ECOLOGIA BALKANICA

International Scientific Research Journal of Ecology

Volume 9, Issue 1
June 2017

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

UNIVERSITY OF PLOVDIV PUBLISHING HOUSE

International Standard Serial Number
Online ISSN 1313-9940; Print ISSN 1314-0213 (from 2009-1015)

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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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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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A Floristic Study of Hamun Lake Basin, South East of Iran

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Abstract. Lake Hamun is the largest freshwater resource in Iran with area of about 3820 km^2. The present study aims to evaluate the floristic elements of the studied site. Plant samples were gathered from nature, from March to July at the growing season. Life form and chorotype of plants in Lake Hamun basin were investigated. Totally 128 plant species belonging to 80 genera and 30 families were identified. Families as *Poaceae*, *Amaranthaceae* and *Fabaceae* were the most dominant and frequent families. Considering biological types revealed that the most frequent forms were therophytes (61%) and hemicryptophytes (17%). Floristic elements of the area were mainly Irano-touranian mixed with Saharo-Arabian and Sindu-Sudanian types, although multi- and bi- regional elements were also frequent. As the lake has recently become an international conserved area, the complete biological and ecological study of the site is a necessity.

Key words: Hamun Lake, Sistan and Baluchestan, Iran.

Introduction

Iran is a country in the south-west of Asia with 1.648 million km^2 area. Sistan and Baluchestan province is located in the south-east of the country. Sistan receives the discharge of the Hamun River in the lower Hamun Basin, and was often described in different centuries as one of the harshest and bare deserts of the world. This large desert basin is mainly known for windstorms, extreme floods, and droughts. The closed basin receives the water of the Hirmand River. This river is the only major river in western Asia between the Tigris-Euphrates and Indus Rivers (SHIRDELI, 2014).

Sistan, which was historically known as the breadbasket of western Asia, is now covered with large sand dunes. The Hamun Basin with area of approximately 310,000 km^2 is limited at the East by Iranian highlands, at the North by the southern Hindu Kush ranges, on the west by the East Iranian ranges; and on the South by mountain ranges in Baluchestan province of Pakistan (WHITNEY, 2006).

This biosphere reservoir includes terrestrial and wetland ecosystems, with desert and semi-desert areas, as well as marshlands and watersheds of the lake. The Hamun have completely dried up at least three times during the 20th century (SHIRDELI, 2014). Floristic elements of the area have not been studied yet, despite some studies with limited scopes (IRANMANESH *et al.*, 2010; ALLAHDOU *et al.*, 2012; JABBARY *et al.*, 2013). A complete botanical study of the area is the first step in choosing conservation strategies.

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Material and Methods

The studied area is located in the south-east of Iran in Sistan and Baluchestan province between 61º, 45′ to 61º, 09′ East longitude and 31º, 17′ to 30º, 47′ North latitude with altitude of 460-590 meters a.s.l. (Fig. 1). The lake basin was considered and about 7000 km² was sampled.

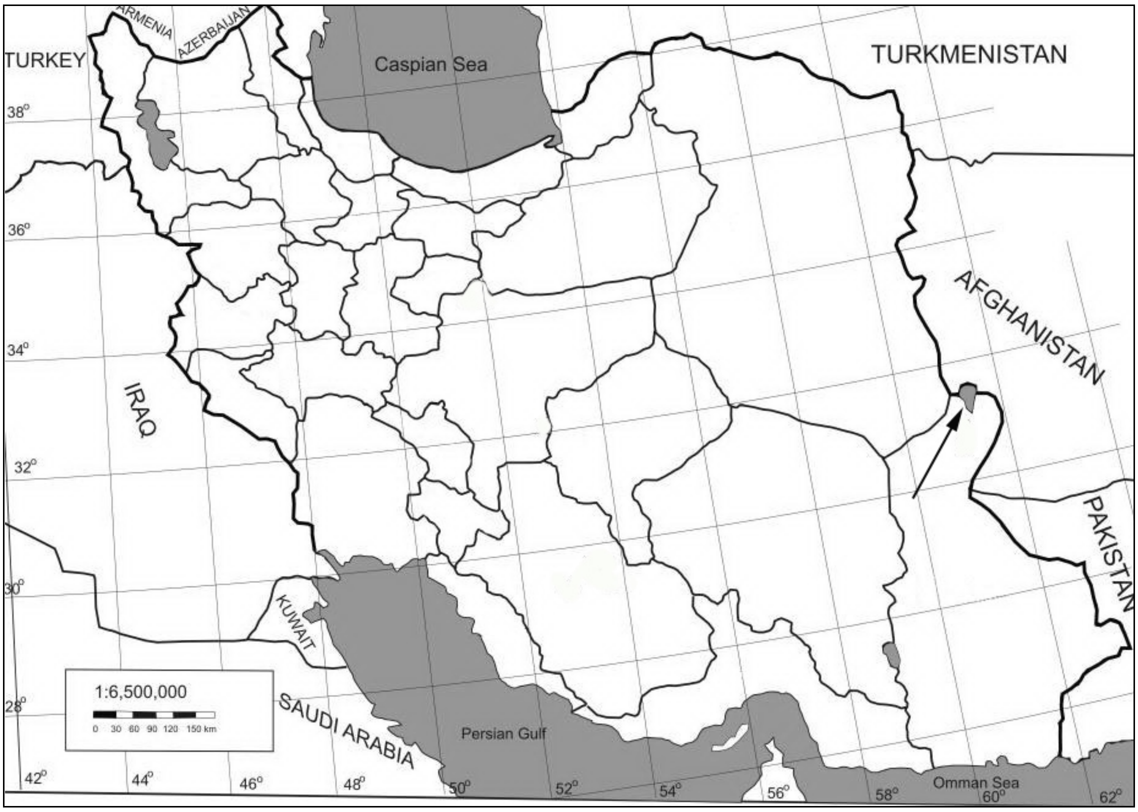

Fig. 1. Geographic map of the studied area.

As the studied area has a desert climate, the rainfall during the year is limited. The average annual temperature is 21.7 °C with average annual rainfall of 53 mm. July with temperatures of 34.4 °C and January with temperatures of 7.9 °C are the warmest and coldest months of the year respectively.

Plant specimens were collected between March to July at growth seasons during 2015-2016 and prepared according to standard herbarium techniques. All taxa were determined using Flora of Iran (ASSADI, 1988-2002), Flora Iranica (RECHINGER, 1963-2010), Flora of Turkey and the East Aegean Islands (DAVIS, 1965-1988), Flora of the USSR (KOMAROV, 1968–2001) and Flora of Pakistan (ALI & NASIR, 1990-1992; ALI & QAISER, 1992-2007). All vouchers were deposited at the herbarium of Alzahra University (ALUH), Tehran, Iran. Geographical data were collected by use of GPS and data of the nearby meteorological station. Ombrothermic diagram was drawn based on the meteorological data from IRIMO (2016) for 14 years (2000 to 2014) (Fig. 2).

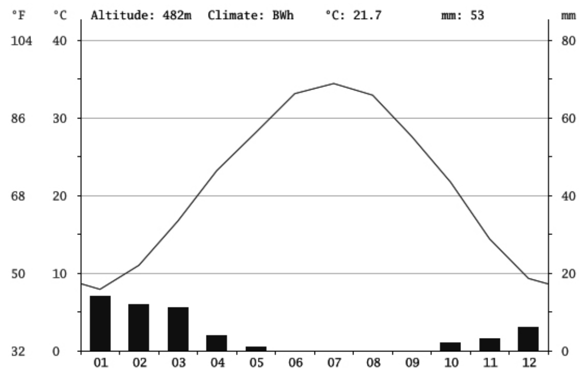

Fig. 2. The ombrothermic diagram of the Hamun Lake.

Life forms were recognized using Raunkiaer's classification (RAUNKIAER, 1934) comprising phanerophytes, geophytes, cryptophytes, hemi-cryptophytes, therophytes and parasites. Chorology and phytogeographic areas of each taxon were determined using ZOHARY (1973), different floras and available literatures.

Results

In the studied area 128 taxa of 80 genera and 30 families were identified. Mainly plants were angiosperms elements except for *Ephedra* (Gnetophytes). Totally 42 samples were monocots and 86 ones were dicots. The largest family in the area was *Poaceae* with 39 species. Beside grass family, *Amaranthaceae* (12 species), *Asteraceae* (10 species) and *Fabaceae* (10 species) were frequent (Fig. 3 and Table 1).

Table 1. Checklist of taxa in the studied area. Abbreviations are based on Fig. 5-6, * - Medicinal plant based on MOZAFFARIAN (2015).

Family	Taxon	Biol. form	Phytochoria	Voucher no.
Amaranthaceae	**Amaranthus viridis* L.	Th	Cosm.	ALH- ha100
	**Amaranthus caudatus* L.	Th	Cosm.	ALH- ha101
	Amaranthus graecizans subsp. *graecizans* L.	Th	ES-M-SS	ALH- ha102
	**Atriplex halimus* L.	Ch	M-SA	ALH- ha103
	**Chenopodium album* L.	Th	IT	ALH- ha104
	Chenopodium badachschanicum Tzvelev.	Th	IT	ALH- ha105
	Chenopodium glaucum L.	Th	IT-ES	ALH- ha106
	**Chenopodium murale* L.	Th	Cosm.	ALH- ha107
	Chenopodium vulvaria L.	Th	IT-M	ALH- ha108
	Salsola aperta Paulsen.	Th	IT	ALH- ha109
	Salsola turkestanica Litv.	Th	IT	ALH- ha110
	**Suaeda fruticosa* Forssk. Ex J.F.Gmel.	Ch	IT	ALH- ha111
Asclepidiaceae	*Cynanchum acutum* L.	Ch	SA	ALH- ha112
Asteraceae	*Carduus hamulosus* Ehrh.	Th	EU	ALH- ha113
	**Cichorium intybus* L.	He	IT-ES-M	ALH- ha114
	**Carthamus oxycanthus* M. Bieb.	He	IT-SS	ALH- ha115
	Centaurea bruguierana subsp. *belangeriana* (DC.) Bornm.	He	IT-OS	ALH- ha116
	**Cirsium vulgare* (Savi) Ten.	He	Pl	ALH- ha117
	Lactuca scariola L.	He	IT-ES-M	ALH- ha118
	**Launaea mucronata* (Forssk.) Muschl.	Th	IT	ALH- ha119
	**Sonchus oleraceus* (L.) L.	Th	IT-M	ALH- ha120
	**Tragopogon graminifolius* DC.	Th	IT	ALH- ha121
	**Xanthium strumarium* L.	Th	IT-M	ALH- ha122
Boraginaceae	*Gastrocotyle hispida* (Forssk.) Bunge	G	IT-SA	ALH- ha123
	Heliotropium lasiocarpum Fisch. & C.A.Mey.	Ch	SA	ALH- ha124
Brassicaceae	*Brassica elongata* Ehrh.	He	IT-M	ALH- ha125
	**Cardaria draba* (L.) Desv.	He	IT	ALH- ha126
	**Descurainia sophia* (L.) Webb ex Prantl	Th	IT-ES-M	ALH- ha127
	**Erysimum repandum* L.	Th	IT-ES-M	ALH- ha128
	Lepidium aucheri Boiss.	He	IT	ALH- ha129
	**Malcolmia africana* var. *africana* (L.) R.Br.	Th	IT- M- SS	ALH- ha130
Capparaceae	**Capparis parviflora* Boiss.	Ch	IT	ALH- ha131

Caryophyllaceae	**Sagina saginoides* (L.) H.Karast.	Th	Cosm.	ALH- ha132
Convolvulaceae	*Convolvulus pentapetaloides* L.	Th	M	ALH- ha133
	**Cressa cretica* L.	He	IT	ALH- ha134
	Cuscuta campestris Yunck.	Parasite	Cosm.	ALH- ha135
Cucurbitaceae	**Citrullus colocynthis* (L.) Schrad.	He	M-SA	ALH- ha136
Cyperaceae	*Cyperus glaber* L.	G	IT-M	ALH- ha137
	Cyperus laevigatus L.	G	Cosm.	ALH- ha138
	Schoenus nigricans L.	G	Sub Cosm.	ALH- ha139
Dipsacaceae	*Pterocephalus brevis* Coult.	Th	IT- ES-M	ALH- ha140
Eleagnaceae	**Elaeagnus ungustifolia* L.	Ph	ES	ALH- ha141
Ephedraceae	**Ephedra strobilacea* Bunge	Ch	IT	ALH- ha142
Euphorbiaceae	**Chrozophora obliqua* (Vahl) A.Juss. ex Spreng.	Th	IT-M	ALH- ha143
	**Chrozophora hierosolymitana* Spreng.	Th	IT	ALH- ha144
	Euphorbia azerbajdzhanica Bordz.	Th	IT	ALH- ha145
	Euphorbia densa Schrenk	Th	IT- SS	ALH- ha146
	Euphorbia humifusa Willd.	Th	IT	ALH- ha147
	Euphorbia inderiensis Less. Ex Kar. & Kir.	Th	IT	ALH- ha148
	Euphorbia petiolata Banks & Sol.	Th	IT-M	ALH- ha149
	Euphorbia turcomanica Boiss.	Th	IT	ALH- ha150
Fabaceae	**Medicago sativa* L.	He	IT	ALH- ha151
	Melilotus dentatus (Waldsst. & Kit.) Pers	Th	M	ALH- ha152
	Melilotus indicus (L.) All.	Th	IT-SA	ALH- ha153
	**Melilotus officinalis* (L.) Pall.	Th	IT-ES-M	ALH- ha154
	**Pisum sativum* L.	Th	IT	ALH- ha155
	**Prosopis farcta* (Banks & Sol.) J.G.Macbor.	Ph	IT- SS	ALH- ha156
	**Trigonella monantha* C.A.Mey.	Th	IT-M	ALH- ha157
	**Vicia ervilia* (L.) Willd.	Th	M	ALH- ha158
	Vicia monantha Retz.	Th	IT	ALH- ha159
	Vicia peregrina L.	Th	IT-M	ALH- ha160
Malvaceae	**Malva pusilla* Sm.	Ch	IT-M	ALH- ha161
	**Malva verticillata* L.	Ch	IT-M	ALH- ha162
Moraceae	**Morus nigra* L.	Ph	IT-ES-M	ALH- ha163
Oleaceae	*Olea aucheri* A.Chev. ex Ehrend.	Ph	SU	ALH- ha164
Orobanchaceae	*Orobanche ramosa* L.	Parasite	IT- ES	ALH- ha165
Papaveraceae	*Papaver hybridum* L.	Th	IT-M	ALH- ha166
Plantaginaceae	**Plantago lanceolata* L.	He	IT-M	ALH- ha167
Poaceae	**Aeluropus lagopoides* (L.)Thwaites	He	IT	ALH- ha168
	Aeluropus littolaris (Gouan)Parl.	He	IT- M-Ss	ALH- ha169
	Aeluropus pungens (M.Bieb.)K.Koch	He	IT-M	ALH- ha170
	**Avena barbata* Pott ex Link	Th	M	ALH- ha171
	**Avena fatua* var. *fatua* L.	Th	Pl	ALH- ha172
	Avena ludoviciana Durieu	Th	IT-M	ALH- ha173
	Bromus arvensis L.	Th	IT	ALH- ha174
	Bromus danthoniae Trin.	Th	IT-M	ALH- ha175
	Bromus fasciculatus C.Presl	Th	M	ALH- ha176

	Bromus japonicus var. *velutinus* (W.D.J.Koch) Asch. & Graebn	Th	Pl	ALH- ha177
	Bromus madritensis L.	Th	IT-M	ALH- ha178
	Bromus tectorum L.	Th	IT- M –SS	ALH- ha179
	**Cynodon dactylon* (L.) Pers.	He	Cosm.	ALH- ha180
	Echinochloa colona (L.) Link	Th	IT-M	ALH- ha181
	**Echinochloa crus-galli* (L.) P.Beauv.	Th	IT	ALH- ha182
	Echinochloa stagnina (Retz.) P.Beauv.	Th	SS	ALH- ha183
	Eremopyrum confusum Melderis	Th	IT	ALH- ha184
	Henrardia pubescens (Bertol.) C.E.Hubb.	Th	SS	ALH- ha185
	Hordeum marinum Huds.	Th	M	ALH- ha186
	Hyparrhenia hirta (L.) Stapf	Ch- G- He	SA-M- IT	ALH- ha187
	Imperata cylindrica (L.) Raeusch.	Th	IT-SS	ALH- ha188
	Piptatherum barbellatum Mez	Th	IT	ALH- ha189
	**Piptatherum gracile* Mez	Th	IT	ALH- ha190
	Piptatherum microcarpum (Pilg.) Tzvelev	Th	IT	ALH- ha191
	Piptatherum molinioides Boiss.	Th	IT	ALH- ha192
	Phalaris brachystachys Link	Th	M	ALH- ha193
	Phalaris minor Retz.	Th	IT-M	ALH- ha194
	Phalaris paradoxa var. *paradoxa* L.	Th	IT	ALH- ha195
	Phalaris paradoxa var. *praemorsa* (Lam.) Coss. & Durieu	Th	IT	ALH- ha196
	Phleum pratense L.	Th	IT-ES-M	ALH- ha197
	**Phragmites australis* (Cav.) Trin. ex Steud.	He	IT	ALH- ha198
	Poa annua L.	Th	Cosm.	ALH- ha199
	**Poa bulbosa* L.	Th	IT-M	ALH- ha200
	Polypogon monspeliensis (L.) Desf.	Th	Pl	ALH- ha201
	Rostraria phleoides (Desf.) Holub	Th	IT-M	ALH- ha202
	**Saccharum griffithii* Munro ex Aitch.	He	IT	ALH- ha203
	Setaria glauca (L.) P.Beauv.	Th	IT-SS	ALH- ha204
	**Setaria viridis* (L.) P.Beauv	Th	IT- ES-M	ALH- ha205
	Vulpia ciliata Dumort.	Th	IT-M	ALH- ha206
Polygonaceae	**Persicaria minor* (Huds.) Opiz	Th	IT- ES	ALH- ha207
	Polygonum argyrocoleon Steud. ex Kunze	Th	IT	ALH- ha208
	**Polygonum aviculare* L.	Th	Cosm.	ALH- ha209
	Polygonum olivascens Rech. F. & Schiman-Czeika	Th	IT	ALH- ha210
	**Polygonum patulum* M.Bieb.	Th	IT	ALH- ha211
	Rumex angulatus Rech. F.	He	IT	ALH- ha212
	**Rumex dentatus* subsp. *halascyi* (Rech.) Rech.f.	He	M-SS	ALH- ha213
	**Rumex dentatus* subsp. *Klotzschianus* (Meisn.) Rech.f.	He	M-SS	ALH- ha214
	**Rumex obtusifolius* L.	He	IT-ES	ALH- ha215
Portulacaceae	**Portulaca oleracea* L.	Th	Cosm.	ALH- ha216
Rubiaceae	*Galium hirtiflorum* Req. ex DC.	Th	IT	ALH- ha217
Salicaceae	**Populus euphratica* Oliv.	Ph	IT	ALH- ha218
Solanaceae	**Solanum nigrum* L.	Th	IT-M	ALH- ha219

Tamaricaceae	**Tamarix aphylla* (L.)H.Karst.	Ph	SU	ALH- ha220
	Tamarix karakalensis Freyn	Ph	IT	ALH- ha221
	Tamarix kotschyi Bunge	Ph	IT-M	ALH- ha222
	**Tamarix stricta* Boiss.	Ph	IT	ALH- ha223
	Tamarix tetragyna Ehrenb.	Ph	IT-M	ALH- ha224
Vitaceae	**Vitis vinifera* L.	Ph	IT	ALH- ha225
Zygophyllaceae	**Peganum harmala* L.	Ch	IT- M- SS	ALH- ha226
	**Tribulus terrestris* L.	Th	IT-M	ALH- ha227

The Largest genera in the area were *Bromus* and *Euphorbia* each with six species and *Chenopodium* and *Tamarix*, each with five species (Fig. 4). In this study *Chenopodium badachschanicum* (KESHAVARZI *et al.*, 2016) and *Henrardia pubescens* were observed for the first time and were recorded for the flora of Iran.

Chenopodium badachchanicum Tzvelev

Annual herb up to 30 cm, sparsely farinose to sub-glabrous, yellowish-green, erect, angular, branched, lower branches sub-opposite. Petiole usually *c.* 1/3 of the length of leaf blade, blade thin, 3-8(-15) cm, lanceolate, with outward-projecting acute basal lobes and 0-2 lobe-like acute teeth on both sides, apex acute to acuminate, base sub-truncate to slightly cordate, bracts narrowly triangular, hastate, entire, uppermost lanceolate. Inflorescence narrow, lax, mostly leafless, terminal and axillary, cymose -dichasial, branches divaricate, solitary or several loosely together. Perianth segments 5, connate to below the middle, partly spreading in fruit, with a strong midrib visible especially inside, back apically keeled. Stamens 5. Stigmas 2-3. Pericarp persisting. Seeds horizontal, black, (1.2-)1.4-1.6(-2.0) mm in diameter, round in outline, margin somewhat acute, testa with large, irregular but mostly radially elongated pits and radial furrows, sometimes almost smooth.

Studied population. IRAN: Sistan & Baluchestan, 7 km south of Hirmand, Barahoii village (ALH – ha105).

General distribution. Central Asia, North-East Afghanistan, North of Pakistan, North of India, China, Nepal.

Henrardia pubescens (Bertol.) C.E.Hubb.

Annual herb up to 40 cm high, culms often decumbent at base, branched at base, linear leaves with 1-3 mm wide and 25-30 mm long, leaf blade linear, flat or rolled, pubescent; Inflorescence a single spike, spikelets 5-6 mm long, 1- flowered, glumes acute, subulate, with long and hispid hairs at the back.

Studied population. IRAN: Sistan & Baluchestan, 7 km south of Hirmand, Barahoii village (ALH – ha185).

General distribution. Syria, Jordan, Lebanon, Iraq.

Biological types of the area: The main biological form of the area was therophytes (61%). The frequency of other types were: hemicryptophytes (17%), phanerophyte (9%), cryptophyes (7%), geophytes (4%) and parasites (2%) (Fig. 5).

Chorotypes of the studied area. Distribution patterns of taxa revealed the power of Irano-Touranian elements in the area (with about 31%). Due to the penetration of the elements of other phytogeographic regions, there are somehow high percentages of multi- and bi-regional elements (33% and 12% respectively). In multiregional forms, the Irano-Touranian affinity is always present. The Hamun basin has also some Sindu Sudanian (3%) and Sahara-Arabian elements (2%). Plants with Mediterranean affiliation showed 6% of chorotypes. Some cosmopolitan plants as *Chenopodium album*, *Portulaca oleracea*, *Cuscuta campestris*, *Cyperus laevigatus*, *Poa annua*, *Polygonum aviculare*, *Cynodon dactylon*, *Amaranths caudatus* and *Amaranths viridis* were also observed (8%). Even Euro-Siberian elements were present in the area (2%) (Fig. 6).

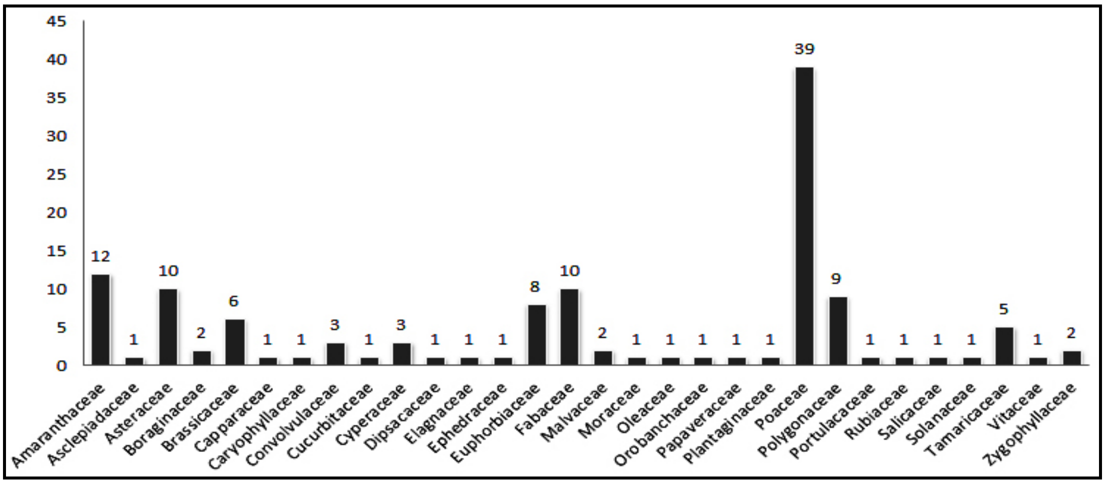

Fig. 3. Frequency of the families in the studied area.

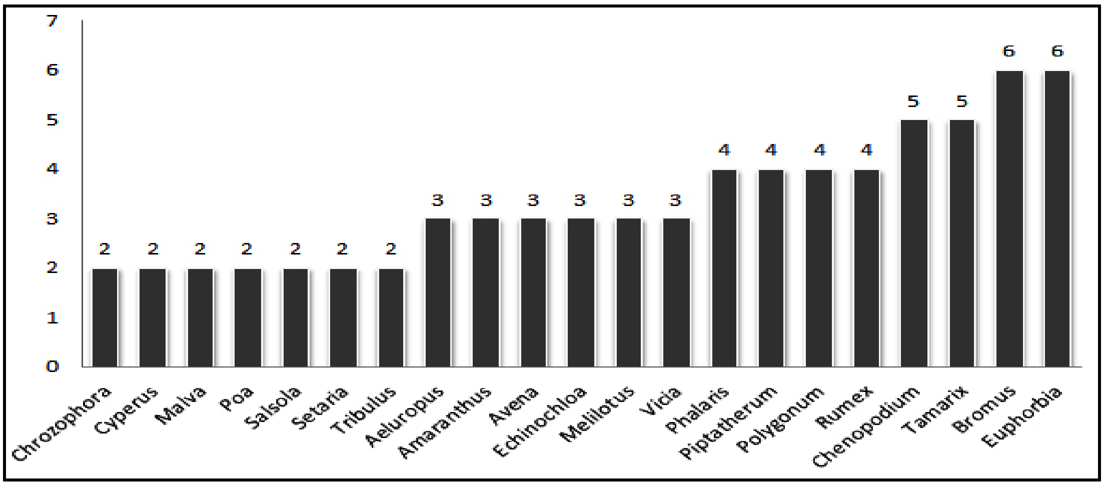

Fig. 4. Frequency of non-monotypic genus in the studied area.

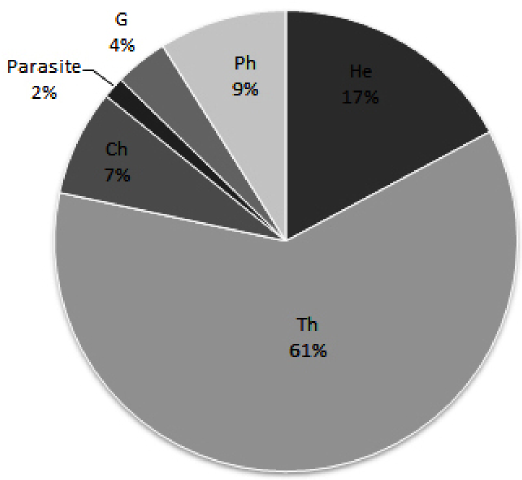

Fig. 5. The percentage of each biological type in the studied area. (Th: Therophyte, He: Hemicryptophyte, Ph: Phanerophyte, G: Geophyte, Ch: Chryoptophyte).

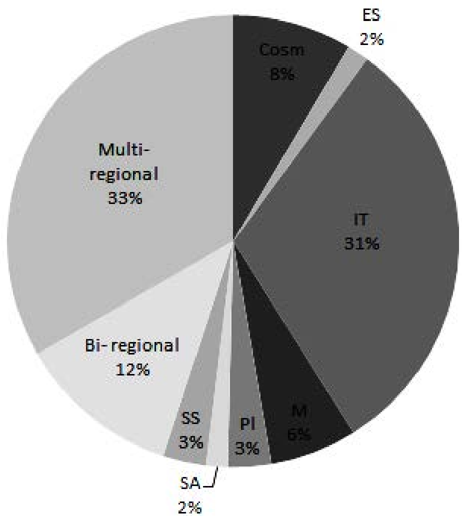

Fig. 6. The percentage of each chorotype in the studied area. (IT: Irano-Touranian; M: Mediterranean; SA: Sahara-Arabian; SS: Sindu-Sudanian; ES: Euro-Siberian; Cosm: Cosmopolite; multi- regional: IT-ES-M, ES-M-SS, IT-M-SS, IT-M-SA; bi- regional: IT-SS, IT-ES, IT-M, IT-SA, M-SA, M-SS).

Discussion

Knowing the floristic composition of a habitat is valuable for environmental and ecological research, and also for choosing management and conservation strategies (DAR & KHUROO, 2013). In the present research, 128 plant species were identified in the south-eastern border of Iran. Studying the chorology and life forms of these taxa is of great importance for the further ecological investigation, conservation and management.

The results of floristic study in Hamun Lake basin showed that the area is located in the transitional zone between the Irano-Touranian and Saharo-Arabain and Sindu-Sudanian regions. The studied area is not rich in endemic species and showed some affinities with the taxa in neighboring floras (Afghanistan and specially Flora of West Pakistan).

The Iranian flora is the intersection of different phytochoria as Irano-Touranian, Euro-Siberian, Sindu-Sudanian, Saharo-Arabian and also Mediterranean elements (ZOHARY, 1973). The chorological studies showed that 31% of species belong to the Irano–Touranian zone and 33% to multi-regional chorotype. In floristic projects in many other parts of Iran with great amount of human disturbance, multi- regional species make a significant portion of the studied flora (SOLTANIPOOR, 2006; NAQINEZHAD *et al.*, 2006; JANKJU *et al.*, 2011). Cosmopolitan, invasive and ruderals or weeds mainly reflect the anthropogenic origin of some habitats in the area. In the present study, *Portulaca oleracea*, *Polygonum aviculare*, *Poa annua* and *Cynodon dactylon* were the famous cosmopolitan taxa.

Life-forms in fact show the strategy of plant for obtaining resources. Therophytes are frequent in desert and arid lands. NAQINEZHAD *et al.* (2006) pointed an increase in therophytes with anthropogenic and grazing effects. The frequency of therophytes in the studied area revealed that Mediterranean climate condition is not dominant in this area (MOBAYEN, 1996). Hemicryptophytes are plants, which are resistant to cold climatic condition and developed their life by using ground water or reduced their water needs by shading. In the studied area, therophytes are plants, which are adapted to the dryness and the shortage of rainfall. The complex presence of therophytes and hemicryptophytes is an indicator of arid lands (ALSHERIF *et al.*, 2013).

The wetlands play essential role of ecology, economy and culture of the inhabitants in the Sistan basin. Drought have made problems more serious in this area and the floristic elements have catastrophically decreased. Many people have lost their income and had to migrate to other areas through drought periods of recent years. Local inhabitants basically rely on Hamun Lake for their food (fishery and agriculture).

The presence of annual (therophytes) is an indicator of anthropogenic pressure and overgrazing. The high amount of *Asteraceae* elements in the area is an alarm for the vegetation destroys.

In the studied area, 59 taxa such as *Melilotus officinalis, Malva pusilla, Cynodon dactylon, Polygonum aviculare* and *Medicago sativa* are of known medicinal importance.

Based on IUCN red list, *Cynanchum acutum, Cressa cretica, Cyperus glaber, Cyperus laevigatus, Ephedra strobilacea, Phragmites australis, Poa annua, Polypogon monspeliensis, Polygonum argyrocoleon, Schoenus nigricans, Tamarix kotschyi, Tamarix tetragyna* and *Vitis vinifera* are in status of least concern (LC).

Low rainfall, high temperature and evaporation and 120-day winds of Sistan are particular factors affecting the floristic composition of the studied area as mentioned by JABBARY *et al.* (2013). The present study is the first floristic study of the Hamun Lake basin and reflects the importance of the plant diversity of this area. Perhaps some plant species were left unrecorded so a comprehensive study in this field will be continued to identify and protect the genetic resource of this area.

Acknowledgements

We are indebted to the Vice President of Research and Technology of Alzahra University for their financial Supports.

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Received: 17.06.2016
Accepted: 02.03.2017

ECOLOGIA BALKANICA
2017, Vol. 9, Issue 1 June 2017 pp. 11-17

Comparative Study on the Landfill Leachate Treatment in a Vertical Flow Wetland System With/Without Phragmites australis

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Abstract: The landfill leachate treatment efficiency in a vertical-flow wetland system with and without planted *Phragmites australis* was investigated. The BOD/COD ratio of the landfill leachate was 0.38. Water samples were taken daily for determination of the COD, BOD, [NH_4-N], [NO_2-N], [NO_3-N] and orthophosphates values. High COD and BOD removal efficiencies and discharge limits in both laboratory systems were achieved. Complete elimination of the ammonium nitrogen from the leachate in the nonvegetated wetland for 13 and 14 days was obtained at a recirculation ratio of 1:1 and 1:2, respectively, and for 9 and 7 days - in the vegetated wetland, at the same recirculation ratios. The initial nitrite nitrogen concentration in the landfill leachate was 17.2 ± 1.1 mg/L, which is 430 times over the discharge limit for these ions. After landfill leachate treatment in the nonvegetated wetland system at the recirculation ratios 1:1 and 1:2, nitrites removal efficiencies of 98.8% and 92.5%, respectively, were achieved. In the vegetated wetland system at the same recirculation ratios, nitrites removal efficiencies of 96.5% and 76.2%, respectively were achieved. Nitrates removal was not observed. The results show that the orthophosphates were assimilated better from the *Phragmites australis* at longer water resting period.

Key words: Landfill leachate, vertical flow constructed wetland, *Phragmites australis*.

