acid.

Table 3. Histopathological lesions in the gills of common carp after 96-h exposure to propamocarb hydrochloride. Severity of the histopathological alterations, according to Saraiva et al. (2015) gradation scale: 0 – none; 1 – very mild alterations; 2 – mild alterations; 3 – moderate/pronounced alterations; 4 – severe alterations; 5 – very severe alterations.

Reaction pattern	Functional unit of the tissue	Alteration	Importance factor	Score value – concentrations of Propamocarb hydrochloride (µg/L)		
				Control	40	80
Changes in the circulatory system	*Filament*	Vasodilation	$W_{GC1} = 1$	0	2	2
	Secondary lamellae	Vasodilation	$W_{GC2} = 2$	0	2	2
	Secondary lamellae	Aneurysms	$W_{GC3} = 2$	0	1	0
Index for changes in the circulatory system				**I_{GC} =0**	**I_{GC} =8**	**I_{GC} =6**
Degenerative changes	*Gill epithelium (filament)*	Necrosis	$W_{GR1} = 3$	0	0	0
	Gill epithelium (secondary lamellae)	Necrosis	$W_{GR2} = 3$	0	0	0
Index for degenerative changes				**I_{GR} = 0**	**I_{GR}=0**	**I_{GR}=0**
Proliferative changes	*Gill epithelium (filament)*	Edema	$W_{GP1} = 1$	0	3	2
		Proliferation of stratified epithelium	$W_{GP2} = 2$	0	5	4
		Proliferation of glandular cells	$W_{GP3} = 1$	0	1	0
		Fusion	$W_{GP4} = 3$	0	4	4
	Gill epithelium (secondary lamellae)	Lamellar lifting	$W_{GP5} = 1$	0	2	2
		Proliferation of stratified epithelium	$W_{GP6} = 2$	0	4	3
Index for proliferative changes				**I_{GP}=0**	**I_{GP}=36**	**I_{GP}=30**
Index for organ I_G				**I_G=0**	**I_G=44**	**I_G=36**

In regard to the alterations in the circulatory system, we found vasodilation of the blood vessels in the filament and in the secondary lamellae expressed in a mild to moderate degree in both concentrations of propamocarb hydrochloride (Table 3, Fig. 3A, D). We found aneurisms only at the lower fungicide concentration (Table 3, Fig. 3C). Concerning the degenerative changes in the filament and in the secondary lamellae, no changes of the epithelial tissue were observed in both tested concentration of the fungicide (Table 1). The proliferative changes were showed in both filament and secondary lamellae of the gills. Edema was observed in a moderate degree in the lower concentration, but in a mild in the higher concentration. We found the similar tendency in the observed proliferation of the stratified epithelium of the filament (Table 3, Fig. 3F). The proliferation of glandular cells in the filament was found only in the lower concentration. As more pronounced proliferative change, fusion was shown at both concentrations in a severe degree (Fig. 3B). Also, the lamellar lifting showed similar degree of expression in both concentrations (Fig. 3E). Proliferation of the stratified epithelium of the secondary lamellae was expressed in a severe degree at concentration of 40 µg/L and in moderate degree at concentration of 80 µg/L (Table 3). The indices of histopathological changes in the circulatory system (I_{GC}) for the concentration of 40 µg/L I_{GC} was 8, while at the concentration of 80 µg/L I_{GC} was 6. In addition to the changes in the circulatory system, the indices of proliferative changes (I_{GP}) showed a tendency to decrease the degree of expression similarly to the circulatory alterations. According to Table 3, at the lower tested concentration I_{GP} was 36, while at the higher concentration we observed I_{GP} 30. The indices for degenerative changes (I_{GR}) after propamocarb hydrochloride exposure were scored as 0 for both fungicide concentrations due to the fact that we found no necrosis in the fish gills after the acute treatment (Table 3). Based on the obtained results and the scale proposed by Zimmerli et al. (2007) we calculated that gill index (I_G) falls into the Class V (index > 41) for the concentration of 40 µg/L propamocarb hydrochloride, which is an indicator of the severe histopathological alterations in the gill tissue caused by the negative effects of propamocarb hydrochloride. The concentration of 80 µg/L falls into the Class IV (index 31-40), which showed pronounced histopathological alterations. Generally, we found a tendency towards decreasing the degree of expression with the increasing concentration of the tested fungicide propamocarb hydrochloride (Table 3) from which can be assumed that the fish treated with lower concentration of the pesticide showed a higher degree of adaptive mechanisms.

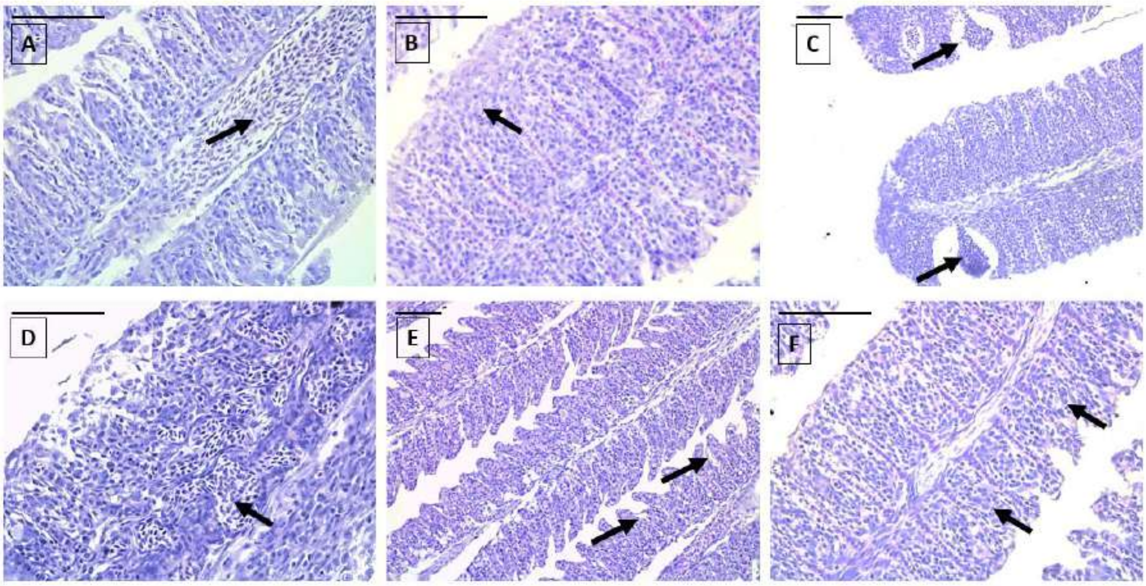

Fig. 3. Histopathological lesions in common carp gills after propamocarb hydrochloride exposure (H&E): A – vasodilatation of the blood vessel in the filament after exposure with 40 µg/L propamocarb hydrochloride, x400; B – fusion in the secondary lamellae after exposure with 40 µg/L propamocarb hydrochloride, x400; C – aneurisms in the secondary lamellae after exposure with 40 µg/L propamocarb hydrochloride, x200; D – vasodilatation of the secondary lamellae after exposure with 80 µg/L propamocarb hydrochloride, x400; E – lamellar lifting of the secondary lamellae after exposure with 80 µg/L propamocarb hydrochloride, x200; F - proliferation of stratified epithelium in the filament after exposure with 80 µg/L propamocarb hydrochloride, x400.

Discussion

Fish gills play an important role in exchange of gas and ionic regulation. Pesticides, and especially organophosphates, have a severe effect on the structure and function of the gills. According to Menezes-Faria et al. (2007) gills are firstly affected from entered pesticides into fish, and in response to the changes in the environment, they may produce adaptive strategies to preserve the physiological function. The histopathological alterations can be used as efficient indicators of toxicity in fish. The high variety of different chemical substances in water is an additional factor influencing their toxicity in aquatic organisms.

In addition, the alterations in the gill architecture are adaptive, necessary to reduce the rate of absorption of toxicants. Epithelia hypertrophy increases the water-blood distance and as a result reducing the rate of absorption of xenobiotics (Agamy, 2013). However, epithelia hypertrophy decreases the respiratory surface area thus, reducing the effectiveness of gas exchange and leading to osmoregulatory dysfunction (Sakuragui et al., 2003).

The obtained results from the present study show a high degree of proliferative changes in the gill epithelium due to the negative effects of the tested pesticides. These alterations serve as an indicator of the occurrence of compensatory-adaptive mechanisms in the organism of the studied individuals under the influence of the applied pesticide concentrations and short-term exposure. Although the experimental concentrations are lower than the LC_{50} their application in the plant protection product leads to an increase in the toxicity of the pesticides. As a result of the toxicant effects, protective mechanisms were activated in the organism and specifically in the gills, which are in direct contact with the contaminated water. An increase in the thickness of the epithelial layer, often leading to fusion of the secondary lamellae, increases the distance between the organism and the toxicant. This statement is also confirmed by the lower expression of degenerative changes in the gill epithelium, which we observed. The highest degree of proliferation was found after the exposure to the tested fungicide, where the indices ranged between 30-36. On the other hand, the lowest degree of proliferative changes was observed after pirimiphos-methyl exposure. In the case of the insecticide exposure, in parallel to the lower values of proliferative changes, the highest values of necrotic changes in the gill epithelium were also found. We can assume that with chronic exposure, the proliferative and degenerative processes could most likely equalize or even lead to a preferential increase in necrobiosis and necrosis.

Unlike the insecticide and herbicide exposures, during the exposure to the tested fungicide necrotic changes were not observed. Moreover, a higher degree of proliferative changes was observed at the lower concentration. In parallel, the degree of changes in the circulatory system was also higher at this concentration. The observed changes could be due to oxidative stress occurring in the organism at a concentration of 40 µg/L, which leads to a higher need for oxygen delivery, which is an indicator for vasodilation and aneurysms. The higher need for oxygen is a result of intense mitoses, which reach a severe and very severe degree of expression with changes, such as proliferation of the epithelium at the filament and fusion of the secondary lamellae. At concentration of 80 µg/L, these values decreased and we could possibly assume that during the chronic exposure, the degree of proliferative changes and those in the circulatory system may equalize, which could also lead to the appearance of degenerative changes in the epithelial cells due to disruption of their metabolism.

Our results on gill histology after pirimiphos-methyl treatment are similar to those reported by Xing et al. (2012), who studied the effect of chlorpyrifos and found varied degrees of epithelial hypertrophy, telangiectasis, oedema with epithelial separation from basement membranes, general necrosis, and epithelial desquamation. Similar changes were observed by Al-Mamoori et al. (2014), who found partial lamellar deformation, abnormal lamellae, partial terminal attachment of the lamellae, marginal dilation, hyperplasia of epithelial cells, marked gill deformation, marked lamellar aneurysm, marked lamellar fusion with epithelial cells hyperplasia, and diffuse mass of the gill lamella after treatment with chlorfos.

We agree with Devi & Mishra (2013) who observed signs of epithelial lesions when fish were exposed to sublethal concentration of chlorpyrifos, which was time dependent even treated with low concentration. Our results are also in agreement with those obtained by Kunjamma et al. (2008) in regards to short-term exposure of pesticides (hyperemia, clubbing and edema).

Our results on gill histology are similar to those reported by Makinde et al. (2015) who stated there was an evidence of lifting of epithelia layer, vacuolization, inter-lamella hyperplasia and mild desquamation of the epithelia lining. The alterations in the gill architecture the authors observed in all fish exposed to the acute concentrations of 2, 4-D amine except the control group. The degenerative lesions (vacuolization, epithelia lifting and desquamation of the epithelia lining) and proliferative response (hypertrophy) in their study were severe in the fish exposed to the highest concentration of 2,4 D - amine.

We agree with Vigario & Saboia-Morais (2014) who found signs of high gill vascularization, epithelial hyperplasia, lamellar fusion and changes in glycoconjugate granules of mucous cells after exposure to 2,4 – D herbicide. As explained by the authors, we agree that lamellar fusion is a defense mechanism that decreases the surface of gas exchange, which is vulnerable to the action of the herbicide. However, such defense responses to pollutant agents are usually irreversible toxic effects (Ortiz et al., 2003).

Furthermore, we agree with Rocha et al. (2015) who found histopathological changes, such as regressive, vascular and progressive disorders, and no neoplastic disorders after a herbicide treatment.

Our result on gill histology after propamocarb hydrochloride treatment are similar to the result reported by Boran et al. (2012) who observed hypertrophy and necrosis of epithelium, separation of epithelium from lamellae (epithelial lifting), lamellar fusion, hyperplasia of lamella and the space under the epithelium filled with eosinophilic material after a captan exposure. In addition, Choudhury (2018) found epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, edema, general necrosis and degeneration of primary and secondary gill lamellae after fungicide treatment.

Conclusions

In sum, we can conclude that both concentrations of each pesticide (pirimiphos-methyl, 2,4 - dichlorophenoxyacetic acid and propamocarb hydrochloride), which were lower than LC_{50} negatively affected the histological structure of common carp gills and also activated compensatory-adaptive mechanisms, resulting in pathological proliferative changes. We consider that these histopathological lesions affect the gills by disrupting their functions. There was a tendency towards enhancing the morphological alterations and their degree of expression was proportional to the increasing pesticide concentrations. Overall, based on our results, the test insecticide showed higher toxicity with more severe irreversible necrotic changes in the common carp gills compared to the herbicide and fungicide exposure.

Acknowledgments

The national program "Young scientists and postdoscs" funded by the Ministry of Education in Bulgaria is highly appreciated. The study is conducted with the financial help of project FP 21 BF 008 – "*Ex situ* assessment of xenobiotic-contaminated aquatic ecosystems by applying multi-biomarkers in selected in bioindicator species" financed by NPD at the University of Plovdiv Paisii Hilendarski.

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Received: 25.08.2022
Accepted: 28.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 161-169

New Data on the Tetrapod Fauna of Lyulin Mts., Bulgaria

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Abstract. Lyulin Mountain is located near the capital city of Sofia, but its fauna is relatively poorly studied. It offers a wide variety of habitats, which suggests the possible presence of many species of vertebrates. Older preliminary studies show that some of them are species of national and European importance. Our survey was conducted in 2021, between April and September. Transects were walked for direct visual or vocal observations of amphibians, reptiles, birds, mammals, or traces of their presence. A total of 12 species of amphibians and reptiles were observed during the current study. The most common species were the green lizard (*Lacerta viridis*), the fire salamander (*Salamandra salamandra*) and the yellow-bellied toad (*Bombina variegata*). 46 species of birds were registered, with the Eurasian blackcap (*Sylvia atricapilla*), Common chaffinch (*Fringilla coelebs*) and Common blackbird (*Turdus merula*) being the most common. 11 species of wild mammals were documented, including the endangered in Bulgaria pine marten (*Martes martes*). The most common and widely distributed mammals were the roe deer (*Capreolus capreolus*) and the red fox (*Vulpes vulpes*). The results of our study demonstrate that Lyulin Mts. hosts a large diversity of vertebrates and needs to be studied in more detail to aid their effective management and conservation.

Key words: diversity, amphibians, reptiles, birds, mammals.

Introduction

Assessing the biodiversity is invariably the first step towards the successful management and conservation of any area of interest. Lyulin Mountain is located near the capital city of Sofia and the much larger protected area Nature Park "Vitosha". Perhaps this is the reason Lyulin is relatively poorly studied in comparison. However, it offers a wide variety of habitats, which suggests the presence of many species of vertebrates. Preliminary studies show that some of them are species of national and European importance (Petrov, 2016).

Lyulin Mountain is not a protected area, which means that various human activities are allowed: tourism, logging, hunting etc. Furthermore, the mountain is bordering numerous human settlements and urbanised areas, which additionally increases anthropogenic disturbance. All of these factors contribute towards the uncertain status of the vulnerable or endangered species that might inhabit it. Thus, the aim of the current study is to assess the vertebrate biodiversity (Tetrapoda) and its conservation status in Lyulin, aiming its effective management and conservation.

Material and Methods

Study area

Lyulin Mountain is in the Southwestern part of Bulgaria, easily accessible, near numerous settlements. The highest peak – Dupevitsa (1256 m) is in the eastern part. The western part is a ridge that rises like an arc to the north (Raylovsko gradishte peak, 1199 m). The rivers that spring from Lyulin Mts. are short and shallow. They dry up at the maximum summer temperatures. Lyulinska River is the largest river in the mountain. Lyulin Mts. has limited water resources based on only 600 mm annual precipitation. Therefore, the mountain is characterised as relatively waterless. The snow cover lasts from 60 to 80 days, and usually it is one to 1.5 meters thick. Steep slopes and low forest cover turn deep ravines and small rivers into destructive torrents (Georgiev, 1985).

The climate is temperate continental. The average annual air temperature for the period 1956-2007 is 9.8^{0}C, the coldest month is January (-1.3^{0}C) and the warmest is July (20^{0}C). Typical for the region is the wind Fyon - strong and stormy south pulsating wind, accompanied by a sharp rise in air temperature and lower humidity (Bondev, 1982).

Nowadays, the woody and shrubby vegetation is of natural origin – mostly coppice. Coniferous trees comprise about 6% of the forest area, and they are artificial plantations. Predominantly the woodlands are aggregation of oak (*Quercus* sp.) trees. Beech (*Fagus sylvatica*) forests are located in the highest parts of the mountain. There are also bushes, represented by hazel (*Corylus avellana*), dogwood (*Cornus* sp.), hawthorn (*Crataegus monogyna)*. Black elderberry (*Sambucus nigra*) and dog rose (*Rosa canina)* are widespread. These shrublands do not occupy large areas but are an integral part of the mountains' landscape (bgjourney.com, 2022).

Methods

Field observations in Lyulin mountain were conducted from 2014. In 2021, between April and September, we conducted a study using camera traps for mammals and periodically (on average twice a month) walked transects for direct visual or vocal observations of amphibians, reptiles, and birds. 10 camera traps were placed on animal paths in three areas of the mountain (Fig. 1). They were set up to take 3 consecutive photos and a 10-second video (5 seconds apart) upon triggering. Additionally, five pre-set (via GIS tools) line transects (overall approx. 15 km) were surveyed in the same areas (Fig. 1) in order to record directly (through observation or capture) species or through traces of their presence (tracks, excrements, markings, etc.). The transect method was applied to register amphibians, reptiles, birds and mammals inhabiting the Lyulin Mountains. The transects were visited periodically (at least once every 30 days, during the active period for amphibians and reptiles, and throughout the whole study period for birds and mammals). All observed or captured amphibians and reptiles were identified to species level (Tsankov et al., 2014) and GPS coordinates of the sites were recorded. During the survey of the transects, all observed mammals or traces of their activity (tracks, excrements, markings, feeding sites, etc.) were also documented.

Results

12 species of amphibians (n = 6, from four families) and reptiles (n = 6, from four families) were observed during the study duration (Table 1). All of these species are under the protection of the Biodiversity Law in Bulgaria or are included in the Appendix II (or III) of the Bern Convention. The most common species were the green lizard *Lacerta viridis,* the fire salamander *Salamandra salamandra* and the yellow-bellied toad *Bombina variegata,* and the rarest was the European copper skink *Ablepharus kitaibelii*.

Forty six species of birds from nine orders were also registered, with the Eurasian blackcap (*Sylvia atricapilla*), Common chaffinch (*Fringilla coelebs*) and common blackbird (*Turdus merula*) being the most common. The observed species are presented in Table 2.

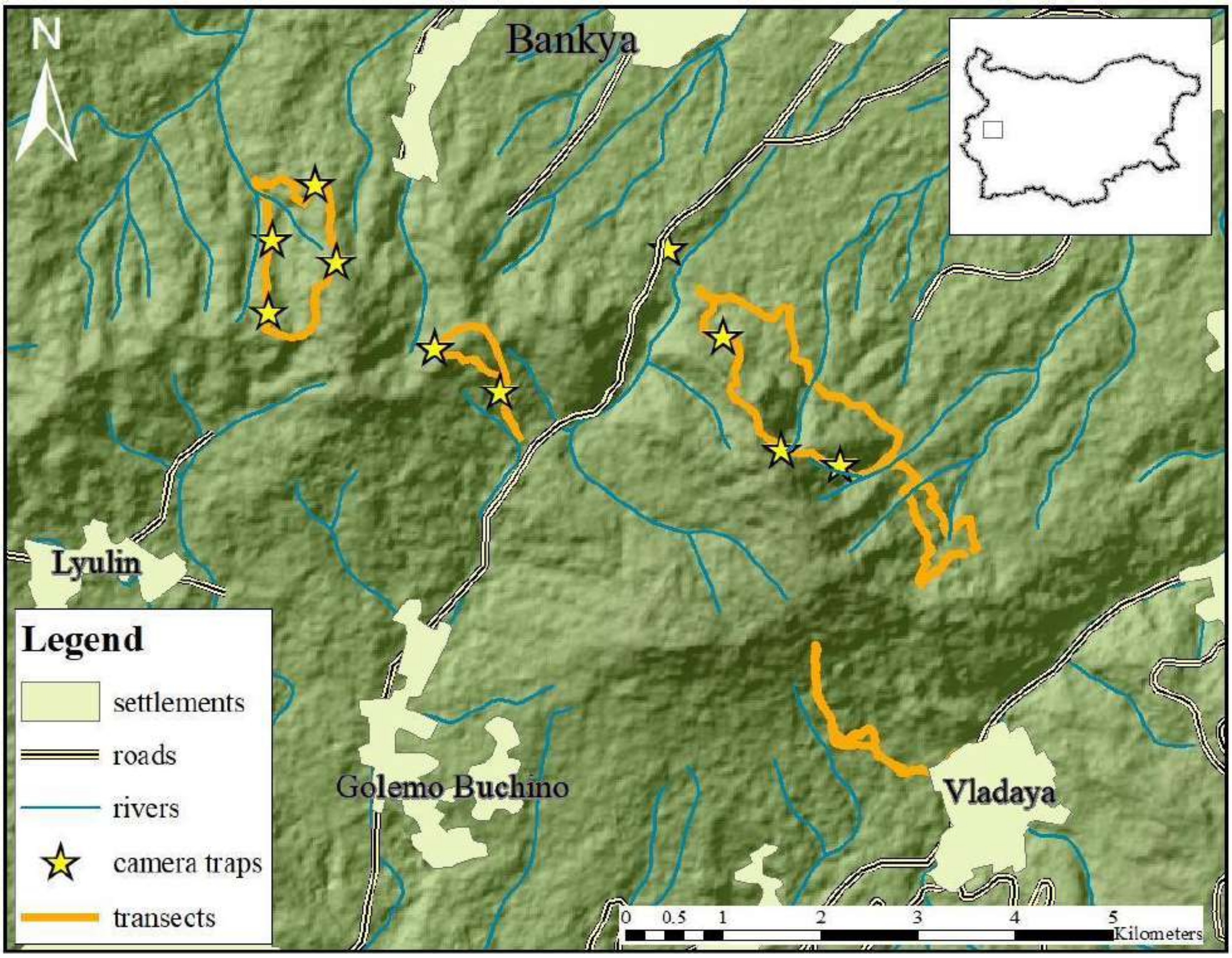

Fig. 1. Map of the study area with camera trap locations and transects.

Thirteen species of mammals (11 wild and 2 domesticated) were documented, including the endangered in Bulgaria pine marten (*Martes martes*) (Table 3). The most common and widely distributed mammals were the roe deer (*Capreolus capreolus*) and the red fox (*Vulpes vulpes*), which were registered on all camera traps and transects.

Table 1. List of the observed amphibians and reptiles, number of locations in which the species were observed and legal and conservation status. *Legend:* II, III, IV, V – the species is listed in Appendix №, 2, 3, 4 and/or 5 respectively; BBA – Bulgarian Biodiversity Act (State Gazzette, 09.08.2002); RDB - Red Data Book of the Republic of Bulgaria, (part 2, 2015) - EN – endangered species, VU – vulnerable species; HD – Habitats Directive (Directive 92/43/EEC); BC – Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention).

Family/species	Latin name	No. of locations	Legal and conservation status			
			BBA	RDB	HD	BC
Bufonidae						
Common toad	*Bufo bufo* L.	2	III			III

Ranidae						
Marsh frog	*Pelophylax ridibundus* Pallas	3	IV		V	III
Agile frog	*Rana dalmatina* Fitzinger	6	II		IV	II
Bombinatoridae						
Yellow-bellied toad	*Bombina variegata* L.	12	II, III		II, IV	II
Salamandridae						
Fire salamander	*Salamandra salamandra* L.	21	III			III
Smooth newt	*Lissotriton vulgaris* L.	1	III			III
Anguidae						
Slow worm	*Anguis fragilis* L.	3	III			III
Scincidae						
European copper skink	*Ablepharus kitaibelii* Bibron & Bory	1	III		IV	II
Lacertidae						
European green lizard	*Lacerta viridis* Laurenti	26			IV	II
Common wall lizard	*Podarcis muralis* Laurenti	3			IV	II
Colubridae						
Grass snake	*Natrix natrix* L.	2				III
Aesculapian snake	*Zamenis longissimus* Laurenti	2	III	EN	IV	II

Table 2. List of the observed birds and number of locations in which the species were observed during the study and legal and conservation status. *Legend:* II, III, IV, V – the species is listed in Appendix №, 2, 3, 4 and/or 5 respectively; BBA – Bulgarian Biodiversity Act (State Gazzette, 09.08.2002); HGPA - I – Hunting And Game Preservation Act (State Gazzette, 26.09.2000) - Appendix № 1; RDB - Red Data Book of the Republic of Bulgaria, (part 2, 2015) - EN – endangered species, VU – vulnerable species; BC – Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention); CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Order/ species	**Latin name**	**No. of locations**	**Legal and conservation status**				
			BBA	**HGPA - I**	**RDB**	**BC**	**CITES**
Accipitriformes							
Common buzzard	*Buteo buteo*	6	III			II	II
European honey buzzard	*Pernis apivorus*	1	II, III		LC	II	II
Falconiformes							
Common kestrel	*Falco tinnunculus*	4	III			II	II
Eurasian hobby	*Falco subbuteo*	1	III		VU	II	II
Piciformes							
Great spotted woodpecker	*Dendrocopos major*	5	III			II	
Black woodpecker	*Dryocopus martius*	2	II, III		VU	II	
European green woodpecker	*Picus viridis*	3	III			II	
Gruiformes							
Corncrake	*Crex crex*	1	II, III		VU	II	
Cuculiformes							

Common cuckoo	*Cuculus canorus*	3	III			III
Columbiformes						
Common wood pigeon	*Columba palumbus*	5	IV, VI	+		
Eurasian collared dove	*Streptopelia decaocto*	1	IV	+		III
Coraciiformes						
European bee-eater	*Merops apiaster*		II			II
Apodiformes						
Common swift	*Apus apus*	1	III			II
Passeriformes						
European skylark	*Alauda arvensis*	1	III			III
Barn swallow	*Hirundo rustica*	1	III			II
Common house martin	*Delichon urbica*	6	III			II
Red-rumped swallow	*Hirundo daurica*	3	III			II
White wagtail	*Motacilla alba*	1	III			II
African stonechat	*Saxicola torquatus*	1	III			II
Common nightingale	*Luscinia megarhynchos*	1	III			II
Song thrush	*Turdus philomelos*	4	III			III
Mistle thrush	*Turdus viscivorus*	4	III			III
Common blackbird	*Turdus merula*	10	III			III
Common chiffchaff	*Phylloscopus collybita*	9	III			II
Semicollared flycatcher	*Ficedula semitorquata*	5	II, III		VU	II
Blackcap	*Sylvia atricapilla*	10	III			II
European robin	*Erithacus rubecula*	5	III			II
Common whitethroat	*Sylvia communis*	4	III			II
Great tit	*Parus major*	6	III			II
Coal tit	*Parus ater*	1	III			II
Sombre tit	*Parus lugubris*	1	III			II
Marsh tit	*Parus palustris*	1	III			II
Eurasian wren	*Troglodytes troglodytes*	5	III			II
Red-backed shrike	*Lanius collurio*	4	II, III			II
Black redstart	*Phoenicurus ochruros*	1	III			II
European goldfinch	*Carduelis carduelis*	2	III			II
European greenfinch	*Carduelis chloris*	5	III			II
Common chaffinch	*Fringilla coelebs*	12	III			III
Brambling	*Fringilla montifringilla*	1	III			III
Eurasian nuthatch	*Sitta europaea*	5	III			II
Hawfinch	*Coccothraustes coccothraustes*	4	III			II
Eurasian bullfinch	*Pyrrhula pyrrhula*	2	III			III
Common starling	*Sturnus vulgaris*	3	IV	+		
Yellowhammer	*Emberiza citrinella*	5	III			II
Corn bunting	*Miliaria calandra*	1	III			III
Eurasian jay	*Garrulus glandarius*	3				III
Common raven	*Corvus corax*	1	III			III

Table 3. List of the observed mammals, number of locations in which the species were observed and legal and conservation status. *Legend:* II, III, IV, V – the species is listed in Appendix №, 2, 3, 4 and/or 5 respectively; BBA – Bulgarian Biodiversity Act (State Gazzette, 09.08.2002); HGPA - I – Hunting And Game Preservation Act (State Gazzette, 26.09.2000) - Appendix № 1; RDB - Red Data Book of the Republic of Bulgaria, (part 2, 2015) - EN – endangered species, VU – vulnerable species; HD – Habitats Directive (Directive 92/43/EEC); BC – Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention); CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Family/ species	Latin name	No. of locations	Legal and conservation status					
			BBA	HGPA - I	RDB	HD	BC	CITES
Canidae								
Red fox	*Vulpes vulpes* L.	7		+				
Golden jackal	*Canis aureus* L.	1		+				
Domestic dog	*Canis familiaris* L.	1						
Felidae								
European wildcat	*Felis silvestris* Schr.	2	III		EN	IV	II	II
Domestic cat	*Felis catus* L.	4						
Suidae								
Wild boar	*Sus scrofa* L.	5		+				
Cervidae								
Roe deer	*Capreolus capreolus* L.	9		+			III	
Leporidae								
European hare	*Lepus europeus* Pallas	2		+			III	
Mustelidae								
European badger	*Meles meles* L.	5		+			III	
Stone marten	*Martes foina* Erxl.	1		+			III	
Pine marten	*Martes martes* L.	1	III		EN	V	III	
Unidentified marten	*Martes* sp.	5						
Sciuridae								
Red squirrel	*Sciurus vulgaris* L.	2		+			III	
Erinaceidae								
Northern white-breasted hedgehog	*Erinaceus roumanicus* Barrett-Hamilton	1	III					

Discussion

Amphibians and reptiles

From a herpetological point of view, Lyulin Mountain is largely understudied. For the identified species, information on population size, distribution, etc. is scarce, which indicates the need for continuous, more detailed studies of the mountain and its surroundings. This study can serve as a basis for such research. In a previous study of the herpetofauna of Lyulin Mountain (Petrov, 2016) the European copper skink (*Ablepharus kitaibelii*) and the slow worm (*Anguis fragilis*) were not observed. Therefore, these two species are newly registered for the mountain in the current study. Meanwhile, during our research, three previously observed species were not found – the Buresch's crested newt (*Triturus ivanbureschi*), sand lizard (*Lacerta agilis*) and smooth snake (*Coronella austriaca*). Thus, the total number of registered amphibians and reptiles from the two studies is 15.

Tsankov et al. (2014), published a detailed identification guide of the amphibians and reptiles in Vitosha Nature Park, part of the Vitosha Mountain. The species composition of this field guide includes all the species from our study. This is so, apart from the topographic features and similar climatic conditions, and also because of the good connectivity between the two mountains, regardless of the presence of the motorway between them (Naumov *et. al.*, 2020)

Although Lyulin Mts. has a limited territory, 39% of the amphibians and 23% of the reptiles inhabiting Bulgaria are found in the mountain.