Introduction

The landfill leachate is a liquid, which is formed as a result of degradation processes of deposited wastes. The quantity of the leachates depends on the wastes composition, the used deposition method, the precipitations amounts, as well as the landfill age. The landfill leachates contain a large variety of contaminants and because of this they have harmful effect on the environment. This is the main reason for their preliminary treatment before discharge. The Landfill Directive and Waste Framework Directive directly influence the practices for leachate management and especially their collection and methods for treatment. This in turn affects the concentrations of BOD and ammonium nitrogen (BRENNAN *et al.*, 2016). The most common practice of the landfill leachates disposal is their mixing with domestic wastewater and their co-treatment in the wastewater treatment plants. Advanced oxidation methods, which are able to degrade wide range of the compounds, contained in the leachates were also used. They are very effective but quite expensive. Therefore, it is necessary to use a combination of chemical and biological methods that complement each other and lead to effective landfill leachate treatment. There are integrated systems including different processes like biochemical oxidation, coagulation, sedimentation and photo oxidation (SILVA *et al.*, 2017). The so-called constructed wetlands for wastewater treatment are used over the past few decades

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in Europe and US (VYMAZAL, 2010). The constructed wetlands can provide effective wastewaters treatment but for longer period of time because of contained toxic and persistent organic pollutants in landfill leachates, which inhibit the biochemical reactions (KADLEC & ZMARTHIE, 2010). Different types of plants in these systems are used. They contribute for treatment efficiency by accumulation of the pollutants in their tissue (LAVROVA & KOUMANOVA, 2008). One of the most important requirements for the effectiveness of these systems is the right choice of vegetation (LAVROVA & KOUMANOVA, 2013). The type of filler media in the systems also plays significant role in the purification process. Materials such as gravel, sand, zeolite, organic matter and other are used for filling the wetlands body (LI *et al.*, 2010; PELISSARI *et al.*, 2016; MOJIRI *et al.*, 2016; DE ROZARI *et al.*, 2016). The removal of organics, nitrogen and phosphorus containing substances and other pollutants in these systems is of a particular interest in research.

The aim of this research was to study the landfill leachate treatment efficiency in vertical-flow wetland system with and without planted *Phragmites australis*.

Materials and Methods

Wastewater characteristics

The landfill leachate was taken from a 10-15 years old landfill situated in the north-western part of Bulgaria. The characteristics of the landfill leachate used in the experiments are presented in Table 1. The values show that the cell from which the leachate was generated is in the methanogenic phase.

The BOD/COD ratio of the landfill leachate was 0.38 which means that the wastewater is fairly biodegradable and can be effectively treated biologically. The landfill leachate contains ammonium ions with high concentration which is directly dependent with the organic matter decomposition (BURTON & WATSON-CRAIK, 1998; LEE *et al.*, 2010). The wastewater contains nitrites and low concentration of nitrates, which is evidence for presence of slight nitrification of the generated ammonium ions.

Table 1. Landfill leachate characteristics.

Parameter	Value (mean ± SD)
COD (mg/L)	2031.3 ± 9.2
BOD (mg/L)	762.2 ± 5.1
NH_4-N (mg/L)	332.3 ± 32.4
NO_2-N (mg/L)	17.2 ± 1.1
NO_3-N (mg/L)	0.3 ± 0.2
Orthophosphates (mg/L)	1.9 ± 0.1

Laboratory system

Two identical laboratory systems (Fig. 1) were used in the experiments. They have one significant difference – in the first laboratory system the reactor type subsurface vertical-flow wetland (SVFW) was unplanted and in the second laboratory system this reactor was planted with *Phragmites australis*. The reactor type SVFW was a column with dimensions 123 mm in diameter and 900 mm in height. It was filled with 35 ÷ 55 mm round gravel as a bottom layer with a height of 300 mm and top layer with a height of 500 mm of 5 ÷ 25 mm gravel.

After clarifying into a sedimentation tank the landfill leachate flows into the SVFW reactor. Peristaltic pump ensures the water movement through the reactor with flow rate 60 ml/min. The system operated continuously in recirculation regime. The recirculation was performed at ratio of 1:1 and 1:2, giving 1 h water movement through the filter media and 1 (2) h resting period of the water into the reactor (contact between the wastewater and the bed matrix). Water samples were taken daily for determination of the COD, BOD, NH_4-N, NO_2-N, NO_3-N and OP values. In a similar laboratory system young *Phragmites australis* (common reed), obtained from comparatively clean area, was planted.

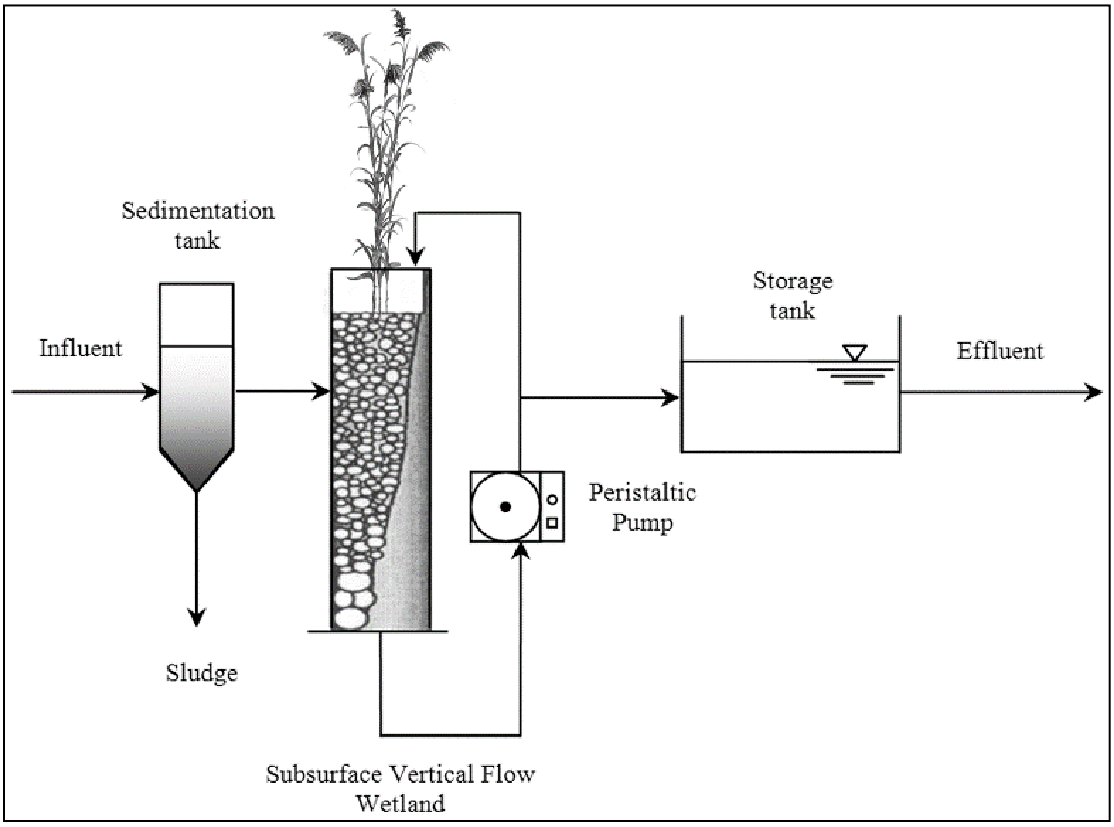

Fig. 1. Scheme of the laboratory system used in the experiments.

Analytical methods and chemicals

The parameters Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Ammonium Nitrogen [NH_4-N], Nitrite Nitrogen [NO_2-N], Nitrate Nitrogen [NO_3-N] and Orthophosphates (OP) were determined by standard methods, using pure reagents for analysis (APHA, 1992).

Removal efficiency

The removal efficiency was determined by using the following formula:

$$E = \frac{C_0 - C_t}{C_0} \times 100, \%$$

where C_o is the initial concentration in mg/L and C_t is the concentration at time t.

Results and Discussion

COD removal

The landfill leachates used in the experiments has high initial COD values (2031.3 ± 9.2 mg/L). The obtained results show that the leachate resting period and the plants presence in the system are important for the treatment efficiency (Table 2).

For 15 days leachate treatment in the laboratory system, consisted of reactor type subsurface vertical-flow wetland without *Phragmites australis*, COD removal efficiency of 81.6% and 92% was achieved at recirculation ratio 1:1 and 1:2, respectively. The longer contact time between the leachate and the reactor's package leads to better results probably due to the vital activity of the microorganisms attached on the gravel surface. For this period of time the COD discharge limit was not met. During the leachate treatment in the system with *Phragmites australis*, faster decreasing of COD was observed. In this system COD discharge limit was achieved with removal efficiency of 96.2% and 96.8% for 12 and 10 days at recirculation ratio 1:1 and 1:2, respectively. The plant presence in the laboratory system contributes to the faster removal of contaminants from the leachate probably due to the well-developed microbial community and root system which release oxygen in the surrounding media. It is also well known that the plants have ability to accumulate different compounds from the aquatic environment in their tissue which also contributes for the COD decreasing.

Table 2. COD and BOD removal efficiency (%).

Days	SVFW without *Phragmites australis*		SVFW with *Phragmites australis*	
	Recirculation ratio			
	1:1	1:2	1:1	1:2
		COD		
3	28.4	38.5	45.8	56.9
6	45.6	63.2	76.1	87.9
10	63.7	80.4	93.9	96.8
		BOD		
3	49.6	55.8	65.1	81.2
5	61.0	69.8	81.5	90.9
8	74.1	79.2	96.5	98.3

BOD removal

The landfill leachate used was with high BOD initial concentration (762.2 ± 5.1 mg/L). During the experiments the BOD values flowingly decreased as in the laboratory system without vegetation the process becomes slower in comparison with laboratory system with *Phragmites australis* (Table 2). In the SVFW without *Phragmites australis* the BOD discharge limit was not met for 15 days treatment. This period of time was insufficient for complete leachate polishing because of high contaminants concentration in it, although the BOD/COD ratio of 0.38, which is an indicator for slight biodegradable compounds. During the experiments in the laboratory system with common reed, the BOD discharge limit was achieved for 9 and 8 days at recirculation ratio 1:1 and 1:2, respectively.

NH_4-N removal

The ammonium nitrogen concentration was reduced gradually in each experiment (Fig. 2). The results show that the concentration of these ions was reduced as slowly as in the wetland system without vegetation at recirculation ratio of 1:2, and most rapidly in the wetland system with vegetation and at the same recirculation ratio. Generally, the natural NH_4-N oxidation by the air has a fundamental influence on the process. In the vegetated wetland it was noted that the process occurs faster than in the nonvegetated system. This is probably due to the release of oxygen from the *Phragmites australis* root system into the rhizosphere. The fact, that the ammonium nitrogen decreases more rapidly at a recirculation ratio 1:2 in the wetland with growing vegetation means that, thelonger the leachate resting period in the system is, the longer the influence of the aerobic environment, formed in the rhizosphere is. Complete elimination of the ammonium nitrogen from the leachate in the nonvegetated wetland for 13 and 14 days was obtained at a recirculation ratio of 1:1 and 1:2, respectively, and for 9 and 7 days in the vegetated wetland at the same recirculation ratios.

NO_2-N removal

The initial nitrites concentration in the landfill leachate was 17.2 ± 1.1 mg/L, which is 430 times over the discharge limit for these ions. As a result of the ongoing nitrification in both laboratory systems, the concentration of these ions reaches even higher values (Fig. 3). For example, the maximum nitrites concentration of 20.9 ± 0.9 mg/L and 24.9 ± 2.2 mg/L at recirculation ratio 1:1 and 1:2, respectively, was achieved at the second day after the treatment process beginning in the vegetated wetland. Then their concentrations are decreasing, reaching values of 0.6 ± 0.25 mg/L and 4.1 ± 0.12 mg/L, at recirculation ratio 1:1 and 1:2, respectively, in the vegetated wetland. Significantly lower are the concentrations of these ions in the nonvegetated system. Perhaps this is due to the weaker nitrification. In the nonvegetated wetland an increase of these ions concentrations was not observed.

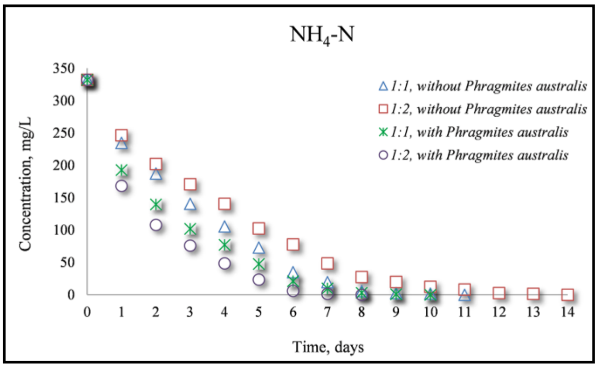

Fig. 2. Ammonium ions concentration decrease in all experiments.

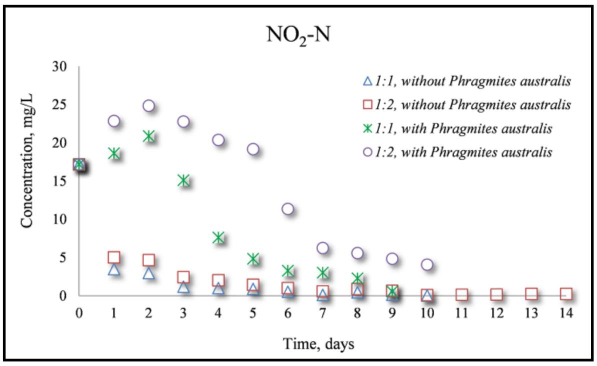

Fig. 3. Nitrite nitrogen concentration decrease.

In the first twenty-four hours in this system a sharp decrease of the nitrite nitrogen concentration was observed, and at recirculation ratio of 1:1 and 1:2 their values reach up to 3.5 ± 0.16 mg/L and 5.03 ± 0.17mg/L, respectively. Thereafter, the concentrations slightly decreased and after 13 days at recirculation ratio of 1:1 the values reach up to 0.2 ± 0.2 mg/L and after 14 days at a recirculation ratio of 1:2 - up to 0.3 ± 0.04 mg/L. In the nonvegetated wetland system at the recirculation ratios 1:1 and 1:2, treatment efficiencies of 98.8% and 92.5%, respectively were achieved. In the vegetated wetland system at the same recirculation ratios, treatment efficiencies of 96.5% and 76.2%, respectively were achieved.

NO_3-N removal

As a result of the nitrification in both laboratory systems an increase of the nitrates concentrations was observed (Fig. 4). From the results it is particularly evident that the vegetation significantly affects the concentration of these ions. As it can be seen from Figure 4, the NO_3-N concentrations in the laboratory system with vegetated wetland rapidly increased as early as the first day of the treatment process and reached values of 14.4 ± 0.4 mg/L and 16.3 ± 0.9 mg/L at recirculation ratio of 1:1 and 1:2, respectively. Then the concentration of these ions began to decrease gradually and after the seventh day almost did not change. The final concentrations of 10.4 ± 0.3 mg/L and 11.4 ± 0.2 mg/L, respectively, were achieved in this laboratory installation during one-hour and two-hour leachate resting. In the nonvegetated wetland a gradual increase of the nitrate nitrogen concentration was observed and from the ninth day until to the end of the experiment almost constant concentration of 8.2 ± 0.1 mg/L and 5.2 ± 0.3 mg/L was observed at recirculation ratio 1:1 and 1:2, respectively. The results show that in both systems at these experimental conditions denitrification was not achieved. In the nonvegetated wetland it could be achieved by increasing of the leachate resting period and additional organic carbon source as a food for denitrifying bacteria.

Orthophosphates removal

The initial orthophosphate concentration in the landfill leachate was 1.9 ± 0.01 mg/L. During the experiments a slight change in their concentration was observed (Fig. 5). In the reactor without vegetation the treatment efficiencies 16.8% at recirculation ratio of 1:1 and 25.9% - at recirculation ratio of 1:2, respectively, were achieved. In the wetland with vegetation, treatment efficiencies of

29.3% and 38.3% were achieved at recirculation ratios 1:1 and 1:2, respectively.

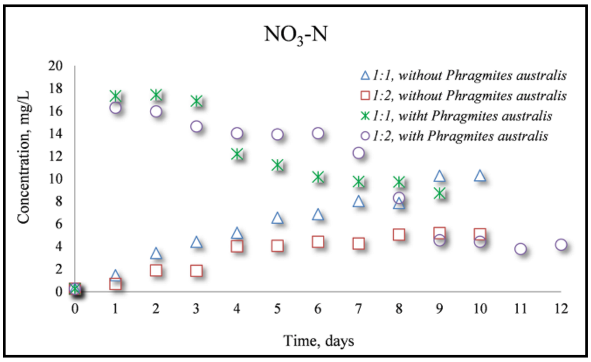

Fig. 4. Nitrate nitrogen concentration variation.

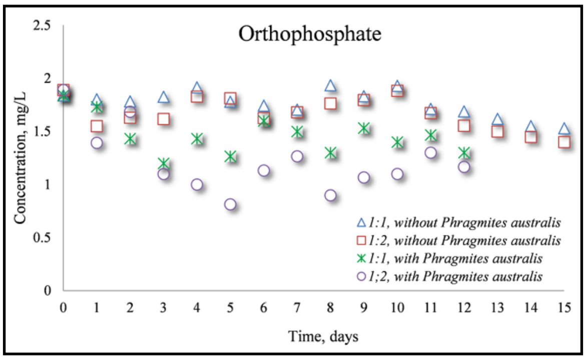

Fig. 5. Orthophosphate concentration variation.

The results show that the orthophosphates were assimilated better from the *Phragmites australis* at longer water resting period (recirculation ratio 1:2).

Conclusions

The landfill leachate treatment efficiency was compared in laboratory systems with and without planted *Phragmites australis*.

The obtained results show that the vegetation significantly affects the treatment process. The concentrations of COD and BOD decreased much faster in the vegetated wetland compared to the nonvegetated wetland. Complete elimination of ammonium nitrogen from landfill leachate was achieved in both wetlands. Complete nitrate nitrogen denitrification was not observed because of the aerobic conditions in both laboratory wetlands. The orthophosphate concentration decreasing in the wetland system with growing *Phragmites australis* was two times faster than that in the nonvegetated system. The presence of *Phragmites australis* significantly increases the treatment efficiency of the vertical-flow wetland systems.

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Received: 02.02.2017
Accepted: 22.03.2017

ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 | June 2017 | pp. 19-27

Assessment of Organic Pollutants Accumulation in Fish from Maritsa River Basin (Bulgaria)

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Abstract. Two selected fish species were sampled from 5 sites within the Maritsa River Basin in the period 2013-2014 with the aim to assess the effects of pesticide industry and intensive agriculture. Sampling sites were located in the middle of the Maritsa River Basin (near Plovdiv City, Bulgaria) and the watersheds of the Chepelarska and Stryama Rivers. Methods for analysis of certain priority substances and specific pollutants were applied in order to establish trends in the accumulation in fish, as required by the Directive 2008/60/EC and Directive 2008/105/EC. The reported data for organic pollutants are the first for the studied river basin. The selection of indicators (priority substances and specific pollutants) provides particular guidelines for planning future monitoring for assessment of river chemical and ecological status.

Key words: fish, Maritsa River, PCBs, OCPs, PAHs**.**

Introduction

Maritsa River is the largest on the Balkan Peninsula; it is the biggest river in terms of discharge volume and the third longest river in Bulgaria. It emerges from springs in the Rila mountain range and its basin is a cross-border watershed for Bulgaria, Greece and Turkey. The number of the tributaries of Maritsa River is about 100. The most significant among them are the rivers Chepinska, Topolnitsa, Luda Yana, Vacha, Chepelarska, Stryama, Sazliyka, Arda, Tundzha, Ergene.

Determining organic compounds in freshwater fish enables the identification of the contaminant origin in local regions. Polychlorinated biphenyls (PCBs) are toxic chemicals which precipitate in soil and water. They are mixtures of synthetic and organic chemical substances which share similar chemical structure. PCBs had been derived for their very high flash points and were widely deployed as fire-extinguishing agents, electrical insulators and plasticizers, mainly in electrical apparatuses. Most often they are introduced into the environment through defective equipment, illegal discharge, scavenge oil from electrical equipment, as well as hazardous waste. They are a family of 209 synthetic molecules composed of a biphenyl nucleus with chlorine at any, or all, of the 10 available sites (McFarland & Clarke, 1989). The ortho-, meta- and para positions are important in determining the chemical properties of PCB, such as high dielectric constants, non-flammability, hydrophobic quality and chemical stability. The usefulness of these properties led PCBs to be used in mixtures for a wide range of

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applications from the 1930s onwards (BREIVIK *et al*., 2002; BEYER & BIZIUK, 2009). However, after their toxicity became recognized, PCBs were progressively banned in most developed countries during the 1980s (LETZ, 1983). Due to their large-scale production, extensive use and environmental persistence, these compounds have accumulated in many ecosystems all over the world; in addition in aquatic environments they are trapped in sediments. PCB contamination continues to be a problem as these compounds can be transferred from the sediment to the lower trophic levels of an ecosystem through microbial and bottom-feeder uptake.

Organochlorine pesticides (OCPs) have been widely used in the past, but because of their high persistency in environment and accumulation in the food chain, they can still arouse topical concerns about human health

Polyaromatic hydrocarbons (PAHs) are a large group of organic substances with two or more benzene cores. They are characterized by low solubility in water, but high solubility in fats. The polycyclic aromatic hydrocarbons are produced mainly by incomplete combustion of coal and diesel fuel. There are several hundred PAHs. These compounds are absorbed by organisms mainly through the respiratory system, but may also be assimilated together with water and food. The most thorough study of the carcinogenic effect is that of inhaled benzo[a]pyrene (BaP), essential source of which is tobacco smoke.

The aim of the research was to identify the extent of accumulation of organic pollutants in "fish" matrix in selected river valleys and monitoring stations along the Maritsa River Basin.

Material and Methods

The monitoring of fish was accomplished in accordance with Guidance document No. 25 on chemical monitoring of sediment and biota to the common implementation strategy for the Directive 2008/105/EC (EC, 2010).

The research was conducted at 5 monitoring stations located in the Maritsa River Basin and two of its major tributaries – Chepelarska and Stryama River (Fig. 1).

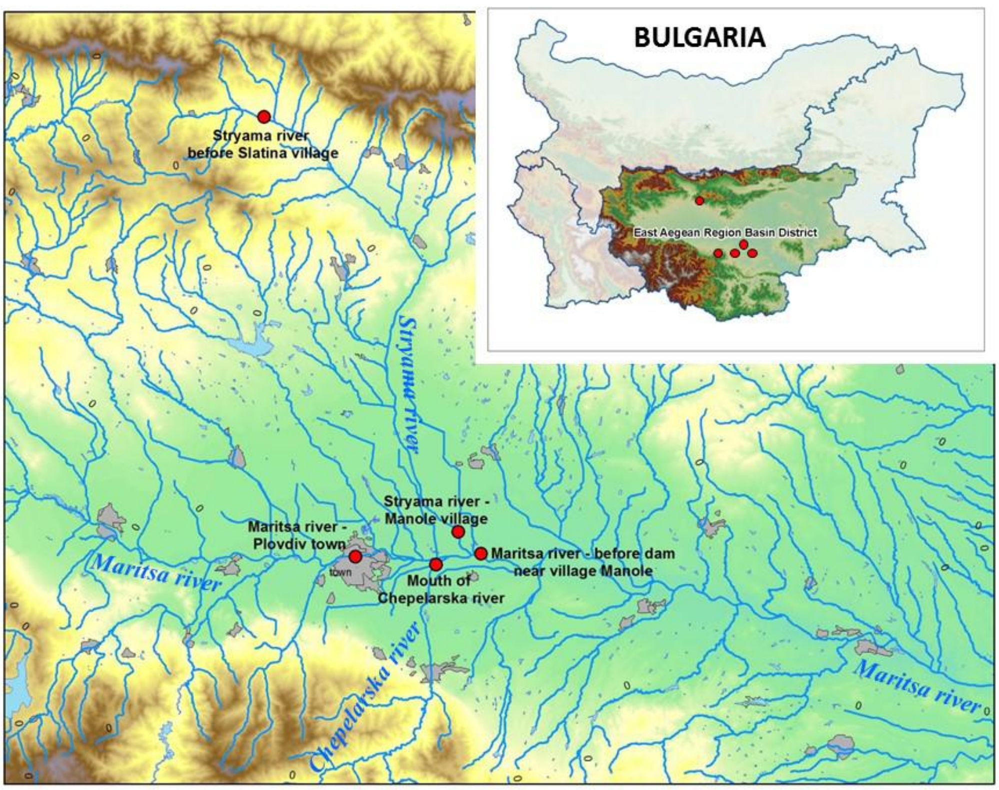

Fig 1. Map of the studied region.

The selection of the stations was made in compliance with the key criteria and the best practices of the strategy for sampling of fish specified in Guidance document No. 7 of the Water Frame Directive (EC, 2003). Thus, the stations were selected upon a preliminary analysis of the information about the anthropogenic pressure from point and diffuse sources of organic pollutants, the typological characteristics of the rivers, the composition of the bottom substrate (pebbles, gravel, sand, slime, clay), the hydrological characteristics (water runoff, water level) and accessibility for sampling. The geographic characteristics of the monitoring stations (watershed, river, location, geographic coordinates) were specified below.

Stryama river, the village of Slatina (N42.6902; E24.56917) was selected as a potential reference site for national type R5 Semi-mountain rivers in Ecoregion 7, slightly influenced by anthropogenic pressure and limited impact on the quality elements: extensive agriculture and forestry in the vicinity of the monitoring station; low pressure from domestic discharges in the catchment area of the site (the town of Klissura); limited automobile traffic; good chemical and good to high ecological status identified as a reference point for type R5 in the RBMP of East Aegean River Basin district. Substratum was consisted mainly by pebbles, gravel and sand, and organic slime where the current is sluggish. Moderate to significant water runoff, moderate to high current velocity, water level was 0.2-0.4 m.

Stryama river, the village of Manole (N42.1871; E24.91314) – type R13 Small and medium-sized lowland rivers in Ecoregion 7, located in a region with settlements in the catchment area of the of monitoring station; intensive agriculture; cumulative pressure from small settlements (<2000 p.e.); automobile traffic; good chemical and moderate ecological status; substrate – mostly sand and at places where the current is sluggish – organic slime. Moderate to significant water runoff, moderate to low current velocity, water level was 0.3-0.6 m.

Chepelarska River mouth, Kemera bridge area (N42.1457; E24.87722) – type R5 Semi-intensive rivers in Ecoregion 7, a site of significant anthropogenic pressure from industrial plants producing pesticides and metals ("Agria" Ltd., "KCM" Ltd.); discharge of untreated waste water from large settlements (Assenovgrad Town > 1000 p.e.); intensive agriculture; automobile traffic; poor chemical status (metals) and poor ecological status; substrate – mostly gravel and sand, and where the current is sluggish – organic slime. Moderate to significant water runoff, moderate to low current velocity, water level: 0.3-0.5 m.

Maritsa River, upstream of Plovdiv City (42.1608; 24.95124) – type R12 Large lowland rivers in Ecoregion 7, site of combined point and diffuse pressure from the upper and middle part of the catchment area of the Maritsa river, before discharge of waste water from Plovdiv City; discharge of treated waste water from large settlements in the catchment area on the monitoring station (Pazardzhik >1000 p.e., Stamboliyski town 2000-10000 p.e.); advanced industry (pulp and paper, food and flavor industry); intensive agriculture; intensive automobile traffic; good chemical and moderate to good ecological status; substrate – mostly sand and gravel with depositions of organic slime at places of sluggish current; significant water runoff, moderate to low current velocity, water level: 0.4-1.2 m.

Maritsa river, dam near the village of Manole (N42.1529; E24.74322) – type R12 Large lowland rivers in Ecoregion 7; a monitoring station of significant cumulative anthropogenic pressure from all the sources in the catchment area of the other monitoring stations (discharge of waste water from large settlements, industrial enterprises and intensive agriculture; discharge of treated waste water) from large settlements in the catchment area of the monitoring station (Plovdiv City – 300000 p.e., Assenovgrad Town >10000 p.e., a large number of settlements <2000 p.e.); combined pressure from developed industry (industrial zones of the city of Plovdiv, "Agria" Ltd., "KCM" Ltd.); intensive agriculture in the catchment areas of the surveyed rivers – the Maritsa, Stryama and Chepelarska rivers; limited automobile traffic; good chemical and moderate ecological status; substrate – massive deposition of organic slime in the area of the monitoring station before the dam near the village of Manole, sand; significant water runoff, low velocity of the current, water level: 1.0-3.0 m.

Selection of biota (fish) for analysis. Two species were selected for their importance to local human fish consumption: *Barbus cyclolepis* Heckel, 1837 - a freshwater fish in the family Cyprinidae. It is found in Bulgaria, Grece, and Turkey. Its natural habitats are river and intermittent rivers. It is not considered a threatened species by the IUCN (Crivelli, 2006). *Squalius orpheus* Kottelat & Economidis, 2006, which belongs to a genus of fish in the family Cyprinidae found in Europe and Asia, with "LC - least concern" status, same as the previous species, according to the IUCN Red List (Freyhof & Kottelat, 2008).

Sampling. The sampling was accomplished three times in the period between June and October 2013-2014. The sampling was done in compliance with the EN 14011 European Standart "Water quality – Sampling of fish with electricity" (CEN, 2003).

Transportation and sieving. Samples (upon sieving) were transferred into preliminary cleaned brown glass containers for organic pollutants. They were transported in a cooled state to the laboratory (at 4°C) within a period not exceeding 6 hours. Cooling was accomplished by using cool boxes and freezer block inserts.

Storage. The sifted samples of fish were kept in containers frozen at -20°C.

Analytical methods. A 10 g sample was extracted with organic solvent hexane: acetone (1:1), by microwave decomposition under a programmed furnace temperature of 120°C detainment time 25. The extract obtained was concentrated, then subjected to a purification procedure with silica gel and again concentrated. The sample was analyzed using gas chromatography – mass spectrometry (GC-MS) equipment.

Gas chromatographic analysis of the OCP, DDTs and PCBs were carried out by GC (Agilent 7890B/5977A/MSD).

Quality control. The procedures for quality assurance include validation of the methods by routine in-house procedures and independent external procedures (participation in inter-laboratory tests). Use was made of certified reference materials: standard referent material 1947 Lake Michigan fish tissue - NIST, BCR 682-70G mussel tissue, standard referent material 2974a-Organics in Freeze-Dried Mussel Tissue - NIST, Pesticide mix 13 - Dr. Ehrenstorfer, PAH - Dr. Ehrenstorfer.

Selection of organic pollutants to be monitored. Analysis of synthetic compounds was made (pesticides, medicinal preparations, industrial pollutants), dissociated in the event of pollution from point and diffusive sources, atmospheric depositions, which are taken into account in the assessment of the chemical and ecological status of the water bodies (e.g. priority substances according to Directive 2008/105/EC (EC, 2008) and Directive 2013/39/EC (EC, 2013) and specific pollutants in compliance with the approved list and the EQS of the Bulgarian legislation.

Results and Discussion

In the fish *Barbus cyclolepis* the concentrations of all tree PCBs (PCB 52, PCB 153, PCB 180) were below the limit of quantification (<1 μg kg^{-1} w.w) at all monitoring stations. In addition, in the he fish *Squalius orpheus* the concentrations of all tree PCBs (PCB 138, PCB 153, PCB 180) were also below the limit of quantification (<1 μg kg^{-1} w.w) at all monitoring stations.

However, the results for 6 PCB_s in all fish showed concentrations exceeding the limit of quantification was - PCB 28, PCB 52, PCB 101, PCB 138 (Table 1) and (Fig. 2, 3).

Table 1. Minimum, average and maximum concentrations of 6 measured PCB in *Barbus cyclolepis* and *Squalius orpheus*, µg kg^{-1} w.w.

PCB	***Barbus cyclolepis*** (n=15)			***Squalius orpheus*** (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
PCB 28	3,38	8,20	5,15	1,74	6,15	3,30
PCB 52		<1		5,82	6,00	5,93
PCB 101		2,37	2,08	1,49	1,94	1,71
PCB 138		2,82	2,58		<1	
PCB 153		<1			<1	
PCB 180		<1			<1	

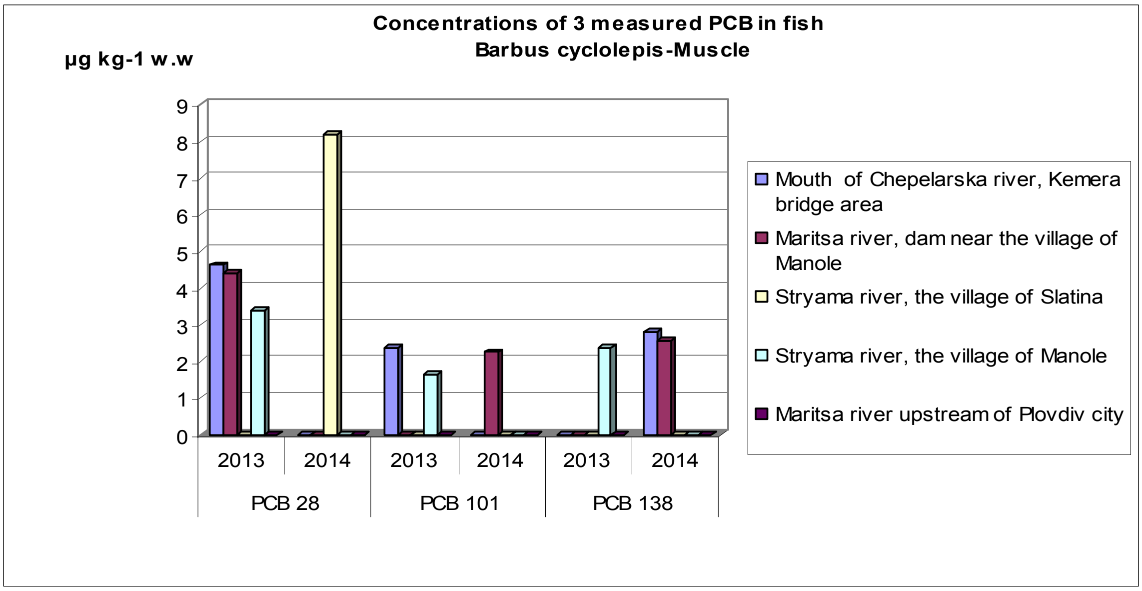

Fig. 2. Concentrations of measured PCB in fish *Barbus cyclolepis* - muscle.