Birds

Among the observed birds, the most common species were typical of forest habitats - the blackcap (*Sylvia atricapilla*), the common finch (*Fringilla coelebs*) and the blackbird (*Turdus merula*). Other forest species, such as Eurasian wren (*Troglodytes troglodytes)* and European robin (*Erithacus rubecula)*, were also well represented. The majority of the observed species are under protection from the Bulgarian biodiversity act.

Of the species with unfavourable conservation status, it is important to note the presence of at least 3 territorial pairs of semi-collared flycatchers (*Ficedula semitorquata*). According to the Atlas of breeding birds in Bulgaria, the species was previously reported in the period 1958-1959 (Iankov, 2007). During the present research, one of the pairs was found in an atypical habitat in a sparse birch (*Betula alba*) plantation, situated on a dry southwestern slope, while the others were in dense forests of beech (*Fagus*) and hornbeam (*Fraxinus*), listed as typical habitats by Iankov (2007). It is likely that additional researches in Lyulin Mountain will reveal more pairs, given the favourable conditions we found in the present study. The species is listed as "vulnerable" in Bulgaria (Golemanski, 2011). In the relatively small but representative wet meadows, a mating song of the corncrake (*Crex crex*) was documented. The species is sensitive to the destruction of its habitats thus categorised as "vulnerable" in Bulgaria (Golemanski, 2011). It was reported for many locations in the vicinity of city of Sofia in the previous decades (Nankinov, 1982), but many of the suitable meadows in the area were turned in urbanised zones, which probably caused the retreat of the species to the surviving favourable habitats in the neighbouring territories such as Lyulin mountain. The meadows in the researched area are also home to species such as the red-backed Shrike (*Lanius collurio*) and the corn bunting (*Miliaria calandra*), and given the findings of amphibians and reptiles in these meadows, special attention should be paid to the conservation of these valuable areas. During the study, another rare observation was made for the country – a male brambling (*Fringilla montifringilla*) in mating plumage in late spring (May). Such observations are very few in the published literature for Bulgaria, as the species has a northern nesting distribution and usually should have left the country much earlier. According to Nankinov (1982) the species could be observed very rarely until mid May, with the latest observation on 13.05.1976 from Rila mountain, which is 5 days earlier than our present one.

The presence of well-preserved forests aged 100 or more years and the large quantities of deadwood are a prerequisite for a good variety of woodpeckers. During the study, Black (*Dryocopus martius*) - listed as "vulnerable" in the Red data book of Bulgaria (Golemanski, 2011), European green (*Picus viridis*) and Great spotted woodpecker (*Dendrocopos major*) were found, as well as numerous hollows and traces of their activity on all walked transects. The hollows that are not used by the woodpeckers provide an opportunity for nesting of many other species, including the semi-collared flycatchers, a species of conservation importance.

Future research of the avifauna is recommended, since no data related to the area was published in the recent decades. Detailed research was found only in unpublished sources (Master's thesis from 1954-1967 and 1989). Compared to the species listed in them, in the current study we observed one additional species, not reported for the mountain so far – the common swift (*Apus apus*).

Mammals

Despite its relatively small territory, Lyulin hosts a large percentage of the middle and large mammals inhabiting Bulgaria (Popov & Sedefchev, 2003) (Table 4). Mammals with diverse legal and conservation status have been registered (Table 3). 73% (n=8) of the observed wild mammals are game species, while only 2.7% (n=3) are protected and 1.8% (n=2) are listed in the Bulgarian Red Data book as endangered. 64% (n=7) are species of European importance, listed in the Bern Convention, including 1 species which is also listed in CITES. The careful management and conservation of the protected and endangered species of national and European importance in the mountain, most of which fall into hunting areas, is of particular importance.

Table 4. Percentage of species documented for Lyulin Mts. in the current study, relative to the known biodiversity for Bulgaria by family.

Suidae	Leporidae	Erinaceidae	Canidae	Felidae	Sciuridae	Mustelidae	Cervidae
100%	100%	100%	50%	50%	50%	38%	33%

For the first time in the present study, the presence of pine marten was detected. The nearest other known records are found in PP "Vitosha", located at a 10 km straight-line distance. The two locations are separated by two major roads, which most likely indicates that they are not connected. Another interesting observation was that of a wildcat (*Felis silvestris*), which is also a protected species. Stray cats were also found in the same area. It is known that this is a prerequisite for the emergence of hybridization and deterioration of the genetic status of the wildcat. The problem has not yet been studied in sufficient detail in Bulgaria, and due to the increased anthropogenic impact, it is probably very pronounced on the territory of Lyulin Mountain. The presence of stray dogs was also documented, which is an important factor in disturbing wildlife. A brown bear Ursus arctos L. footprint was documented in 2016 during preliminary studies (Petrov, pers. comm.). However, we did not detect its presence during the current research. One of the camera traps was placed on the wildlife overpass on the Lyulin Motorway near the village of Malo Buchino. The presence of red fox, roe deer, European badger, European hare and an unidentified species from the *Martes* genus (most likely a stone marten) was established, indicating that the passage at least partially fulfils its intended functions.

Conclusions

Even with the strong anthropogenic influence due to the proximity of many human settlements, the old forests of the mountain give home to many vertebrates. The results of our study demonstrate that Lyulin mountain hosts a large diversity of vertebrates and needs to be studied in more detail, aiding its effective management and conservation.

Acknowledgments The study was conducted within the project: „First detailed study of the vertebrate fauna (Tetrapoda) on the territory of Lyulin Mountain" (№ 80.10-75/25.03.2021) funded by The Scientific Research Fund of SU „St. Kliment Ohridski".

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Received: 27.05.2022
Accepted: 30.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 171-189

Comparative Analysis of the Water Policies and the Systems for Ecological Status Assessment of the Running Waters in Bulgaria and South Korea: Case study on Maritsa River and Han River

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Abstract. Rivers are the main resource for water supply in Bulgaria and South Korea, thus the ecological status of the running water bodies is important for the ecosystem integrity, as well as for the health and life standard of the population in both countries. This study presents and compares the water management policies, the water legislations and the systems for ecological status assessment of the running waters in both countries. In Bulgaria, the Ministry of Environment and Water provides the water management policy and the national water legislation is fully harmonized with the European requirements. Korean government has strategically been enforced water management plans since the passing of the Environmental Pollution Prevention Act in 1963. The water quality criteria follow the regulations in both countries - European Union Water Framework Directive (2000/60/EC) in Bulgaria and the Environmental Policy Act and the Water Environment Conservation Act in South Korea. The evaluation of the ecological status of the running waters in both countries is based on biological, physico-chemical and hydro-morphological quality elements, and each criterion is specifically defined. Both classification systems for the status evaluation of flowing water bodies by biological quality element are composed of five-level scales, which makes them relatively comparable. Ecological status evaluation based on the macrozoobenthos, according to the classification systems of the Bulgarian and South Korean water legislations was determined for 30 study sites located in the basins of Maritsa River in Bulgaria and Han River in South Korea. The study showed relatively good comparability of the obtained assessments.

Key words: water resource, water management policy, water quality elements, macrozoobenthos.

Introduction

Water related policies have been changed historically depending on the natural and socio-economic conditions and human demands. In recent years, as the anthropogenic pressure and the climate change impacts are increasing worldwide, the ecologically related approaches and environmentally friendly technologies for sustainable water management are highlighted.

Bulgaria joined the European Union (EU) in 2007. Due to the long transitional period from socialism towards democracy and the innovative changes in the economy structure, the implementation of the regulations is comparatively slow and lengthy. The government has updated regulations in line with the EU Water Framework Directive (WFD) (2000/60/EC) and other key documents in the field of the European water legislation. Currently, the strategic aim of Bulgaria in the water management policy is optimal provision of water in the required quantity and good quality for the population and the economy, and enough natural water for the normal functioning of the aquatic ecosystems (Water Act, 1999). Bulgaria is facing environmental issues in the field of water management due to the country's urbanization, infrastructure development and unsustainable agriculture. Especially, during the economic crisis in 2010, water abstraction increased significantly for industry, production and distribution of electricity, heat and gas, and irrigation (Sharkov, 2017). The main significant pollutants of the surface water bodies are the nutrients (40%) and the organic pollution (25%), and in rivers they come mainly from unknown anthropogenic sources (23%), from urban wastewater (22%) and agriculture (19%) (EC, 2019). Moreover, the insufficient number and capacity of wastewater treatment plants delay the purification of the polluted waters. Thus, the main challenges for the water management in Bulgaria have become the appropriate collection and treatment of urban wastewater, and implementation of proper water protection legislations (Water Act, 1999 and its accompanying regulations).

In the period of 1880s and 1960s, South Korea had poor quality of the surface waters because of the lack of water and insufficient sanitation infrastructures, so people suffered from waterborne infectious diseases (Ministry of Environment (ME), 2017). The water infrastructure was reconstructed according to a post-war restoration project funded by international aids and municipal bonds in 1945-1950s (Choi et al., 2017; ME, 2017). After the Korean War (1950-1953), Korea experienced rapid industrialization, urbanization, and economy growth as well as rapid population increase. The massive scales of the developments straightened and covered many urban streams for the extension of land use. Furthermore, untreated industrial wastewater and sewage discharged directly into the rivers especially in rainy seasons. The remarkable population growth and movements particularly to Seoul (from 2.44 million in 1960 to 8.36 million in 1980) increased the contamination even more (KRIHS, 2012; Choi et al., 2017). That resulted in serious urban stream pollution. In 1991, phenol solution from the storage tanks of an electronic factory leaked to the Nakdong River, and the odour was detected in the tap water. That came as a shock to the public and became a huge social issue (KRIHS, 2012; ME, 2017). It raised the awareness of the water quality protection and the drinking water safety in the public. In the twenty-first century, South Korea have faced increased drought and flood risks caused by the climate change, but also issues related to the aging water infrastructure. The importance of the co-relationships between human and nature are also highlighted, as well as the need of sustainable water management systems.

The paper aims at presenting and comparing the water management policies and the water legislation in Bulgaria and South Korea, as well as the systems for the ecological status assessment of the running waters through a study of model rivers in both countries.

Material and methods

Water management policies in Bulgaria and South Korea

The land area (around 100,000 km^2) and the distinct topography of Bulgaria and South Korea are similar regarding the mountains, which pass through the middle of their territories (Balkan, Pirin and Rila Mountains in Bulgaria, and Taebaek and

Sobaek Mountains in South Korea) and the existence of maritime borders.

Bulgaria is located in the central part of the Balkan Peninsula and is divided into four River Basin Districts (RBDs) for basin management: Danube RBD, Black Sea RBD, East Aegean Sea RBD and West Aegean Sea RBD (Fig. 1) (DANGO, 2014; RBMP, 2016-2021; EC, 2019). The Danube RBD is the largest one (42.5% of the country territory) and is subdivided into eleven unites, which are covering the water catchment areas of Iskar, Erma, Nishava, Ogosta, West of Ogosta, Vit, Osam, Yantra, Rusenski Lom, Danubian and Dobrudjan rivers (DRBMP, 2016-2021). The Black Sea RBD is subdivided into several river basins, which are the basins of Shablenska, Batova, Provadiyska, Kamchia, North-Bourgas rivers (Fandakliiska, Panair dere, Dvojnitsa, Vaya, Drashtela, Hadjiyska, Aheloy, Curbandere, Aitoska, Chukarska), Mandrenski rivers (Rusokastrenska, Sredetska, Fakijska, Izvorska), South-Bourgas rivers (Ropotamo, Dyavolska, Karaagach and Lisovo dere), Veleka and Rezovska rivers (BSRBMP, 2016-2021). The East Aegean Sea RBD is subdivided into four river region units: Tundja, Maritsa, Arda and Byala (EARBMP, 2016-2021). The West Aegean Sea RBD is subdivided into three river basins, which are the basins of Mesta, Struma and Dospat rivers (WARBMP, 2016-2021). The river network, hydro-geographical regions and the main catchment areas are described by Hristova (2012). Bulgaria shares three major river basins with neighbouring countries (Serbia and Macedonia to the west, Greece and Turkey to the south, and Romania to the north) and 84.3 % of the renewable freshwater resources are external inflow from the neighbouring territories (EEA, 2018). Thus, their water resources are interdependent and interconnected.

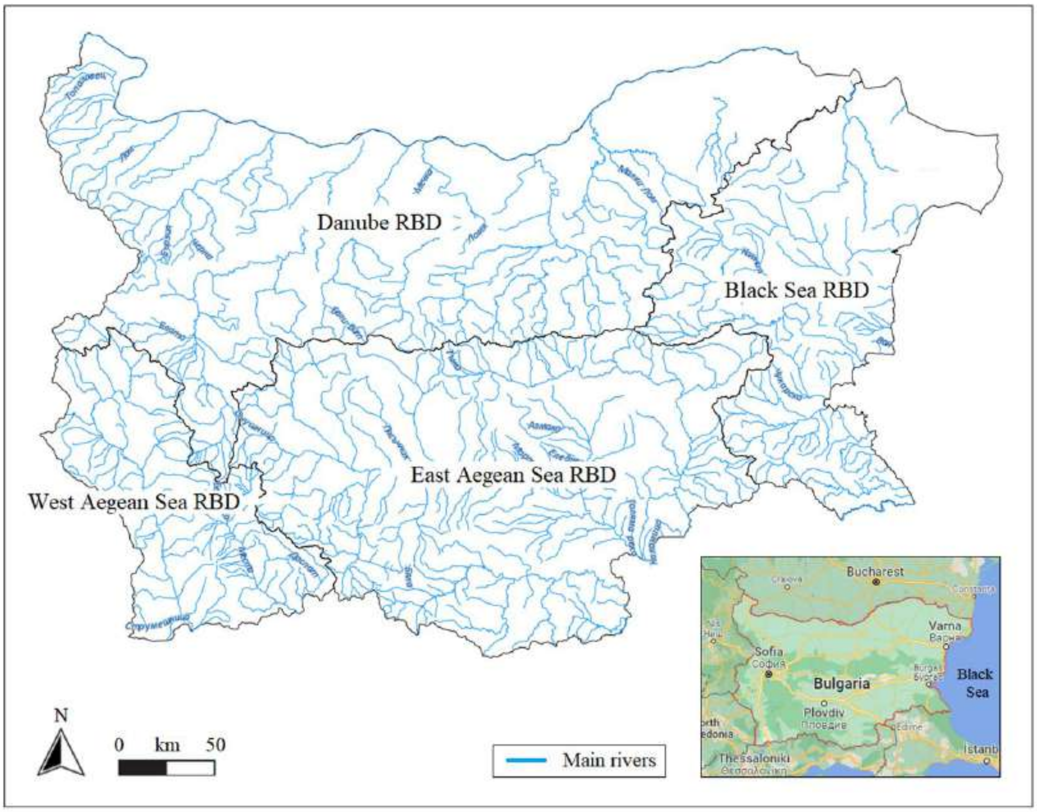

Fig. 1. The four major River Basin Districts (RBDs) in Bulgaria.

In Bulgaria, the Ministry of Environment and Water and the Executive Environmental Agency provide the national water management policy. On a territorial basis, water management is carried out by the four major River Basin Directorates. The first and second River Basin Management Plans (RBMP) have been developed and implemented for the periods of 2010-2015 and 2016-2021. Currently, the third RBMP (2022-2027) is in action. In the recent years, because of climate change and unreasonable human intervention, periods of extremely high tide of water have been observed, causing problems in floodplains and endangering people's lives. In connection with these adverse events, the first Flood risk management plans (FRMP, 2016-2021) had been developed and implemented and the second one is under the preparation. Totally, 16 Regional Inspectorates of Environment and Water monitor the superficial waters and control the wastewaters in their respective territorial scope. The National Institute of Meteorology and Hydrology manages the hydrological and hydro-geological station networks and monitors hydrological and hydro-geological elements.

South Korea is located on the southern part of the Korean Peninsula, which is surrounded by three seas: East Sea, South Sea and Yellow Sea (Fig. 2). About 64% of the territory of the country is covered by mountainous terrains, and most rivers begin from the mountains and flow into the South and Yellow Seas (ME, 2015; Kim et al., 2018). South Korea defines five major River Watershed Regions (RWRs); Han, Geum, Nakdong, Yeongsan and Seomgin, and according to the River Law all rivers in the RWRs are classified in three river types (62 national, 3,773 local and 22,664 small rivers) for efficient management (Fig. 2). The Han RWR is the largest one and the capital city of Seoul is located in it (river length of 514.8 km, basin area of 26,018 km^2; WAMIS, 2021). The Geum RWR is located in the central western area (river length of 395.9 km, basin area of 9,810 km^2; WAMIS, 2021). The Nakdong RWR is located in the eastern part of the country and the total length of the river is the longest (river length of 521.5 km, basin area of 23,817 km^2; WAMIS, 2021). The Yeongsan RWR (river length of 136.0 km, basin area of 3,371 km^2) is located in the south-western area, and the Seomgin RWR (river length of 222.1 km, basin area of 4,897 km^2) is located in the south-central part of the country (WRMIS, 2021).

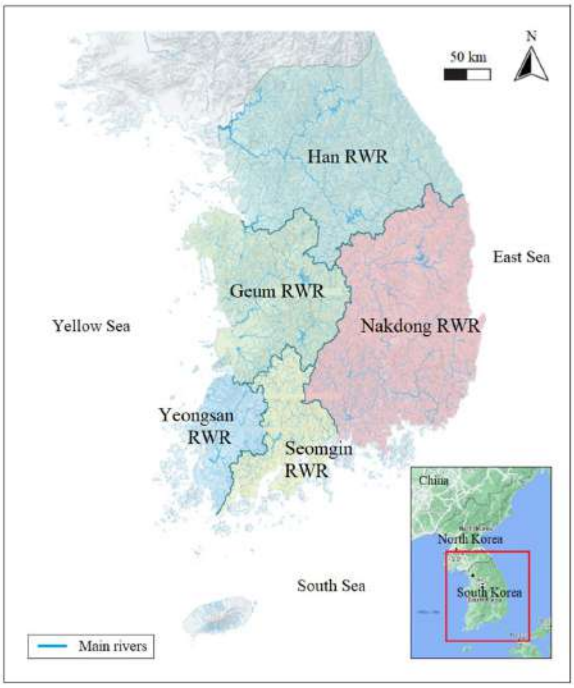

Fig. 2. The five major River Watershed Regions (RWRs) in South Korea.

In South Korea, water management was organised by various water-related ministries until 1990s: Ministry of Environment (ME); Ministry of Land, Infrastructure and Transport; Ministry of Agriculture, Food and Rural Affairs; Ministry of the Interior and Safety; Ministry of Trade, Industry and Energy (Lee and Kim, 2009; KRIHS, 2012; ME, 2017, 2020). The ministries managed and controlled the total 293 of water source protection and special measure areas based on administrative regions; one capital, one special autonomous and six metropolitan cities, and nine provinces including one special self-governing autonomous province (ME, 2021a; MLIT, 2021). However, the administrative district system differs with the RWRs, so conflictions occur between up and down streams in different administrative districts. Moreover, similar plans on same RWRs by various ministries promoted the necessity of the unification of the water management plans for the efficient actions and budget consumptions. Although, the integrations were not straightforward due to the different opinions between the ministries, governments phased to unify them into the ME in the period of 1990-2018 (ME, 2020). In present, the ME is the integrated national water management organisation as the single authority.

Study sites

Two main rivers were chosen for the case study: Maritsa River in the East Aegean Sea RBD from Bulgaria and Han River in the Han RWR from South Korea (Fig. 3). For the purpose of representativeness of the assessment, the sampling sites – 15 in each region, were selected in different river sections along the main rivers and their tributary systems. They represent unaffected reference sites and sites subjected to various types of anthropogenic pressure (Table 1 and 2).

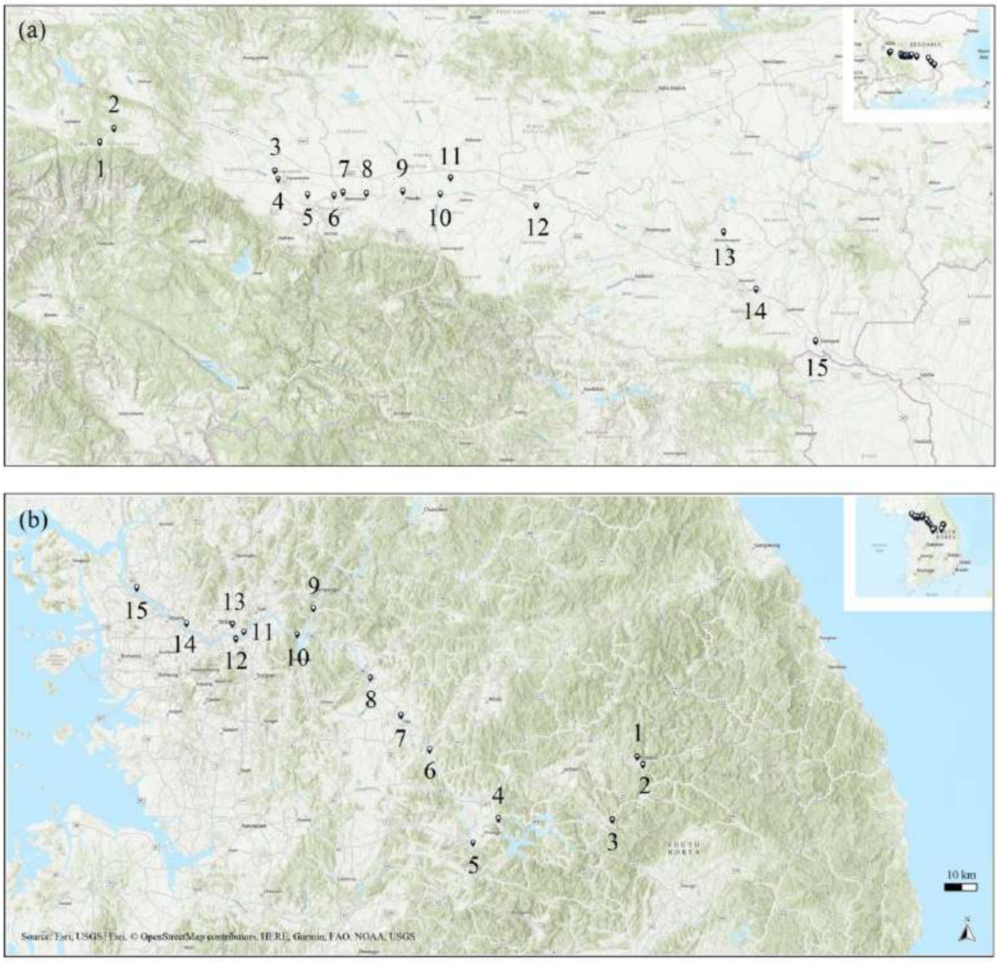

Fig. 3. Maps of the case study sites along Maritsa River in Bulgaria (a) and Han River in South Korea (b).

Table 1. The list and localities characteristics of the 15 case study sites in the Maritsa River Basin.

Site no.	Name of the site	Coordinates (N, E)	Characteristics
1	Maritsa River, Raduil	42.2795, 23.6872	Near the Raduil village (no data for significant anthropogenic pressure, reference site)
2	Maritsa River, Dolna Banya	42.3159, 23.7330	Before the Dolna Banya town (low anthropogenic pressure, mainly from diffuse sources, reference site)
3	Topolnitsa River, Pazardzhik, before estuary	42.2062, 24.2954	Near the bridge for Boshulia village (illegal waste disposal at the banks)
4	Maritsa River, Pazardzhik, Atlantic Motorway Bridge	42.1843, 24.3082	Urbanized area, before the bridge (factories)
5	Maritsa River, Ognyanovo	42.1438, 24.4115	3.7 km downstream from the estuary of Luda Yana River
6	Maritsa River, Govedare	42.1420, 24.5040	Near the village (agricultural land use)
7	Maritsa River, Stamboliyski	42.1498, 24.5349	City centre of Stamboliyski, 1.45 km before the road bridge and weir (agricultural land use and factories)
8	Maritsa River, after Vacha River	42.1477, 24.6174	240 m downstream from the estuary of Vacha River
9	Maritsa River, Plovdiv	42.1536, 24.7450	City centre of Plovdiv (domestic waste disposal at the banks)
10	Chepelarska River, Kemera Old Bridge	42.1456, 24.8772	2.2 km upstream from the estuary (agricultural land use)
11	Stryama River, Manole	42.1874, 24.9131	Representative site for the lower Stryama River, near the Manole village, under the road bridge (agricultural land use, fishing and poaching)
12	Maritsa River, Parvomay	42.1160, 25.2132	Representative site for the middle course of Maritsa River, under the old wooden bridge (significant biogenic and industrial pollution)
13	Sazliyka River, before estuary	42.0478, 25.8702	2.07 km upstream from the estuary of Sazliyka River, near the bridge for Svirkovo and Troyan villages (cow farms, heavy biogenic and organic pollution)
14	Maritsa River, Dositeevo	41.8980, 25.9840	Representative site for the lower Maritsa River, City centre of Dositeevo, after stone weir
15	Maritsa River, Svilengrad	41.7634, 26.1928	Representative site for the lower Maritsa River, City centre of Svilengrad

Table 2. The list and localities characteristics of the 15 case study sites in Han River Watershed Region (RWR).

Site no.	Name of the site	Coordinates (N, E)	Characteristics
1	East River, Yeongwol bridge	37.181944, 128.475556	Representative site for the Upper South Han River (recreational area - resorts, fishing spots, camping sites - on river sides)
2	West River, Palheung Bridge	37.160222, 128.495806	Representative site for the Upper Han River (1.63 km upper - water and sewerage facility, 582 m upper - hydro and nuclear power plant)
3	South Han River, Deokcheon Bridge	37.005528, 128.389167	Representative site for the Dal Stream and Han RWR (no data for significant anthropogenic pressure, reference site)
4	South Han River, after Chungju Dam	37.009222, 127.981806	After Chungju Lake (recreational area at the lake side)
5	Dal Stream, Danwol Bridge	36.941508, 127.899844	Danwol River Beach (livestock logistics centres on the left bank)
6	South Han River, SHR Bridge	37.201889, 127.747556	Representative site for the downstream of Chungju Dam (camping sites at the lake side, a cow farm 1.74 km away from the right bank)
7	South Han River, Yeoju Bridge	37.296722, 127.647889	Parks, gardens, tracking courses on river sides
8	South Han River, Ipo Bridge	37.398083, 127.541389	Representative site for the Ipo Weir (350 m downstream from Ipo Bridge) (camping sites on right side)
9	North Han River	37.594225, 127.341306	Representative site for Cheongpyeong Dam and Han RWR (farms on right side, recreational area on river sides)
10	Han River, after Paldang Dam	37.521394, 127.283339	Representative site for Paldang Dam and Han RWR, before the Paldang Dam with hydro power plant (major water supply to the capital city), Geomdan mountain on left side
11	Han River, Jamsil Railway Bridge	37.528986, 127.097153	City centre of Seoul, representative site for Han River Jamsil, parks on river sides
12	Han River, Tan stream, before estuary	37.510208, 127.070622	City centre of Seoul, Tan stream passes through most uptown areas and flows into Han River, parks on river sides
13	Han River, Cheonggye stream	37.549322, 127.055453	City centre of Seoul, significant urban environment infrastructure in downtown (restored in 2005)
14	Han River, before Seongsan Bridge	37.553022, 126.896244	City centre of Seoul, parks and recreational areas on river sides
15	Han River, Ilsan Bridge	37.651869, 126.721647	Representative site for Han River_Goyang, parks on river sides

Data analysis

Data about the macrozoobenthos collected from Maritsa River in July 2021 (Varadinova et al., 2022) and from Han River (WEIS, 2022) in September - October 2021 were used.

Two indices: Biotic index (BI) (Cheshmedjiev & Varadinova, 2013) and Benthic Macroinvertebrate Index (BMI) (Guidelines, 2019-52) were calculated for the biological quality assessment based on the macrozoobenthos. For the calculation of the BMI, 190 indicator species have been defined with saprobic values (s) and indicator weight values (g). The index is calculated according to the formula:

$$\mathrm{BMI} = 4 - \frac{\sum_{i=1}^{n} si\ hi\ gi}{\sum_{i=1}^{n} hi\ gi}\ x\ 25$$

i: number assigned to the species,
n: number of indicator species,
s_i: saprobic value of the species i,
g_i: indicator weight value of the species i
h_i: frequency of the species i.

The value of h changes from 1 to 5 according to R_i (1 (> 80%), 2 (60% < ~ ≤ 80%), 3 (40% < ~ ≤ 60%), 4 (20% < ~ ≤ 40%) and 5 (≤ 20%)). The R_i is calculated as a percentage of the ranking of the abundance (1, 2, 3 .... in the order of the highest abundance) for each appeared species (Guidelines, 2019-52).

The assessments of the ecological status of all study sites were done according to the Bulgarian (Regulation H-4/2012) and the Korean (Water Environment Conservation Act (WECA, 2022, Article 9) water legislations. Data about the ecological status assessment of Maritsa River, according to the Bulgarian legislation, published by Varadinova et al. (2022), were used in this article.

Results and Discussion

Comparative analysis of the water legislation in Bulgaria and South Korea

Although water management is organized differently, restrictive water legislation is implemented in Bulgaria and South Korea, which aims at the water resources conservation, as well as the preservation of its quality.

Since Bulgaria's accession to the EU, the water legislation of the country has been fully harmonized with the European one. The Water Act has been adopted in accordance with EU WFD (2000/60/EC). Bulgaria has been included in two Ecoregions (7 Eastern Balkans and 12 Pontic Province) and both surface (running and standing) and underground water bodies have been identified and characterised in accordance with national river typology (Cheshmedjiev et al., 2013). Achieving good (ecological and chemical) status of the waters in the country has been defined as a main goal. Gradually, the relevant ordinances concerning various aspects of water protection have been adopted. Regulations have been developed for water monitoring, characterisation of the superficial waters, quality of drinking and bathing water, emission standards for the permissible content of harmful and dangerous substances in wastewater discharged into water bodies, for preservation of waters from pollution by nitrates from agricultural sources, for groundwater protection and others (Water Law). Areas with a special status of water protection according to the European (Directive 92/43/EEC; Directive 2009/147/EC) and national (Protected Areas Act, 1998; Biodiversity Act, 2002) legislation have been introduced. The Regulation H-4/2012 of the surface waters characterisation and its amendments and additions plays a key role to define the quality elements, methods for sampling, analysis and assessment of the ecological status of the surface water bodies in Bulgaria.

The geographical, economic and political features contribute to the major difference in the water resource management approach in Bulgaria and South Korea. Bulgaria efforts on the harmonious cooperation with neighbouring countries which are not members of the EU to maintain and protect the water quality from industrial, agricultural, and domestic waste pollutants. The Bulgarian government has made bilateral agreements and partnerships in several international conventions such as the Convention on Co-operation for the Protection and Sustainable Use of the River Danube, the Convention on the Protection of the Black Sea against Pollution and the Convention on the Protection and Use of Transboundary Water courses and International Lakes (RBMP, 2016-2021).

Korean government has strategically been enforced legislation on water environment to protect water quality and provide safe drinking water. The water-related policies have been evolved from pollution control to cleaner production and eco-efficiency management highlighting the importance of the environmental issues. In 1963, the Environmental Pollution Prevention Act was imposed as the first water related law to protect people's health from industrial wastewater. During the period of 1970-1980s, many urban rivers were covered or reformed, and their channels were straightened for the needs of transportation infrastructure and rapid urbanization in accordance with the River Management Policy. However, as the issues of environmental degradation of rivers raised, Ecological Stream Restoration Project (ESRP) has implemented to restore urban rivers by replacing artificial infrastructures with natural conditions in 1987 (ESRP, 2020). This enhances the self-purifying capacity of the rivers, improves the water quality, and provides better habitats for aquatic flora and fauna. In 1990, WECA (1990) was implemented in order to manage the complex environmental problems more effectively. In line with the ESRP, the 'Four Major River Restoration Project' was enforced to secure the water resources and to enhance the water quality of the rivers; the Acts on the River Watershed Management and Community Support were enacted for Han RWR in 1999 and for Nakdong, Geum and Yeongsan RWRs in 2002 (Kim et al., 2007; KRIHS, 2012; WEPA, 2012; Choi et al., 2017). Until 2019, about 97 of water-related plans existed by seven ministries in accordance with 29 Acts. Since the ME became the central government administration, the ministry has defined a new Water Management Act (WMA, 2019). The Act focuses not only on the unification of the water managements but also on the extended applications of the present legislations, which used to be applied mainly for major RWRs, for more comprehensive and effective achievements. In accordance with the WMA, the first National Water Management Plan (NWMP) (2021-2030) and the Comprehensive Watershed Management Plan (2021-2030) are in action.