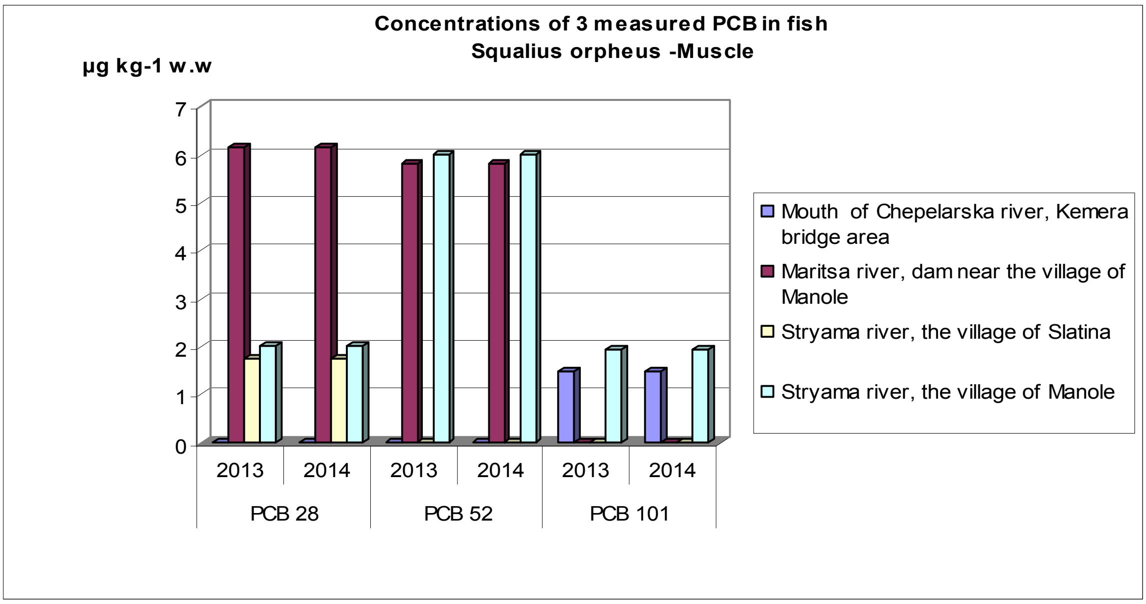

Fig. 3. Concentrations of measured PCB in fish *Squalius orpheus* - muscle.

Table 2. Detected minimum, maximum and average values of studied 24 OCPs in *Barbus cyclolepis* and *Squalius orpheus*, µg kg^{-1} w.w.

OCP	***Barbus cyclolepis*** (n=15)			***Squalius orpheus*** (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
HCB		<1			<1	
α-HCH	2,41	2,63	6,86	2,63	6,86	3,95
β-HCH	9,96	3,32	41,31	3,32	41,31	19,99
γ-HCH	7,71	4,51	22,08	4,51	22,08	12,59
δ-HCH	3,40	3,48	5,97	3,48	5,97	3,05
ε-HCH		<1		3,00	3,39	3,00
Heptachlor		<2			<2	
Aldrin		<2			<2	
Isodrin	12,90	12,42	66,98	12,42	66,98	38,59
Dieldrin	4,42	4,36	73,00	4,36	73,00	24,14
Endrin		<2			<2	
Chlordan		<2			<2	
trans-chlordan		<2			<2	
Metoxychlor		<2			<2	
Mirex		<2			<2	
α-Endosulfane		<3			<3	
β-Endosulfane		<3			<3	
Heptachlor		<2			<2	
o,p-DDE		<1			<1	
p,p-DDE		<1			<1	
o,p-DDT		<1			<1	
p,p-DDT		<2			<2	
o,p-DDD	3,72	4,21	32,85	4,21	32,85	11,32
p,p-DDD		<3		5,71	18,45	5,71

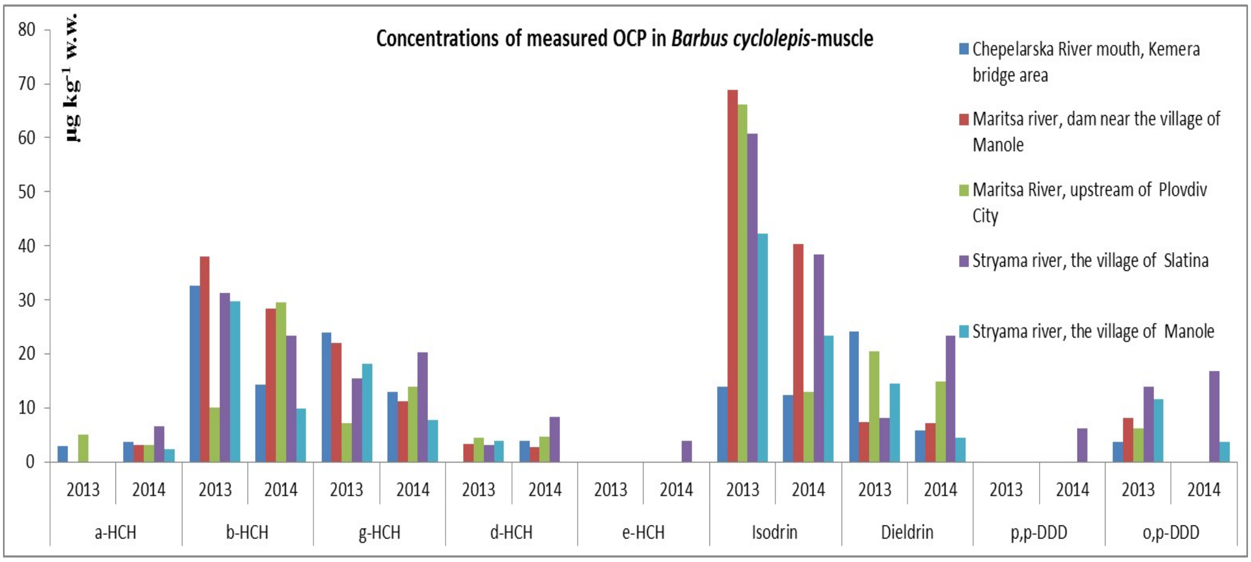

Fig. 4. Concentrations of measured OCP in *Barbus cyclolepis* - muscle.

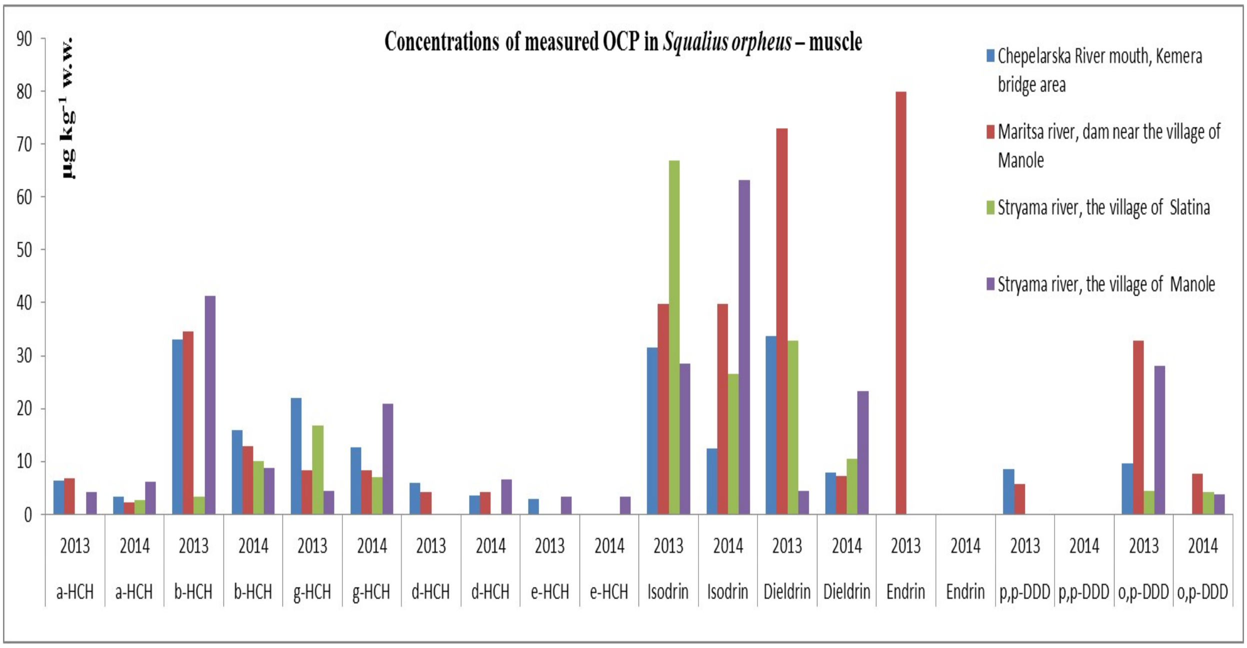

Fig. 5. Concentrations of measured OCP in *Squalius orpheus* - muscle.

Table 3. Minimum, average and maximum concentrations of 16 measured PAH in *Barbus cyclolepis* and *Squalius orpheus*, µg kg^{-1} w.w.

PAH	*Barbus cyclolepis* (n=15)			*Squalius orpheus* (n=15)		
	min	**max**	**average**	**min**	**max**	**average**
Naphthalene	23,88	30,64	27,33	25,48	34,65	30,11
Acenaphthene		<10			<10	
Acenaphthylene		<20			<20	
Fluoranthene		<20			<20	
Phenanthrene	10,18	34,75	16,11	3,73	32,54	17,92
Anthracene		<2			<2	
Flurene	3,11	4,56	3,78	3,59	4,10	3,85
Pyrene	8,91	20,60	18,64		<2	
Benzo(a)anthracene		<1			<1	
Chrysene		<1			<1	
Benzo(b)fluoranthene		<1			<1	
Benzo(k)fluoranthene		<1			<1	
Benzo(a)pyrene		<1			<1	
Indeno(1,2,3cd)pyrene		<3			<3	
Dibenz(a,h)anthracene		<3			<3	
Benzo(g,h,i)perylene		<3			<3	

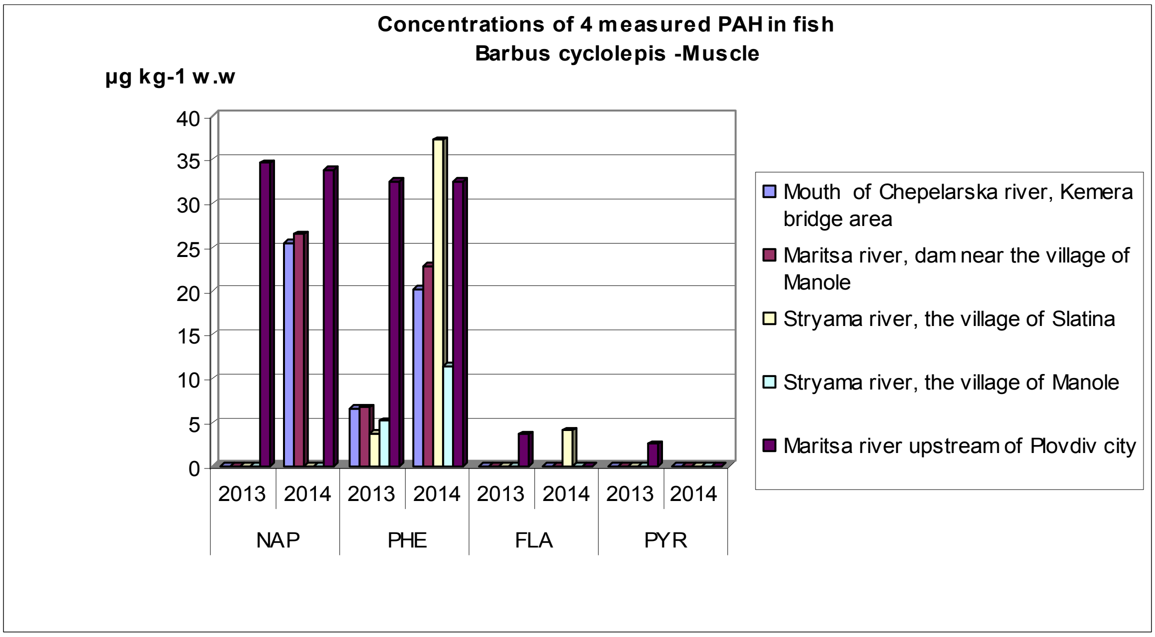

Fig. 6. Concentrations of measured PAH in *Barbus cyclolepis* - muscle.

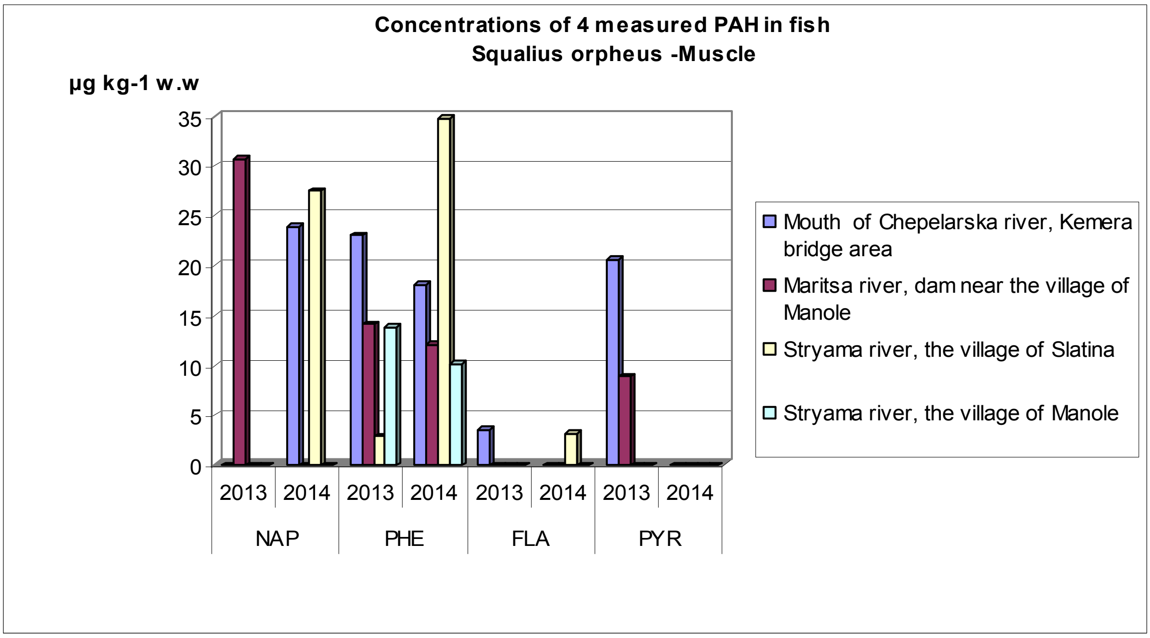

Fig. 7. Concentrations of measured PAH in *Squalius orpheus* – muscle.

The results for 24 OCPs in all fish showed concentrations exceeding the limit of quantification - α-HCH, β-HCH, γ-HCH, δ-HCH, Isodrin, Dieldrin, o,p-DDD (Table 2) and (Fig. 4, 5). The lowest concentrations were detected in the Stryama River, Slatina Village – a site slightly influenced by anthropogenic pressure.

Exceeding the limit of quantification for 16 PAHs analyzed was recorded for Naphthalene, Phenanthrene, Flurantene, Pyrene, (Table 3) and (Fig. 6, 7). The lowest concentrations were ascertained again in the Stryama River, Slatina Village, while the highest concentrations were in the Chepelarska River, Kemera Village – a site of significant anthropogenic pressure due to discharge of waste waters from settlements (Assenovgrad Town) and industrial enterprises ("KCM" Ltd. and "Agria" Ltd.).

Conclusions

At all monitoring stations PCB, OCPs and PAHs above the LOQ were detected, which was probably due to diffuse and point pollution in the area of the surveyed river sections. The most persistent PCB congener (PCB 138) were found only in *Barbus cyclolepis*, defined by WHO as important for evaluating the risk to human health. Ten substances from the group of OCPs were established. Moreover, six of them were found constantly in all five monitoring stations (α-HCH, β-HCH, γ-HCH, δ-HCH, Isodrin, Dieldrin, o,p-DDD). In the environment DDT metabolized slowly and the metabolite DDE is particularly persistent compound. The metabolite p,p′-DDE constituted more than 67% of the ΣDDTs for each species, followed by p,p′-DDD. In our study another metabolite of DDT, p,p′-DDD and o,p′-DDD, was also found, but in lower amounts. The p,p-DDT, o,p-DDT, p,p-DDE and o,p DDE in all analyzed fish species were found under limit of detection of the method.

The reported results could be a basis for initial examination of the level of pollution in the surveyed water bodies and identification of the sources of anthropogenic pressure. The data from the analysis conducted may be used for the purpose of monitoring the tendencies in the pollution of water bodies in terms of the examined indicators.

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Received: 31.05.2017
Accepted: 20.06.2017

ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 June 2017 pp. 29-37

Notes on Seasonal Dynamics of Paederus fuscipes Curtis, 1826 in Western Anatolia, Turkey (Coleoptera: Staphylinidae: Paederinae)

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Abstract. The seasonal activity of *Paederus fuscipes* Curtis, 1826 was studied between May-November in 2013 and 2014 in ten localities situated of five provinces in western Anatolia, Turkey. In generally, their number start to increase from May, reaches a high level in early and mid-June, decreases in July and August, which is the dry season, and reaches the highest level in early and mid-September. It was determined that *P. fuscipes* reach large populations particularly wet and hot weather and after rain.

Key words: Staphylinidae, Paederinae, *Paederus fuscipes*, seasonal dynamics, western Anatolia, Turkey.

Introduction

Species of the genus *Paederus* Curtis, 1826 are generally 6-10 mm in length, remarkable with the coloring of their body. These species can be found in almost any habitat depending on humidity and water. However, they are more common in the banks of rivers, creeks, lakes and dams, wetlands and wet agricultural areas. According to FRANK (1988), about 600 species are known worldwide. In the Palearctic Region, 95 species are distributed belonging to 9 subgenera (SCHÜLKE & SMETANA, 2015). Six *Paederus* species currently occur in Turkey (ANLAŞ, 2009). At least, 20 species of the genus *Paederus* are known to contain the unique hemolymph toxin pederin, which causes Paederus dermatitis (FRANK & KANAMITSU, 1987).

This dermatitis has been described by VORDERMANN (1901) for the first time, and PAVAN & BO (1953) named this toxic substances causing dermatities "Pederin", which is very complex and a non-protein substance (QUILICO *et al.*, 1961; VIEIRE *et al.*, 2014). Its importance comes from its very powerful cytotoxic contents, so that it has effects that are more powerful that the toxins of Black Widow Spider (=*Latrodectus sp.*) and Cobra (=*Naja sp.*), about 15 folds (FRANK, 2008). It blocks the protein and DNA syntheses in eukaryotic cells, while it does not impair the prokaryotic cells; however, although it inhibits the DNA synthesis, it does not affect the RNA synthesis (KELLNER & DETTNER, 1996; FRANK & KANAMITSU, 1987; PAVAN, 1963, 1975; SOLDATI *et al.*, 1966). Pederin also cause various lesions on the human skin. Existence and effects of pederin in Chinese sources goes back to past 1300 years; in these sources, it is mentioned that pederin causes swelling and peeling of the skin. It has been reported that pederin was used to function

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as caustic (NaOH), against boiling toxic substances and in the treatment of nasal polyp and ringworm. (FRANK & KANAMITSU, 1987; NARQUIZIAN & KOCIENSKI, 2000).

Paederus fuscipes Curtis, 1826 one of the most common and best known species in the genus *Paederus* in the world. This species is commonly spread in Afrotropical, Australia and Oriental regions apart from the Palearctic region. *P. fuscipes* is common in settlement and agricultural areas, and they are known as beneficial because it feeds on some insects, e. g. species of *Corcyra sp., Heliothis sp.* and *Aphis sp.,* that cause significant damages particularly in agricultural landscapes, e. g. in cotton, wheat, rice, maize and vegetable fields (BERGLIND *et al.,* 1997; KRAKERB *et al.,* 2000; DEVI *et al.,* 2003; KOMALA *et al.,* 2003; NASIR *et al.,* 2012). Their light orientation behavior allows them to be observed when flying around bright and powerful light sources at nighttime.

The cases with lesions caused by *Paederus* species in the world were first reported from India (STRICKLAND, 1924), and hundreds, even from some places, thousands of cases have been reported from South America, Africa, Mediterranean Countries, Pakistan, Iraq and Iran. Of these, a very significant portion of these are epidemics caused by *Paederus fuscipes*. There are also some cases reported from Turkey and known as caused by *P. fuscipes.* Likewise, 46 cases have been reported from Aydın province (ŞENDUR *et al.,* 1999). 204 patients have been reported from Çukurova University Hospital, Adana who were affected from this dermatitis and applied between 1995 and 1997 (USLULAR *et al.,* 2002). 16 patients have been recorded in Denizli province (ERDOĞAN *et al.,* 2006). However, it is believed that there are many other unknown cases because adequate records for these cases are not kept in Turkey, and also public health specialists do not know this dermatitis.

Too little studies have been carried out on the ecology and phenology on *Paederus fuscipes*, which is important in both public health and agricultural senses up to now. Knowing about the seasonal activity of this species will be beneficial for the future ecological studies on this species.

Up to now, there is no comprehensive data on seasonal dynamics of *Paederus fuscipes* have been published in Turkey. In this study, seasonal activity of *Paederus fuscipes* was determined with a study spread to two years, for the first time in the world.

Material and Methods

Study Area. To determine the seasonal activity of *P. fuscipes,* ten light traps were laid in two areas in each of the five provinces; Izmir: Menderes and Seferihisar; Manisa: Alaşehir and Turgutlu; Aydın: Söke and central district; Denizli: Buldan and Tavas; Muğla: Fethiye and Köyceğiz (Table 1; Fig. 1); in western Anatolia between May and November in 2013 and 2014. The maps (Fig. 1) were generated using Earth Explorer 6.1 and the online mapping tool of the Seaturtle website (Seaturtle.org, 2002).

Western Anatolia includes in the Mediterranean climate, which is characterized by dry, hot summers and mild, moist winters. But, variation in temperatures and average rainfalls different from study areas. In generally, the rainy season from November to May and from June to late October there is a rain-less period at these localities. Average temperature and average rainfall amount of studied localities are provided in Fig. 2.

Sampling. For this study, ten light traps were settled in 1 May 2013 and all localities were visited in ca. 14-day intervals from mid May to the ending of November during 2013 and 2014. All insect samples were placed into a plastic jars and transported to the laboratory, where *Paederus* species were separated from insect material. The specimens were preserved in 70 % alcohol. The morphological studies were conducted using a Stemi 2000-C microscope (Zeiss, Germany). The collected specimens referred to in this study are deposited in the collection of the Alaşehir Zoological Museum, Manisa (AZMM) of the Celal Bayar University.

Table 1. Provinces and localities of light trapping study area.

Provinces	Localities	2013-2014
Izmir	1. Seferihisar, 38°12'10"N, 26°50'23"E 2. Menderes, Tahtalı Dam, 38°05'52"N, 27°01'55"E	1 May-1 November
Manisa	3. Turgutlu, Çıkrıkçı, 38°28'06"N, 27°49'06"E 4. Alaşehir, Baklacı, 38°22'23"E, 28°31'36"E	1 May-1 November
Aydın	5. Central district, Adnan Menderes University, Agriculture Faculty garden, 37°45'37"N, 27°45'27"E 6. Söke, Güllübahçe, 37°40'02"N, 27°18'59"E	1 May-1 November
Denizli	7. Buldan, Yenicekent, 38°02'48"N, 28°56'13"E 8. Tavas, 37°34'05"N, 29°03'18"E	1 May-1 November
Muğla	9. Köyceğiz, Yaylaköy, 37°01'50"N, 28°45'02"E 10. Fethiye, Girmeler, 36°36'51"N, 29°22'11"E	1 May-1 November

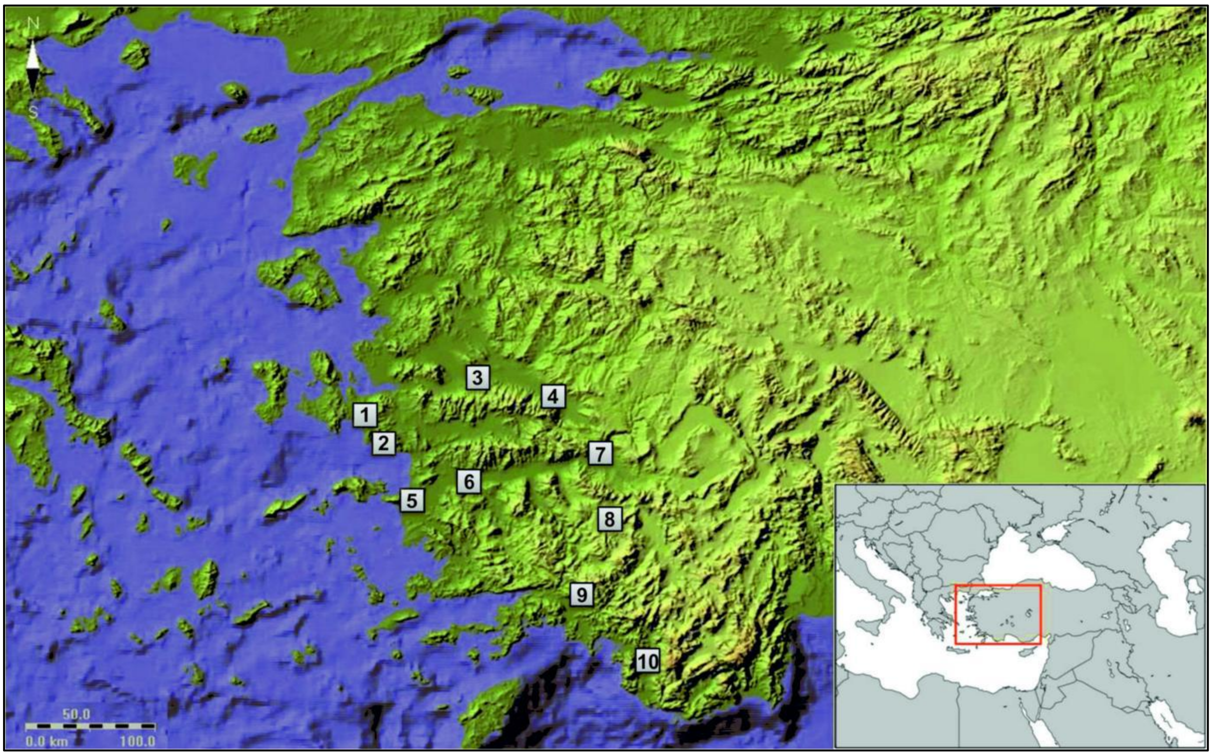

Fig. 1. The localities of light trapping study area in western Anatolia, Turkey.

Results and Discussion

Based on the results obtained, 10.332 specimens of *Paederus fuscipes* were collected by the ten light traps were laid in two areas in each of the five provinces in western Anatolia between May and November in 2013 and 2014 (Table 2). The location that specimens of the *P. fuscipes* species were found most intensely was determined as the central district of Aydın province with 3.410 specimens, followed by Turgutlu (Manisa province) with 1.404 specimens and Buldan (Denizli) with 1.392 specimens. The location that specimens with smallest number were collected was Köyceğiz (Muğla) with 165 specimens. Total 2.671 and 7.651 specimens were collected in the years 2013 and 2014, respectively.

The seasonal activities of *P. fuscipes* in the areas that light traps were laid were evaluated separately below (Fig. 3A-J): in the locality of Menderes (Izmir), this specimen reached the highest number in the first half of June; number of specimens collected in 2014, with 764 specimens, are greater than seven folds of the number collected in 2013, with 105 specimens. (Fig. 3A). 549 specimens in total were collected from Seferihisar (Izmir) locality within both years. Considering the activities of the *P. fuscipes* caught urban area, it is understood that they exist throughout the collection period. Based on this, it was found that they continued their activities during July and August, even with low levels. The reason for this was related to the presence of wet or damp areas (parks and gardens) continuously within the city (Fig. 3B).

Central District of Aydın province was the area that the seasonal activity of this species was studied the best. While 1.167 specimens have been collected within the first year; specimens in a record number, that is, 2.243 specimens were caught within the second year. While the numbers were less throughout July and August, they were seen throughout the entire collecting period. They were collected in great numbers particularly throughout May and the first half of June and within September. The number reached 613 particularly in the first half of September, 2014 (Fig. 3E). In the light trap laid in Söke (Aydın), *P. fuscipes* species first appeared in the first period of May 2013; however, it then started to become gradually lower in number, and it was never collected between the second half of July and early September. After this time, the number increased gradually and reached the highest number in the first half of October with 43 specimens, and then the number started to decrease again. It was never seen after the second half of October. Activity of this species in 2014 is similar to that of 2013. However, in contrast with 2013, intensity of the species continued till the mid-June (Fig. 3F).

Buldan (Denizli) is one of the locations that this species is found abundantly. While 389 specimens have been collected in 2013, 1003 specimens were caught in 2014. Seasonal activity of the species resembles the activity in central district, Aydın province (Fig. 3G). Tavas (Denizli) is a location that specimens were found in small numbers, and it has been found that the highest number was reached in the second half of May. Specimens were either not collected at all, or collected in very small numbers (Fig. 3H).

Turgutlu (Manisa) is the locality that specimens in the greatest numbers were collected after the central district of Aydın. 274 specimens were collected in the first year of the sampling period, and 1.130 specimens were collected in the second year. It is seen that the seasonal activity of the species resembles the activities in localities in Aydın and Denizli provinces (Fig. 3C). This species reached the highest numbers in May and in the first half of May in 2013 in Alaşehir (Manisa) locality. While only 9 specimens were collected in July and August 2013, total 128 specimens were found in 2014. In addition, presence of specimens in a significant number in July, 2014 is related with the precipitations in this period (Fig. 3D).

The light trap laid in Fethiye (Muğla) is the only locality that *P. littoralis* species was caught together with *P. fuscipes*; however, only three specimens were caught. When the seasonal activity of *P. fuscipes* species is considered, it is seen that while 66 specimens were collected in 2013, 731 specimens were collected in 2014. Although the number of specimens collected in 2013 is small, it was possible to collect specimens throughout the year. However, no specimens were caught in August, 2014. The greatest number of specimens was found in the second half of May and first half of September in 2014 (Fig. 3J). Köyceğiz (Muğla) locality was the area that specimens of this species were caught in the smallest numbers. It was possible to collect only 30 specimens in 2013 and 135 specimens in 2014. It is thought that the altitude of the village that the trap was set was about 1.000 meters and therefore temperatures at the evening and night are low. The small number of the specimens of the species caught does not allow the evaluation of the seasonal activity (Fig. 3I).

When the seasonal activity of *P. fuscipes* is considered in the general sense, it is seen that, despite the small differences between areas, their number start to increase from May, reaches a high level in early and mid-June, decreases in July and August, which is the dry season, and reaches the highest level in early and mid-September. It is also seen that their numbers decrease with the lowering temperatures, and reach the lowest number in November. Based on both the specimens caught in the light trap and our observations, it was determined that *P. fuscipes* reach large populations particularly wet and hot weather and after rain.

There are only a few publications about, even partially, the intensities and seasonal activities of *Paederus* species. In a study, on the three *Paederus* species found in Iran, it has been reported that these species are active between May and September, and the *P. fuscipes* species is mostly active between 09:00 p.m. and 10:00 p.m. (ABBASIPOUR & TAGHAVI, 2005). In another study, ZAGARI *et al.* (2003), *Paederus* species were more active in hot and damp weather and were seen between May and September in Iran. Similar findings are seen in some other studies also (e. g. MANLEY, 1977; NASIR *et al.*, 2012). A study carried out on insects of Iran with medical significance have shown that *Paederus* species are most commonly seen between May and August based on the specimens collected with the help of a light trap (NIKBAKHTZADEH & TIRGARI, 2008). AL-DHALIMI (2008) found that this species was mostly active in May based on the cases seen in Najaf City in Iraq.

All those said in the studies cited above related to the seasonal activities of species are basically observational and short-term data, and they have been taken here without being adequately based on digital and/or statistical data. However, it has been seen upon comparison with our study, it was seen that they were generally consistent with our results.

Table 2. Number of *Paederus fuscipes* specimens collected in the studied localities in western Anatolia during 2003 and 2004 for this study.

Provinces	Localities	Year	Collecting dates												Total
			15.V	01.VI	15.VI	01.VII	15.VII	01.VIII	15.VIII	01.IX	15.IX	01.X	15.X	01.XI	
Izmir	Menderes	2013	28	16	16	10	2	3	2	-	22	2	4	-	105
		2014	88	123	165	98	17	27	7	43	109	45	35	7	764
	Seferihisar	2013	26	15	10	15	14	11	8	2	34	11	2	2	150
		2014	27	23	44	51	27	22	18	11	89	78	8	1	399
Manisa	Turgutlu	2013	27	22	19	36	11	6	-	6	96	40	8	3	274
		2014	45	86	187	144	32	-	1	105	342	124	56	8	1.130
	Alaşehir	2013	102	81	12	17	-	-	-	9	36	9	3	-	269
		2014	75	74	67	89	65	12	8	43	168	43	29	23	696
Aydın	Central District	2013	144	97	66	43	74	60	54	48	377	114	78	12	1.167
		2014	201	312	399	127	108	96	100	143	613	107	34	3	2.243
	Söke	2013	25	16	14	3	7	-	-	-	43	27	2	-	137
		2014	37	45	57	12	5	1	-	12	65	23	-	-	257
Denizli	Buldan	2013	55	74	38	37	23	17	4	-	127	14	-	-	389
		2014	89	128	155	111	34	2	6	53	312	108	5	-	1.003
	Tavas	2013	14	14	8	11	-	-	-	-	37	-	-	-	84
		2014	27	97	15	44	15	3	-	15	48	17	13	2	296
Muğla	Köyceğiz	2013	7	8	4	1	-	-	-	1	8	1	-	-	30
		2014	12	20	24	18	9	-	5	16	18	11	-	2	135
	Fethiye	2013	12	8	6	6	4	1	3	1	12	5	3	2	63
		2014	127	133	100	87	33	4	-	-	169	29	27	22	731
TOTAL		2013 and 2014	1.168	1.392	1.406	960	480	265	216	508	2.725	808	307	87	10.322

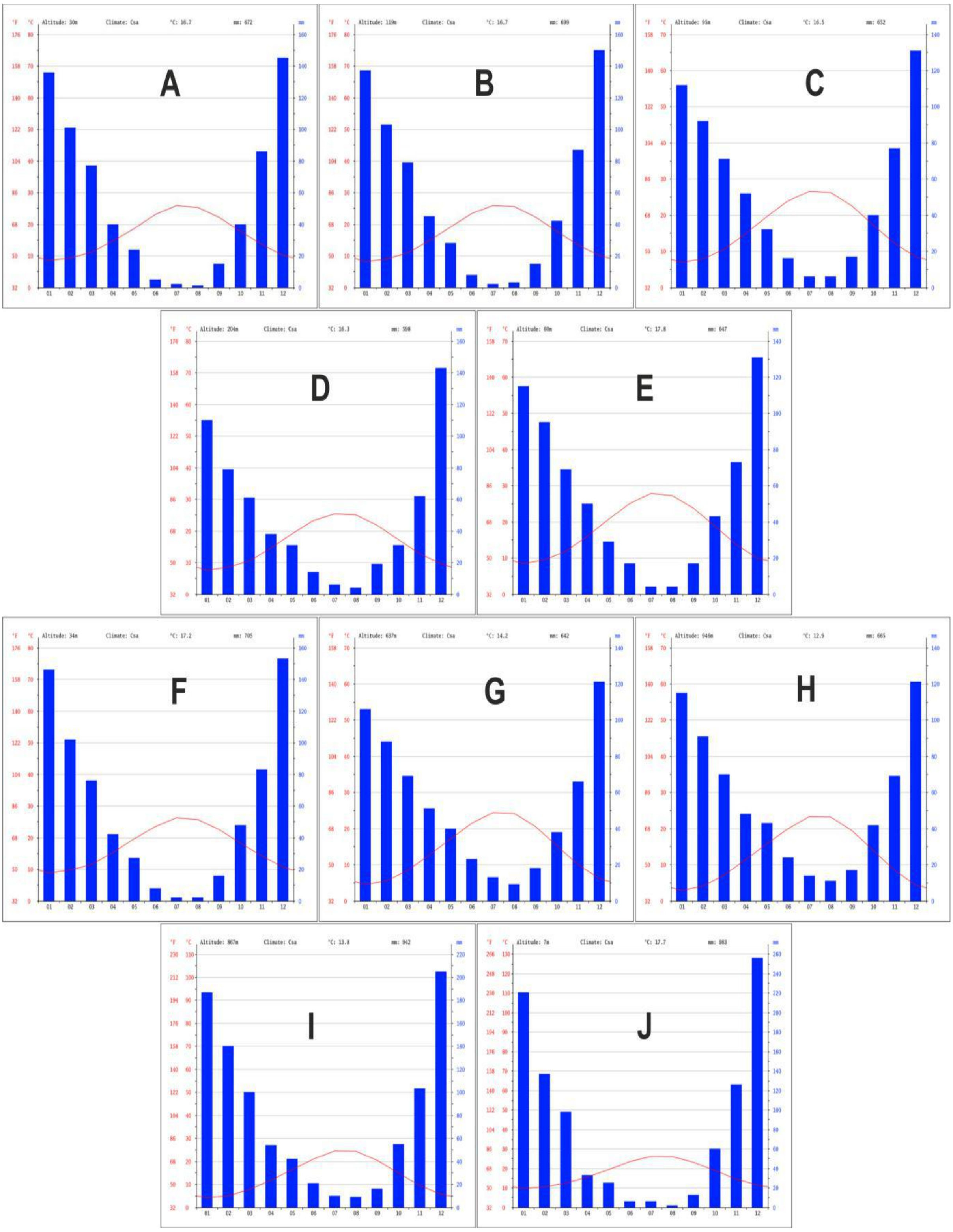

Fig. 2A-J. Avarage temperature and average rainfall amount in the studied localities in western Anatolia: A - Seferihisar, B - Menderes, C - Turgutlu, D - Alaşehir, E - Aydın, central district, F - Söke, G - Buldan, H - Tavas, I - Köyceğiz, J - Fethiye. (Source: tr.climate-data.org).

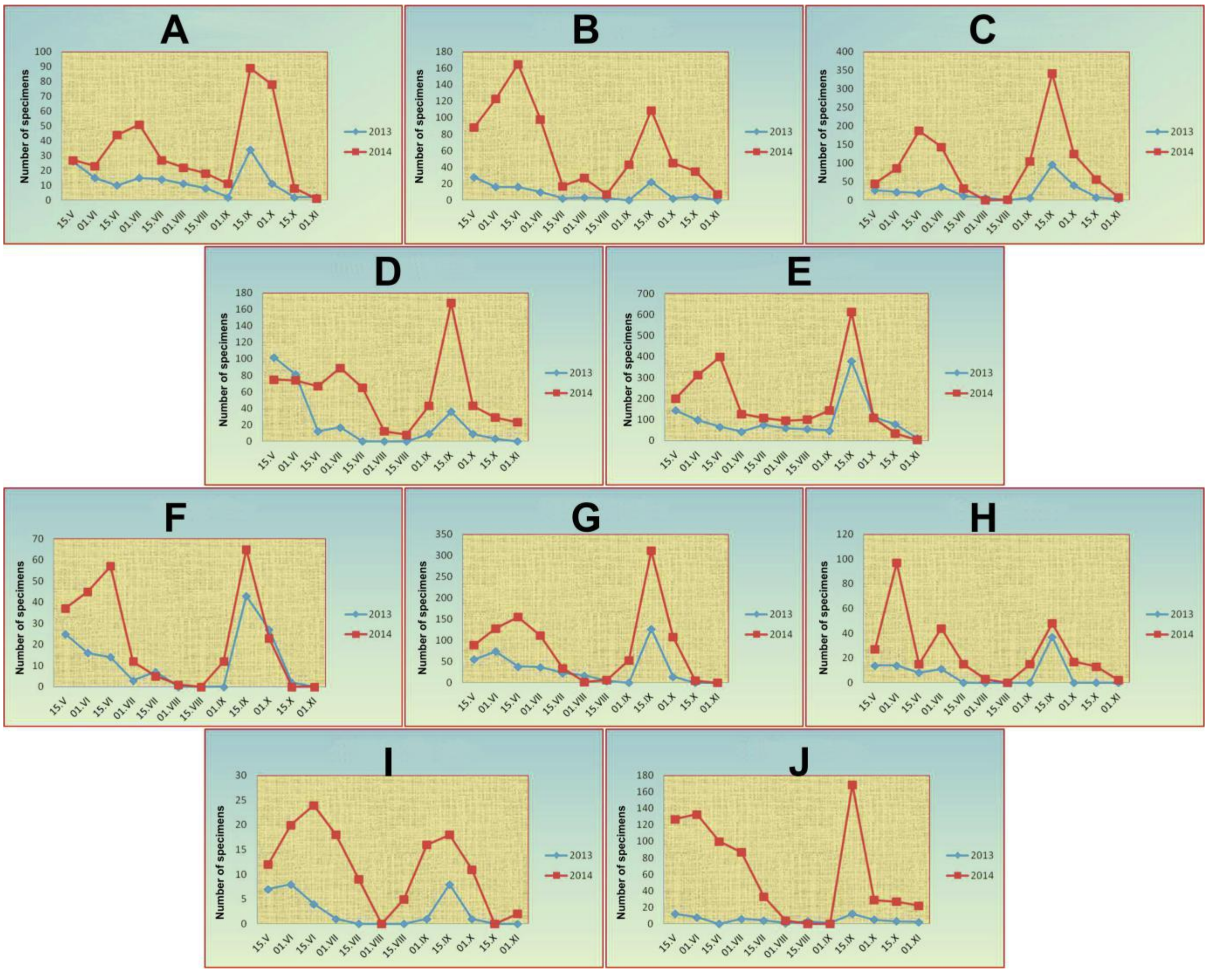

Fig. 3A-J. Seasonal dynamics of *Paederus fuscipes* on the studied localities in western Anatolia during 2003 and 2004. A - Seferihisar, B - Menderes, C - Turgutlu, D - Alaşehir, E - Aydın, central district, F - Söke, G - Buldan, H - Tavas, I - Köyceğiz, J - Fethiye.

Conclusions

The data obtained in the current study showed that the most dominating and common species in the western Anatolia is *Paederus fuscipes*, with 10.322 specimens. This, also indicates that it can reach high numbers large enough to threaten public health.

According to our findings, the months that have high risk of epidemics caused by Paederus dermatitis in the western Anatolia includes May, June and September. The most risky province is the Aydın province. Particularly, the large number of specimens trapped in the traps we have laid in the Adnan Menderes University, Agriculture Faculty garden located in the center of Aydın province had led to the conclusion that Aydın province can encounter the risk of Paederus epidemics from time to time. However, their presence in large numbers in the agricultural lands in Aydın (particularly in cotton fields), as seen in our field studies, is a positive sign for agriculture, because *Paederus fuscipes* is the predator of many harmful species in the agricultural sense. We estimate that specimens collected in 2014 being larger in number as compared to those collected in 2013 is related to the heavy showers recorded in June and September following the winter and spring seasons that were dry. However, no such intense rain was recorded in these months of 2013. *P. fuscipes* species had reached large numbers especially following the showers, and therefore they were caught in our traps. Seasonal activities of *Paederus* species are closely related with the humidity level.

Based on our observations, their population reached large numbers particularly in humid weathers and following rain in all light trapping localities.

Although the *Paederus* species and pederin substance are important in medicine and agriculture, they have not been investigated adequately yet. The reasons for this can be listed as the difficulties of the identification of *Paederus* species, taxonomic confusions in their classification, inadequacies in the information related to their distribution and inadequate information about their seasonal activities. On the other hand, the statements related to *Paederus* studies made in the few studies that have been carried out have been reported without being supported sufficiently with digital and/or statistical data. With all these reasons, as a result of this study aiming at removing some deficiencies of knowledge in the literature, the species known in Turkey and the Aegean Region have been determined, and the seasonal activity of the *P. fuscipes* species with a study spread to two years as a first in the world. Based on our observations, *Paederus* species do not hide below under stones or in the soil in contrast with other staphylinds, and wander around borders of damp places, and their flights and being oriented to light (fluorescent lamps, specially) coincide with evening and night hours. Thus, they reach large numbers particularly at evening hours in hot and moist spring and summer months.

Global warming and changes in precipitation regimes specific to the climatic changes in the world that have been speeded up especially within the last 30 to 40 years have started to affect Turkey and adjacent countries also. With this reason, we foresee that changes will be seen in the seasonal activities of *Paederus* species, and their numbers can increase rapidly in relation with sudden showers during the hot summer months with the possibility of causing significant health problems. Likewise, the variations in the numbers of specimens trapped between 2013 and 2014 according to both years and months and the numbers of specimens trapped after rain in summer months in our study confirm this foresight.

More studies on the ecology and phenology of *Paederus* species are needed, which is important in both public health and agricultural senses. It is hoped that current data on the species of *Paederus* will be contributed more studies that will be carried out in other countries.

Acknowledgments

This study was supported by the Scientific and Technological Research Council of Turkey (TUBITAK, project no. 112T907).

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Received: 03.02.2017
Accepted: 26.06.2017

ECOLOGIA BALKANICA
2017, Vol. 9, Issue 1 June 2017 pp. 39-51

Distribution and Resources of the Medicinal Plant Colchicum autumnale L. in Bulgaria

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Abstract. *Colchicum autumnale* (Colchicaceae) is a perennial geophyte and a medicinal plant. Its biomass is collected for industrial uses for obtaining the alkaloids colchicine and demecolcine. The objective of the present study was to estimate the distribution and potential resources of *C. autumnale* populations in Bulgaria in terms of their sustainable use. Monitoring of habitats was carried out in concrete harvesting areas. The distribution of the populations and the amount of drug production in specific sites and conditions were studied. In 2014-2015, eleven localities were established in seven floristic regions, spread on an area of 498000 m². Seed resources obtained from the different populations ranged from 3.57 g to 12225 g. The seed yield depends on the environmental conditions, the number of plants per m², the number of fruit capsules per plant and the weight of the seeds contained in them. Changes in the management approach to habitats occupied by *C. autumnale* caused degradation of the areas, resulting in the decrease of the population density of the species.

Key words: *Colchicum autumnale*, meadow saffron, autumn crocus, resources, medicinal plant, Bulgaria.

Introduction

Colchicum autumnale (meadow saffron, autumn crocus) is a valuable medicinal plant containing over 20 alkaloids, such as colchicine, demecolcine, colchicoside, etc. Colchicine is among the major alkaloids and it is an acid amide derivative of tropolone (Boichinov & Ahtardzhiev, 1969). At lower doses (0.0001 g) it is used for treatment of gout and it was proven to have an antitumor effect (Asenov et al., 1998). In the world literature it has been announced that the drugs obtained from the plant species are used in veterinary medicine for arthritis and as a diuretic (Jaeger & Flesch, 1990).

In world literature studies of the species were most often directed to: 1) investigating the species as a raw material for pharmaceutical industry and 2) investigating the species as poisonous for livestock after grazing on pastures, in which there are populations of *C. autumnale*.

A significant share of the research studies in world literature were aimed at determining the chemical composition of the species. Some of them refer to the territory of Turkey where representatives of *Colchicum* genus are spread, including endemic species (KAYA *et al.*, 2013). Colchicine dynamics at different periods of collecting specimens of the genus were studied in a series of research experiments, including species not found in our flora (NOGHONDAR *et al.*, 2012; AKRAM *et al.*, 2012; MORTEZA *et al.*, 2013; METIN *et al.*, 2014). POUTARAUD & GIRARDIN (2003) estimated seed alkaloid yield of the species in natural and cultivated conditions. The authors reported an increase of the seed yield under cultivation conditions, due to the increase in the number of capsules per plant. They established that the alkaloid content was stable over the years. MROZ (2008) carried out a study on the effect of the mineral soil content on the population density of the species. The author established differences in the reproductive and vegetative performance of plants in 25 populations as a result of the influence of the different chemical composition of soil.

In different studies on the population density of the species in Central Europe, a number of authors recommended measures for grassland management with the aim of protecting animals against poisoning (JUNG *et al.*, 2011; WINTER *et al.*, 2011, 2014; PERATONER *et al.*, 2014). The authors found out that the early cut, before the formation of the fruit capsules of *C. automnale*, reduced poisoning in animals, regulated the population density of the studied plant species, but also reduced hay yield.

Developing a strategy for limiting the species distribution in Germany, SEITHER & ELSÄSSER (2014) recommended a number of measures. The authors established that mulching treatment (in April-May), mowing or herbicide application reduced the population density of the species.

Scientific literature review shows that there are no current studies on the species in Bulgaria, both on its phytochemistry and on its resources and distribution. The earliest data about the distribution of *C. automnale* in our country were presented in the publications of VELENOVSKY (1892), who mentioned that the species was found in the regions of Lovech, Troyan, Yablanitsa, Knyazhevo, etc.

URUMOV (1906; 1908; 1917; 1929) announced that the species was found in the region of Krapetz and between Sevlievo and Lovech, in grassy habitats. The same author noted that the species prefers the meadow communities on the slopes of the village of Shtraklyuvo and the dry pastures in Gorna Klisura, Bratsigovo, etc.

During the same period TOSHEV (1895; 1902; 1903) expanded the knowledge about the distribution of *Colchicum* in the Thracian lowland – Haskovo district, Tatarevo, around Stara Zagora, Kobilin dol, etc.

STRANSKY (1921) and YORDANOV (1923-1924) identified *C. automnale* in dry habitats in the areas of Batak, Beglika, Seminska Kula in the Rhodope Mountains. They also emphasized that the species prefers the grassy communities in Lozen Mountain, Kocherinovo, Govedarnika, around Dupnitsa, Yakorouda, etc.

In "Flora of the Republic of Bulgaria" KOUZMANOV & KOZHUHAROV (1964) mentioned that the species required moist, grassy habitats and it was spread all over the lowland and mountainous parts of the country.

DIMITROV *et al.* (1967) recorded that populations of the species in the region of Kardzhali, the village of Razdel, Knyazhevo and Elhovo.

The revised herbarium specimens and literature showed that the information on the distribution of the species is outdated, it has not been updated over the last 50-60 years and data about the resources were not found. That determined the aim of the present study, namely to investigate the distribution, as well as to establish the resources of *C. autumnale* L. in Bulgaria.

Material and Methods

Colchicum autumnale L. (Meadow Saffron, Autumn Crocus) of Liliaceae/Colchicaceae family was the object of the present study. The investigation was carried out in the period 2014-2015 at mass flowering and fruit-bearing of the species.

The starting point of the study on the species distribution was a review of herbarium specimens in the herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM), the herbarium of Sofia University "St. Kliment Ohridski" (SO) and the herbarium of the Agricultural University, Plovdiv (SOA). The available horological information, the inspected herbarium specimens, personal collections and field inspection were the basis for studying the distribution and current status of *C. autumnale* populations.

Three hundred and thirty two herbarium specimens were studied. Seventy six specimens from the herbarium of the Institute of Biodiversity and Ecosystem Research (SOM), 39 from the herbarium of Sofia University "St. Kliment Ohridski" (SO) and 117 from the herbarium of the Agricultural University, Plovdiv (SOA).

Mapping was conducted following the methodology of KOZHUHAROV *et al.* (1983) by using the route and the point (stationary) methods. The climatic characteristics of the habitats were described according to KOPRALEV *et al.* (2002). The soil types were identified according to "Soil Atlas of Bulgaria" of KOYNOV *et al.* (1998) and according to "Soil Map of Bulgaria" (1968) in scale 1:400 000.

The exact location, the geographical coordinates and altitude of the habitats were determined by GPS (Garmin Dakota 20) and the data are presented in Table 1 and Fig. 1.

The following populations were selected in the study for evaluating the diversity of climatic factors and the relief of the different floristic regions:

Floristic region of Black Sea Coast (South) – Arkutino, climate region of Burgas Lowlands, continental and Mediterranean Climatic Zone.

Floristic region of Thracian lowland (southernmost point) – Mezek, climate region of East-Rhodope river valleys, continental and Mediterranean Climatic Zone.

Floristic region of Struma valley – M. Pole, Kulata, Kresna gorge, located in Petrich-and-Sandanski continental and Mediterranean Climatic Zone.

Floristic region of the Balkan mountain range (Western part) – Balyuvitsa, Balkan foothill climate region of the European Continental Climatic Zone.

Fig. 1. Indicative map of the studied populations.

Table 1. Coordinates and resource of *C. autumnale* in the studied populations (2014-2015).

No./Location/ coordinates	Altitude in m	Net area in %	Total area in m²	Average number of plants per m² X±SE	Weight in g/m²	Resource in g
1.Chirpanskata koriya N42^{0}13'579", E25^{0}15'444"	170	1.5	1000	5.3±0.49	2.96	27.03
2. Kresna Gorge N41046'765", E23009'231"	142	2	20 000	7±0.90	4.76	952
3. Marino pole/Kulata N41024'963", E23021'336"	135	1.5	50 000	3.6±0.37	1.94	729
4. Observatory Rozhen N41041'747" E24044'134"	1774	0.5	1000	2.6±0.30	1.43	3.57
5. Rozhen grasslands N41040'331", E24043'867"	1423	4	100 000	10.4±0.13	5.51	11024
6. Chokmanovo N41031'887", E24043'793"	1090	50	5000	9.8±0.41	5.48	6850
7. Smilyan N41030'650" E24045'291"	799	50	10 000	8.9±0.52	4.89	12225
8. Mezek N41043'875" E26006'020"	132	1	100 000	2.8±0.90	1.56	784
9. Arkutino N42019'931" E27043'263"	29	1	1000	4.0±0.25	2.20	11
10. Elhovo/Balabana N42084'830" E26032'207"	108	2	200 000	10.4±0.68	5.40	10800
11.Balyuvitsa N43029'897" E23020'117"	270	40	10 000	10.8±0.40	5.61	11232

Floristic region of Thracian lowland – Chirpanska Koria, climate region of East Central Bulgaria of the European Continental Climatic Zone.

Floristic region of the Rhodope Mountains (Central part) – Rozhen, in the high mountainous part of the Mountain climate region of the European Continental Climatic Zone.

Floristic region of the Rhodope Mountains (Middle part) – for evaluating the effect of the altitude on the distribution of the species, specimens were collected from the middle mountainous and high mountainous parts of the Mountain climate region of the European Continental Climatic Zone. The resource was developed at four different altitudes: 799 m a.s.l. (Smilyan), 1090 m a.s.l (Chokmanovo), 1423 m a.s.l (the low part of Rozhen) and 1744 m a.s.l (Rozhen Observatory).

Floristic region of Tundzha hilly valley – Elhovo, Balabana locality, in the climate region of East Central Bulgaria of the European Continental Climatic Zone.

The present research study on *C. autumnale* resources was carried out following the methodology of the Russian School by the methods of SHRËTER *et al*. (1986) in concrete habitats (harvesting areas). This methodology has been officially adopted in Bulgaria. It is applied in order to determine the resource potential stock of the medicinal plants in the available populations.

The total area of all the habitats was measured in m^2 by longitudinal transect routes.

Determining the yield harvested from the habitats was performed by setting control sites in accordance with the requirements of the methodology. As the studied species is spread in spots (colonies), the area of the entire territory was determined first and then the percentage of the net area occupied by the species was calculated. The yield obtained was measured by collecting the capsules with seeds, following the instructions for gathering and drying of herbs. The dried seeds were weighed in grams with a precision of ±5%. The results obtained were processed by the methods of variation statistics. Mean arithmetic values (M) of the studied indicators and standard errors of the mean arithmetic (SE) were calculated.

The possible annual resource was estimated, as follows: the net area occupied by the species was multiplied by the lowest seed yield (M-2SE) and then the obtained exploitation stock was divided by the number of years necessary for the population to be restored according to the requirements of the methodology. When estimating the resources of the studied medicinal species, the plants found in settlements, along railways and highroads and in contaminated areas, were not taken into consideration, following the methodology used.

Phytocenological description of the habitats was made when evaluating the resource, as the plant species are an indicator of the conditions existing in the biotope and an evidence of the balance in the environment. Descriptions were made following the principles of the Western phytocenological school of BRAUN-BLANQUET (1964). The projective cover of the vegetation from each species was visually assessed. Five-point cover-abundance scale was used in the fieldwork, which is of a mixed type – numeric and symbolic. Natural habitats were determined according to phytocenological descriptions, following the "Guidelines for determining the habitats of European importance in Bulgaria" (KAVRAKOVA *et al*., 2009).

Results and Discussion

According to the distribution of the deposited herbarium specimens, the largest amount of material of *C. autumnale* was collected from the Floristic Region of the Rhodopes (Western, Central and Eastern parts) – 26.58%. The species was found in grassy habitats in the region of Batak dam, the villages of Startsevo, Sivino, Smilyan, Byala Cherkva, etc.

Second came the Floristic Region of the Balkan Mountain Range (Eastern, Central, Western) – 22.78%. The species is distributed in Kalofer Dzhendem, above Karlovo, above the town of Berkovitsa, etc.

According to the deposited herbaria, the next group comprised the Floristic Region of Sofia (13.29%) and the Floristic Region of the Upper Thracian lowland (10.12%). The presented herbaria were collected from Mezek, Elin Pelin, the district of Stara Zagora, Panagyurishte, etc.

Single deposited specimens had been collected in the Floristic Regions of the Danube, Black Sea Coast, North-East Bulgaria, Rila Mountain, Slavyanka Mountain, Struma valley. It should be mentioned that all the deposited specimens were collected in the period 1921-1967. In the first year of the study, 11 populations of the species were established, distributed in 7 Floristic Regions of Bulgaria. *C. autumnale* was found at the Black Sea Coast, in the lowlands of the Thracian, Struma and Tundzha valleys, as well as in the highlands of the Rhodopes and the Balkan Mountains. The altitude varies from 29 to 1744 meters above sea level (Table 1). The soil types, on which the populations were mapped, also varies – leached cinnamon forest (Cromic Cambisols/FAO), gray forest, alluvial-meadow (Fluvisols/FAO), forest brown (Cambisols/FAO).

The first studied population was established in the protected area Chirpanska Koria in Thracian Floristic Region. The area is located on the border between Pazardzhik and Plovdiv districts and Stara Zagora district. It is characterized by transitory-continental climate and the influence of the

Mediterranean Sea is also felt (KOPRALEV *et al.*, 2002). Soils are typical Pellic Vertisols/FAO and Fluvisols/FAO, characterized by varying content of humus and total major elements (KOINOV *et al.*, 1998). The total area of the habitat is 1000 m^2 and the net area of distribution is 1.5%. The average number of plants of the studied species is 5.3 per square meter. The possible resource potential per sq. m. is insignificant – 3.60 g (Table 1). The total population resource is scarce (27.3 g) and it has no practical value.

The phytocenological description (Table 2) shows that the dominating species are *Cynodon dactylon*, *Potentilla erecta* and *Dactylis glomerata*, the *Poaceae* family being most represented.

The second studied population occupies Kresna gorge in the region of "Kresna Hanche" in the Floristic Region of the Struma valley. The gorge connects Simitly valley to the north and Petrich-Sandanski valley to the south (KOPRALEV *et al.*, 2002) and the climate is characterized by mild winters and dry summers. The soil types, on which the species grow in that region, are shallow leached Cromic Cambisols/FAO (Soil Map of Bulgaria 1968; KOINOV *et al.*, 1998). The total area occupied by the species is 20000 m^2. The mean number of plants per sq. m. is 7 with a yield of 4.76 g/m^2. The net area occupied by the species is 2%. The total population resource is 952 g. The other species found are mainly *Juniperus oxycedrus*, *Paliurus spina-christi* and *Jasminum fruticans* (Table 2). Those plants are diagnostic of habitat 5210 "Scrubs with *Juniperus sp*." (KAVRAKOVA *et al.*, 2009).

The third population was established on the territory of the village of Marino Pole (Petrich municipality). It is located in Sandanski-Petrich valley along the left bank of the Struma river, also in the Floristic Region of the Struma valley. The climate is continental Mediterranean, with hot and dry summers. The soil types, on which the *C. autumnale* grows, are eroded Cambisols/FAO and Fluvisols/FAO (KOINOV *et al.*, 1998). The population consists of single plants, 3.6 per m^2. in average. The species is scattered among light-permitting shrubs, mainly *Paliurus spina-christi*, *Carpinus orientalis*, *Crataegus monogina* (Table 2). The habitat could be defined as 5210 "scrubs with *Juniperus sp*." (KAVRAKOVA *et al.*, 2009), which is the final stage of degradation of xerothermic oak forests in South Bulgaria. Total area of the habitat is 50000 m^2. The net area occupied by the species is 1.5%. The estimated yield is 1.94 g/m^2 and the resource potential is 729 g.

Four of the studied populations were found in the Floristic Region of the Rhodope Mountains. It includes the central mountainous and the high mountainous parts of the Mountain Climate Region of the European Continental Climatic Zone. The resources of the species were assessed at four different altitudes: 799 m a.s.l. (Smilyan), 1090 ma.s.l. (Chokmanovo), 1423 m a.s.l. (the low part of Rozhen) and 1744 m a.s.l. (Rozhen Observatory).

The population growing at the highest altitude (1744 m a.s.l) occupies an area of 1000 m^2 around the Rozhen Observatory. The soil types are mountain meadow, secondary grassed brown forest (Cambisols/FAO) (KOINOV *et al*., 1998). The mean number of plants is 2.6 pcs./m^2 with a yield of 1.43 g/m^2. The established total resource is 3.57 g, the net area being 0.5 %. Observations on the development of the species in that population showed that a large number of the fruit capsules were underdeveloped and they had 3-4 seeds. Probably the reason is the higher altitude, cooler climate and smaller number of insects for pollination. The phytocenological description (Table 2) shows that the vegetation is typical of habitat 6520 "Mountain hay meadows" (KAVRAKOVA *et al*., 2009), with a large number of representatives from *Fabaceae* family.

The next habitat is located at 1423 m a.s.l. in the locality Rozhen Meadows, situated in Rozhen saddle. It is the climatic border of the Mediterranean influence. The area is characterized by the rapid drop of temperatures and a precipitation increase with height. The soil types are mountain meadow, secondary grassed brown forest (Cambisols/FAO). They are characterized by low natural fertility due to the processes of acidification and erosion (KOINOV *et al*., 1998). Comparatively large number of plants per m^2 (10.4) was registered in that population, with a yield of 5.51 g. Our observations revealed that the fruit capsules per plant were 3-4 in average. Weight and number of seeds in the capsules varied significantly.

The area occupied by the species is 100000 m² and the net area is 4%. The total resource is 11024 g. The species is concentrated at the edges of the meadows along the border with the forest. The population of the species (Table 2) is found in a typical habitat 6520 "Mountain hay meadows" (KAVRAKOVA *et al.*, 2009). Grasslands are dominated by *Agrostis capillaris*, *Calamagrostis arundinaceae* and *Dactylis glomerata*.

The next habitat of the species in the Floristic Region of the Rhodopes is distributed at the outskirts of the village of Chokmanovo at 1090 m a.s.l. Soil types are brown forest (Cambisols/FAO), with a light mechanical composition, acid in nature, with a low content of mineral substances (KOINOV *et al.*, 1998). The established population density is comparatively high – 9.8 pcs./m² and the yield is 5.48 g. The total area of the habitat is 5000 m², the net area being 50%. The possible resource of the habitat is 6850 g. The phytocenological evaluation of the population (Table 2) showed that the vegetation in the habitat was strongly influenced by the anthropogenic activity and it has lost its natural appearance. A significant number of ruderal species are found, typical of abandoned agricultural ecosystems (*Daucus carota*, *Cynodon dactylon*).

The last population of *C. autumnale* in the Floristic region of the Rhodopes was found on the territory of the village of Smilyan at 799 m a.s.l. The species is spread on mountain meadows used for hay production. The soil types are brown forest (Cambisols/FAO), (KOINOV *et al.*, 1998). The average number of plants per sq. m. is 8.9, the yield is 4.89 g. The total area occupied by the population is 10000 m², the net area being 50%. The possible annual resource of that population is 12225 g. The phyto-cenological evaluation of the population (Table 2) showed that vegetation in the habitat was strongly influenced by the anthropogenic activity.

The next studied population of *C. autumnale* was established at the southernmost point of the Floristic region of Thracian lowland (Mezek). The species was found in a plant community typical of 5210 "scrubs with *Juniperus sp*." (KAVRAKOVA *et al.*, 2009). It is characterized by shrubs and grasses dominated by *Festuca sp.*, *Clinopodium vulgare* and *Dactylis glomerata* (Table 2). The area is influenced by Continental Mediterranean climate and the soil types are leached cinnamon (Chromic Cambisols/FAO). They are characterized by a high content of clay, a low content of mineral substances and comparatively high humus content (KOINOV *et al.*, 1998). The total area occupied by the plant is 100000 m² and the net area of the studied species is 1%. The average number of plants per m² is 2.8, but due to the large area of the habitat the obtained resource is 784 g.

The ninth population was found in the Floristic region of the Black Sea Coast (South) close to the state hunting area 'Ropotamo' – Arkutino. The population is located at the edges of meadows bordered by dense longos forests. The soil types in that region are alluvial-meadow, delluvial (Fluvisols/FAO). They are characterized by shallow ground waters and a low content of nitrogen and phosphorus and well supplied with potassium (KOINOV *et al.*, 1998). The total area of the habitat is 1000 m² and the net area occupied by the species is 1%. The established yield is 2.2 g/m² and the possible resource of the population is 11 g. The vegetation is described in Table 2.

The tenth studied population of *C. autumnale* is located in lowland meadows close to the maintained reserve "Balabana" in the Floristic Region of Tundzha hilly valley. The area of its distribution in that Floristic Region is influenced by transitional continental climate, characterized by hot summers and mild winters, but strong north-east winds (KOPRALEV *et al.*, 2002). The species is distributed in groups or as single plants. The total area occupied by the *C. autumnale* population is 200000 m² and the net area is 2%. The established resource is 10800 g and the yield is 5.4 g/m² (Table 1). Plant vegetation is dominated by wheat grass species and it is characterized by rich diversity of species (Table 2). The habitat is attributed to 6510 "Lowland hay meadows" (KAVRAKOVA *et al.*, 2009).

Balyuvitsa is the eleventh studied population (Table 2), found in the Floristic region of the West Balkan mountain range of the Balkan foothill climate region. The climate is characterized by comparatively cool summers, increased amounts of summer rainfalls and the influence of the Balkan mountain range. The population is spread on an area of 10000 m². The soil types are typical of the region – gray forest to light gray forest soils (Ortic luvisols/FAO). They are characterized by a low content of humus, low contents of total nitrogen and phosphorus (KOINOV *et al.*, 1998). The net area occupied by *C. autumnale* is 40%, which is a comparatively high population density. The average number of plants is 10.8 pcs./m² and the yield is 5.61 g. The resource potential is 11232 g.

From the literature review it becomes clear that the species was widely distributed in Bulgaria in the period of 1923-1964 (KOUZMANOV & KOZHUHAROV, 1964). High plant populations were established. As a result of the present research and the conducted field observations, a number of widespread localities of the species were not found in some floristic regions (Northeast Bulgaria, Eastern Balkan Mountain, etc.). Most often single plants were found in herbaceous communities, which do not constitute an industrial interest. The observations showed that the anthropogenic impact, namely the urbanization of some of the habitats, had a negative effect on the population density of the species (construction, drainage, etc.). We assume that the individual plants, found in the habitats, remained from the larger populations occupied by the species in previous years. That suggestion confirmed the results obtained in the study of ADRIAENS *et al.* (2009). Those authors noted that environmental changes decreased the survival rate of the plants. They responded by reduced reproductive capacity (generative and vegetative).