Assessment of the ecological and chemical status of the surface waters in Bulgaria and South Korea

Bulgarian authorities follow the EU WFD for the assessment of the ecological and chemical status of surface waters. The ecological status is evaluated by biological, physico-chemical and hydro-morphological quality elements. Five obligatory biological quality elements are used – phytoplankton, phytobenthos, macrophytes, macro-zoobenthos and fish. For each element, specific indices have been developed with corresponding type-specific scales for assessing the status (Regulation H-4/2012). The evaluation is expressed as 'Ecological Quality Ratios (EQR = observed value / reference value) and is performed through a five-step type-specific classification scale (high, good, moderate, poor and bad status) (Table 3). The ratio has a numerical value that varies between zero and one, which expresses high status. The classification guidance separates three levels in the biological assessment: the quality element level, the parameter level, and the status classification. The main conclusion is that the WFD requires classification of water bodies at the quality element level, and that the worst of the relevant quality elements determines the final classification (the "one out, all out" principle) (Van de Bund & Solimini, 2007). The physico-chemical quality elements for rivers include basic parameters dissolved oxygen (DO), conductivity, pH, nutrients (N-NH_4, N-NO_3, N-NO_2, total nitrogen (TN), P-ortho-PO_4 and total phosphorus (TP)) and biological oxygen demand (BOD), and specific pollutants (Regulation H-4/2012). The assessment is characterised with a three-step scale (high, good and moderate status). Hydro-morphological quality elements determine the status 'good' (native or unaffected conditions) or 'deviations from natural conditions' (CIS-WFD, 2003). The physico-chemical and hydro-morphological quality elements play a supporting role in the ecological assessment of the water bodies. The chemical status of the water bodies is set in the Regulation on environmental quality standards for 45 priority substances and specific pollutants (Directive 2013/39/EU), and the criteria is whether 'good' or 'failing to achieve good' status (Regulation of 09.11.2010) (Table 3). In this way, the general status of a surface water body is determined by its worse ecological or chemical status.

In South Korea, the water quality standards are classified into surface, ground, coastal and drinking waters based on the Environmental Policy Act (EPA, 2022) and the WECA. For the surface water in rivers, there are two quality standards – one of them is applied for 'Conservation of the Living Environment (CLE)' and the other one is for 'Protecting Human Health' (EPA, Article 2). In terms of the CLE, the water status in rivers is determined by biological, hydro-morphological, and physico-chemical quality elements, and hazardous chemical materials, same categories as in Bulgaria.

Table 3. The ecological and chemical status and the quality elements for surface water assessment of the rivers in Bulgaria (The colour-code of each status classification is indicated).

	Ecological status			Chemical status
Quality elements	**Biological (incl. macrozoo-benthos)**	**Physico-chemical**	**Hydro-morpho-logical**	**Priority substances /specific pollutants**
Grade	High	High	Good	Good
	Good	Good	Deviation from the natural conditions	Failing to achieve good
	Moderate	Moderate		
	Poor			
	Bad			

The biological and hydro-morphological quality is surveyed and evaluated in accordance with the Guidelines No. 2019-52. The indices based on the quality elements are Trophic Diatom Index (TDI), Benthic Macroinvertebrate index (MBI), Fish Assessment Index (FAI), Riparian Vegetation Index (RVI) and Habitat and Riparian Index (HRI) (Table 4). Each element quantitatively evaluates the ecosystem health into five grades (very good (A), good (B), average (C), poor (D) and very poor (E). The colour-coding of the ecological status classification is the same in both countries (Table 3 & 4). The physico-chemical quality is characterised with eight elements which are pH, BOD, Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Suspended Solids (SS), DO, TP and Coliforms, with seven grades (very good (Ⅰ$_a$), good (Ⅰ$_b$), somewhat good (Ⅱ), average (Ⅲ), somewhat poor (Ⅳ), poor (V) and very poor (Ⅵ)) (Table 5). For the measurement, Water Environment Measurement Network monitors the physico-chemical quality elements monthly at 691 sites and the biological quality elements at 3,035 sites (TDI, BMI and FAI are twice a year, RVI and HRI are once a year) on rivers (ME, 2021a-297). Currently, advanced water quality standards including water quantity and waterside environment (history, culture, scenery etc.) are preparing for the 2nd NWMP (2031-2040).

Table 4. The biological and hydro-morphological quality elements and the criteria for Conservation of the Living Environment in rivers in South Korea (The colour-code of each status classification is indicated).

Grade		TDI	BMI	FAI	RVI	HRI
Very good	A	≥ 90	≥ 80	≥ 80	> 65	> 80
Good	B	≥ 70	≥ 65	≥ 60	> 50	> 60
Average	C	≥ 50	≥ 50	≥ 40	> 30	> 40
Poor	D	≥ 30	≥ 35	≥ 20	> 15	> 20
Very poor	E	< 30	< 35	< 20	≤ 15	≤ 20

Table 5. The physico-chemical quality elements and the criteria for Conservation of the Living Environment in rivers in South Korea (The colour-code of each status classification is indicated).

Grade		Elements								
		pH	BOD	COD	TOC	SS	DO	TP	Coliforms (groups/100mL)	
			(mg/L)						Total	Fecal
Very good	Ia	6.5-8.5	≤ 1	≤ 2	≤ 2	≤ 25	≥ 7.5	≤ 0.02	≤ 50	≤ 10
Good	Ib		≤ 2	≤ 4	≤ 3		≥ 5.0	≤ 0.04	≤ 500	≤ 100
Somewhat good	II		≤ 3	≤ 5	≤ 4			≤ 0.1	≤ 1,000	≤ 200
Average	III		≤ 5	≤ 7	≤ 5			≤ 0.2	≤5,000	≤ 1,000
Somewhat poor	IV	6.0-8.5	≤ 8	≤ 9	≤ 6	≤ 100	≥ 2.0	≤ 0.3	-	-
Poor	V		≤ 10	≤ 11	≤ 8	No floating garbage, etc.		≤ 0.5	-	-
Very poor	VI	-	> 10	> 11	> 8	-	< 2.0	> 0.5	-	-

Current water quality status and future of the water management in Bulgaria and South Korea

Water management should be considered in two aspects: improving water quality and sustainable use of the water resources. These are especially important against the background of the growing needs of people and the increasing impacts of the global climate change.

Bulgaria is obliged by the European Structural and Investment Fund (ESIF) rules and should improve the necessary infrastructures to comply with the Urban Waste Water Treatment Directive. The government has increased the number of wastewater treatment plants (79 pcs in 2010 to 174 pcs in 2019; EEA, 2021), and the compliance levels have increased in recent years (90-100% in the improvement of surface water quality in EU; EEA, 2016 & 2018).

According to the RBMPs reports, Bulgaria has improved the water quality overall in rivers. The high or good ecological status in the four RBDs have increased from 43.2% in the 1st to 52.6% in the 2nd RBMP (WISE). The assessment of the main physico-chemical indicators (DO, BOD, ammonium, nitrogen, and phosphates) showed that the water bodies had on average 66.9% of high or good ecological status (1996-2019), with the lowest values in the Black Sea RBD (51.2%) and the highest ones in the in Danube RBD (74.6%) (Table 6) (EEA, 2021). The assessment of the main biological quality elements (phytoplankton, macrophytes, phytobenthos, benthic invertebrates and fish) showed that on average 62.7% of rivers had high or good ecological status (Table 7) (EEA, 2021). Despite of the achievements, further improvements are required because of the low values of some quality elements (Table 6 and 7). Thus, strategic documents are developing such as the National Strategy for Management and Development of the Water Sector and the Action Plan until 2037, and the Implementation of Directive 91/271/EEC on the treatment of urban wastewater. In addition, a new classification system has been proposed and the scales for the biological status of different types of water bodies have been specified. A system for evaluation of the status by hydro-morphological quality elements is also to be developed. The government is forcing conservation laws and environmental regulations for effective water management and efficient spending of available finances.

Table 6. The percentages of the water bodies with high or good status according to the main physico-chemical indicators in the four major RBDs in Bulgaria for the period of 1996-2019 (EEA, 2021).

Physico-chemical indicators		**DO**	**BOD**	**Ammonium**	**Nitrogen**	**Phosphate**
RBDs	Danube	86	77	85	61	64
	Black Sea	57	53	46	50	50
	East Aegean Sea	84	53	74	75	53
	West Aegean Sea	90	68	81	80	51

Table 7. The percentages of the water bodies with high or good status according to the main biological indicators in the four major RBDs in Bulgaria based on the 2nd RBMP report.

Biological indicators		**Phyto-plankton**	**Macro-phytes**	**Phyto-benthos**	**Benthic invertebrates**	**Fish**
RBDs	Danube	50	72	76	67	61
	Black Sea	57	73	85	57	56
	East Aegean Sea	38	65	72	45	71
	West Aegean Sea	-	77	60	67	57

The implementation of the water management policy has improved water quality and ecosystem integrity of surface waters overall in South Korea. Especially, the BOD decreased significantly from maximum 130 mg/l in 1981 to 20 mg/l in 1995, and during the recent decades it has maintained average values of 1 mg/l (ME, 2021a). However, other elements have not improved as much as the BOD.

In the basis of the WECA (Article 9-2), the government defines targeting grades for physico-chemical quality elements at 35% of very good (Ia), 43% of good (Ib), 13% of somewhat good (Ⅱ), 10% of average (Ⅲ), and 1% of somewhat poor (Ⅳ) of all rivers in RWRs (ME Notice No. 2018-6). However, for the period 2016-2020, the assessment based on the physico-chemical quality elements showed that 63.7% of rivers were in a very good (Ia) or good (Ib) status, which is lower than the targets (ME, 2021b) (details in Table 8). The lowest value was in the Geum RWR (59.9%) and the highest one was in the Nakdong RWR (66.1%). The assessment of the biological and hydro-morphological indicators showed that overall 40.0% of rivers in RWRs were in a very good (A) or good (B) status (TDI: 38.3%, BMI: 52.0%, FAI: 42.1%, RVI: 33.5% and HRI: 30.7%) (ME, 2021b) (Table 9). The lowest value was in the Geum RWR (32.3%) and the highest one was in the Han RWR (43.6%). Thus, in order to improve the water quality in RWRs, the government is investigating not only a highly advanced water management system but also doing structural changes. For example, the 'Four Major River Restoration Project' restored 1,813 rivers from 1987 to 2015, as a result, the percentage of ecologically degraded rivers of total rivers decreased from 55% in 2009 to 35% in 2015 (ESRP-MLP, 2016-2020; ESRP, 2020). The project is continuing with Comprehensive Mid- (2020) to Long-term (2025) Plan (ESRP, 2020). Also, weirs in the RWRs are gradually opening since 2017 for the restoration of natural conditions (ME, 2020). The most significant changes are an increase in flow rate and a decrease in residence time, and consequently a significant decrease in tidal flow. However, the water quality did not improve significantly after the weir opened, and the cause is analysed by external variables (precipitation and upstream pollutants). Therefore, long-term monitoring is required for the effectiveness of the weir openings in the water quality.

Table 8. The percentages of the rivers with very good or good status according to the physico-chemical quality elements in the five major River Watershed Regions (RWRs) in South Korea for the periods of 2016-2020 (ME, 2021b).

RWRs	Quality Elements								
	pH	BOD	COD	TOC	SS	DO	T-P	Coliforms (groups/100mL)	
								Total	Fecal
Han	95.3	64.1	52.5	65.7	97.2	100	46.1	24.7	45.6
Nakdong	98.6	76.7	32.3	49.8	98.2	100	57.7	37.3	44.6
Geum	99.6	46.1	34.3	44.1	88.5	100	99.1	12.4	15.1
Yeongsan and Seomgin	100	65.9	37.8	49.6	91.1	100	33.3	43.2	46.2

Table 9. The percentages of rivers with very good or good status according to the biological and hydro-morphological quality elements in the five River Watershed Regions (RWRs) in South Korea for the period of 2016-2020 (ME, 2021b).

RWRs	Elements				
	TDI	BMI	FAI	RVI	HRI
Han	43.9	58.7	51.2	25.4	38.7
Nakdong	49.0	56.3	46.2	29.6	27.1
Geum	24.1	40.3	31.4	38.2	27.8
Yeongsan and Seomgin	36.0	52.6	39.5	41.0	29.4

Bulgaria and South Korea aim to achieve and maintain at least good ecological status of rivers. For the recent five years (2016-2020), both countries showed average achievements – 16th (50.5%) and 14th (51.8%) place among 30 countries, although the specifications of the criteria are different, the main quality elements are the same (Fig. 4). These are higher than the average to the countries, 2nd RBMPs (44.0% of all rivers, WISE).

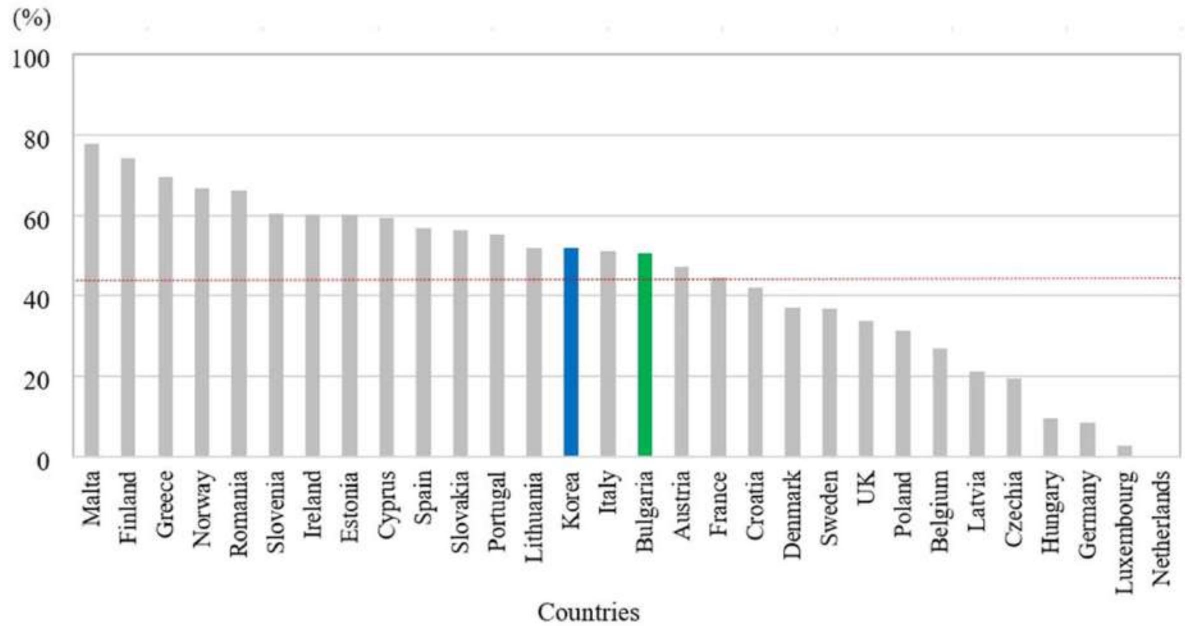

Fig. 4. The percentages of high/very good or good ecological status in rivers in European countries and South Korea for the periods of 2016-2020 (the red dash line indicates the average value for all the countries; the data are based on 2nd RBMPs reports (WISE) and ME (2021b).