During the study carried out on the distribution of *C. autumnale* in seven Floristic Regions of Bulgaria, it was found out that the species form small (2.6-5.3 plants per m² in average) to large groups (7-10.8 plants per m² in average). It was established that the number of fruit capsules varied from 1-2 to 4-5 in the separate plants in the different habitats. Probably the reason for the variation is complex (the mineral composition of soil, climatic characteristics of the region, etc.). Often some of the fruits were underdeveloped and seedless. Seed amount and weight per plant also showed variable values (0.35-0.56 g). The analysis of the results shows that the population showing the highest resource capacity (12225 g) is the one growing in the territory of the village of Smilyan. That resource is a result of the high percentage of net area occupied by the species and the number of plants per m². Next in the ranking come the populations located in Balyuvitsa (11232 g). The population in Elhovo region, which is close to 'Balabana' maintained reserve, also has a high resource capacity (10800 g). That could be explained by the fact that it is the largest area, on which the species is found. The average number of plants is high (10.4 pcs./m²), and hence, the harvested yield. Similar resource potential was also established in the populations spreading in Rozhen meadows (11024 g) and Chokmanovo (6.850 g).

The lowest resource was established in the habitats of the species in the vicinity of Arkutino (11 g), Rozhen observatory (3.57 g) and Chirpanska Koria (27.3 g) and those habitats have no practical value. From the field study it became clear that the distribution of the species in some populations (Chirpan, M. pole, Arkutino) is on abandoned areas, heavily affected by anthropogenic activities in the past. Usually plant species diversity is confined in those areas and it is rich in ruderal species (*Prunus spinosa*, *Cynodon dactylon*, etc.). Processes of secondary succession are going on there, which have an adverse effect on the development and distribution of the species. Changes in the regime of pasture management are likely to cause degradation of the areas and a reduction of the population density, which determines the limited resource.

In highland populations (Rozhen Meadows), traditionally used as meadows for hay production, *C. autumnale* appears as a species causing poisoning of the animals and the farmers destroy the species in that area. Therefore it is found at the edges of the meadows along the border with the forest. Evaluating the effect of the altitude on the formation of the resource, it was established that out of the four studied populations in the Floristic Region of the Rhodopes, the resource of the population located at the highest altitude (Rozhen Observatory 1744 m a.s.l.) is insignificant, as only single plants were found. Similar resource was established for the other three populations.

Table 2. Cover-abundance in the studied areas with *C. autumnale* L. populations (2014-2015).

Studied area	Species	Assessment Braun-Blanquet	Species	Assessment Braun-Blanquet
Chirpanskata koriya	*Poa bulbosa*	1	*Centaurea sp.*	+
	Cynodon dactylon	2	*Inula conyza*	+
	Potentilla erecta	+	*Plantago lanceolata*	1
	Dactylis glomerata	1	*Polygonum aviculare*	1
	Potentilla argentea	1	*Epilobium parviflorum*	+
	Convolvulus arvensis	+	*Colchicum autumnale*	1
	Achillea millefolium		*Ulmus minor*	r
	Pyrus pyraster	r	*Rosa canina*	r
	Inula conyza	+		
Kresna Gorge	*Jasminum fruticans*	1	*Plumbago europea*	+
	Juniperus oxycedrus	2	*Achilea millefolium*	+
	Fraxinus ornus	2	*Verbascum sp.*	r
	Clematis vitalba	+	*Gypsofila sp.*	r
	Quercus polycarpa	+	*Teucrium polium*	+
	Colchicum autumnale	1	*Asparagus sp.*	r
	Paliurus spina-christi	3	*Chrysopogon gryllus*	+
Marino pole/Kulata	*Carpinus orientalis*	2	*Brachypodium sylvaticum*	1
	Juniperus oxycedrus	+	*Poa bulbosa*	r
	Fraxinus ornus	1	*Quercus pubescens*	r
	Crataegus monogina	2	*Colchicum autumnale*	+
	Cornus mas	r	*Asparagus sp.*	r
Observatory Rozhen	*Festuca rubra*	3	*Trifolium pratense*	1
	Trifilium repens	2	*Nepeta sp.*	r
	Lathyrus sp.	2	*Mentha sp.*	r
	Heracleum sibiricum	1	*Silene bupleroides*	+
	Trifolium latinum	1	*Ranunculus sp.*	r
	Erodium cicutarium	+	*Myosotis sp.*	r
	Vicia sp.	r	*Fragaria vesca*	+
	Alchemilla sp.	r	*Pastinaca sativa*	r
	Stachys sp.	+	*Colchicum autumnale*	+
	Taraxacum sp.	+	*Viola sp.*	r
Rozhen grasslands	*Calamagrostis arundinaceae*	2	*Euphorbia helioscopia*	1
	Agrostis capillaris	2	*Carduus sp.*	r
	Dactylis glomerata	2	*Thlaspi arvense*	+
	Festuca rubra	1	*Sanguisorba officinalis*	+
	Teucrium chamaedrys	1	*Galium sp.*	+
	Potentilla reptans	+	*Fragaria vesca*	+
	Trifolium repens	1	*Jniperus communis*	r
	Verbascum thapsiforme	+	*Picea abies*	r
	Lotus corniculatus	1	*Achillea sp.*	r
	Plantago lanceolata	+	*Thamus sp.*	+
	Potentilla ternata	+	*Salvia pratensis*	+
	Colchicum autumnale	2	*Rumex sp.*	r

Chokmanovo	*Bromus mollis*	1		
	Festuca rubra	1	*Daucus carota*	2
	Prunella vulgaris	1	*Agremonia eupatoria*	+
	Lotus corniculatus	1	*Cynodon dactylon*	2
	Potentilla reptans	+	*Colchicum autumnale*	1
	Plantago lanceolata	1	*Asparagus sp.*	r
	Clinopodium vulgare	+	*Viola odorata*	r
	Teucrium chamaedrys	1	*Trifolium repens*	1
	Centaurium erythraea	r	*Thamus sp.*	+
	Veronica officinalis	r		
Smilyan	*Festuca sp.*	3	*Colchicum autumnale*	1
	Poa sp.	2	*Prunus spinosa*	r
	Plantago lanceolata	1	*Potentilla argentea*	1
	Teucrium chamaedris	1	*Inula sp.*	+
	Achillea millefolium	r	*Cynodon dactylon*	1
	Daucus carota	+	*Sonchus sp.*	r
	Ononis sp.	r	*Setaria pumila*	r
Mezek	*Juniperus oxycedrus*	3	*Plantago lanceolata*	1
	Rosa canina	1	*Cirsium sp.*	+
	Paliurus spina-christ	2	*Centaurea stoebe*	+
	Pyrus amygdaloides	+	*Eringium campestre*	r
	Rubus sp.	1	*Dianthus campestris*	r
	Scabiosa sicula	1	*Sanguisorba minor*	1
	Festuca sp.	3	*Cichorium intybus*	+
	Dactylis glomerata	2	*Echium italicum*	+
Arkutino	*Clinopodium vulgare*	2	*Prunus cerasifera*	r
	Potentilla reptans	2	*Prunus spinosa*	r
	Hieracium hoppeanum	3	*Pyrus bulgarica*	r
	Campanula sparsa	r	*Colchicum autumnale*	1
	Linaria sp.	+	*Agrimonia eupatoria*	1
	Galium verum	+	*Hypericum perforatum*	1
	Taraxacum sp.	2	*Cichorium intybus*	+
	Cynodon dactylon	3	*Trifolium repens*	2
	Alliaria petiolata	+	*Daucus carota*	1
	Inula sp.	+	*Plantago lanceolata*	1
	Rumex sp.	r	*Colchicum autumnale*	+
Balabana	*Agrostis stolonifera*	2	*Ranunculus acris*	2
	Festuca arundinacea	3	*Convolvulus arvensis*	+
	Trifolium ptatense	1	*Sanguisorba minor*	r
	Alopecurus pratensis	2	*Cynodon dactylon*	1
	Carex caryophyllea	+	*Tragopogon pratensis*	r
	Dactylis glomerata	2	*Lysimachia nummularia*	+
	Cirsium conium	1	*Prunella vulgaris*	1
	Cynosurus cristatus	1	*Daucus carota*	r
	Stellaria graminea	+	*Colchicum autumnale*	1
Balyuvitsa	*Dactylis glomerata*	2	*Thlaspi arvense*	+
	Festuca rubra	1	*Sanguisorba officinalis*	+
	Teucrium chamaedrys	1	*Galium sp.*	+
	Potentilla reptans	+	*Fragaria vesca*	+
	Trifolium repens	1	*Jniperus communis*	r

Lotus corniculatus	1	*Achillea sp.*	r
Plantago lanceolata	+	*Thamus sp.*	+
Potentilla ternata	+	*Salvia pratensis*	+
Colchicum autumnale	2	*Rumex sp.*	r
Bromus mollis	1	*Cynodon dactylon*	r

Despite of the relatively high resource obtained in some habitats, it should be emphasized that the changes in land management, drainage of many territories and changes in climatic environmental factors are the most likely reason for the disappearance of the species or for the reduction of the population density.

Conclusions

Depending on the amount of the obtained resource, the populations of *C. autumnale* can be grouped, as follows:

Areas with a large resource potential – from 6850 to 12225 g. That group includes 5 populations (around Smilyan, Balyuvitsa, Rozhen Meadows, Balabana, Chokmanovo), representing 45.45% of all the populations. The total exploitation resource of that group is 50131 g.

Areas with a medium resource potential – from 729 to 952 g. That group includes three populations (Kresna, M. pole, Mezek), representing 27.27%. The total exploitation resource of that group is 2465 g.

Areas with a low resource potential – from 0.01 to 27.3 g. That group includes three populations (Chirpanska Koria, Arkutino, Rozhen Observatory), representing 27.27%. The total exploitation resource of that group is 41.6 g.

We recommend collecting plant resources of the species from populations with a large resource potential – around Balabana, Smilyan, Rozhen Meadows, Balyuvitsa. Collecting the fruit capsules will free the meadows from seeds and prevent poisoning of animals.

Acknowledgements. The present study was supported by the Scientific Research Centre of the Agricultural University Plovdiv: Project №12-15: "Biological and phytochemical study of some species of the genus *Colchicum* L. (Liliaceae Hall.) and genus *Galanthus* L. (Amaryllidaceae Lindl.). The authors are thankful to Prof. PhD Malgorzata Berova Agricultural University-Plovdiv for the support of the activities of this research group.

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Received: 28.11.2016
Accepted: 20.02.2017

ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 June 2017 pp. 53-62

Photosynthetic Pigments as Parameters/Indicators of Tree Tolerance to Urban Environment (Plovdiv, Bulgaria)

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Abstract. As polluted air is a stress factor that contributes to the decline of trees in urban areas, we aimed to investigate the complex impact of anthropogenic activity on chlorophylls and carotenoids content of four tree species (*Acer heldreichii* Boiss., *Tilia tomentosa* Moench, *Fraxinus excelsior* L. and *Pinus nigra* L.). Seedlings were purchased from certified greenery and planted by us at four selected sites in the city of Plovdiv (Bulgaria) during spring of 2015. Leaf samples were taken monthly and photosynthetic pigments content was measured immediately after sampling. Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. Although an extension to the above work is necessary to quantify possible differences between the levels at which photosynthetic components are affected, it is obvious that both ratios chlorophyll a/b and chlorophylls/carotenoids could serve as very useful indicators of stress level. Because of the non-specificity in pigment reaction to different type of anthropogenic impact, we recommended to apply a combination among pigments concentration and another parameters (morphological, biochemical, physiological) for the targets of biomonitoring.

Key words: photosynthetic pigments, air pollution, urban environment, *Acer heldreichii, Tilia tomentosa, Fraxinus excelsior, Pinus nigra*.

Introduction

Over billions of years, green plants have formed as a perfect system for absorbing and transforming solar radiation. Radiation absorbed by plant communities is included as a driving force of all life processes or remains stored in the accumulated biomass, defining the overall productivity of plants. Productivity is a complex and multi-step process that involved many different interconnected and interdependent processes. In the ontogenetic development plants are subjected to the continuous impact of environmental factors, including the atmospheric anthropogenic pollution. Exceeding values above the permissible level, and the continued operation of the average concentrations of toxic gases, aerosols and dust negatively affect the status and operation of the plant organism. Green plant as a complex integrated system operating on the principle of feedback and self-regulation is particularly vulnerable to any extreme influence.

Some authors have discussed that the plants have growing under conditions of constant "re-adaptation" to the dynamic

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presence of various contaminants in the environment. According to Levitt (1972) sustainability of the plant organism to the impact of extreme factors is determined by two basic reactions - "ability to avoid stress" and "resistance to stress". Stress effects on plants have three stages: 1) primary stress reactions; 2) adaptive responses; 3) weight loss and death. In a strong but short-term stress exhibit the nonspecific adaptive responses while in a prolonged impact – the specific mechanisms. It has been shown that repeated high doses of stress cause a kind of "hardening" of the plant to stress impact and that "curing" to one stress factor may lead to adjustments of the body and to other stress factors.

Chlorophyll content is an important parameter to evaluate the effect of air pollutants on plants as it plays an important role in plant metabolism. For example, it can be used as an index of the photosynthetic potential as well as of the plant productivity (Carter, 1998) and is closely related to various types of plant stresses and senescence (Gitelson & Merzlyak, 1994). Carotenoids are accessory pigments and essential structural components of the photosynthetic antenna and reaction centers in higher plants. As non-enzymatic antioxidants, their main function is to protect the photosynthetic apparatus, dissipating energy to avoid harmful photooxidative processes.

Aim of this study is to make field evaluation of air pollution tolerances of three deciduous (*Acer heldreichii, Tilia tomentosa, Fraxinus excelsior*) and one coniferous (*Pinus nigra*) trees as reflected by their photosynthetic pigments content.

Material and Methods

Sampling plots. For the purposes of our study, four sampling plots were selected on the basis of typology of urban environment they represent and the type of anthropogenic impact (Fig. 1). Seedlings of selected tree species were purchased from certified greenery and planted in the plots during the spring of 2015.

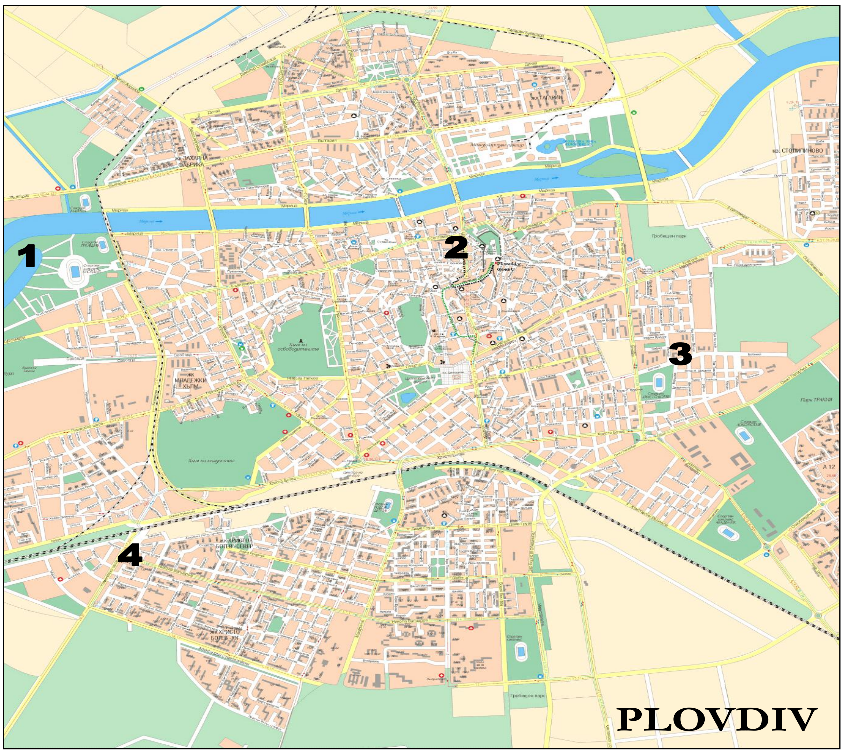

Fig. 1. Map of Plovdiv and locations of the four selected plots: Plot 1 – at City park "Recreation and Culture" (Control); Plot 2 – near Automatic station for environmental monitoring "Banya Starinna; Plot 3 – at Automatic station for environmental monitoring "Kamenitza"; Plot 4 – at Komatevo Road Junction.

Plot 1 was chosen in the West part of the city (NW direction), in the big city park "Recreation and Culture", subjected to a low anthropogenic impact. Trees were planted in a large green area, close to the city outskirts and we aimed to use them as a "provisional control" of urban environment.

Plot 2 was chosen in the Central part of the city (NE direction), along to a busy road junction and close to the Automatic station for environmental monitoring, named "Banya Starinna". Trees were planted in a large green area, located at a distance of about 5 m of the junction.

Plot 3 was also situated in the Central part of the city (SE direction), close to the Automatic station for environmental monitoring, named "Kamenitza" and was characterized by moderate household pressure. Trees were planted in small green patches, 8–10 m away from tall buildings.

Plot 4 was chosen in the West part of the city (SW direction), "Smirnenski"suburb and the trees were planted in a large green area, situated between two railroad tracks and the "Komatevo" road junction.

Plant species. Based on our previous researches (PETROVA *et al.*, 2014) and on the literature review (PICZAK *et al.*, 2001; ROMANIC & KRAUTHACKER, 2004; AKSOY & DEMIREZEN, 2006; SERBULA *et al.*, 2013), three deciduous and one coniferous tree species were selected.

Acer heldreichii, common names Heldreich's maple, Greek maple and Balkan maple, is a European species of maple. It is native to Greece, Albania, Bulgaria, Macedonia, Montenegro, Kosovo, Serbia, and Bosnia-Herzegovina. Tree is up to 20 m, with broad, spherical crown. Leaves are 5–14 cm long, deeply 5-lobed with middle lobe free nearly to base; lobes acute, with 2 or 3 large teeth on each side, dark green, glabrous above, light green and hairy on veins beneath. Fruits are glabrous, with arcuate wings usually diverging at an obtuse angle. *Acer platanoides* is well known biomonitor of air pollution (SAEBO *et al.*, 2012; PETROVA *et al.*, 2014), but we could not find any information about *Acer heldreichii* as an object of biomonitoring studies. That was the reason for choosing this species.

Tilia tomentosa, common names Silver lime or Silver linden, is native to southeastern Europe and southwestern Asia, from Hungary and the Balkans east to western Turkey, occurring at moderate altitudes. *T. tomentosa* is a deciduous tree growing to 20–35 m tall, with a trunk up to 2 m in diameter. Leaves are alternately arranged, rounded to triangular-ovate, 4–13 cm long and broad with a 2.5–4 cm petiole, green and mostly hairless above, covered by densely white tomentose with white hairs below and with a coarsely toothed margin. This species is approved as efficient biomonitor in many studies because of its bioaccumulation capability and sensitivity to air pollution (BRAUN *et al.*, 2007; ANICIC *et al.*, 2011; PETROVA *et al.*, 2014).

Fraxinus excelsior, known as European ash or Common ash, is native to most of Europe from Portugal to Russia, with the exception of northern Scandinavia and southern Iberia. It is also considered native in southwestern Asia from northern Turkey east to the Caucasus and Alborz mountains. It is a large deciduous tree, growing to 20–35 m tall with a trunk up to 2 m diameter, with a tall, domed crown. Bark is smooth and pale grey on young trees, becoming thick and vertically fissured on old trees. Shoots are stout, greenish-grey, with jet black buds (which distinguish it from most other ash species, which have grey or brown buds). Leaves are 20–35 cm long, pinnate compound, with 7-13 leaflets, the leaflets 3–12 cm long and 0.8–3 cm broad, sessile on the leaf rachis and with a serrated margin. Leaves are often among the last to open in spring and the first to fall in autumn if an early frost strikes; they have no marked autumn colour, often falling dull green. This species have not received very attention as potential biomonitor, only few studies were conducted (AKSOY & DEMIREZEN, 2006).

Pinus nigra, common names Austrian pine or Black pine, is a moderately variable species of pine, occurring across southern Mediterranean Europe from Spain to the eastern Mediterranean on Anatolian peninsula of Turkey and on Corsica/Cyprus, including Crimea. It is a large coniferous evergreen tree, growing to 20–55 m tall at maturity. Bark is grey to yellow-brown, and is widely split by flaking fissures into scaly plates, becoming increasingly fissured with age. It is also well known biomonitor of air pollution by organic substances and toxic elements (TSIKRITZIS *et al*., 2002; ROMANIC & KRAUTHACKER, 2004; LEHNDORFF & SCHWARK, 2008; SAWIDIS *et al*., 2011).

Leaf sampling and pigment analysis. Samples were taken monthly through the vegetation period when the leaf petiole was fully developed (July, August and September) and photosynthetic pigments content was measured immediately after sampling. In order to obtain a homogeneous sample, a large number of one–year–old leaves, comparable in size and shape, were taken by hand from the branches, taking care to minimize contact with the leaf surface. Usually 80–100 fully expanded leaves per tree were collected and a composite sample was prepared for analyses. All the samples were stored in clean, labeled, polyethylene bags, closed tightly to avoid contamination during transport.

Pigment analysis followed SHLYK (1965). Spectrophotometric reading of photosynthetic pigments was performed after extraction with 90% acetone at 440.5 nm for carotenoids, 644 nm for chlorophyll b and 662 nm for chlorophyll a. Concentrations of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids were calculated for each sample and presented in mg g^{-1} fresh weight and then were calculated the ratios chlorophyll a/b and total chlorophylls/ carotenoids (PETROVA, 2011).

Data processing. All data presented in the present study were an average of triplicate analysis of three separate subsamples. The concentrations were expressed as arithmetic means and standard deviations (mean±SD). For the statistical evaluation of the data obtained, the raw values of 3 subsamples per species per site were used. T-test was performed for detecting significant differences between sampling plots and between studied plant species ($p<0.05$). All statistical analyses were made with the STATISTICA 7.0 statistical package (STATSOFT, 2004).

Results and Discussion

Our studies on determining the tree tolerance to the impact of urban environment were focused on the quantities of the main components of the pigment complex – chlorophyll a, chlorophyll b, total chlorophylls and carotenoids as chlorophylls and carotenoids are the main photosynthetic pigments in plants.

Total chlorophylls amount normally decreases from June to September and together with leaf ageing the decomposition of chlorophyll a is faster than chlorophyll b (WAGH *et al*., 2006). Our results showed a complex situation in this respect when a comparison was made between selected plant species and sampling sites. Chlorophyll b was found as more susceptible to pollution compared to chlorophyll a, corresponding to the findings of PAVLOVIC *et al.* (2017). A similar trend was found by GAJIĆ *et al.* (2009) at polluted sites (urban parks of Belgrade), where higher content of chlorophyll b in *Ligustrum ovalifolium* Hassk. was measured in June than in October. In our study, the highest concentrations of chl b were determined in July in leaves of *Fraxinus excelsior* and *Tilia tomentosa*, followed by *Acer heldreichii* and *Pinus nigra*. However, the different responses of chlorophyll a and chlorophyll b are not unexpected, since different types of pollution exert different effects on the pigment content, hence the response of a plant can be attributed to the interaction between various types of pollutants as well as abiotic factors (high temperature, drought, intense insolation, etc.) (BRUGNOLI *et al*., 1994; PAVLOVIC *et al*., 2017).

In July and August, a general tendency towards increasing the amount of total chlorophylls and chlorophylls/carotenoids ratio in the three deciduous tree species was established in Plot 3 (moderate household pressure). Tendency to reduce pigments content and chlorophyll a/b ratio was observed in Plot 4 (heavy traffic). According to many authors (WAGH *et al*., 2006; JOSHI & SWAMI, 2009; PAVLOVIC *et al*., 2017) the changes in the pigment content are one of the first signs of the harmful effect on the plants caused by atmospheric pollutants. Toxic gases penetrate in leaves, accumulate in the chloroplasts and damage their structure leading to reduce of the pigment content. Although, many authors have shown that an increased content in polluting agent leads to chlorophyll inhibition either through direct inhibition of several enzymatic steps (JOSHI & SWAMI, 2009) or as a result of substitution of the central Mg ion (GAJIĆ *et al.*, 2009), several studies have shown that exposure to heavy metals induces oxidative stress which is accompanied by an increase in the chlorophyll content (PETROVA, 2011; PAVLOVIC *et al.*, 2017). Our results obtained by the studied coniferous species are in good agreement with these findings as in the same period, maximum content of photosynthetic pigments and ratio values in *Pinus nigra* were observed in the leaf samples from Plot 4 (heavy motor and railroad traffic) (Fig. 5, 9, 13).

In September, we found very different changes in the four studied plant species, as explained below. Maximum total chlorophylls (2.08 mg kg^{-1}) and carotenoids (0.32 mg kg^{-1}) content in *A. heldreichhi* leaves was measured in Plot 1 (control), while minimum content was obtained in Plot 3 (1.26 mg kg^{-1} and 0.27 mg kg^{-1}, respectively) (Fig. 2). Highest chlorophyll a/b ratio (1.81) was calculated for Plot 3 and lowest (1.37) – for Plot 4 (Fig. 6). When comparing with the previous months, it is obvious that the chlorophyll a/b ratio has decreased by 25%, probably due to the fast decomposition of chlorophyll b and its transform to chlorophyll a. Highest chlorophylls / carotenoid ratio was obtained in Plot 2 (7.65) and lowest (3.78) – in Plot 3 (Fig. 10). As a whole, we found an increment of this ratio which could be explained by both the increment of total chlorophylls content accompanied by a decrement of carotenoid content.

Tendency to maintain highest pigment content and ratios values in Plot 3 and lowest ones in Plot 4 during the all studied period was found in *T. tomentosa* leaves (Fig. 3, 7, 11). Data showed almost a twice increase in chlorophylls/carotenoids ratio (from 4.04-4.83 to 7.21-8.45) which is probably due to the reduced carotenoid synthesis (Fig. 3) in September.

F. excelsior showed similar pigment content in both 3 months of the study: chlorophyll a varied from 0.63 to 1.29 mg kg^{-1}; chlorophyll b from 0.53 to 0.94 mg kg^{-1}; carotenoids varied from 0.17 to 0.52 mg kg^{-1} (Fig. 4). Results from September revealed a significant increment of chlorophylls/ carotenoids ratio in Plot 3 (28%) and Plot 1 (261%) ($p<0.05$) (Fig. 12).

In *P. nigra* leaf samples the most pronounced change was the decrease of carotenoids within the vegetation period in all sampling plots, as follows: Plot 1 – 2.0 fold; Plot 2 – 2.66 fold; Plot 3 – 1.75 fold; Plot 4 – 2.42 fold ($p<0.05$) (Fig. 5). This reducing has led to the increased chlorophylls/ carotenoids ratio values reaching up to 13.57 in Plot 2 (Fig. 13).

SILLANPÄÄ *et al.* (2008) measured elevated carotenoid level in *B. pendula* leaves sampled from pollutes area in comparison with samples from unpolluted one. They indicated that carotenoids perform many important physiological functions in plants like influencing development and adaptation mechanisms, suggesting coordination of their synthesis in different physiological processes, but mostly serve as antioxidants against endogenous and exogenous oxidative stress. Oxidative stress caused by pollutants occurs when the amount of oxidants in the cell exceeds that of antioxidants. In our study, we found an increment in carotenoids content in September only in *F. excelsior* leaves from Plot 4 (Fig. 4), which was in agreement with the findings of SILLANPÄÄ *et al.* (2008) as this plot was characterized as most polluted. The low carotenoids values in the other plots and their dynamics could be explained by JOSHI *et al.* (2009), WAGH *et al.* (2006), JOSHI & SWAMI (2009) findings that the changes in pigment content can characterize the resistance and the degree of adaptation of plants to constant and high level of atmospheric pollution in the environment.

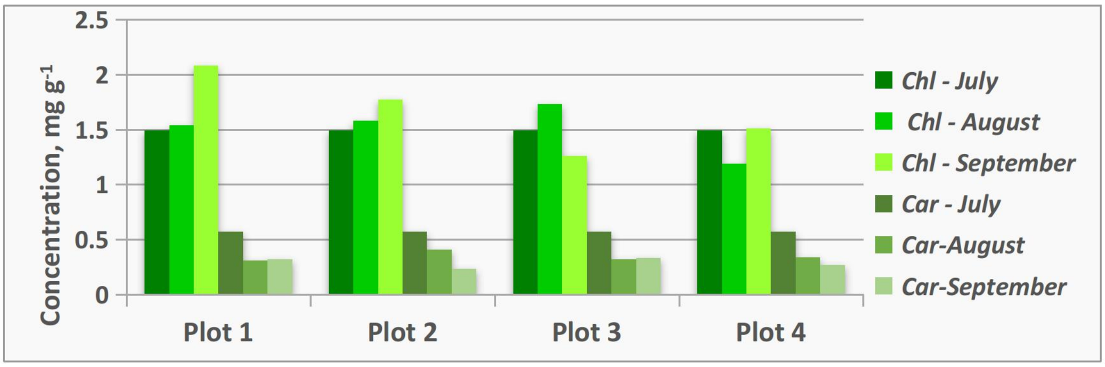

Fig. 2. Total chlorophylls and carotenoids content in *Acer heldreichii* leaf samples.

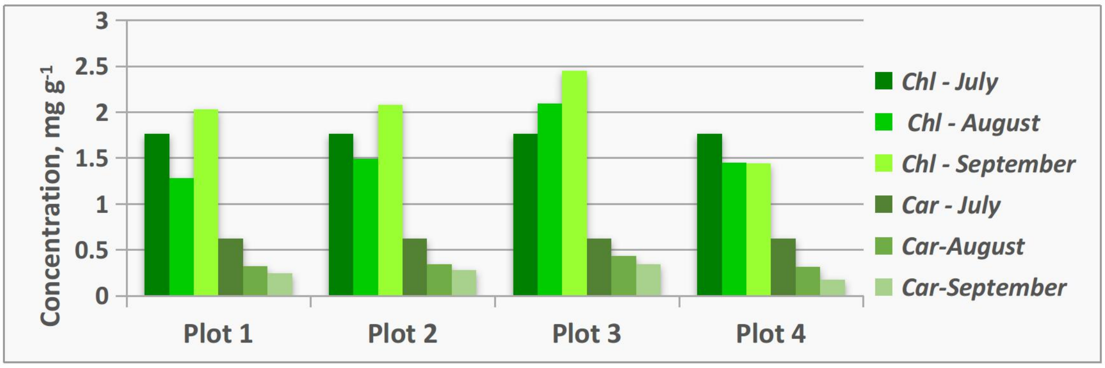

Fig. 3. Total chlorophylls and carotenoids content in *Tilia tomentosa* leaf samples.

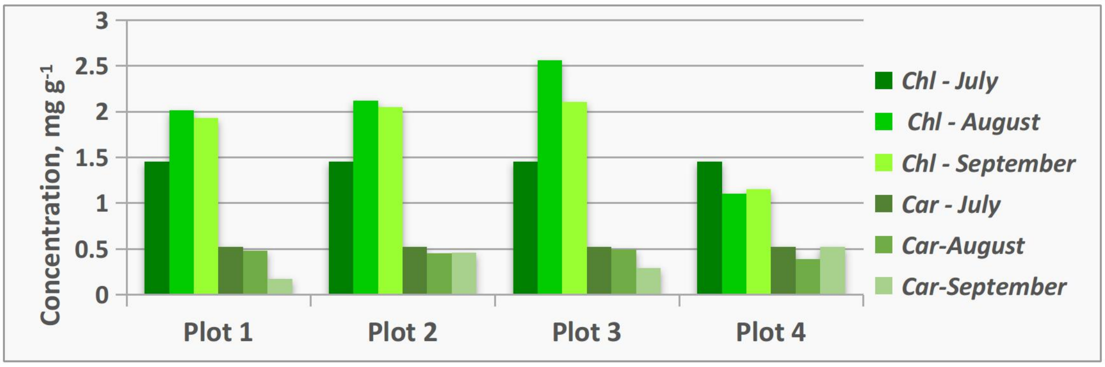

Fig. 4. Total chlorophylls and carotenoids content in *Fraxinus excelsior* leaf samples.

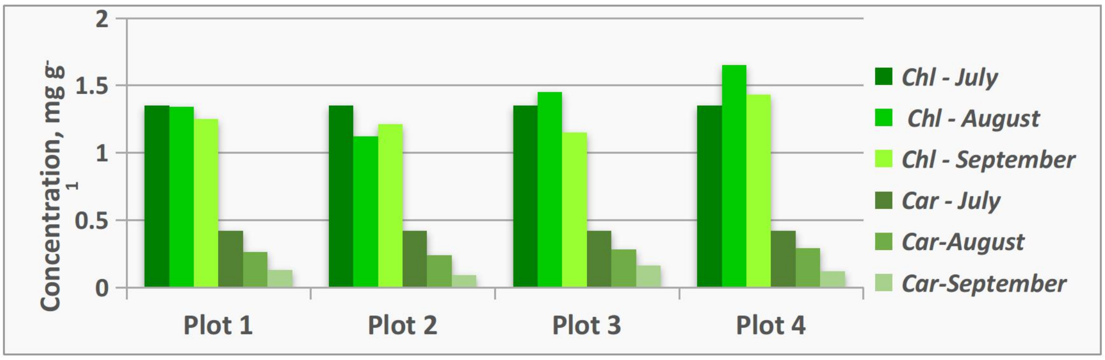

Fig. 5. Total chlorophylls and carotenoids content in *Pinus nigra* leaf samples.

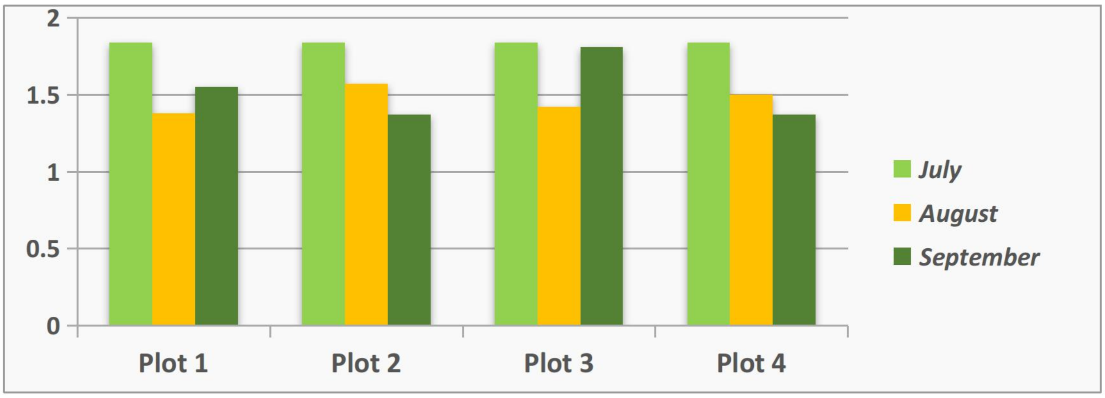

Fig. 6. Chlorophyll a/b ratio in *Acer heldreichii* leaf samples.

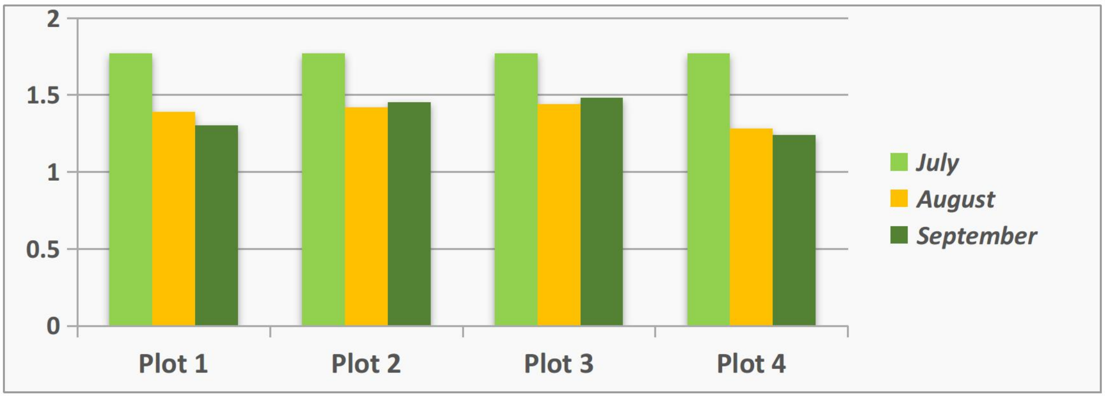

Fig. 7. Chlorophyll a/b ratio in *Tilia tomentosa* leaf samples.

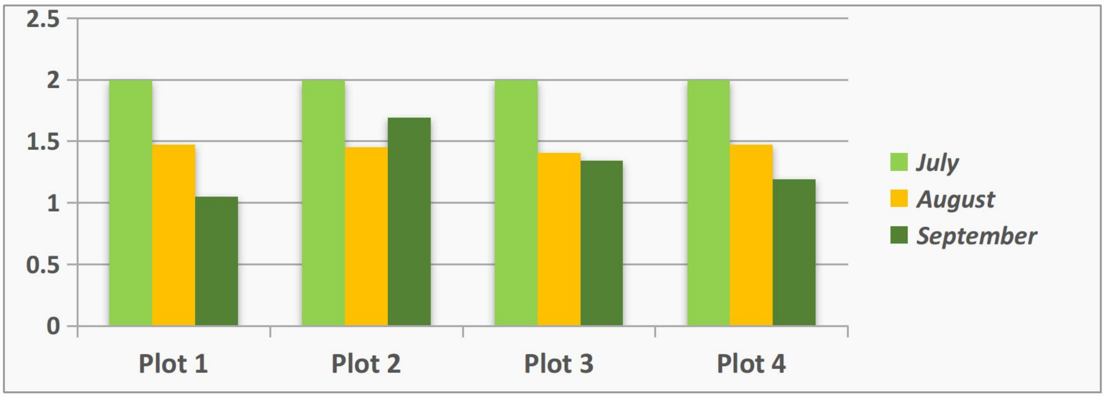

Fig. 8. Chlorophyll a/b ratio in *Fraxinus excelsior* leaf samples.

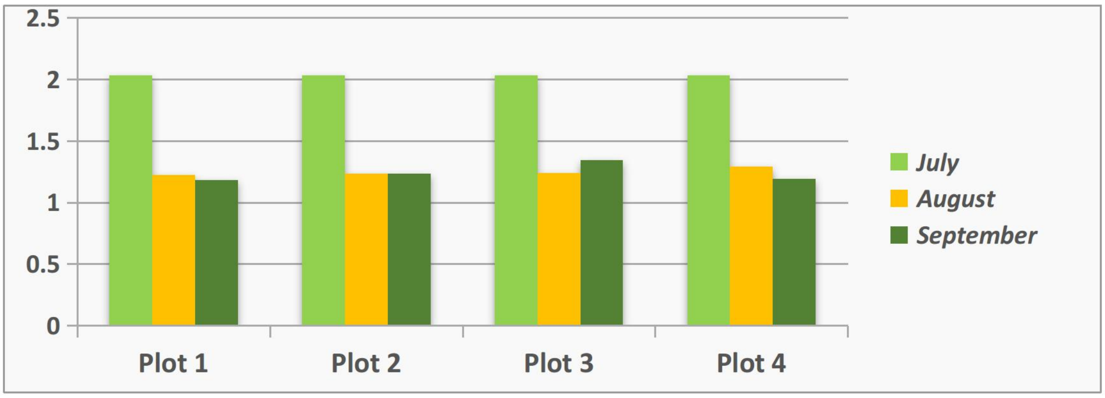

Fig. 9. Chlorophyll a/b ratio in *Pinus nigra* leaf samples.

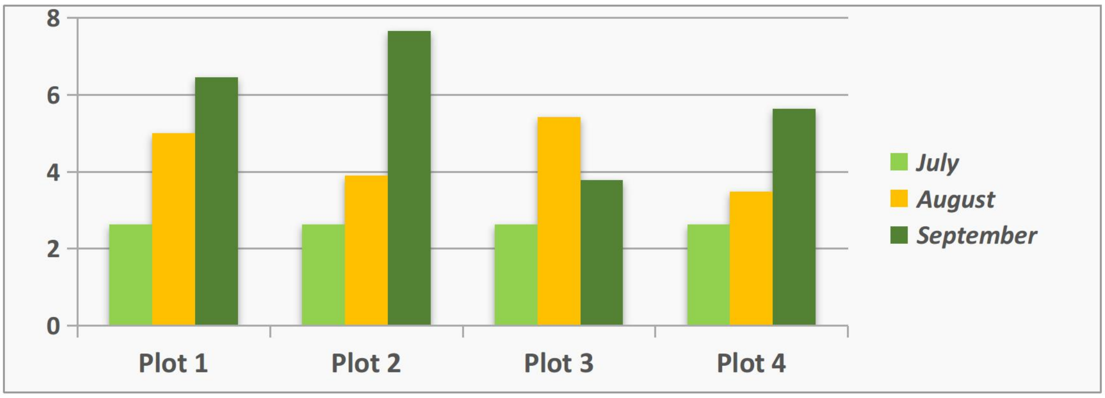

Fig. 10. Chlorophylls/carotenoids ratio in *Acer heldreichii* leaf samples.

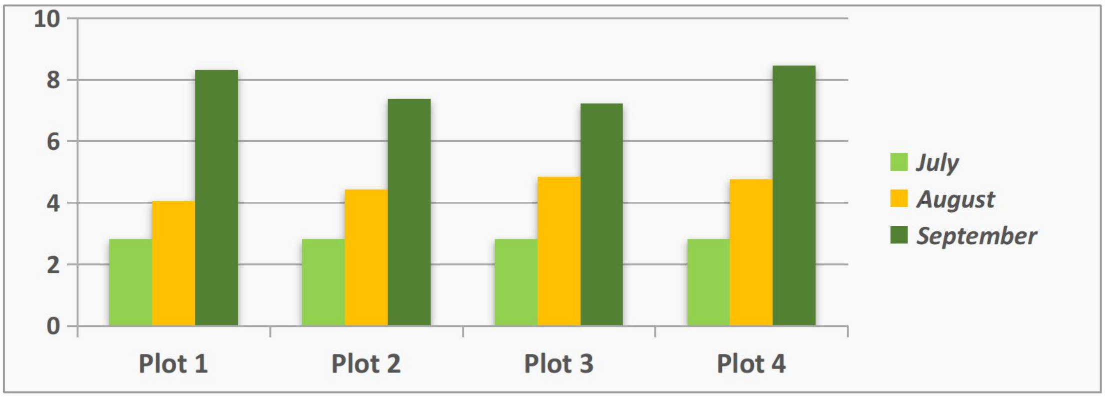

Fig. 11. Chlorophylls/carotenoids ratio in *Tilia tomentosa* leaf samples.

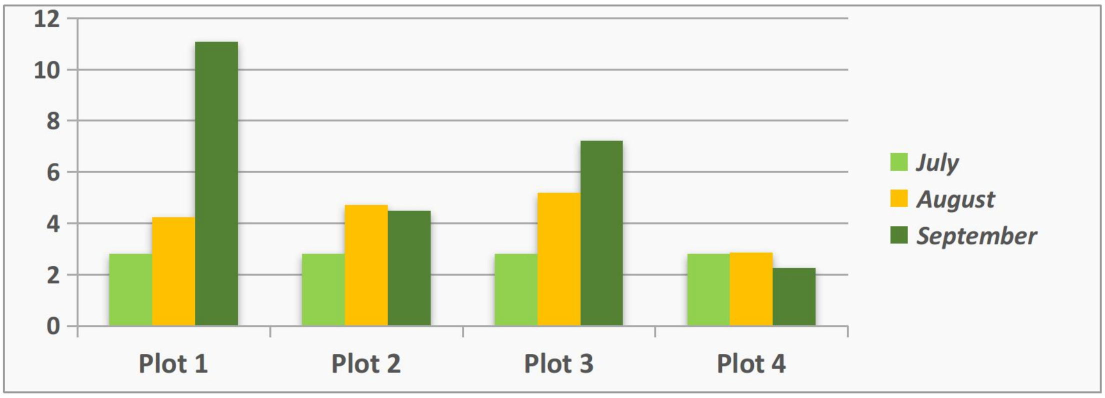

Fig. 12. Chlorophylls/carotenoids ratio in *Fraxinus excelsior* leaf samples.

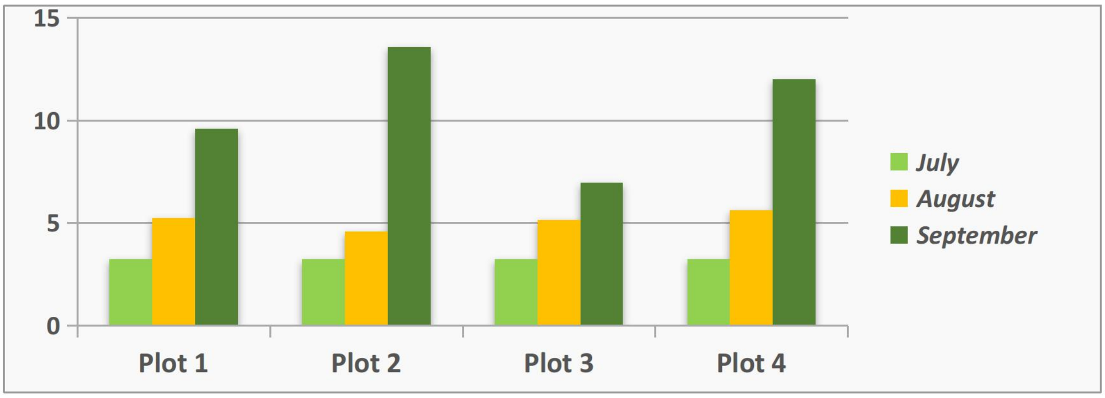

Fig. 13. Chlorophylls/carotenoids ratio in *Pinus nigra* leaf samples.

Conclusions

Results of this preliminary study confirmed that pigment levels in plants varied between species, locations and seasons. We could not found some significant differences between the pigment content of all studied trees from Plot 1, selected as a "provisional control" of urban environment and the trees in the other three plots ($p<0.05$). As more efficient parameters in this aspect were proven the chlorophyll a/b ratio and the chlorophylls/carotenoids ratio, where we found a general trend towards lower values at all studied plots and tree species compared to the control due to the anthropogenic impact on trees. These ratios could be used as more informative indicators in ecological investigations than pigment content. They have also the advantage to be dimensionless parameters, so they could be calculated and used for evaluation of plant status in different studies, regardless the extraction procedure of pigments and the analytical methods.

This study clearly indicates the great relevance of the problem of the connection of the host plant with the environment, both theoretically and in practice. This actuality is necessary not only to the requirements for environmental protection, but also in relation to the establishment of species specificity in the response of different plant species and their tolerance to pollution. This is crucial to forecasting the green system in urban areas and the selection of appropriate species for landscaping activities.

Acknowledgements. We are grateful to Fund "Scientific research" at the Plovdiv University "Paisii Hilendarski" for financial support by contract SP15-BF14, managed by Prof. Iliana Velcheva.

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Received: 07.12.2016
Accepted: 02.06.2017

ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 June 2017 pp. 63-77

Ground Beetles (Coleoptera: Carabidae) and Some Other Invertebrates from the Managed Nature Reserves "Dolna Topchiya" and "Balabana" (Lower Valley of the River of Tundzha, Bulgaria)

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Abstract. The invertebrate fauna of the "Balabana" and "Dolna Topchiya" managed nature reserves is studied, with particular consideration to the ground beetles. The area of study is interesting from a biological point of view, as the Tundzha River constitutes a corridor of penetration of southern and thermophilic elements. On the other hand, the specifics of the territory predetermine the presence of many typically forest and some mountain species, as well as a lot of inhabitants of open biotopes, in particular – steppe forms. During the study, altogether 2041 specimens of carabid beetles belonging to 88 species are captured, as well as 76 other invertebrate species, some of which are with a conservation significance – new, endemic, rare, protected or endangered. Forty-six carabid species are reported for the first time for the Sakar-Tundzha region. Ground beetles are characterized and classified according to their zoogeographical belonging and the life forms they refer to.

Key words: Carabidae, invertebrates, "Balabana", "Dolna Topchiya", Tundzha River, Bulgaria.

Introduction

The investigation of the biota of the protected natural areas is an important component of their functioning and allows the assessment of the value of the given territory and its representativeness as a repository for the gene pool of the particular ecosystems. The work concerning the invertebrates at European level lies in the principles of Natura 2000 and consists in monitoring and conservation of a number of species and, on the other hand, those animals are used as indicator group in the monitoring programs of the targeted habitats.

Efforts in protecting the communities of invertebrates as potential indicators for the state of the environment and as economic biological agents can be successful only if a sufficient amount of information about their structure and functioning in the different habitats is available. This fact underlines the need for further studies, especially at regional and national levels, and also emphasizes the importance of this study.

Up to this point only partial and insufficient investigations on the invertebrate fauna in the territory of the "Balabana" and "Dolna Topchiya" managed nature reserves have been conducted. Complete and profound studies are lacking, which premises the aim of the present research. The main objective of the study is to determine the species composition of

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Carabidae, existing in the research area. The study will be a preliminary step in order to detect the Coleoptera fauna of the Lower valley of the Tundzha River, where this group is poorly studied. The additional notes about the other established invertebrates supplement the faunistic knowledge about the region.

Material and Methods

Study area. "Balabana" and "Dolna Topchiya" managed nature reserves are situated in south-eastern Bulgaria, near the town of Elhovo, along the Lower valley of the river of Tundzha (Fig. 1). The reserves are under the jurisdiction of the Regional Inspectorate of Environment and Water – Stara Zagora. The coordinates of the considered reserves' centers are: 42°08′48′′N, 26°32′23′′E (average altitude 104 m a.s.l.) for "Balabana" and 42°12′07′′N, 26°33′58′′E (average altitude 107 m a.s.l.) for "Dolna Topchiya".

Geographical location, climate features and natural landscapes in the region are a prerequisite for the great faunistic diversity. The combination of relatively high annual average temperatures with high humidity predetermines the shaping of longose habitats with high conservation significance along the banks of the river. The valley of the Tundzha River in turn represents an original corridor for the penetration of some Mediterranean and Neareastern invertebrate species. Weak exploration work in the area, however, does not allow the evaluation of its conservational value and the degree of the influence of the human factors. The territory of the two reserves includes variety of natural habitats, maintaining diverse animal species composition and faunistic complexes (TEOFILOVA *et al.*, 2016).

The area has not been subjected to detailed faunistic investigations. The lack of reliable faunistic studies impedes the overall assessment of the species abundance, size of the populations, nature of their spatial distribution, biodiversity and extent of anthropogenic impact.

For the whole Tundzha-Sakar region around 80 species of ground beetles (Coleoptera: Carabidae) are known so far (GUÉORGUIEV & GUÉORGUIEV 1995). A large part of them are typical hygrophilous and mesophilous forms, attached to coastal and forest habitats (mostly representatives of the genera *Bembidion*, *Chlaenius*, *Agonum*, *Carabus*, etc.).

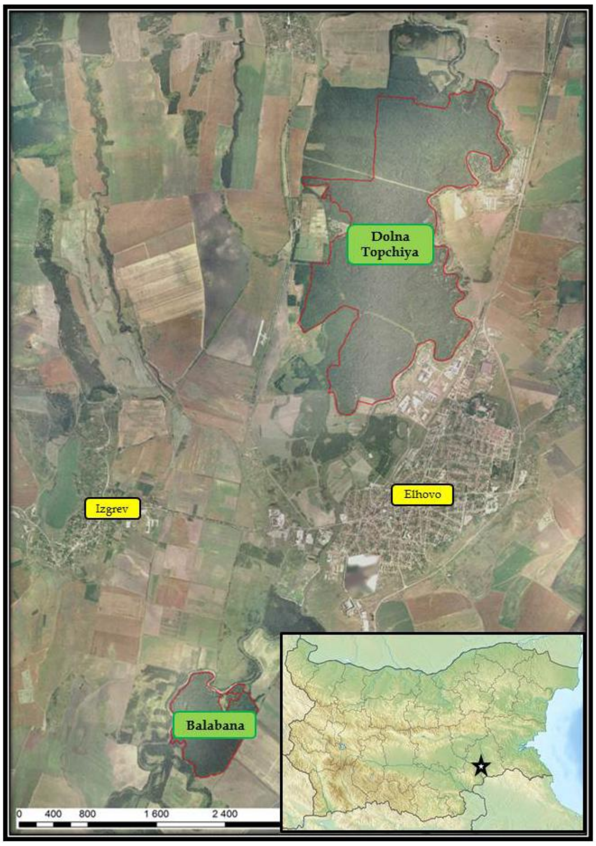

Fig. 1. Map of the location of the two reserves.

Field work and determination of the material. Field work was carried out in the periods: 8 – 12 May, 9 – 14 June and 1 – 5 September 2014. It included: 1) transect method with observations *in situ* or collection of material; 2) stationary method with „pitfall" traps made of plastic bottles, buried at the level of the ground surface, with a 4% solution of formaldehyde as a fixation fluid; this method is suitable for ecological research on adult beetles, and mainly reflects their activity (LÖVEI & SUNDERLAND, 1996); there are no reasonable alternatives to this type of traps in the study of epigeic arthropod communities (SPENCE & NIEMELA, 1994); it is considered that the application of this method allows approximately 95% of the species active in radius of 50 m around the traps to be caught (BAARS & VAN DIJK, 1984); 3) handpicking and shaking of branches, capturing with a standard entomological sack; 4) collection of standard hydrobiological samples (ISO 10870, 2012) for determining the taxonomic composition of the benthic fauna in the Tundzha River and the channel Dipsiza.

Sampling areas in the "Balabana" Reserve were three: (I) Mesophilous oak (*Quercus* spp.) forest (12 traps); (II) Open habitat – meadow (8 traps); (III) Humid coastal forest with dominant tree species from the genera *Ulmus* and *Fraxinus* (11 traps). Sampling areas in the "Dolna Topchiya" Reserve were also three: (I) Open habitat – meadow (6 traps); (II) Ecotone (8 traps); (III) Humid coastal forest with dominant tree species from the genera *Ulmus* and *Fraxinus* (11 traps).

Captured animals were determined with the help of several main literary sources (BEI-BIENKO, 1965; HŮRKA, 1996; HARDE, 2000; MARINOV *et al*., 2000; REITTER, 2006; PEHLIVANOV *et al*., 2010; ARNDT *et al*., 2011; STERI, 2000; Kryzhanovskij, 2017, unpublished data), and with the kind assistance of relevant specialists in the individual groups of invertebrates: Lyubomira Lyubomirova (aquatic invertebrates; Sofia University, Sofia), Dr. Dragan Chobanov (Orthoptera), Dr. Toshko Ljubomirov (Hymenoptera) (Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences, Sofia), Dr. Nikolay Kodzhabashev (Forestry University, Sofia) and Dr. Mihail Danilevskiy (Cerambycidae; Institute of Ecology and Evolution – Russian Academy of Sciences, Moscow).

Materials are deposited in the collections of the Institute of Biodiversity and Ecosystem Research (Bulgarian Academy of Sciences, Sofia), Forestry University and Sofia University.

The systematic list of Carabidae follows KRYZHANOVSKIJ *et al*. (1995).

Data analysis. According to their zoogeographical belonging ground beetles were separated in zoogeographical categories and faunal types according to VIGNA TAGLIANTI *et al*. (1999) with some changes (KODZHABASHEV & PENEV, 2006): *I. Northern Holarctic and Euro-Siberian faunal type:* OLA – Holarctic; PAL – Palaearctic; W-PAL – Western Palaearctic; E-SI – Eurosiberian; E-WSI – Euro-Westsiberian. *II. European faunal type:* EUR – European; E-PAS – European-Neareastern; CE-PAS – Central European and Neareastern; CE-E – Central and Eastern European; BAL-K – Balkan-Carpathian. *III. Euroasiatic faunal type:* E-AS – Euroasiatic steppe complex; E-CAS – European and Central Asian; B-CAS – Balkan and Central Asian; B-PAS – Balkan-Neareastern (+ Balkan-Anatolian). *IV. Mediterranean (sensu lato) faunal type (species of the Ancient Mediterraneum):* E-CA-M – European-Centralasian-Mediterranean; E-PA-M – European-Neareastern-Mediterranean; CA-MED – Mediterranean-Centralasian; MED-PA – Mediterranean-Neareastern; MED – Mediterranean; E-MED – Eastmediterranean; NMED – Northmediterranean; NM-CAS – Northmediterranean-Centralasian. *V. Endemic complex:* BAL - Balkan endemic.

Categorization of the species in respect of their life forms follows the classification of SHAROVA (1981). The following codes were used: *Life form class 1. Zoophagous*. Life form subclass: 1.2 – Epigeobios; 1.3 – Stratobios;. 1.4 – Geobios. Life form groups: 1.2.2 – large walking epigeobionts; 1.2.2(1) – large walking dendroepigeobionts; 1.2.3 – running epigeobionts; 1.2.4 – flying epigeobionts; 1.3(1) – series crevice-dwelling stratobionts; 1.3(1).1 – surface & litter-dwelling; 1.3(1).2 – litter-dwelling; 1.3(1).3 – litter & crevice-dwelling; 1.3(2) – series digging stratobionts; 1.3(2).1 – litter & soil-dwelling; 1.4.2(1) – small digging geobionts; 1.4.2(2) – large digging geobionts. *Life form class 2. Mixophytophagous*. Life form subclass: 2.1 – Stratobios; 2.2 – Stratohortobios; 2.3 – Geohortobios. Life form groups: 2.1.1 – crevice-dwelling stratobionts; 2.2.1 – stratohortobionts; 2.3.1 – harpaloid geohortobionts; 2.3.2 – zabroid geohortobionts; 2.3.3 – dytomeoid geohortobionts. The first digit in the index shows the class of life form, the second – the subclass, the third – the life form group. In brackets after the subclass the series is shown, when it exists.

Results and Discussion

Faunistic and taxonomic data about the ground beetles

During the study, altogether 2041 specimens of ground beetles belonging to 42 genera and 88 species are captured – 860 specimens and 61 species in "Balabana" and 1181 specimens and 67 species in "Dolna Topchiya" (Appendix 1). This represents 34% of the genera and 12% of the species occurring in Bulgaria. Full list of the established carabids is given in Appendix 1.

The distribution of the species and specimens among the different sampling sites is shown on Table 1. Forests seem to maintain a greater abundance and diversity of carabids than the open habitats. Given the fact that the natural river valleys and the periodically inundated coastal forests are becoming rarer across Europe (TOMIAŁOJĆ & DYRCZ, 1993) efforts for the conservation of the biological diversity should be directed towards preservation and restoration of this type of habitats and restriction of their anthropogenization.

Representatives of 20 tribes are captured. The most species rich in both of the reserves is the tribe Harpalini, probably due to the presence of inhabitants of the open habitats. Same pattern is also noticed with the abundance of the ground beetles – the largest number of specimens was established for the same tribe in both of the reserves (Table 2).

As a result of the field work 46 species are reported for the first time for the Sakar-Tundzha region, where "Balabana" and "Dolna Topchiya" are located. Those are: *Elaphrus aureus*, *Scarites terricola*, *Clivina fossor*, *Apotomus clypeonites adanensis*, *Asaphidion flavipes*, *Bembidion lampros*, *Bembidion properans*, *Bembidion lunulatum*, *Poecilus cupreus*, *Poecilus cursorius*, *Pterostichus ovoideus*, *Pterostichus melas*, *Abax carinatus*, *Calathus fuscipes*, *Calathus melanocephalus*, *Calathus cinctus*, *Laemostenus venustus*, *Limodromus assimilis*, *Anchomenus dorsalis*, *Amara tricuspidata*, *Amara aenea*, *Amara convexior*, *Amara communis*, *Anisodactylus binotatus*, *Diachromus germanus*, *Parophonus laeviceps*, *Parophonus mendax*, *Parophonus hirsutulus*, *Ophonus laticollis*, *Ophonus cribricollis*, *Harpalus honestus*, *Harpalus atratus*, *Harpalus subcylindricus*, *Harpalus tardus*, *Harpalus latus*, *Carterus dama*, *Dixus eremita*, *Chlaenius nitidulus*, *Chlaenius nigricornis*, *Badister bullatus*, *Philorhizus notatus*, *Syntomus obscuroguttatus*, *Microlestes fissuralis*, *Microlestes minutulus*, *Microlestes negrita negrita*, *Brachinus psophia*. Eleven species could also be pointed out as new for the region, although they were noted in another study, concerning the area of the Lower valley of Tundzha (TEOFILOVA *et al*., 2016): *Leistus ferrugineus*, *Notiophilus palustris*, *Notiophilus rufipes*, *Calosoma sycophanta*, *Carabus granulatus*, *Bembidion decolor*, *Bembidion inoptatum*, *Bembidion castaneipenne*, *Stomis pumicatus*, *Amblystomus niger*, *Microlestes fulvibasis*. The presence of *Harpalus rubripes* in the studied region is confirmed.

Zoogeographical peculiarities of the ground beetles

The analysis of the data shows that the Northern Holarctic and European-Siberian complex (species distributed mainly in the northern regions of the Holarctic, mostly in Europe and Siberia) prevails, consisting of 24 species (27% of all species). Mediterranean (*s. lato*) complex is immediately after it with 22 species (25%). European-Asiatic type (species ranges lie between the Eurosiberian and Mediterranean zones) includes 21 species (24%). European faunal type (mostly forest dwelling species connected to the middle and southern part of Europe) consists of 17 species (19%). Only 4 (5%) Balkan endemics are found (Fig. 3A). In terms of the quantitative relations, however, European complex is dominant with 50% of all captured ground beetle specimens (Fig. 3B).

Table 1. Number of specimens and species of Carabidae, captured in the different biotopes in both studied reserves.

"Balabana"			*"Dolna Topchiya"*		
Sampling site	***Number of specimens***	***Number of species***	***Sampling site***	***Number of specimens***	***Number of species***
Mesophilous oak forest	314	30	Meadow	320	28
Meadow	240	29	Ecotone	381	32
Humid coastal forest	303	35	Humid coastal forest	477	36
hand catch	3	3	hand catch	3	3
Total:	*860*	*61*	***Total:***	*1181*	*67*

Table 2. Number of specimens and species in the different tribes of Carabidae, captured in both studied reserves.

Tribe	*"Balabana"*		*"Dolna Topchiya"*		*Both reserves*	
	Number of specimens	***Number of species***	***Number of specimens***	***Number of species***	***Number of specimens***	***Number of species***
Cicindelini	-	-	35	2	35	2
Nebriini	8	2	32	1	40	2
Notiophilini	46	2	36	1	82	2
Carabini	197	7	101	4	298	8
Elaphrini	4	1	-	-	4	1
Scaritini	-	-	1	1	1	1
Clivinini	1	1	-	-	1	1
Dyschiriini	4	1	8	1	12	1
Apotomini	-	-	2	1	2	1
Trechini	59	1	19	1	78	1
Bembidiini	61	5	68	4	129	7
Pterostichini	84	6	82	6	166	7
Sphodrini	14	3	94	4	108	4
Agonini	41	2	92	2	133	2
Amarini	29	5	27	4	56	6
Harpalini	292	16	541	23	833	27
Callistini	-	-	5	3	5	3
Licinini	2	2	1	1	3	2
Lebiini	5	4	30	6	35	6
Brachinini	13	3	7	2	20	4
Total:	***860***	***61***	***1181***	***67***	***2041***	***88***

Representatives of 23 zoogeographical categories are established during the study. The largest number of species is found in the following categories: Palearctic (10 species), European and Neareastern (10 species), European and Central Asian (10 species) and European-Centralasian-Mediterranean (8 species) – one category from each of the faunal complexes. Similar pattern is observed also in the quantitative relations (Table 3).

Physiographic conditions and habitat peculiarities of the research area determine the presence of various zoogeographical elements. Mesophilous and longose forests keep many nemoral (primarily European and European-Siberian forest species) elements. At the same time, the valley of the river of Tundzha helps the penetration of some Mediterranean and Neareastern forms. The endemic complex is less covered, probably due to the dynamic conditions caused by the unstable hydrological regime of the river.

Life forms of the ground beetles

The 88 ground beetle species and subspecies, established for the area of "Balabana" and "Dolna Topchiya" reserves, relate to two classes and 17 life form groups proposed by SHAROVA (1981), with clear predominance of class Zoophaga, presented by 55 species (62.5% of all species). Mixophyitophagous are 33 species (37.5%). According to SHAROVA (1981) similar distribution is typical for the forest-steppe zones. The most numerous are the surface and litter-dwelling stratobionts (15 species; 17%) and litter and crevice-dwelling stratobionts (10 species; 11%) from class Zoophaga and the harpaloid geohortobionts (15 species; 17%) from class Mixophytophaga (Fig. 4A). These numbers and polidominant pattern of the life form groups are typical for the forests (SHAROVA 1981).

In quantitative relations is noticeable the significant percentage of the mixophytophagous harpaloid geohorto-bionts, mainly due to the increased presence of species from the genus *Harpalus* in the open parts of the reserves (Fig. 4B).

Notes on the other invertebrate groups

During the field observations are identified 76 invertebrate species (except the ground beetles), under 46 families, 22 orders and 6 classes: Bivalvia – 3 species (3 in "Balabana" and 2 in "Dolna Topchiya"), Gastropoda – 8 species (4 in "Balabana" and 7 in "Dolna Topchiya"), Clitellata – 3 species (1 in "Balabana" and 3 in "Dolna Topchiya"), Malacostraca – 5 species (2 in "Balabana" and 5 in "Dolna Topchiya"), Insecta – 57 species (38 in "Balabana" and 38 in "Dolna Topchiya").

In the "Balabana" Reserve altogether (with the ground beetles) 108 invertebrate species are found and in "Dolna Topchiya" Reserve – 125 species. Full list of the established invertebrate animals are given in Appendix 2. A total of 145 insect species are captured. Most of them are coleopterans and ants (Fig. 5). A number of species with conservation significance – protected, rare, endemic, or species with restricted distribution and species with scientific value – were found during the study (TEOFILOVA *et al.*, 2016).

Conclusions

As a result of the present study 88 species of ground beetles were established. Four Balkan endemics were found among them. Seventy-six other invertebrates were recorded. The real state of the diversity of the invertebrates in the area could be revealed only after future investigations and discovery of additional new species for the region.

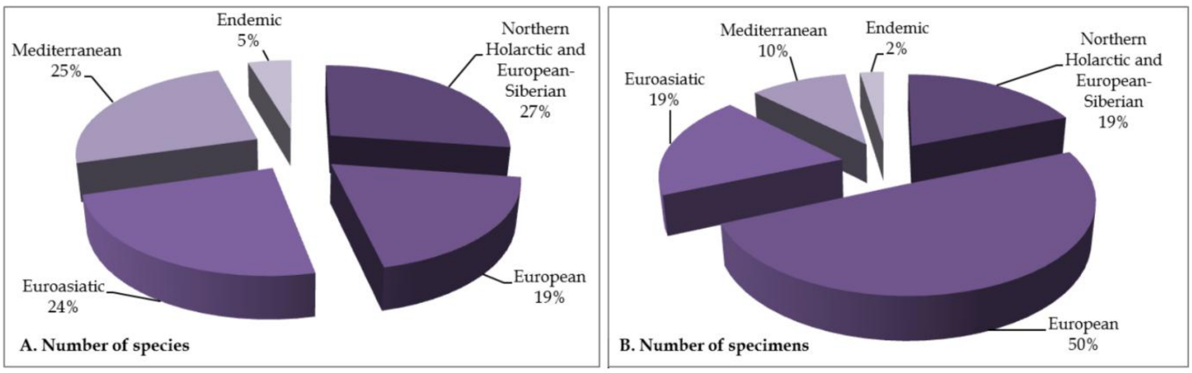

Fig. 3. Qualitative and quantitative zoogeographical peculiarities of the ground beetles from the two studied managed nature reserves.

Table 3. Zoogeographical categories of the ground beetles.