Comparison of ecological status assessment in Bulgaria and South Korea: Case study of Maritsa River and Han River

Both classification systems for the status evaluation of running water bodies by biological quality element are composed of five-level scales (Tables 3 and 4), which makes them relatively comparable. Ecological status assessment of the study sites located in the basins of Maritsa and Han rivers according to the classification systems of the two water legislations

(Bulgaria and South Korea) is presented in Table 10.

In total, the ecological status of Maritsa River evaluated by two classification scales coincides at seven sites: sites 2, 3, 8 and 11 – good; site 10 and 15 – moderate / average; and site 13 – bad / very poor. The assessment of the status of Han River shows a match at eight sites: sites 2 and 3 – high / very good; sites 6 and 12 – moderate / average; sites 8, 13 and 14 – poor; and site 15 – bad / very poor. There is a discrepancy in the ecological status of Maritsa River by one class at 8 sites: at three sites (1, 7 and 14) lower, and at four sites (4, 5, 6 and 12) higher status, and only at site 9 the condition is higher by two classes in accordance with the Korean water legislation. The ecological status assessment of Han River differs at seven sites: at four sites (5, 7, 9 and 11) by one class lower, at two sites (1 and 4) by one class higher, and at site 10 by two classes lower according to the Bulgarian water legislation.

Table 10. Ecological status assessment of the study sites in Maritsa and Han rivers based on the macrozoobenthos according to the Bulgarian and Korean water legislations in 2021 (The colour-code of each status classification is indicated).

River	Maritsa River		Han River	
Assessment	Bulgarian water legislation (Varadinova et al., 2022)	Korean water legislation	Bulgarian water legislation	Korean water legislation
Site 1	High	Good	High	Good
Site 2	Good	Good	High	Very good
Site 3	Good	Good	High	Very good
Site 4	Moderate	Good	Moderate	Poor
Site 5	Moderate	Good	Good	Very good
Site 6	Moderate	Good	Moderate	Average
Site 7	Good	Average	Moderate	Good
Site 8	Good	Good	Poor	Poor
Site 9	Moderate	Very good	Moderate	Good
Site 10	Moderate	Average	Poor	Good
Site 11	Good	Good	Bad	Poor
Site 12	Moderate	Good	Moderate	Average
Site 13	Bad	Very poor	Poor	Poor
Site 14	Moderate	Poor	Poor	Poor
Site 15	Moderate	Average	Bad	Very poor

Both assessments determined one and the same ecological status almost at the half of the sites in both rivers, and only one level of discrepancy for most of the rest. The assessment of the condition of the rivers by the two classification systems shows that the Bulgarian assessment scale is stricter for both rivers. The number of sites with good or high ecological status determined according to the values of the BI used in Bulgaria is less in both rivers (6 in Maritsa River and 4 in Han River) than the number of sites with the same status, but defined through the Korean BMI (10 in Maritsa

River and 7 in Han River) (Table 10). It should be noted that the ecological assessment of the conditions in Bulgarian rivers is being determined according to type-specific scales, in which the boundaries between individual classes are determined in terms of the type of running water body (Regulation H-4/2012). Unlike Bulgaria, Korean assessment does not apply river types and reference sites for the evaluation. The biological quality assessment of running waters in South Korea is based on defined indicator species with their own index values and only those species are used for the evaluation (Guidelines, 2019-52). However, the species composition of the macrozoobenthos, and the indicator species depend on the complex action of the specific environmental factors in the river habitats. In this case study, 40 out of 110 taxa found in the Maritsa River survey were not included in the calculation of BMI, while only 2 taxa from Han River were excluded. However, at site 4 in Maritsa River where all taxa were used in the calculation, the status differ by one class. On the other hand, 6 taxa with total abundance of 60 individuals at site 3 and 8 taxa with total abundance of 532 individuals at site 8 in Maritsa River were excluded, but the two assessments determined the same status. This case study demonstrated that the calculation approaches of the assessment methods are of essential importance in determining of the ecological status. Thus, in Bulgaria, the evaluation of the BI is based on taxa, which belong to certain indicator groups, differing in degree of sensitivity. The calculation of the South Korean index (BMI) is based on the saprobic values and indicator weights of each individual species.

Conclusion

Despite of the specific differences in the water legislation in Bulgaria and South Korea, the basic national policies in the field of surface running waters are aimed at protection and sustainable use of the limited water resources affected by the growing freshwater requirements and global climate change. Both countries apply scientifically based methods in the water resources management and basin approach for comprehensive and integrated water management taking into account the specifics of the geographical locations. The ecological status of rivers (water bodies) in Bulgaria and South Korea is evaluated by the same groups of quality elements - biological, physico-chemical and hydro-morphological. The results from the study of Maritsa and Han rivers showed that the assessments based on one of the key biological quality elements macrozoobenthos and made according to the classification systems of Bulgarian and South Korean water legislations determined the same or similar ecological status. More detailed studies based on long data set received from more rivers in the two countries has to be conducted in order to receive more reliable results.

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Received: 10.11.2022
Accepted: 05.12.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 191-193

Short note

Fatal Attraction - a Case of Multiple Amplexus and Some Breeding Peculiarities in the European Green Toad (Bufotes viridis) from the City of Plovdiv (Bulgaria)

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Abstract. A previously unreported case of multiple amplexus and ampleaxus with dead female, as well as some peculiarities of the breeding of a *Bufotes viridis* population from the city of Plovdiv in Bulgaria are presented. A discussion of their potential causes and comparison with similar cases are also given.

Key words: *Bufotes viridis*, breeding peculiarities, multiple amplexus, Plovdiv, Bulgaria.

Amplexus is the mating position adopted by many amphibians for breeding. In anurans, amplexus is dorsal: the male grasps the female on the back, either at the armpits - axillary amplexus or at the waist - lumbar amplexus (Dufrenes, 2019). Despite the fact that quite a lot of studies on the breeding behavior and the aberrations from the normal amplexus of anuran amphibians are conducted in Bulgaria (for comprehensive review see Mollov et al., 2010) there are still some aspects that are not well studied.

The Green Toad (*Bufotes viridis*) is regarded as near synanthropic species in most parts of Europe, occurring mainly in agricultural landscapes with a warm climate (Stöck et al. 2009). It also inhabits gardens, parks and ruderal areas and often in urban environments (Kaczmarski et al. 2019). Its ecology and breeding phenology is well described from the city of Plovdiv by Mollov (2019). In the current short note, new data about the breeding phenology of the species and a case of multiple amplexus and amplexus between alive male and dead female is reported for the first time.

In March 2022 at nature monument "Mladezhki halm" Hill in Plovdiv City, in a small temporary pond (42°08'08.5"N 24°43'42.5"E) at the south side of the hill (Fig. 1A) an unusual breeding behavior of *B. viridis* was observed. On 24.03.2022 a multiple amplexus between four males and a female green toads were successfully photographed (Fig. 1B). The female was laying on its dorsal side in the water and was dead, at the time of observation, probably drowned by the clasping males. Several other pairs in normal amplexus as well as laid cords of eggs were also recorded (Fig. 1C). Few days later (29.03.2022) the dead body of the same female toad was found in the pond as well as three dead males, scattered at different parts of the pond, who probably died due to exhaustion.

A - The study site (temporary standing water basin in the south side of NM "Mladezhki Halm" Hill.

B - Four males grasping a dead female at the study site (24.03.2022).

C - a pair in normal amplexus and layed eggs (24.03.2022).

D - hatched larvae at the study site (29.03.2022).

Fig. 1. Study site and and observed individuals of *B. viridis* in the city of Plovdiv.

Newly hatched larvae (few days old) were also recorded in the pond (Fig. 1D). By this time all adult toads have already left the water.

At the same site in 2021 the first mating calls of the males were recorded in March 2021 (surveys on March 14 and 26). Shortly after, the first cords with eggs were also registered. At the beginning of April (05.04.2021), tadpoles in an early stage of metamorphosis were already registered. Later towards the middle of April 2021, there was an unusual for this time of the year cold weather and even snow fall. Although it lasted only for two days, the bad weather probably interrupted the metamorphosis and greatly hindered toads' reproduction. After that, there was no water left in the pond in May and no tadpoles or adult frogs were recorded in the pond area. In 2022 most of the larvae managed to complete their the metamorphosis and in May 2022 there were numerous newly metamorphosed toads and almost no water left in the pond. As this was observed many times before at the same place - metamorphosis for this species in Plovdiv City finishes very fast (about 30 days) (see Mollov, 2019).

A multiple amplexus (several males on one female) was previously reported for *Bombina variegata, Rana temporaria* and *R. dalmatina* from Bulgaria even for a closely related to *B. viridis* species as the Common Toad (*Bufo bufo*) (see Mollov et al., 2010; Covaciu-Marcov & Sucea, 2021), but this is the first reported sighting of a multiple amplexus between several males and one female for *B. viridis,* as well as amplexus between alive male and dead female.

On 02.03.2008 in the same pond another peculiar aberration from the normal amplexus was observed, namely an amplexus between two males (Mollov et al., 2010), which is also reported by Huebauer (2019) from Austria.

The reason for all of these aberrations from the normal breeding behavior is most like due to the very skewed sex ratio towards the males. Thus the operational sex ratio may be strongly male biased in these cases resulting in an intense competition between males to obtain a mate. From a previous study in 2007-2008 at the same site a sex ration (males:females) of 3.68:1 was recorded (Mollov, 2019). Sistani et al. (2021) registered a sex ration for a B. viridis population from Vienna (Austria) of approximately 4:1. Multiple amplexus is especially common in explosive breeding amphibians, such as *B. viridis* where a large number of breeding adults can be present in the breeding site for a short time period (days to weeks). In these cases, females are important resources for males, because the males are present in larger numbers than females. This competition may result in multiple amplexus, takeovers, and frequently, the death of females, as it was previously observed for example in *B. bufo* (Mollov et al., 2010).

In conclusion an emphasis on the importance of recording and describing observed changes in the phenology of anurans should be made, which will result in enhancing the effectiveness of conservation or monitoring activities undertaken for management of these vulnerable species.

Acknowledgements. This research was carried out with the financial support of NPD at University of Plovdiv "Paisii Hilendarski", Grant No MU21-BF-021, "Population and feeding ecology of the green toad, *Bufotes viridis* (Laurenti, 1768) (Amphibia: Anura) in urban environments" A permit by MOEW No 866/22.02.2021 was issued for work with this species during the current study.

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Received: 22.05.2022
Accepted: 12.08.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 195-198

Short note

First Record of Therrya fuckelii (Rhytismatales, Ascomycota) in Bulgaria, with Ecological Remarks

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Abstract. The short note presents the first record in Bulgaria of *Therrya fuckelii,* an ascomycetous fungus developing on hosts of the genus *Pinus* L. The Bulgarian collection was found on the relict and subendemic Bosnian Pine (*Pinus heldreichii*). Brief description and illustrations of the Bulgarian specimen are provided and some aspects of the ecology of the species are briefly discussed.

Key words: Bulgarian mycota, endophytic fungi, Leotiomycetes, forest pathogen, Rhytismataceae.

Introduction

In 2021 during studies of the fungal diversity in stands of *Pinus heldreichii* H. Christ. the authors collected a remarkable member of the family *Rhytismataceae* Chevall., preliminary identified in the field as a member of the genus *Therrya* Sacc. Further study of this collection revealed that it belongs to *T. fuckelii* (Rehm) Kujala, a fungal pathogen new for the Bulgarian mycota. This finding is presented herein.

Material and Methods

The examined specimens were photographed and documented in the field, as well as *ex-situ*. The microscopic study was held with an AmScope T360B light microscope with an AmScope MU900 digital camera. All microscopic observations were held in cotton blue in lactoglycerol (lactic acid : glycerol : water = 2 : 1 : 1 v/v) after heating. Melzer's reagent was used for testing the amyloid reaction of asci. Measurements of microscopic structures were obtained from pre-calibrated digital images with the aid of Piximetre 5.10 software. Air-dried voucher specimens are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

Results and Discussion

Therrya fuckelii (Rehm) Kujala, Comm. Inst. Forest. Fenn. 38(4): 48 (1950); *Coccophacidium fuckelii* (Rehm) Krieg., Fung. Saxon. Exsicc., Pilze Sachsen's 15: no. 735 (1892); *Coccophacidium pini* var. *fuckelii* Rehm in Rabenh. Krypt.-Fl., Edn 2, 1.3(lief. 29): 99 (1888)/[1896].

Macroscopic features. Ascomata in groups or scattered on dead, attached branches and twigs, initially covered with thin bark layer, then erumpent from bark, rounded or irregular in top view, at first covered by black fungal layer, which later disrupts by radial splits into several coriaceous rays, curving backwards, producing star-like structure and revealing up to 4 mm broad, hazel, sepia to blackish brown coloured disk with somewhat waxy appearance.

Fig. 1. Ascomata of *Therrya fuckelii* on branch of *Pinus heldreichii*. Scale bar = 10 mm.

Microscopic features. Ascospores 103.5–121.7 × 4.2–5.5 µm, straight or to a different degree curved, 7–10-septate and slightly constricted at septa, hyaline, smooth, at the apices tapering into thread-like tips. Asci cylindrical, 128–175 × 10–17 µm, 4-spored with parallel spore arrangement, clavate, with flattened apex, thin-walled, inamyloid, without visible predefined opening structures. Paraphyses slightly longer than asci, filiform, 2–3.5 µm wide, with widened tips (up to 8 µm) at the apex, thin-walled, septate, hyaline, enveloped in mucous sheath.

Specimen examined. Bulgaria: Znepole Region, Konyavska Mt, below Viden Peak (Kyustendil Province), 42°20'47.6"N, 22°50'21.4"E, elev. ca 1455 m, on branches of *Pinus heldreichii* in an artificial plantation (Fig. 3), 30.09.2021, B. Assyov (SOMF 30423).

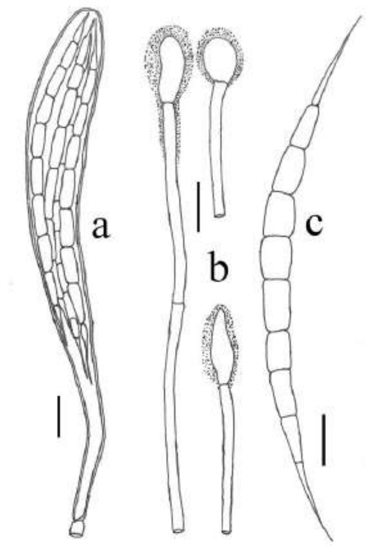

Fig. 2. Microscopic features of *Therrya fuckelii*: a – ascus with ascospores; b – paraphyses (mucous sheaths presented as dotted outline); c - ascospore. Scale bars = 10 µm.

The morphological features of the Bulgarian collection of *T. fuckelii* (Fig. 1–2) are consistent with the characters, accounted by Reid & Cain (1961) and Minter (1996). The only notable difference is that in our collection of *T. fuckelii* on *P. heldreichii* we have not observed the presumed characteristic bright red-brown colouration of the colonized twigs (also illustrated in Kwaśna & Lakomy, 2011) and this is a character that might be related to some peculiarities of the host tree species. The closest European species to *T. fuckelii* is *T. pini* (Alb. & Schwein.) Höhn., which has considerable similarity in gross morphology. The former species is however easily distinguished upon microscopic observations by the strictly four-spored asci and ascospores, which are 7- to 11-septate and with tips narrowing in thread-like appendages (Reid & Cain, 1961; Minter, 1996). *Therrya pini* has 8-spored asci and very different, predominantly 3-septate (by exception 7-septate) ascospores, with abruptly tapered apices, not elongated in thread-like tips (Reid & Cain, 1961; Minter, 1996).

Fig. 3. The habitat of *Therrya fuckelii* on the slopes of Mount Vezhen in Konyavska Mts.