Faunal type	*Zoogeographical element*	*Number of species*	%	*Number of specimens*	%
Northern Holarctic and European-Siberian	OLA	3	3.4	73	3.6
	PAL	10	11.4	180	8.8
	W-PAL	4	4.5	92	4.5
	E-SI	4	4.5	40	2.0
	E-WSI	3	3.4	4	0.2
European	EUR	1	1.1	4	0.2
	E-PAS	10	11.4	499	24.5
	CE-PAS	3	3.4	189	9.3
	CE-E	2	2.3	201	9.8
	BAL-K	1	1.1	118	5.8
Euroasiatic	E-AS	6	6.8	50	2.4
	E-CAS	10	11.4	325	15.9
	B-CAS	1	1.1	1	0.05
	B-PAS	4	4.5	7	0.3
Mediterranean	E-CA-M	8	9.1	125	6.1
	E-PA-M	4	4.5	12	0.6
	CA-MED	2	2.3	7	0.3
	MED-PA	1	1.1	3	0.15
	MED	1	1.1	4	0.2
	E-MED	2	2.3	4	0.2
	NMED	3	3.4	44	2.2
	NM-CAS	1	1.1	8	0.4
Endemic	BAL	4	4.5	51	2.5

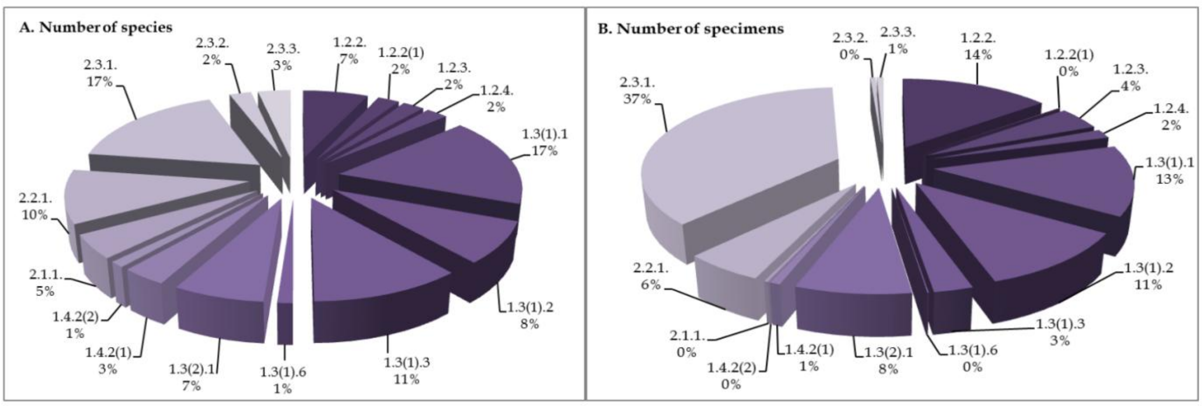

Fig. 4. Qualitative and quantitative peculiarities of the life forms of the carabids from the two studied managed nature reserves.

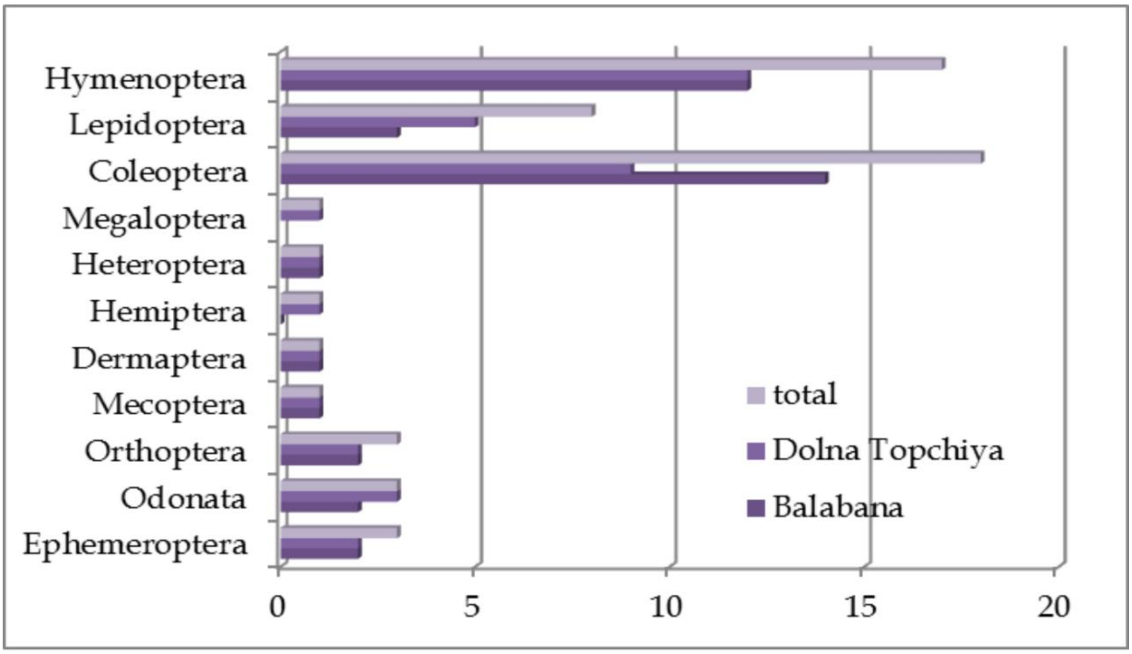

Fig. 5. Number of species of insects from the different orders. For order Coleoptera the data about the ground beetles (Carabidae) are not included.

It should be noted the importance of the territory for the preservation of many southern thermophilic faunistic elements with limited distribution in Europe and Bulgaria. Especially great is the role of the dense longose forests as refugia of mesophylous and hygrophilous mountain faunistic elements.

Mesophylous and humid forests maintain a significant abundance and diversity of invertebrates with specific ecological requirements. A major factor in the conservation of these stenotopic species is the preservation of their original primary habitats.

It is necessary the abiding of all restrictions and prohibitions currently in force within the territory of the reserves to be ensured, as well as the conservation of the natural habitats in unaltered state, which would provide a possibility for the fulfilment of the natural successional changes.

The humidification of the dried up eastern part of the "Balabana" Reserve is advisable. The presence of introduced tree species in both reserves is undesirable, because not characteristic edificators are a prerequisite for unpredictable succession.

Conservation of the biological diversity cannot be achieved without sustainable development of socio-economic conditions. In view of the low level of these conditions in the area, the emergence of conflict is inevitable.

Acknowledgments. The study was carried out thanks to the financial aid of the Ministry of Environment and Water and Regional Inspectorate of Environment and Water – Stara Zagora. The author expresses gratitude to Lyubomira Lyubomirova, Dr. Dragan Chobanov, Dr. Toshko Ljubomirov, Dr. Nikolay Kodzhabashev, Pencho Pandakov and Mihail Danilevskiy for their assistance during the field work and the determination of some taxa.

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Received: 11.12.2012
Accepted: 02.03.2013

Appendix 1. Checklist of the carabid beetles, found in the two reserves (codes for the zoogeographical categories, life forms and sampling sites are given in Material and Methods). Species marked with asterisk (*) are reported for the first time for the Sakar-Tundzha region where "Balabana" and "Dolna Topchiya" are located. Species marked with (#) are noted in another study, concerning the area (TEOFILOVA *et al.*, 2016).

№	Species	Range type	Life form	Material	
				"Balabana"	"Dolna Topchiya"
	Tribe Cicindelini				
1.	*Cylindera (Cylindera) germanica germanica* (Linnaeus, 1758)	E-PAS	1.2.4	-	16♀, 17♂ (II)
2.	*Cicindela (Cicindela) campestris campestris* Linnaeus, 1758	PAL	1.2.4	-	2♂ (II)
	Tribe Nebriini				
3.#	*Leistus (Leistus) ferrugineus* (Linnaeus, 1758)	E-SI	1.3(1).2	2♂ (III)	-
4.	*Nebria (Nebria) brevicollis* (Fabricius, 1792)	E-PAS	1.3(1).2	1♀, 1♂ (I); 2♀, 2♂ (III)	15♀, 17♂ (III)
	Tribe Notiophilini				
5.#	*Notiophilus palustris* (Duftschmid, 1812)	E-SI	1.3(1).1	2♀, 3♂ (I); 1♀, 3♂ (III)	-
6.#	*Notiophilus rufipes* Curtis, 1829	E-PAS	1.3(1).1	13♀, 22♂ (I); 2♂ (III)	22♀, 14♂ (III)
	Tribe Carabini				
7.#	*Calosoma (Calosoma) sycophanta* Linnaeus, 1758	PAL	1.2.2(1)	1♀ (I); 1♂ (III)	-
8.	*Calosoma (Acalosoma) inquisitor* (Linnaeus, 1758)	W-PAL	1.2.2(1)	1♂ (III)	-
9.	*Carabus (Eucarabus) ullrichi rhilensis* Kraatz, 1876	CE-E	1.2.2	1♂ (hand); 33♀, 25♂ (I); 1♀ (II); 18♀, 19♂ (III)	2♀, 1♂ (II); 10♀, 16♂ (III)
10.	*Carabus (Tachypus) cancellatus intermedius* Dejean, 1826	BAL	1.2.2	1♀ (I)	-
11.#	*Carabus (Carabus) granulatus granulatus* Linnaeus, 1758	E-AS	1.2.2	1♀ (I)	-
12.	*Carabus (Tomocarabus) convexus gracilior* Géhin, 1885	BAL-K	1.2.2	15♀, 13♂ (I); 25♀, 20♂ (III)	2♀, 1♂ (II); 26♀, 16♂ (III)
13.	*Carabus (Procrustes) coriaceus kindermanni* Waltl, 1838	BAL	1.2.2	6♀, 2♂ (I); 8♀, 4♂ (II); 2♀ (III)	1♀ (hand); 3♀, 2♂ (I); 6♀, 3♂ (II); 8♀, 3♂ (III)
14.	*Carabus (Procerus) scabrosus scabrosus* Olivier, 1789	BAL	1.2.2	-	♀ (hand)
	Tribe Elaphrini				
15.*	*Elaphrus (Elaphroterus) aureus aureus* P. W. J. Müller, 1821	EUR	1.2.3	2♀, 2♂ (III)	-
	Tribe Scaritini				
16.*	*Scarites (Parallelomorphus) terricola* Bonelli, 1813	PAL	1.4.2(2)	-	1 ex. (II)

	Tribe Clivinini				
17.*	*Clivina (Clivina) fossor fossor* (Linnaeus, 1758)	PAL	1.4.2(1)	1♀ (III)	-
	Tribe Dyschiriini				
18.	*Dyschiriodes (Eudyschirius) globosus* Herbst, 1784	PAL	1.4.2(1)	4 ex. (III)	8 ex. (III)
	Tribe Apotomini				
19.*	*Apotomus clypeonites adanensis* Jedlicka, 1961	E-MED	1.4.2(1)	-	1♀, 1♂ (II)
	Tribe Trechini				
20.	*Trechus (Trechus) quadristriatus* (Schrank, 1781)	E-CA-M	1.3(1).2	19♀, 17♂ (I); 14♀, 9♂ (III)	8♀, 11♂ (III)
	Tribe Bembidiini				
21.*	*Asaphidion flavipes* (Linnaeus, 1761)	W-PAL	1.2.3	16♀, 24♂ (I); 11♀, 3♂ (III)	17♀, 12♂ (III)
22.*	*Bembidion (Metallina) lampros* (Herbst, 1784)	OLA	1.3(1).1	1♀, 3♂ (I)	12♀, 25♂ (III)
23.*	*Bembidion (Metallina) properans* (Stephens, 1828)	E-WSI	1.3(1).1	-	1♂ (III)
24.#	*Bembidion (Philochthus) decolor* Apfelbeck, 1911	BAL	1.3(1).1	1♂ (I)	-
25.#	*Bembidion (Philochthus) inoptatum* Schaum, 1857	CE-PAS	1.3(1).1	1♀ (III)	-
26.*	*Bembidion (Philochthus) lunulatum* (Geoffroy in Fourcroy, 1785)	W-PAL	1.3(1).1	-	1♀ (I)
27.#	*Bembidion (Peryphanes) castaneipenne* Jacquelin du Val, 1852	B-PAS	1.3(1).1	1♂ (I)	-
	Tribe Pterostichini				
28.#	*Stomis (Stomis) pumicatus* (Panzer, 1796)	E-PAS	1.3(1).2	1♀ (I); 1♀, 2♂ (III)	-
29.*	*Poecilus (Poecilus) cupreus* (Linnaeus, 1758)	E-AS	1.3(2).1	1♀ (hand); 1♀, 2♂ (I)	1♀ (hand); 2♀ (III)
30.*	*Poecilus (Poecilus) cursorius* (Dejean, 1828)	E-PA-M	1.3(2).1	3♀, 2♂ (III)	1♀ (III)
31.	*Pterostichus (Pseudomaseus) anthracinus* (Illiger, 1798)	E-PAS	1.3(2).1	-	12♀, 12♂ (III)
32.*	*Pterostichus (Phonias) ovoideus* (Sturm, 1824)	E-SI	1.3(2).1	1♂ (I); 6♀, 11♂ (III)	1♂ (II); 1♀, 2♂ (III)
33.*	*Pterostichus (Feronidius) melas* (Creutzer, 1799)	E-PAS	1.3(2).1	1♀ (I); 3♂ (III)	12♀, 12♂ (III)
34.*	*Abax carinatus* (Duftschmid, 1812)	CE-E	1.3(2).1	25♀, 18♂ (I); 3♀, 3♂ (III)	17♀, 7♂, 2 ex. (III)
	Tribe Sphodrini				
35.*	*Calathus (Calathus) fuscipes* (Goeze, 1777)	PAL	1.3(1).2	2♀ (II)	22♀, 21♂ (I); 21♀, 18♂ (II); 2♀, 4♂, 1 ex. (III)
36.*	*Calathus (Neocalathus) melanocephalus* (Linnaeus, 1758)	PAL	1.3(1).2	4♀, 3♂ (II); 1♂ (III)	1♀ (I)
37.*	*Calathus (Neocalathus) cinctus* Motschulsky, 1850	E-CAS	1.3(1).2	1♀, 1♂ (I); 2♀ (II)	1♀ (I); 1♂ (II)

38.*	*Laemostenus (Laemostenus) venustus* (Dejean, 1828)	NMED	1.3(1).6	-	2♂ (III)
	Tribe Agonini				
39.*	*Limodromus assimilis* (Paykull, 1790)	PAL	1.3(1).1	3♀, 2♂ (I); 5♂ (III)	23♀, 15♂ (III)
40.*	*Anchomenus (Anchomenus) dorsalis* (Pontoppidan, 1763)	E-CAS	1.3(1).1	5♀ (I); 1♀ (II); 10♀, 15♂ (III)	19♀, 35♂ (III)
	Tribe Amarini				
41.*	*Amara (Zezea) tricuspidata* Dejean, 1831	E-CA-M	2.2.1	1♀ (II)	-
42.*	*Amara (Amara) aenea* (De Geer, 1774)	OLA	2.3.1	3♀ (II)	3♀, 1♂ (I); 8♀, 1♂ (II)
43.*	*Amara (Amara) convexior* Stephens, 1828	E-CAS	2.3.1	2♂ (I); 2♀, 13♂ (III)	4♀, 6♂ (III)
44.*	*Amara (Amara) communis* (Panzer, 1797)	E-SI	2.3.1	7♂ (III)	-
45.	*Amara (Amara) lucida* (Duftschmid, 1812)	E-PA-M	2.3.1	1♀ (II)	1♀ (II)
46.	*Zabrus (Zabrus) tenebrioides* (Goeze, 1777)	E-CAS	2.3.2	-	2♀, 1♂ (I)
	Tribe Harpalini				
47.*	*Anisodactylus (Anisodactylus) binotatus* (Fabricius, 1787)	E-AS	2.3.1.	1♀, 1♂ (III)	1♂ (I); 4♂ (III)
48.	*Gynandromorphus etruscus etruscus* (Quensel en Schönherr, 1806)	NMED	2.2.1	18♀, 15♂ (II)	1♂ (I);1♂ (II)
49.*	*Diachromus germanus* (Linnaeus, 1758)	E-MED	2.2.1	-	1♀ (I); 1♂ (III)
50.*	*Parophonus (Parophonus) laeviceps* (Ménétriés, 1832)	B-PAS	2.1.1	-	1♀ (I)
51.*	*Parophonus (Parophonus) mendax* (P. Rossi, 1790)	B-PAS	2.1.1	-	2♂ (I)
52.*	*Parophonus (Ophonomimus) hirsutulus* (Dejean, 1829)	CA-MED	2.1.1	-	1♂ (I)
53.*	*Ophonus (Metophonus) laticollis* Mannerheim, 1825	E-CAS	2.2.1	3♀, 1♂ (I); 1♀, 1♂ (II); 4♀, 4♂ (III)	4♀, 6♂ (III)
54.	*Ophonus (Metophonus) rufibarbis* (Fabricius, 1792)	W-PAL	2.2.1	-	1♂ (II); 5♀, 1♂ (III)
55.	*Ophonus (Metophonus) melleti melleti* (Heer, 1837)	E-PAS	2.2.1	2♂ (II)	-
56.	*Ophonus (Hesperophonus) azureus* (Fabricius, 1775)	E-CA-M	2.2.1	1♀ (I); 9♀, 16♂ (II)	4♂ (II)
57.*	*Ophonus (Hesperophonus) cribricollis* (Dejean, 1829)	E-CAS	2.2.1		1♀ (I); 3♂ (II)
58.	*Ophonus (Ophonus) sabulicola* (Panzer, 1796)	CE-PAS	2.2.1	1♀, 1♂ (II)	2♀, 4♂ (I)
59.*	*Harpalus (Harpalus) honestus* (Duftschmid, 1812)	E-WSI	2.3.1	-	2♂ (II)
60.	*Harpalus (Harpalus) rubripes* (Duftschmid, 1812)	E-AS	2.3.1	4♀, 3♂ (II)	10♀, 10♂ (I); 4♀, 1♂ (II); 1♀ (III)
61.*	*Harpalus (Harpalus) atratus*	E-PAS	2.3.1	-	2♀, 7♂ (III)

	Latreille, 1804				
62.	*Harpalus (Harpalus) flavicornis flavicornis* Dejean, 1829	CE-PAS	2.3.1	3♀, 2♂ (II)	40♀, 50♂ (I); 28♀, 57♂ (II)
63.*	*Harpalus (Harpalus) subcylindricus* Dejean, 1829	E-CAS	2.3.1	7♀, 22♂ (II)	14♀, 14♂ (I); 14♀, 39♂ (II)
64.*	*Harpalus (Harpalus) tardus* (Panzer, 1797)	E-CAS	2.3.1	7♀, 10♂ (I); 3♀, 2♂ (II); 6♀, 7♂ (III)	3♀, 13♂ (I); 2♀, 4♂ (II); 3♀, 4♂ (III)
65.	*Harpalus (Harpalus) albanicus* Reitter, 1900	E-PAS	2.3.1	1♀ (II); 1♀ (III)	-
66.*	*Harpalus (Harpalus) latus* (Linnaeus, 1758)	E-AS	2.3.1	2♂ (I); 1♂ (II); 18♀, 18♂ (III)	-
67.	*Harpalus (Harpalus) cupreus fastuosus* Faldermann, 1836	B-PAS	2.3.1	1♀ (hand); 1♂ (I); 1♂ (II)	-
68.	*Harpalus (Harpalus) dimidiatus* (P. Rossi, 1790)	E-PAS	2.3.1	2♀, 2♂ (I); 32♀, 48♂ (II); 2♀, 4♂ (III)	44♀, 36♂ (I); 38♀, 38♂ (II); 1♂ (III)
69.	*Acinopus (Oedematicus) megacephalus* (Rossi, 1794)	NMED	2.3.2	-	2♂ (I); 2♀, 3♂ (II)
70.*	*Carterus (Carterus) dama* (P. Rossi, 1792)	MED	2.3.3	1♀, 1♂ (II)	2♀ (I)
71.*	*Dixus eremita* (Dejean, 1825)	B-CAS	2.3.3	-	1 ex. (I)
72.	*Dixus obscurus* (Dejean, 1825)	NM-CAS	2.3.3	1♀ (II)	2 ex. (I); 3♀, 2 ex. (II)
73.#	*Amblystomus niger* (Heer, 1841)	E-PA-M	2.1.1	-	1♀ (I)
	Tribe Callistini				
74.	*Chlaenius (Dinodes) decipiens* (L. Dufour, 1820)	E-CA-M	1.3(1).1	-	1♀, 1♂ (II)
75.*	*Chlaenius (Chlaeniellus) nitidulus* (Schrank, 1781)	E-CAS	1.3(1).1	-	1♂ (III)
76.*	*Chlaenius (Chlaeniellus) nigricornis* (Fabricius, 1787)	E-AS	1.3(1).1	-	1♂ (II); 1♀ (III)
	Tribe Licinini				
77.	*Licinus (Licinus) depressus* (Paykull, 1790)	E-WSI	1.3(1).1	1♀ (III)	-
78.*	*Badister (Badister) bullatus* (Schrank, 1798)	PAL	1.3(1).1	1♂ (III)	1♀ (III)
	Tribe Lebiini				
79.*	*Philorhizus notatus* (Stephens, 1827)	E-CA-M	1.3(1).3	1♂ (I)	1♀, 3♂ (II); 1♂ (III)
80.*	*Syntomus obscuroguttatus* (Duftschmid, 1812)	E-PA-M	1.3(1).3	1♀, 1♂ (III)	1♀ (III)
81.*	*Microlestes fissuralis* (Reitter, 1901)	E-CAS	1.3(1).3	-	1♀ (II)
82.#	*Microlestes fulvibasis* (Reitter, 1901)	CA-MED	1.3(1).3	1♂ (II)	1♂ (I); 2♀, 2♂ (II)
83.*	*Microlestes minutulus* (Goeze, 1777)	OLA	1.3(1).3	1♂ (II)	4♀ (I); 5♀, 6♂ (II)
84.*	*Microlestes negrita negrita* (Wollaston, 1854)	MED-PA	1.3(1).3	-	2♀, 1♂ (II)
	Tribe Brachinini				
85.	*Brachinus (Brachinus) crepitans*	PAL	1.3(1).3	9♀, 1♂ (II);	1♀ (III)

	(Linnaeus, 1758)			1♀ (III)	
86.*	*Brachinus (Brachinus) psophia* Audinet-Serville, 1821	E-CA-M	1.3(1).3	1♀ (II)	-
87.	*Brachinus (Brachynidius) bodemeyeri* Apfelbeck, 1904	E-CA-M	1.3(1).3	1♂ (II)	-
88.	*Brachinus (Brachynidius) explodens* Duftschmid, 1812	E-CA-M	1.3(1).3	-	1♂ (I); 2♀, 3♂ (II)

Appendix 2. Species of invertebrates, other than Carabidae, found during the field work in the two reserves: B – "Balabana"; DT – "Dolna Topchiya".

№	Species	Family	Order	B	DT
	Phylum Mollusca				
	Class Bivalvia				
1.	*Anodonta anatina* (Linnaeus, 1758)	Unionidae	Unionoida	+	+
2.	*Unio crassus* Philipson, 1788	Unionidae	Unionoida	+	-
3.	*Unio pictorum* (Linnaeus, 1758)	Unionidae	Unionoida	+	+
	Class Gastropoda				
4.	*Ferrissia clessiniana* (Jickeli, 1882)	Planorbidae	Basommatophora	-	+
5.	*Valvata piscinalis* (O. F. Müller, 1774)	Valvatidae	Heterostropha	+	+
6.	*Physa fontinalis* (Linnaeus, 1758)	Physidae	Hygrophila	-	+
7.	*Radix balthica* (Linnaeus, 1758)	Lymnaeidae	Hygrophila	-	+
8.	*Stagnicola palustris* (O. F. Müller, 1774)	Lymnaeidae	Hygrophila	-	+
9.	*Helix lucorum* Linnaeus, 1758	Helicidae	Sigmurethra	+	-
10.	*Helix pomatia* Linnaeus, 1758	Helicidae	Sigmurethra	+	+
11.	*Xerolenta obvia* (Menke, 1828)	Hygromiidae	Sigmurethra	+	+
	Phylum Annelida				
	Class Clitellata				
12.	*Lumbricus terrestris* Linnaeus, 1758	Lumbricidae	Haplotaxida	+	+
13.	*Erpobdella monostriata* (Lindenfeld & Pietruszynski, 1890)	Erpobdellidae	Hirudinea	-	+
14.	*Piscicola geometra* (Linnaeus, 1758)	Piscicolidae	Hirudinea	-	+
	Phylum Arthropoda				
	Class Malacostraca				
15.	*Gammarus arduus* G. S. Karaman, 1975	Gammaridae	Amphipoda	-	+
16.	*Gammarus komareki* Schaferna, 1922	Gammaridae	Amphipoda	-	+
17.	*Asellus aquaticus* (Linnaeus, 1758)	Asellidae	Isopoda	+	+
18.	*Limnomysis benedeni* Czerniavsky, 1882	Mysidae	Mysida	-	+
19.	*Potamon ibericum* (Bieberstein, 1809)	Potamonidae	Decapoda	+	+
	Class Insecta				
20.	*Cloeon dipterum* (Linnaeus, 1761)	Baetidae	Ephemeroptera	+	-
21.	*Heptagenia flava* Rostock, 1878	Heptageniidae	Ephemeroptera	-	+
22.	*Potamanthus luteus* (Linnaeus, 1767)	Potamanthidae	Ephemeroptera	+	+
23.	*Calopteryx virgo* (Linnaeus, 1758)	Calopterygidae	Odonata	-	+
24.	*Calopteryx splendens* (Harris, 1782)	Calopterygidae	Odonata	+	+
25.	*Platycnemis pennipes* (Pallas, 1771)	Platycnemididae	Odonata	+	+
26.	*Gryllus campestris* Linnaeus, 1758	Gryllidae	Orthoptera	+	+
27.	*Gryllotalpa gryllotalpa* (Linnaeus, 1758)	Gryllotalpidae	Orthoptera	+	-
28.	*Aiolopus strepens* (Latreille, 1804)	Acrididae	Orthoptera	-	+
29.	*Panorpa communis* Linnaeus, 1758	Panorpidae	Mecoptera	+	+
30.	*Forficula auricularia* Linnaeus, 1758	Forficulidae	Dermaptera	+	+

31.	*Palomena prasina* (Linnaeus, 1761)	Pentatomidae	Hemiptera	-	+
32.	*Aphelocheirus aestivalis* (Fabricius, 1794)	Aphelocheiridae	Heteroptera	+	+
33.	*Sialis lutaria* (Linnaeus, 1758)	Sialidae	Megaloptera	-	+
34.	*Anthelephila caeruleipennis* (LaFerté-Senéctère, 1847)	Anthicidae	Coleoptera	+	+
35.	*Byturus tomentosus* (De Geer, 1774)	Byturidae	Coleoptera	+	+
36.	*Cerambyx cerdo* Linnaeus, 1758	Cerambycidae	Coleoptera	+	-
37.	*Dorcadion pedestre* (Poda, 1761)	Cerambycidae	Coleoptera	+	+
38.	*Neodorcadion bilineatum* (Germar, 1824)	Cerambycidae	Coleoptera	+	-
39.	*Coccinella septempunctata* Linnaeus, 1758	Coccinelidae	Coleoptera	+	-
40.	*Hispa atra* Linnaeus, 1767	Chrysomelidae	Coleoptera	-	+
41.	*Lilioceris lilii* (Scopoli, 1763)	Chrysomelidae	Coleoptera	+	-
42.	*Sermylassa halensis* (Linnaeus, 1767)	Chrysomelidae	Coleoptera	+	-
43.	*Otiorhynchus rugostriatus* (Goeze, 1777)	Curculionidae	Coleoptera	-	+
44.	*Geotrupes stercorarius* (Linnaeus, 1758)	Geotrupidae	Coleoptera	-	+
45.	*Dorcus parallelipipedus* (Linnaeus, 1758)	Lucanidae	Coleoptera	+	+
46.	*Lucanus cervus* (Linnaeus, 1758)	Lucanidae	Coleoptera	+	+
47.	*Dendroxena quadripunctata* (Scopoli, 1772)	Silphidae	Coleoptera	+	-
48.	*Nicrophorus vespillo* (Linnaeus, 1758)	Silphidae	Coleoptera	+	-
49.	*Silpha obscura* Linnaeus, 1758	Silphidae	Coleoptera	-	+
50.	*Scaphidium quadrimaculatum* Olivier, 1790	Scaphidiidae	Coleoptera	+	-
51.	*Protaetia aeruginosa* (Linnaeus, 1767)	Scarabeidae	Coleoptera	+	-
52.	*Arctia villica* (Linnaeus, 1758)	Arctiidae	Lepidoptera	-	+
53.	*Brenthis daphne* (Bergsträsser, 1780)	Nymphalidae	Lepidoptera	+	-
54.	*Maniola jurtina* (Linnaeus, 1758)	Nymphalidae	Lepidoptera	-	+
55.	*Melanargia galathea* (Linnaeus, 1758)	Nymphalidae	Lepidoptera	+	-
56.	*Pararge aegeria tircis* (Godart, 1821)	Nymphalidae	Lepidoptera		+
57.	*Zerynthia polyxena* (Denis & Schiffermüller, 1775)	Papilionidae	Lepidoptera	-	+
58.	*Zerynthia cerisy* (Godart, [1824])	Papilionidae	Lepidoptera	-	+
59.	*Pieris rapae* (Linnaeus, 1758)	Pieridae	Lepidoptera	+	-
60.	*Apis mellifera* Linnaeus, 1758	Apidae	Hymenoptera	+	+
61.	*Camponotus piceus* (Leach, 1825)	Formicidae	Hymenoptera	+	+
62.	*Formica cunicularia* Latreille, 1798	Formicidae	Hymenoptera	+	-
63.	*Formica rufa* Linnaeus, 1761	Formicidae	Hymenoptera	+	+
64.	*Formica rufibarbis* Fabricius, 1793	Formicidae	Hymenoptera	+	-
65.	*Lasius alienus* (A. Förster, 1850)	Formicidae	Hymenoptera	+	+
66.	*Lasius citrinus* Emery, 1922	Formicidae	Hymenoptera	-	+
67.	*Lasius niger* (Linnaeus, 1758)	Formicidae	Hymenoptera	+	+
68.	*Lepisiota frauenfeldi* (Mayr, 1855)	Formicidae	Hymenoptera	-	+
69.	*Leptothorax affinis* Mayr, 1855	Formicidae	Hymenoptera	-	+
70.	*Leptothorax recedens* (Nylander, 1856)	Formicidae	Hymenoptera	+	-
71.	*Liometopum microcephalum* (Panzer, 1798)	Formicidae	Hymenoptera	-	+
72.	*Plagiolepis pygmaea* (Latreille, 1798)	Formicidae	Hymenoptera	+	-
73.	*Ponera coarctata* (Latreille, 1802)	Formicidae	Hymenoptera	+	-
74.	*Tetramorium caespitum* (Linnaeus, 1758)	Formicidae	Hymenoptera	+	+
75.	*Scelio fulvipes* A. Förster, 1856	Scelionidae	Hymenoptera	-	+
76.	*Vespula germanica* (Fabricius, 1793)	Vespidae	Hymenoptera	+	+

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2017, Vol. 9, Issue 1 June 2017 pp. 79-89

New Data on the Distribution and Seasonal Flight of the Vine Bud Moth Theresimima ampellophaga (Bayle-Barelle, 1808) in Bulgaria - Investigations By Pheromone-Baited Traps

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Abstract. The vine bud moth, *Theresimima ampellophaga* is considered as a pest species of the grapevine *Vitis vinifera* in Bulgaria. Delta sticky traps baited with the main synthetic sex pheromone compound, (2*R*)-butyl (7*Z*)-tetradecenoate, of *Th. ampellophaga* were used for detection and seasonal monitoring of this species in vineyards at four sites in Bulgaria – Lozitsa village (Municipality Nikopol) (northern Bulgaria) and Gornoslav village (Municipality Asenovgrad) in 2015 and 2016, and Plovdiv town and Krumovo village (Municipality Rodopi) (southern Bulgaria) in 2015. Catches of *Th. ampellophaga* males were recorded only in the villages of Lozitsa and Gornoslav. These are new distributional records for this species in Bulgaria. At these two sites, only one generation of *Th. ampellophaga* was established in 2015. In 2016, one generation of this species was recorded in Lozitsa and two generations – in Gornoslav. The flight period of the moths of the first generation was from the end of May (Gornoslav) – middle of June (Lozitsa) to the second half of July at both sites. The moths of the second generation in Gornoslav occur at the second half of August.

Key words: *Theresimima ampellophaga*, pheromone traps, Bulgaria, new records, seasonal flight.

Introduction

Viticulture is a traditional subsector of Bulgarian agriculture. As a result of a massive organizational restructuring and economic problems in this subsector, the total area harvested, yield and grape production has been reduced throughout the last twenty years. However, recently, despite the continuous reduction in harvested area of vines grown by the conventional method, the areas with organic grapes production grew steadily. The age structure of vineyards (many vineyards are old), selection and investment in quality grapevine varieties with high adaptation to the environmental conditions and tolerance to pests / diseases, improvement of soil fertility and application of sustainable pest control methods are important factors for viticulture development in Bulgaria (MOULTON *et al.*, 1994; DIMITROVA *et al.*, 2013; DYAKOVA *et al.*, 2014; TSVETKOV & DZHAMBAZOVA, 2014; ROYCHEVA, 2015). According to HARIZANOV *et al.* (2006), the economically important insect pests of *Vitis vinifera* L. in Bulgaria are the European grapevine moth, *Lobesia botrana*

([Denis & Schiffermüller], 1775), the vine moth, *Eupoecilia ambiguella* (Hübner, 1796), grape leafroller, *Sparganothis pilleriana* ([Denis & Schiffermüller], 1775) (Tortricidae), the vine bud moth, *Theresimima ampellophaga* (Bayle-Barelle, 1808) (Zygaenidae), species belonging to the genera *Agrotis* Ochsenheimer, 1816, *Euxoa* Hübner, 1821, *Xestia* Hübner, 1818, *Mamestra* Ochsenheimer, 1816, *Heliothis* Hübner, 1818 (Noctuidae), the cottony vine scale, *Pulvinaria vitis* (Linnaeus, 1758) (Coccidae). In different years damages caused by other insect pests like scales *Parthenolecanium corni* (Bouché, 1844) and *P. persicae* (Fabricius, 1776), the hazel leaf-roller, *Byctiscus betulae* (Linnaeus, 1758) (Rhynchitidae), the Western grape rootworm, *Bromius obscurus* (Linnaeus, 1758) (Chrysomelidae), and the tarnished plant bugs, *Lygus* Hahn, 1833 (MAF, 2017; KOSTADINOVA *et al.*, 2009) have been also reported. Recently, the spotted wing Drosophila, *Drosophila suzukii* (Matsumura, 1931) (Drosophilidae), a major invasive pest of small and stone fruits including grapes in America and Europe (ASPLEN *et al.*, 2015), was caught for the first time in a trap in 2014 in the southwestern part of Bulgaria (regions of Blagoevgrad, Kyustendil and Plovdiv) and Varna (EPPO, 2015; LAGINOVA & IVANOVA, 2015) and later established in new localities in southern Bulgaria (KARADJOVA *et al.*, 2016). In addition to this, *Scaphoideus titanus* Ball, 1932 (Cicadellidae), a vector of Flavescence dorée, was found in Varna, Veliko Tarnovo and Ruse regions (AVRAMOV *et al.*, 2011; GJONOV & SHISHINIOVA, 2014).