Therrya fuckelii seems to be a widespread species in Europe and has been previously reported to occur on several native species of two-needled pines, but most records are said to be in association with *P. sylvestris* L. (Minter, 1996). Previous findings of *T. fuckelii* with *P. heldreichii* (as *P. leucodermis* Antoine) are of unspecified origin (Minter, 1996) and may originate from Italy or Greece. Furthermore, the Bulgarian collection seems to be the second record of the species on the Balkan Peninsula after the mention from Greece (Minter, 1996).

Therrya fuckelii is the second rhytismataceous fungus to be reported on *P. heldreichii* in Bulgaria, the first being *Zeus olympius,* an uncommon species, as far as currently known strictly confined to this particular host (Minter, 1996; Stoykov et al., 2014). The two species share the presumed endophytic lifestyle, as well as localization of the ascomata, which are believed to appear after the death of host tissues (Minter, 1996). It was noted that *T. fuckelii* and another European species, *T. pini*, occur on dead fallen twigs (Minter, 1996). On the contrary, we observed ascomata of *T. fuckelii* only on branches and twigs still attached to the tree, including (albeit rarely) on not yet defoliated twigs. Fallen branches had merely remnants of ascomata, possibly developed in previous seasons and prior to branch shedding. Furthermore, such remnants were observed on still attached twigs. Ascomata or remnants of them were seen on nearly every tree in the plantation at Mount Viden. This seems to be consistent with the proposed role of the fungus in the trees self-pruning (Minter, 1996) and the high incidence of *T. fuckelii* in the studied area might be related to the high density of the pine plantation. Kwaśna & Lakomy (2011) studied the colonization of fungi of *P. sylvestris* following lightning damage. They found *T. fuckelii* to be the first colonizer and noted the co-occurrence of necrotic conductive tissues in the invaded branches. The colonization by *T. fuckelii* was followed by several other fungi with known pathogenicity. Similarly to our observations, Solheim et al. (2013) noted two cases of occurrence of the fungus on living branches and proposed that the members of this genus would have endophytic growth phase. This habit, along with the observed in the Bulgarian locality ascomata on non-defoliated, living twigs, suggests that the species discussed here may be an opportunistic pathogen, capable of developing ascomata on host individuals weakened by damage or physiological stress.

Acknowledgements. This study was supported by the Bulgarian Science Fund (Grant no. KP-06-N36/13/17.12.2019). The authors appreciate the useful suggestions of the anonymous reviewers.

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Received: 16.05.2022
Accepted: 14.08.2022

ECOLOGIA BALKANICA
2022, Vol. 14, Issue 2 December 2022 pp. 199-203

Short note

First Insights on the Herpetofauna of Ammouliani Island, Chalkidiki, Greece

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Abstract. Ammouliani is a small inhabited island of an area about 6.9 km^2, located in the Gulf of Agion Oros, Chalkidiki, Greece. Hitherto, there have been no herpetological surveys on the island and thus its herpetofaunal composition is still unknown. In the current short note, records of amphibians and reptiles from Ammouliani Island are presented for the first time. A two-day expedition in Spring 2022 resulted in one amphibian and nine reptile species records, all found to occur in relatively low densities. The small number of species of the island and the low densities are discussed.

Key words: Ammouliani, herpetofauna, reptiles, amphibians, fauna, Chalkidiki, Greece.

Introduction

Ammouliani Island is located in the northern part of the Gulf of Agion Oros, Chalkidiki, Greece (Fig. 1). The shortest distance between Ammouliani and the mainland is about 2.4 km (across from Tripiti Village). The island lies within an isobath shallower than 10 meters, within the continental shelf of Chalkidiki Peninsula. It has a total area of about 6.9 km^2, a highest elevation about 90 m.a.s.l. in its northern part and it hosts about 530 residents according to the 2011 census (Hellenic Statistical Authority, 2011). The prevalent type of vegetation of Ammouliani is maquis and phrygana, while agriculture is also noticeable, especially at the center of the island with many orchards, pastures and fields (Georgiev et al., 2018). A small brackish wetland is located in the center of the island and small seasonal streams are running through the island in several locations. On the southwestern margin of the main village there is also a small residual and degraded wetland that is drained through an artificial canal that empties in the village's port, being underground in most of its length. A few artificial ponds also occur scattered on the island. Ammouliani hosts a huge population of European herring gulls (*Larus argentatus*; Laridae) whose nests can be found almost everywhere in big numbers, even on the sides of the main asphalt roads.

To date, there are only a few publications on Ammouliani's fauna, focusing on Gastropoda (Georgiev et al., 2018) and Psocoptera (Insecta; Georgiev & Ivanova, 2019) and there are no published studies on the herpetofauna of the island, or other vertebrates in general. In this document, records of reptile and amphibian species from Ammouliani Island are presented for the first time.

Materials and Methods

A two-day herpetological survey on the island of Ammouliani was conducted between 14th and 16th May, 2022. Field work took place during both day and night, using several methodologies as described in Heyer et al. (1994), Krebs (2014) and Wilkinson (2015), in order to inspect the presence and activity of all diurnal, crepuscular and nocturnal herpetofaunal species. The survey regarding amphibians was conducted with the use of the transect method and road cruising, mostly during the night. Scanning for amphibians' eyeshine using strong head torch was also performed, a technique that allows amphibian detection from a long distance and in a wide range.

As for reptiles, both transect and point count methods were used, as well as road cruising and inspection for dead on road animals. Species identification was performed with the use of both visual (morphological characters) and acoustic (Anuran breeding calls) techniques.

Results and Discussion

In total, one amphibian and nine reptile species were recorded during the short expedition on the island (presented in Table 1). Photographic vouchers were deposited in Natural History Museum of Crete (NHMC), while tissue samples from dead on road animals were obtained. The exact locations are herein withheld to protect these populations and a grid is used instead (Fig. 1) as a reference to the wider areas where the animals were found.

Species Account

Amphibians

Green Toad (GR: Πρασινόφρυνος), *Bufotes viridis* (Laurenti, 1768); Bufonidae. Many adult individuals were found active by night near and in water bodies, as well as a large number of tadpoles in freshwater reservoirs, canals and brooks. The Green Toad was also the only amphibian of which breeding calls could be heard near all water bodies of the island.

Reptiles

Hermann's Tortoise (GR: Μεσογειακή Χελώνα), *Testudo hermanni* Gmelin, 1789; Testudinidae. Only one dead juvenile specimen and a single adult individual were found in the west and in the center of the island, respectively. It seems that the species is quite rare with a sparse population and therefore it is likely endangered on the island.

Fig. 1. Map of Ammouliani island, Chalkidiki, Greece. A grid of 500x500 m covers the map of the island to be used as a reference for the localities of the findings. The location of the island is shown within the red frame in the coloured map showing the Chalkidiki Peninsula. Both maps were generated using QGIS software, version 3.22.9 – "Białowieża".

Table 1. Full list of the amphibians and reptiles found on the island of Ammouliani in the two-day herpetological expedition. The number of individuals and the grid cells (in accordance with Figure 1) within which the species were observed, are also shown. The voucher numbers are assigned to photos that were taken during the survey and deposited in the Natural History Museum of Crete (Greece).

Family	Common name	Scientific name	Observed individuals	Grid cell	N.H.M.C. voucher number
Bufonidae	Green Toad	*Bufotes viridis*	>30 (adults)	C3, C4, E2, E3, F2, F3, G3	80.2.8.1130
Testudinidae	Hermann's Tortoise	*Testudo hermanni*	2	C4, F5	80.3.18.33
Geoemydidae	Balkan Terrapin	*Mauremys rivulata*	13	G2, G3	80.3.15.2003
Gekkonidae	Kotschy's Gecko	*Mediodactylus kotschyi*	11	F4, G2, G4, I6	80.3.85.2007
	Turkish Gecko	*Hemidactylus turcicus*	6	G2, G4	80.3.87.290
Lacertidae	Green Lizard	*Lacerta viridis*	5	F4, G3, G4	80.3.61.154 & 80.3.61.155
	Erhard's Wall Lizard	*Podarcis erhardii*	15	F2, F4, F5, G4	80.3.51.3061
Scincidae	Snake-eyed Skink	*Ablepharus kitaibelii*	1	C4	80.3.82.397
Colubridae	Large Whip Snake	*Dolichophis caspius*	1	E2	80.3.117.66
Viperidae	Nose-horned Viper	*Vipera ammodytes*	2	F3	80.3.40.56 & 80.3.40.57

Balkan Terrapin (GR: Γραμμωτή Νεροχελώνα), *Mauremys rivulata* (Valenciennes, 1833); Geoemydidae. The Balkan Terrapin was only recorded in an artificial canal near the main village (grid cell G3), but its presence in brooks and their estuaries is also very likely. Only a dozen of individuals could be seen in the canal and one dead on road specimen was found on the port's road.

Kotschy's Gecko (GR: Σαμιαμίδι/ Κυρτοδάχτυλος) *Mediodactylus kotschyi* (Steindachner, 1870); Gekkonidae. In total, 11 individuals were observed active both day and night at several spots, often sharing the same microhabitats with Turkish Geckos (see below). By day Kotschy's Geckos could be seen basking on rocks and ruins and their calls could be heard inside abandoned houses (grid cells F4, G2, G4 and I6).

Turkish Gecko (GR: Σαμιαμίδι/ Μολυντήρι), *Hemidactylus turcicus* (Linnaeus, 1758); Gekkonidae. *H. turcicus* was found foraging on walls and ruins inside the main village and in an abandoned house in the center of the island (grid cells G2 and G4), co-existing with Kotschy's Gecko.

Green Lizard (GR: Πρασινόσαυρα), *Lacerta viridis* (Laurenti, 1768); Lacertidae. Four Green Lizards were found in two spots in the center of the island (three adults and one juvenile; F4, G3 and G4) and one adult male right outside of the main village.

Erhard's Wall Lizard (GR: Αιγαιόσαυρα), *Podarcis erhardii* (Bedriaga, 1886); Lacertidae. The Erhard's Wall Lizard was observed basking and hunting on cut slopes of dirt roads, ruins and house fences (F2, F4, F5 and G4). 15 adult individuals were spotted in total during the two-day survey.

Snake-eyed Skink (GR: Αβλέφαρος), *Ablepharus kitaibelii* (Bibron & Bory de Saint-Vincent, 1833); Scincidae. Only one adult individual was spotted moving in low vegetation Near Karagatsia (grid cell C4) and it was captured, photographed and released shortly after. Snout to vent length was at 5 cm and the skink had small but visible ear openings.

Large Whip Snake (GR: Έφιος), *Dolichophis caspius* (Gmelin, 1789); Colubridae. A single adult individual was spotted crossing the road while road cruising in the north of the island (grid cell E2), showing the typical color phenotype as those from nearby mainland and central Makedonia in general. No photographs or other evidence of the species' occurrence could be obtained during the survey, except for a photograph of a dead young specimen that was taken by an Ammouliani resident, Mr. Ilias Rodokalakis (pers. com.). The photograph was obtained and deposited in NHMC (Photographic voucher number 80.3.117.66). The dead snake of the photograph was also found in the north part of the island.

Nose-horned Viper (GR: Οχιά), *Vipera ammodytes* (Linnaeus, 1758); Viperidae. Two adult individuals were found, a dead on road male and a live female, both very close to the main village (F3) and to each other (photographic vouchers 80.3.40.56 and 80.3.40.57, respectively). The male specimen had a total length (TL) of 44.5 cm, 23 rows of dorsal scales across the middle of the body, 144 ventral scales and 34 pairs of subcaudal scales. The female (Fig. 2D) had a TL of 55.5 cm, 23 rows of dorsal scales across the middle of the body and 144 ventral scales. The very end of the tail tip was missing and the remaining subcaudal scales were arranged in 15 pairs.

Fig. 2. Four out of the ten herpetofauna species that were found on the island of Ammouliani, indicatively. A) A male Green Toad, *Bufotes viridis*, B) a male Erhard's Wall Lizard, *Podarcis erhardii,* C) a male Green Lizard, *Lacerta viridis*, and D) a female Nose-horned Viper, *Vipera ammodytes*. Photos are deposited in the Natural History Museum of Crete (for voucher numbers see Table 1).

Other noteworthy animal records made on the island were that of the Northern white-breasted hedgehog, *Erinaceus roumanicus* (Erinaceidae) and scorpions of the genus *Euscorpius* (Euscorpiidae).

Judging by the habitats and the herpetofaunal composition of nearby areas, other amphibians that could possibly occur on the island are *Pelophylax* spp. (Ranidae), *Rana dalmatina* (Ranidae), *Hyla arborea* (Hylidae), *Bufo bufo* (Bufonidae), *Bombina variegata* (Bombinatoridae) and *Lissotriton graecus* (Salamandridae). However, no adults, juveniles, eggs or tadpoles/larvae from other amphibian species were recorded on the island. The fact that *Pelophylax* and *Hyla* frogs' breeding calls are very distinguishable and a lot louder than those of Green Toads, in conjunction with the season and the ideal weather conditions during which the survey took place, indicates that those species are probably absent from Ammouliani island, as they were not seen, nor heard. On the other hand, the presence of the rest of the species cannot be excluded with confidence at the moment.

In general, densities of all species found on the island seem to be very low, despite that the habitats appear to be in good condition with only a few cases of degradation. One possible explanation to this could be the really high numbers of Herring gulls as the whole island constitutes a large and remarkably dense gull colony. European herring gulls (*L. argentatus*) are top opportunistic predators, not only of marine invertebrates and fishes, but also of birds, small mammals, amphibians and reptiles (Martín & López, 1990; Ewins et al., 1994; Prakas et al., 2020). The large number of Herring gulls, in opposition to the low density of amphibians and reptiles on the island, could indicate a high level of predation pressure on herpetofaunal species, however, further research is needed to verify this hypothesis.

This is the first time a herpetological survey is conducted on the island of Ammouliani, offering the first insights on its herpetofaunal composition, adding new insular populations to the currently known species distribution. These findings show once again that there are areas in Greece that are still underexplored (or even completely unexplored) regarding their herpetofauna (e.g. Kalaentzis et al., 2018; Strachinis et al., 2019; Strachinis, 2021), or other important taxa. The present study is preliminary; more thorough faunistic surveys on this underexplored island may reveal the presence of more species and their so far unknown conservation status.

Acknowledgments

I would like to thank Mr. Ilias Rodokalakis for his photograph of a *D. caspius* from Ammouliani. I am also grateful to Dr. Petros Lymberakis (NHMC) for his assistance and fast communication and to Mr. Matt Wilson for his linguistic review of the manuscript.

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Received: 30.09.2022
Accepted: 21.11.2022

ECOLOGIA BALKANICA

2022, Vol. 14, Issue 2 December 2022 pp. 205-208

Short note

On the Circadian Activity of Red Fox (Vulpes vulpes) and Stone Marten (Martes foina) in Agricultural Landscape of Northwestern Bulgaria During Spring-Summer Period

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Abstract. A total of 5 camera traps were set in protected area "Zlatiyata" to record the circadian activity of the Red Fox (*Vulpes vulpes*) and the Stone Marten (*Martes foina*) in agricultural habitats of Northwestern Bulgaria. During spring-summer period the Red Fox exhibited predominantly nocturnal bimodal activity with first peak during twilight (18:00-20:00) and second higher peak after midnight (00:00-02:00). Only one peak (22:00-00:00) was registered in the Stone Marten's activity.

Key words: activity, camera trapping, agricultural regions, Bulgaria.

Introduction

Among the threats to mesopredators in the European lowlands are habitat fragmentation and agricultural expansion (Faaborg et al., 1993; Rodríguez-Refojos & Zuberogoitia, 2011; Vitousek et al., 1997). The animals have to change their activity in order to adapt to the growing human influence on wildlife (Gaynor et al., 2018). Few studies on the circadian activity of Red Fox (*Vulpes vulpes*) and Stone Marten (*Martes foina*) have been conducted in agricultural (Dudin & Georgiev, 2015; Dudin, 2017) and in mountainous (Petrov et al., 2016; Tsunoda et al., 2020) regions of Bulgaria. Our study aimed to provide new data on the behavioral ecology of both species in agricultural landscape in Northwestern part of the country.

Material and Methods

The study was conducted in protected area "Zlatiyata" located in Northwestern Bulgaria (for map and more information on the study area, please see Petrov et al. (2022)). The area is a plateau with bushy slopes and intensive agriculture on the surface, ensuring an open habitat for the wild animals.

The study was conducted from 1-Mar-22 to 31-Aug-22 A total of 5 camera traps (BolyGuard BG590-K2) were set up on threes along the predators' trails. No baits or lures were used. The devices were angled at 45-90 degrees to the trails. The height the devices were mounted on the trees was tailored to the size of the studied species, the slope of the terrain and the available vegetation. The cameras were set to take 3 consecutive photos with 5 minutes delay. The images of a particular species separated by thirty-minute interval were treated as an independent observation (one event).

Results and Discussion

The Red Fox (Fig. 1- left) demonstrated bimodal nocturnal activity and the Stone

Marten (Fig. 1 - right) - unimodal nocturnal activity.

The smaller peak in the activity of *V. vulpes* was around twilight 18:00-20:00 and the second was after midnight (00:00-02:00). *M. foina* exhibited one peak before midnight 22:00-00:00 during the study period (Fig. 2).

Fig. 2. Red Fox (*Vulpes vulpes*) - in the left and Stone Marten (*Martes foina*) - in the right, photographed in protected area "Zlatiyata", Northwestern Bulgaria.

Compared to the previous study (Petrov et al., 2022) the Red Fox has changed the peaks of its activity. During the spring-summer period, the peaks of the bimodal fox activity shifted approximately four hours earlier than those during the autumn-winter period (Fig. 3). The Stone Marten maintained the same peak before midnight in spring-summer period as in the autumn-winter period revealed by Petrov et al. (2022), but no second peak was observed (Fig. 4).

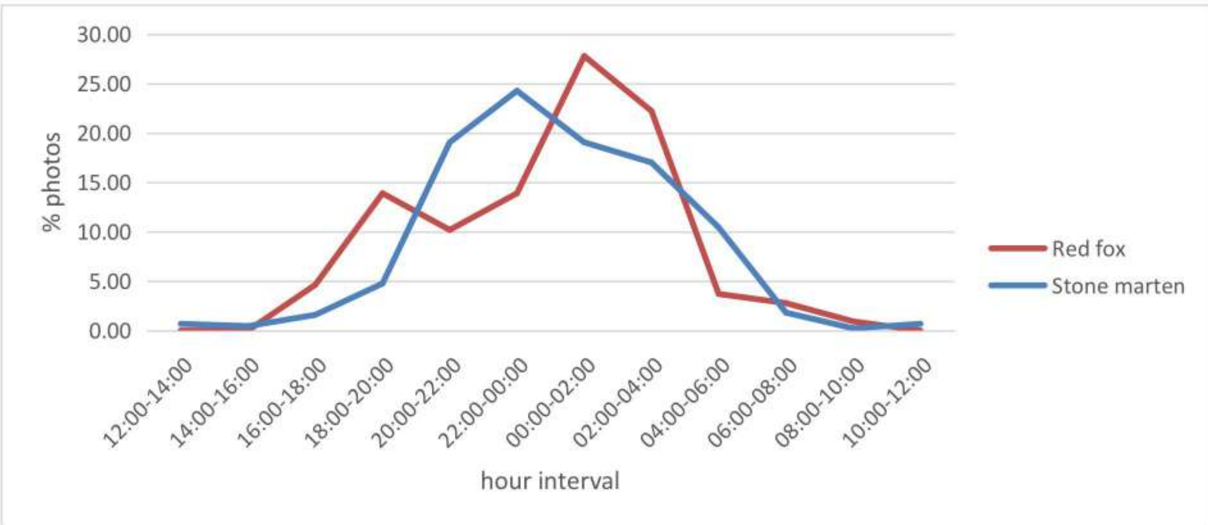

Fig. 2. Activity of the Red Fox (*V. vulpes*) and the Stone Marten (*M. foina*) during spring-summer period in protected area "Zlatiyata", Northwestern Bulgaria.

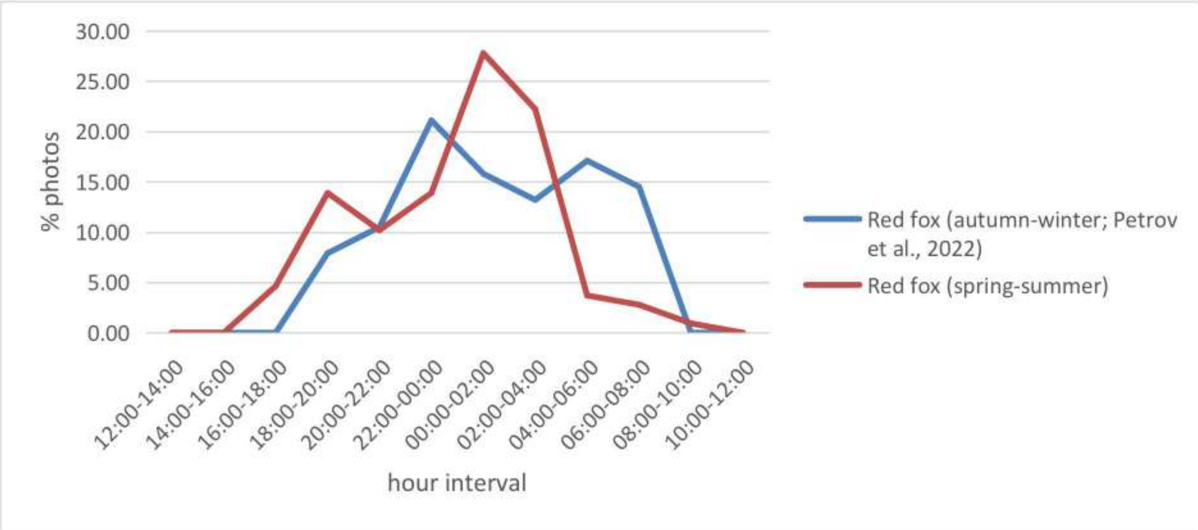

Fig. 3. Season comparison of the activity of the Red Fox (*V. vulpes*) in protected area"Zlatiyata", Northwestern Bulgaria.

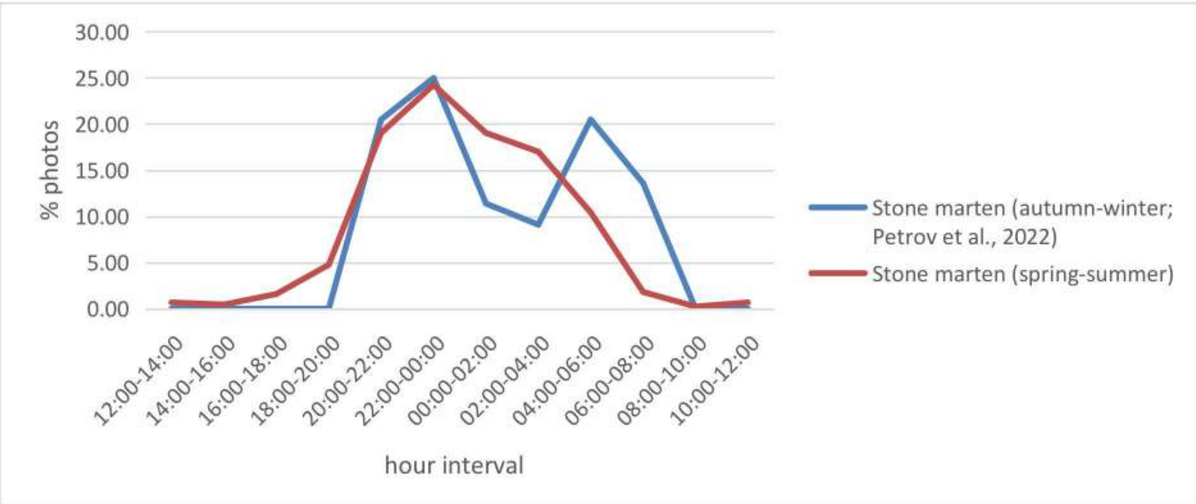

Fig. 4. Season comparison of the activity of the Stone Marten (*M. foina*) in protected area "Zlatiyata", Northwestern Bulgaria.

Compared to the mountain regions (Petrov et al., 2016; Tsunoda et al., 2020) both studied predators in agricultural habitats exhibited similar predominantly nocturnal activity during spring-summer period. While the activity of *V. vulpes* peaked twice in the mentioned habitats, *M. foina* demonstrated unimodal activity in agricultural region during investigated period, different to the bimodal in the mountains.

Acknowledgements. The study is financed by project MU21-BF-016 "Seasonal and daily activity of the Red fox (*Vulpes vulpes* L.) and the Stone marten (*Martes foina* Erxl.) in the protected area "Maritsa - Parvomay" (Upper Thracian lowland) and protected area "Zlatiyata" (Northwest Danube Plain)" under the program "Young Scientists" of the University of Plovdiv "Paisii Hilendarski".

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Received: 03.11.2022
Accepted: 24.11.2022

Reviewers Acknowledgment

Peer reviewers are key to advancing scholarship and contributing to the quality of scholarly journals. We would like to thank the following reviewers who have taken part in the peer-review process for vol. 14, iss. 2, 2022 of Ecologia Balkanica.

Anton Shiryaev - Institute of Plant & Animal Ecology (IPAE); Ural Branch of the Russian Academy of Sciences (UrB RAS) (RUSSIA)
Boris Assyov - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (BULGARIA)
Borislav Naumov - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (BULGARIA)
Esra Akat Comden - Ege University, Faculty of Science, Department of Biology, İzmir (TURKEY)
Diyan Georgiev - Trakia University, Stara Zagora (BULGARIA)
Gana Gecheva - University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and EC (BULGARIA)
Iliana Velcheva - University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and EC (BULGARIA)
Ivan Minchev - Ss. Cyril and Methodius University in Skopje, Faculty of Forest Science, Skopje (NORTH MACEDONIA)
Ivelin Mollov - University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and EC (BULGARIA)
Kerim Çiçek - Ege University, Faculty of Science, Biology Department, Bornova/Izmir (TURKEY)
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Mariyana Lyubenova - Sofia University "St. Kliment Ohridski", Faculty of Biology, Department of Ecology and Environmental Protection, Sofia (BULGARIA)
Ognyan Todorov - Regional Natural History Museum - Plovdiv (BULGARIA)
Olga Shiryaeva - Vegetation & Mycobiota Biodiversity Department, Institute of Plant & Animal Ecology Ural Branch of the Russian Academy of Sciences, Ekaterinburg (RUSSIA)
Petlovanyi Mykhailo - Dnipro University of Technology, Department of Underground Mining, Dnipro (UKRAINE)
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Tatiana Ivanova - Agricultural institute of Shumen (BULGARIA)
Vladimir Ilinkin - Agricultural Academy, Institute of Soil Science (BULGARIA)
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Manuscript preparation

Language
The manuscripts must be written in English. *Contributors who are not native English speakers are strongly advised to ensure that a colleague fluent in the English language*, if none of the authors is so, *has reviewed their manuscript*. Spelling should be British or American English and should be consistent throughout the text. All abbreviations and acronyms should be defined at first mention. To facilitate reader comprehension, abbreviations should be used sparingly.

Technical information
Submissions must be in **electronic version only**, as well as the original figures and tables, implemented in the text. Figures must be sent as separate files as well (see more information below). The manuscript text should be **prepared in MS Office format (.docx)**, justified, font size 11, font "Book Antiqua", without footnotes, column or page breaks, single spaced (about 60 lines per page), on A4 (210 x 297 mm) paper, with margins of exactly 2.5 cm on each side. Pages and lines should not be numbered!

The manuscripts should conform to the following format:
Title: Provide a title that is concise, but also an informative synthesis of the study. Where appropriate, include family or higher taxon.
Author(s): Full first name(s), middle initials and surname(s) in ***bold italic.*** The corresponding author should be marked with the *-symbol. **Please provide the ORCID IDs for ALL authors and co-authors.**
Affiliation(s) with postal address: As complete as possible. Affiliation should be given in the following order – University (Institute), Faculty, Department, postal address, city, COUNTRY (in capital letters). E-mail address is given only for the corresponding author!
Abstract: Maximum length 250 words. The abstract should state briefly the objective of the research, the primary results and major conclusions, with no description of methods, discussions, references and abbreviations.
Key words: Usually 3–10 words suitable for information-retrieval system.

The standard order of sections should be: Abstract, Key words, Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusions (optional), Acknowledgments (optional) and References.

The *Introduction* has to explain the actuality of the researched problem and give the aim of the study.
Materials and Methods have to provide sufficient information to permit repetition of the experiment and/or fieldwork. The technical description of study methods should be given only if such methods are new; otherwise a short presentation is enough.
The *Results* section must be a concise presentation of the finding of the study. **<u>Presentation of the same information as text and/or figure and/or table is forbidden!</u>**
The *Discussion* section should be separated from the results section at full-length papers and should deal with the significance of the results and their relationship to the aims of the paper. Also include how the findings of the paper will change or influence the state of our knowledge about the topic at hand. In separate cases a joint section "Results and Discussion" is allowed, but not preferable.
The *Conclusions* should shortly describe the main contributions and recommendations of the study without including citations and statistics.
In the *Acknowledgments* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

Short Notes (generally less than four-five manuscript pages) should be produced as continuous text, preceded by an abstract of no more than 150 words.

Tables: The tables must not repeat information already presented in the figures or in the text. Each table must be self-explanatory and as simple as possible. Avoid large landscape oriented tables! Tables must be numbered consecutively. **<u>They should be placed within the text at the desired position by the author(s).</u>** An explanatory caption, located on the top of the table, should be provided.

Example:

Table 1. Shannon-Wiener indexes in the burned ($\mathbf{H}_{\text{burned}}$) and control ($\mathbf{H}_{\text{control}}$) territory for the total duration of the study (2004–2006).

Figures: They must not repeat information already presented in the tables or in the text. Lines and letters in figures must be able to be enlarged or reduced without reduction in quality. They should conform to the size of the type area (up to 16 × 24 cm) which is the limit for all illustrations. Magnification should be shown by scale bars. All illustrations must be sharp, of high quality with at least 300 dpi. The following formats are acceptable: JPEG, PNG, TIFF, EPS. The figures must be numbered consecutively and should be provided with an explanatory legend below them. *When the figures present maps of the studied area, we recommend using some kind of*

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Example:

Fig. 1. Indicative map of the study area.

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Citations and references
From January 2020, Ecologia Balkanica adopts the APA (American Psychological Association) bibliographic style (7th edition – 2020).

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In-text references must be included following the use of a quote or paraphrase taken from another piece of work. **Direct copy-paste from another source is not acceptable!** Submitted manuscripts will be pre-checked for plagiarism and auto-plagiarism. In-text citations are citations within the main body of the text and refer to a direct quote or paraphrase. They correspond to a reference in the main reference list. These citations include the surname of the author and date of publication only. For example: Smith (2017) states… Or …(Smith, 2017). In case of two authors: the surname of both authors is stated with an ampersand between. For example: Smith & Smith (2017) state… Or …(Smith & Smith, 2017). In case of three or more authors add „et al." after the first author's surname (*et alii*, from Latin means „and others"): Smith et al. (2017) state… Or …(Smith et al., 2017).

If the author of the cited source is unknown, the first few words of the reference should be used. This is usually the title of the source. If this is the title of a book, periodical, brochure or report, is should be italicised. For example: (*A guide to citation*, 2017). If this is the title of an article, chapter or web page, it should be in quotation marks. For example: ("APA Citation", 2017).

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EC. (2010). Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. *Official Journal of the European Union, L276,* 33-79. Retrieved from https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

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Official laws, orders etc. should be cited as follows (see examples).
Biological Diversity Act. (2002). *State Gazzette, 77*, 09.08.2002. (In Bulgarian).
Medicinal Plants Act. (2000). *State Gazette, 29,* 07.04.2000. (In Bulgarian).
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Korovin, V. (2004). Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural. Ekaterinburg, Russia: Published by Ural University. (In Russian).

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