The vine bud moth, *Th. ampellophaga* is distributed in south eastern France, Italy, Vatican, Slovenia, Hungary, Croatia, Serbia, Albania, Macedonia, Romania, Moldova, Bulgaria, Greece (including islands), Cyprus, Ukraine (region of Odessa), South Russia, Georgia, Azerbaijan, Turkey, Cyprus, Syria, Lebanon, Israel and Algeria (TARMANN, 1998; 2003). The host plants for the larvae of this species are *V. vinifera* and *Parthenocissus* spp. In the last century, periodic and local outbreaks of *Th. ampellophaga* associated with the primary host, grapevine, were reported in several countries, Bulgaria, Italy and Hungary (REICHART & TASNADY, 1967; ANASTASOVA & GEORGIEVA, 1975; PUCCI & DOMINICI, 1986; HARIZANOV *et al.*, 1994). Currently in some regions/ countries the vine bud moth is considered as a pest and control measures were applied (CAN *et al.*, 2010; LEBEDEV, 2011; MAF, 2017), but in other ones it is relatively rare (SUBCHEV *et al.*, 2008a; NAHIRNIĆ *et al.*, 2015) and even endangered species (SHCHUROV & ZAMOTAJLOV, 2006; ZAMOTAJLOV, 2007).

Sex pheromones are an integral part of integrated pest management (IPM) programs in agriculture, particularly for monitoring abundance and distribution of insect pest populations (RODRIGUEZ-SAONA & STELINSKI, 2009). The main sex pheromone compound of *Th. ampellophaga* females, released from a gland at 3rd -5th abdominal tergites (HALLBERG & SUBCHEV, 1997), was identified as (2*R*)-butyl (7*Z*)-tetradecenoate (SUBCHEV *et al.*, 1998). Later, the synthetic sex pheromone was used for detection and monitoring of the seasonal flight of this species in several European countries and in Asiatic part of Turkey (reviewed in SUBCHEV, 2014; RAZOV *et al.*, 2017). Attraction of conspecific males to the opposite enantiomer, (2*S*)-butyl (7*Z*)-tetradecenoate, and 2-butyl 2-dodecenoate has been documented (EFETOV *et al.*, 2010; 2014).

The aim of the current study was to establish the presence and to monitor seasonal flight of *Th. ampellophaga* in four sites in Bulgaria by pheromone-baited traps.

Materials and Methods

Pheromone baits and traps: for preparing pheromone lures, the synthetic sex pheromone, (2*R*)-butyl (7*Z*)-tetradecenoate (SUBCHEV *et al.*, 1998), in a dose of 100 µg was applied onto serum bottle vials of grey rubber as a hexane solution. Home-made sticky Delta traps of transparent PVC foil were used for all field investigations.

Monitoring sites:

1. Lozitsa village (Nikopol Municipality, northern Bulgaria) is situated on Nikopol Plateau, which is representative place for the priority habitats (PEEV *et al.*, 2012) and is designated as Special Protection Area within NATURA 2000 ecological network. Twenty five years ago, the total area of cultivated vineyards of Lozitsa village was

more than 500 ha. Now the cultivated vineyards are about 10 % of this area, and the rest of plantations are abandoned or eradicated and changed into arable lands (PETKOV, pers. comm.). In 2015 and 2016, one pheromone trap in a vineyard with Mavrud variety (0.15 ha) (43°36'33.93"N; 24°59'21.45"E) and one trap in a vineyard with Muscat Ottonel variety (0.6 ha) (43°36'23.65"N; 24°58'26.67"E) were set up on 12 June, 2015 and 28 May, 2016, respectively and removed on 19 September, 2015 and 17 September, 2016, respectively. Distance between the vineyards was about 0.5-0.8 km.

2. Gornoslav village (Asenovgrad Municipality, southern Bulgaria). Currently the total vineyard area of the village is 90 ha. The pheromone traps (one in 2015 and two in 2016) were set up in a private vineyard with Pamid variety (0.7 ha) (41° 55″54.38″N; 24° 57″47.97″E). The monitoring periods were 7 June – 14 September, 2015 and 10 May – 16 September, 2016.

3. Plovdiv town (Plovdiv Municipality, southern Bulgaria). In 2015, one trap in a vineyard belonging to Agricultural University (Plovdiv) with Pleven variety (0.2 ha) (42°07″53.73″N; 24°48″19.09″E) was set up on 7 June and removed on 14 September, 2015.

4. Krumovo Village (Rodopi Municipality, southern Bulgaria). In 2015, one trap in a vineyard with Velika variety (0.5 ha) (42° 04″54.54″N; 24° 48″27.94″E) was set up on 7 June and removed on 14 September, 2015.

In all vineyards, no chemical insecticides were used in the years of our investigations, and in the vineyard in Gornoslav village only organic manage-ment practice was applied. Traps were inspected at least once per week (in exception of the period 15-28 August, 2016 in Gornoslav village) and the sticky layers with insects caught were collected and replaced with clear ones. During the monitoring periods, lures were replaced with fresh ones at 6-8 weeks. The determination of the species was done by examination of the genitalia structure of moth caught.

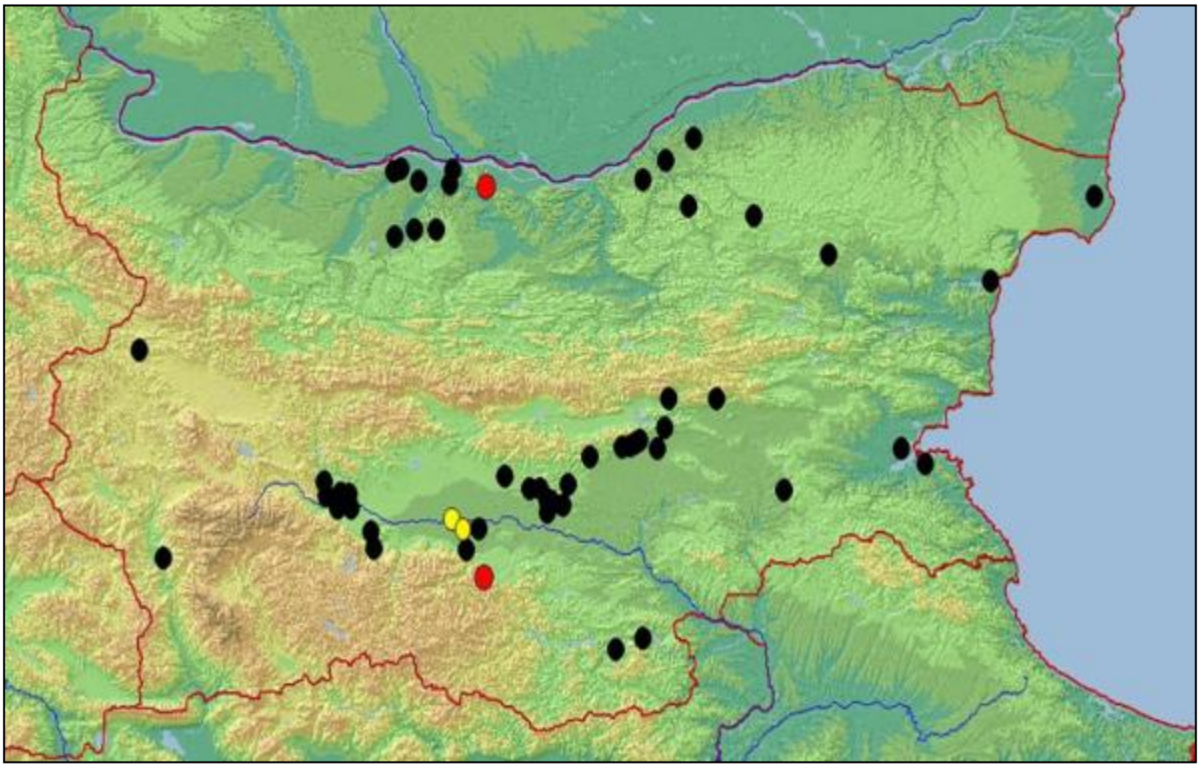

Fig. 1. Map of Bulgaria with localities (black and red dots) where *Th. ampellophaga* has been recorded so far. Black dots – published records, red dots – new records, yellow dots – absence of *Th. ampellophaga* catches during our study.

Results

Th. ampellophaga catches were recorded only in two of the four investigated sites - Lozitsa and Gornoslav. Fig. 1 represents the distribution of *Th. ampellophaga* in Bulgaria based on literature data and the results of the current study (BioOffice database).

The patterns of the seasonal flight of this species in 2015 and 2016 in the studied sites are presented on Fig. 2 and 3.

In 2015, the first catches of male moths in Lozitsa were registered at the second half of June and the last ones at the middle of July. The maximum of the flight was at the end of June – beginning of July. In Gornoslav village, *Th. ampellophaga* males were recorded at the first inspection date after the traps were set up and probably the beginning of the flight was missed; catches were recorded in the period of 7 June to 20 July. The population density of the vine bud moth in 2015 was relatively low at both sites.

In 2016, the flight of the vine bud moth in Lozitsa was from the middle of June (during the period of 12-18 June) to the middle of July (during the period of 10-16 July). In the same year, two well-expressed peaks of catches showed presence of two generations of *Th. ampellophaga* in the vineyard in Gornoslav. The flight of the first generation was in the period of the end of May to the beginning of July. Catches indicating the presence of a second generation of the pest were found in the second half of August, and the total catch of the second generation was higher than the first one (ratio 1.4: 1). Relatively higher population density of *Th. ampellophaga* was observed in Gornoslav in comparison with Lozitsa.

Discussion

In Bulgaria, *Th. ampellophaga* was firstly recorded in the region of Sliven by LEDERER (1863) and REBEL (1903). According to the "System from the Really Defined Natural Territories" proposed by HUBENOV (1997) this species was reported in all main geographic regions of Bulgaria. BURESH & LAZAROV (1956) summarized the records about the distribution of the vine bud moth in Bulgaria published after LEDERER (1863) and REBEL (1903).

After the second half of 20th century many records appeared considering the pest status of the vine bud moth in this country (ANASTASOVA & GEORGIEVA, 1975; HARIZANOV *et al.*, 1980; 1994; 2006; HARIZANOV & HARIZANOV, 1983; HARIZANOV & HARIZANOVA, 1991; HARIZANOVA, 1996; KOSTADINOVA *et al.*, 2009; MAF, 2017 - Agricultural report for 2010), and also some faunistic records (DE FREINA & WITT, 2001; BESHKOV & LANGOUROV, 2004) are available. In Bulgaria, before the current study, *Th. ampellophaga* was found by means of pheromone traps only in the southern part of the country (summarized by SUBCHEV *et al.*, 2008b). To the best of our knowledge, this species has not been reported in Lozitsa and Gornoslav, although it is known from the regions of Pleven and Plovdiv, respectively. A possible reason for absence of catches of the pest in the vineyard in Plovdiv and Krumovo in 2015 can be the fungicide application or an influence of climatic conditions or their complex effect. The intensive use of fungicides and insecticides to control the pests in the crops in the last 50 years has brought to the rapid decline of the populations of this pest and exploitation of wild and decorative vines of *Parthenocissus* spp. as larval host plants (DOMINICI & PUCCI, 1989; EFETOV, 1990; EMBACHER & TARMANN, 2002). DOMINICI & PUCCI (1989) reported that high temperatures, up to 30°C can kill first instar larvae (40%) and wind and rain can cause the death of 3rd, 4rd and 5th instar larvae.

Different factors influence the diapause mechanism and the production of an additional generation of an insect species in the same year – global warming, local weather factors, photoperiod, habitat and microhabitat, host plant, food availability, altitude and latitude, and the number of generations is different from one country to another and even in a particular country (HUNTER & MCNEIL, 1997; BRYANT *et al.*, 2002; CURTIS & ISAAC, 2015; EL IRAQUI & HMIMINA, 2016).

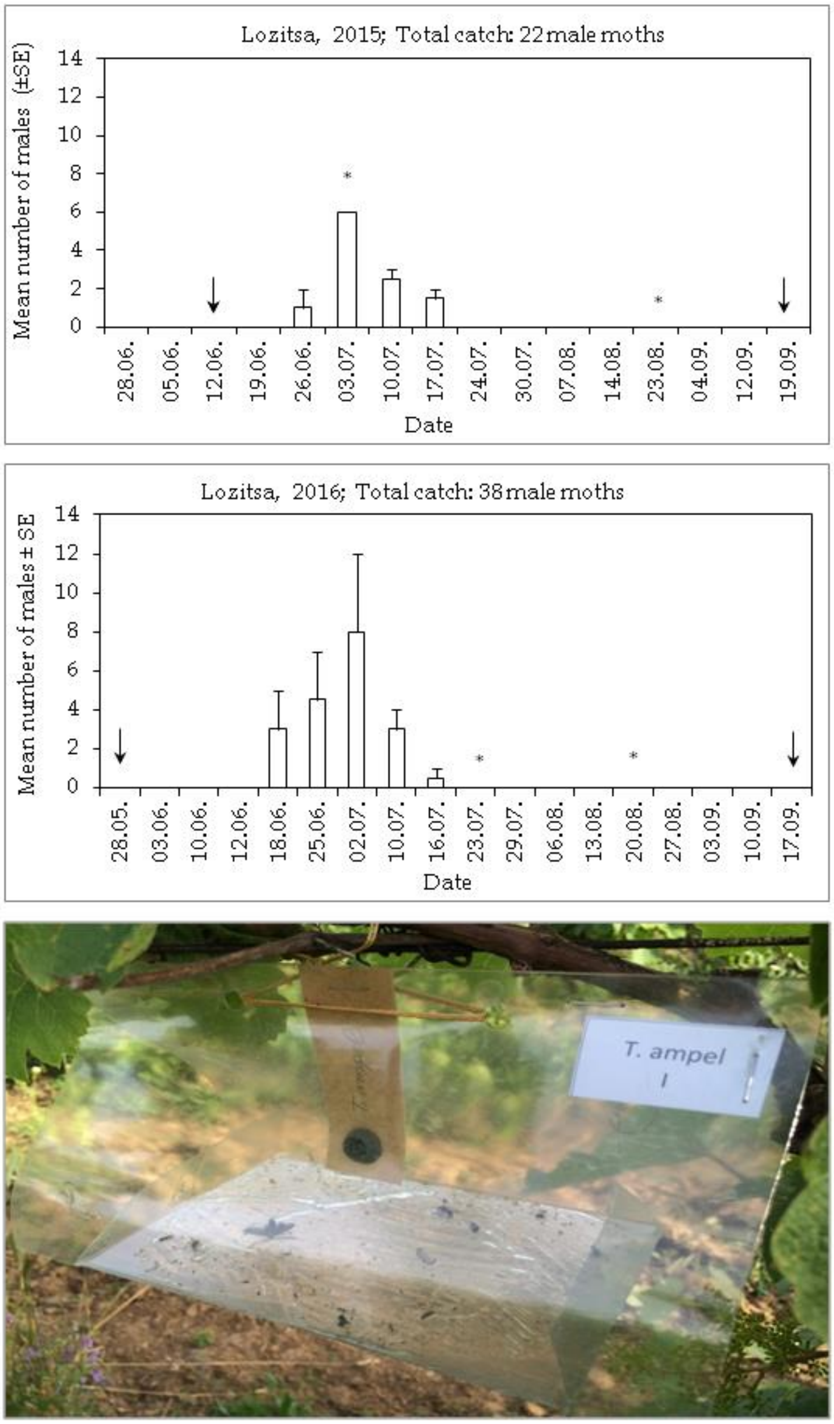

Fig. 2. Catches of *Th. ampellophaga* males in sticky traps baited with (2*R*)-butyl (7*Z*)-tetradecenoate in Lozitsa, 2015 and 2016 (two traps each year). For each year, arrows (↓) show the starting and finishing date of the investigation, and asterisks (*) the date when baits were renewed. The photograph shows a trap with a male moth captured in the period of 12-18 June, 2016.

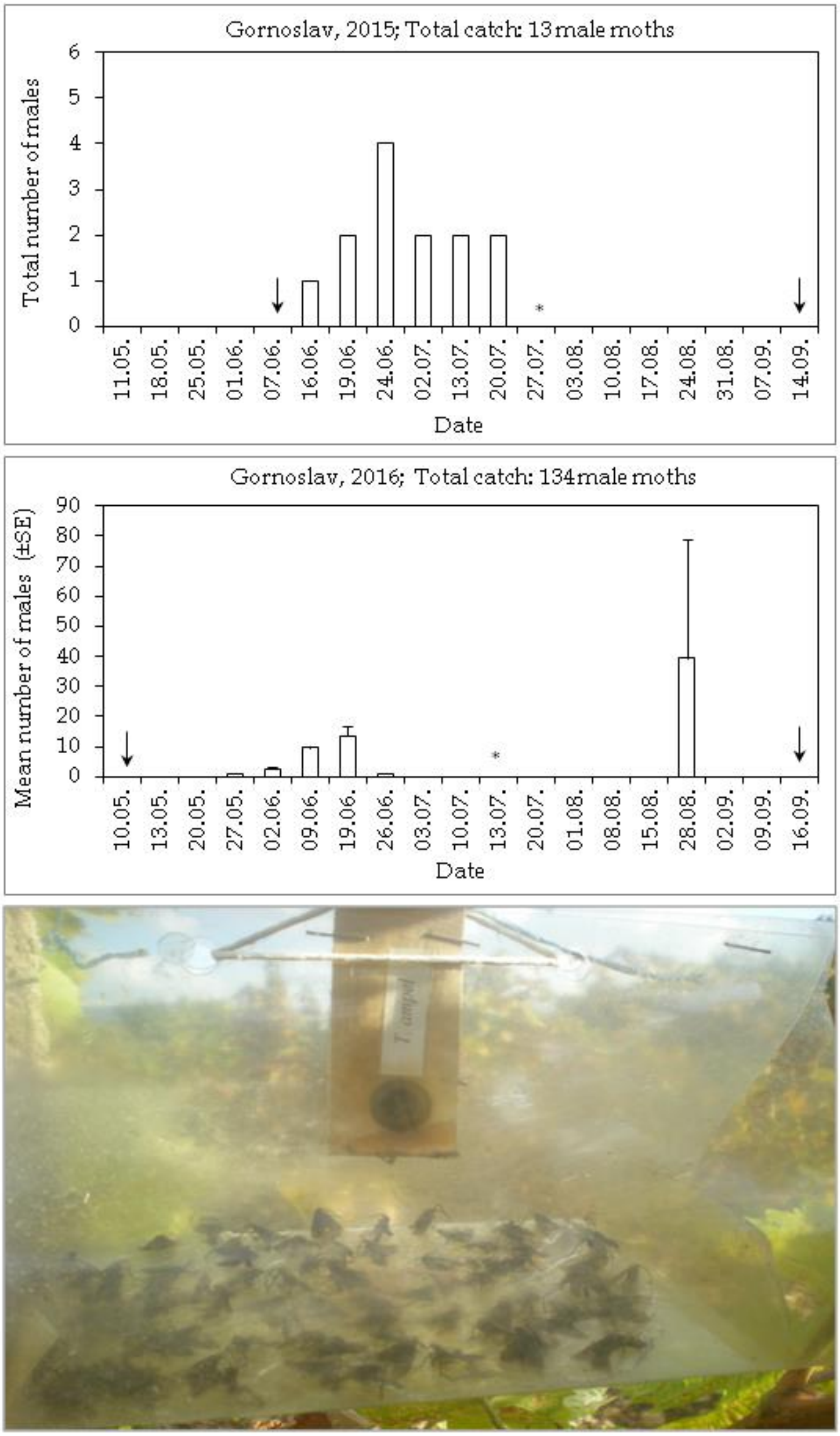

Fig. 3. Catches of *Th. ampellophaga* males in sticky traps baited with (2*R*)-butyl (7*Z*)-tetradecenoate in Gornoslav, 2015 (one trap) and 2016 (two traps). The photograph shows catches of male moths captured in the period of 15-28 August, 2016. For the legend, see Fig. 2

According to Pucci & Dominici (1986) and Efetov (2005), the vine bud moth has one generation in Central Italy and the Crimean Peninsula, respectively. Depending on climatic factors, in some years this species can develop partial second generation in South Russia (Krasnodar region), Georgia and Azerbaijan (Lipetskaya & Ruzaev, 1958; Devyatkin *et al.*, 2012). Two generations of this species were reported for the East Mediterranean countries, Syria and Lebanon (Talhouk, 1969).

Large-scale monitoring / detection investigations by sex pheromone traps in Europe and Turkey showed that *Th. ampellophaga* develops one generation per year in Hungary (Voigh *et al.*, 2000), Central Greece (Subchev *et al.*, 2006), Romania (Subchev *et al.*, 2008a), France (Rymarczyk & Drouet, 2006; Drouet & Lambert, 2010), Serbia (Nahirnić *et al.*, 2015) and some localities of the eastern Mediterranean part of Turkey (Can *et al.*, 2010) while second generation of this species was established in Rhodos Island (Greece) (Subchev *et al.*, 2006), other localities of the eastern Mediterranean part of Turkey (Can *et al.*, 2010) and Aegean Turkey (Can Cengiz *et al.*, 2012).

In Bulgaria, it is considered that *Th. ampellophaga* produces one full and a partial second generation per year (Harizanov *et al.*, 1994; Harizanov *et al.*, 2006). However, using enclosure field cage method for studying the life cycle of the pest in Novo selo (Ruse region) in 1974 Anastasova & Georgieva (1975) reported that this species is bivoltine, and the flight of the first generation moths started at the end of June with a peak of the flight – at the beginning of July (information about the flight of the second generation is not given). Using sex pheromone-baited traps Toshova & Subchev (2002) established only one generation of the vine bud moth in southern Bulgaria in 2000 – 2001 with flight from the beginning of June till the end of July – beginning of August.

During the current study we registered the flight of moths of one generation in northern Bulgaria (Lozitsa) in both 2015 and 2016 while in southern Bulgaria (Gornoslav) we recorded one and two flight periods in 2015 and 2016, respectively, which corresponded to the number of generations. The flight period of the first generation varies from the end of May – middle of June to the beginning – middle of July. In 2016, the early spring flight period of *Th. ampellophaga* in Gornoslav starting about three weeks earlier than the beginning of the flight in Lozitsa (20-28 May as compared to 12-18 June) favored development of a second generation in Gornoslav. In Greece (Subchev *et al.*, 2006) and Turkey (Can *et al.*, 2010), in the years and sites where the second generation of the vine bud moth was established and details are available, the flight of the second generation was from the end of July to the end of August.

Based on the results obtained by means of pheromone traps (Toshova & Subchev, 2002; current study) we can conclude that in Bulgaria *Th. ampellophaga* develops one generation and a second generation can occur in years with favorable conditions.

Conclusions

Th. ampellophaga was newly recorded in two sites in Bulgaria – Lozitsa (northern Bulgaria) and Gornoslav (southern Bulgaria) villages.

For first time by means of pheromone traps we registered two generations of the vine bud moth in Bulgaria.

The flight of the first generation of *Th. ampellophaga* was from the end of May - middle of June up to the second half of July at the investigated sites and years. A second generation at Gornoslav village in 2016 was registered at the second half of August.

Acknowledgments. Thanks are due to Prof. Mitko Subchev (Sofia, Bulgaria) and Prof. Konstantin A. Efetov (Simferopol, Crimea) for valuable comments. The study was supported by the Bulgarian Academy of Sciences, projects ANIDIV-2 and ANIDIV-3.

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Received: 13.03.2017
Accepted: 02.06.2017

ECOLOGIA BALKANICA
2017, Vol. 9, Issue 1 June 2017 pp. 91-102

Evaluation of Adsorption Capacity of Chitosan-Citral Schiff Base for Wastewater Pre-Treatment in Dairy Industries

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Abstract. In this study, we aimed to evaluate the adsorption capacity of the Schiff base chitosan-citral for its application in dairy wastewater pre-treatment. Chemical oxygen demand (COD) reduction was the factor used to evaluate the adsorption efficiency. The maximum COD percentage reduction of 35.3% was obtained at 40.0 °C, pH 9.0, adsorbent dose 15 g L^{-1}, contact time 180 min and agitation speed 100 rpm. It was found that the Langmuir isotherm fitted well the equilibrium data of COD uptake (R^2 = 0.968), whereas the kinetic data were best fitted by the pseudo-second order model (R^2=0.999). Enhancement of the adsorption efficiency up to 29.8% in dependence of the initial COD concentration of the dairy wastewater was observed by adsorption with the Schiff base chitosan-citral adsorbent compared to the non-modified chitosan at the same experimental conditions. The results indicated that the Schiff base chitosan-citral can be used for dairy wastewater physicochemical pre-treatment by adsorption, which might be applied before the biological unit in the wastewater treatment plant to reduce the load.

Key words: Chitosan-citral, Schiff base, adsorption, wastewater, COD removal efficiency.

Introduction

Natural biopolymers attract much attention due to their biocompatibility and biodegradability (Henriksen *et al.*, 1993; Ohkawa *et al.*, 2000; Peter, 1995). Chitin is the most abundant nitrogen containing biopolymer in nature and it is found widely in the shells of crabs, lobster, krill and shrimp. Chitin is used commercially to produce chitosan, a positively charged biopolymer with applications in food processing waste recovery (Peter, 1995; Savant & Torres, 2000; Torres *et al.*, 1999; Ahmed & Pyle, 1999; Shahidi *et al.*, 1999), chemical industries (Chen, 1998), biomedical and pharmaceutical industries (Lee *et al.*, 2001), and biotechnology (Peter, 1995). Chitosan can be easily characterized as a promising material not only due to its physical properties and applications to many fields, but also for its adsorption potential (Kyzas & Bikiaris, 2015).

Since the primary research work of Muzzarelli & Tubertini (1969), who described the synthesis and adsorption evaluation of chitosan for the removal of metal ions from organic and sea waters, numerous papers have been published regarding the use of chitosan as adsorbent for decontamination of wastewaters (or effluents, sea waters, drinking water samples, etc.) from various pollutants, either organic or inorganic species (KYZAS & BIKIARIS, 2015). The investigation of DEVI *et al.*, (2012) showed that low molecular weight crab shell chitosan (MW 20 kDa) could be effectively used as adsorbent in the treatment of dairy wastewater.

In more recent years, researchers have attempted to prepare chitosan-based adsorbent materials modifying the molecules of chitosan. ZHOU *et al.*, (2013) synthesized chitosan-modified biochars in efforts to produce a low-cost adsorbent for heavy metal environmental remediation. Characterization results showed that the coating of chitosan on biochar surfaces could improve its performance as a soil amendment or an adsorbent and had great advantage over many traditional adsorbents. Other researchers synthesized chitosan–zinc oxide nanocomposite materials for the application in milk processing industry wastewater (THIRUGNANASAMBANDHAM & SIVAKUMAR, 2016). As a result, the turbidity and COD were reduced after treatment with these materials.

The major advantage of chitosan is the existence of modifiable positions in its chemical structure. Several workers have reported the use of cross-linking treatments (via Schiff base formation) using several chemical reagents such as glutaraldehyde (SUGUNA *et al.*, 2010; WAN NGAH *et al.*, 2005), epichlorohydrin (WAN NGAH *et al.* 2005; VIEIRA & BEPPU, 2006), *etc.* for removal of metal ions, preventing the dissolution of chitosan in acidic solutions and to improve its metal ion adsorption properties.

The modifications of chitosan with grafting (inserting functional groups) or cross-linking reactions (joining the macromolecular chains each other) lead to the formation of chitosan derivatives with superior properties (enhancement of adsorption capacity and resistance in extreme media conditions, respectively). In the case of grafting reactions, the addition of extra functional groups to chitosan increases the number of adsorption sites and consequently the adsorption capacity. On the other hand, the cross-linking reactions slightly decrease the adsorption capacity, because some functional groups of chitosan (i.e., amino or hydroxyl groups) are bound with the cross-linker and cannot interact with the pollutant (KYZAS & BIKIARIS, 2015).

Dairy industry is one of the largest types of food industry, contributes to a great extent to pollution. In dairy industries, water has been a key processing medium. Water is used throughout all steps of the dairy industry including cleaning, sanitization, heating, cooling and floor washing — and naturally, the requirement of water is huge. Dairy wastewater is distinguished by the high biological oxygen demand (BOD) and chemical oxygen demand (COD) contents, high levels of dissolved or suspended solids including fats, oils and grease, nutrients such as ammonia or minerals and phosphates and therefore requires proper attention before disposal (SARKAR *et al.*, 2006). Dairy wastewater generally does not contain conventional toxic chemicals like those listed under EPA's Toxic Release Inventory. However, it has high concentration of dissolved organic components like whey proteins, lactose, fat and minerals (MUKHOPADHYAY *et al.*, 2003) and it is also malodorous, because of the decomposition of some of the contaminants causing discomfort to the surrounding population.

In general, dairy wastewaters coming from different sources are mixed, pH adjusted and then directed to biological unit for treatment. The higher loading of the biological unit leads to operational difficulties. Therefore, the wastewater physicochemical pre-treatment such as coagulation, flocculation and adsorption, which might be applied before the biological treatment unit in the wastewater treatment plant to reduce the load, became the main focus of the researchers (Chi & Cheng, 2006; Sarkar *et al.*, 2006; Kushwaha *et al.*, 2010).

Coagulation and flocculation processes are commonly used to remove suspended solids and organics. Many studies were performed to investigate the effective removal of COD and suspended solids by optimizing coagulant dosage and pH in the treatment of wastewater (Devi *et al.*, 2012; Amudaa & Amoob, 2007). Some authors reported that low molecular weight crab shell chitosan (Devi *et al.*, 2012) and rice husk (Pathak *et al.*, 2016) could be applied as effective adsorbents for removal of pollutants from dairy wastewater.

There are no reports in the literature about the application of the Schiff base chitosan-citral for pollution removal, therefore the aim of this work is to evaluate the adsorption capacity of the Schiff base chitosan-citral and to investigate the possibility of its applications in dairy industries for wastewater pre-treatment. Chemical oxygen demand (COD) reduction was the factor used to evaluate the adsorption efficiency.

Materials and Methods

Chitosan with a molecular weight of 100000 – 300000 Da and degree of deacetylation 78,98% (calculated in previous study by conductometric titration) was purchased from Across Organics (Belgium); Citral was obtained from Givaudan (France); other chemicals (methanol, ethanol) were of reagent grade and were used without further purification; Synthetic wastewater was prepared from whey obtained from yellow cheese processing plant by dilution with distilled water to the usual raw dairy wastewater pollution measured by COD.

Schiff base chitosan-citral preparation

The Schiff base chitosan-citral was synthesized as per method described earlier with slight modifications (Jin *et al.*, 2009). Chitosan was dispersed in 50 mL of methanol. Then aldehyde dissolved in anhydrous ethanol (20 mL) was added dropwise to the solution under high-intensity ultrasound at 30 – 40 °C for 5 h. When the reaction ended, the product was filtered, and the unreacted aldehyde was extracted in a Soxhlet apparatus with anhydrous ethanol for 12 h. The resulting Schiff base chitosan-citral was dried at 40 °C for 24 h and stored in desicator with sillica gel.

FTIR-spectroscopy

The FTIR spectra of chitosan and Schiff base chitosan-citral were recorded by Nicolet Avatar 330 FT - IR, Termo Science, (USA) spectrophotometer in KBr pellets. The scanning range was 400 – 4000 cm^{-1}.

Batch adsorption experiments

All experiments were carried out at a temperature of 40.0 ± 0.5 °C (based on preliminary experiments) in batch mode. The batch experiments were conducted in different flasks of 100 mL capacity using a water bath shaker at constant agitation speed (100 rpm) in order to ensuring constant mixing. Adsorption experiments were conducted in different batches for all the experimental conditions like pH of the solution, adsorbent contact time, adsorbent dose and initial COD concentration. The desired pH was maintained using diluted NaOH (0.1 mol L^{-1}) or HCl (0.1 mol L^{-1}) solutions. Each flask was filled with a known volume of sample having desired pH and stirring was initiated. The samples were withdrawn from the shaker at predetermined time intervals, filtered through filter paper and analyzed for COD concentration.

All experiments were performed in triplicate. The data were analyzed and presented as mean values.

The effect of pH was studied with constant initial concentration (3750 mg L^{-1}), adsorbent dose (0.1 g 10 mL^{-1}) and constant time, but varying the pH values from 6.0 to 9.0 using diluted NaOH or HCl solutions. The samples were agitated for 60 min, filtered and then analyzed for residual COD concentration.

To determine the contribution of the adsorbent dose on COD reduction, 10 mL of sample were treated with different doses of adsorbent ranging from 0.05 to 0.2 g 10 mL^{-1}. The other conditions were: contact time 60 min, pH 9.0 ± 0.2, and initial COD concentration 3750 mg L^{-1}.

Kinetics experiments

Batch kinetic experiments were carried out at pH 9.0 ± 0.2 and 40.0 ± 0.5 °C. For this purpose, 0.15 g of adsorbent were contacted with 10 mL of wastewater with initial COD concentration 3750 mg L^{-1} in 100 mL Erlenmeyer glass flasks on a water bath shaker at 100 rpm. At different time intervals ranging from 60 to 240 min the treated samples were withdrawn, filtered and was analyzed for residual COD concentration.

Equilibrium experiments

The equilibrium adsorption experiments were carried out by keeping all other conditions constant (40.0 ± 0.5 °C, 10 mL solution, 0.15 g adsorbent, pH 9.0 ± 0.2 and treatment time 240 min), except changing the initial COD concentration as follow: 3750, 2500, 1875, and 1500 mg L^{-1}.

Analytical methods

In this research, we studied the reduction in COD only. The COD of the wastewater samples was measured spectrophotometrically in accordance to the standard method ISO 15705 (2002) before and after treatment with adsorbent. The water sample is oxidized with a hot sulfuric solution of potassium dichromate, with silver sulfate as the catalyst. Chloride is masked with mercury sulfate. The concentration of green Cr^{3+} ions is than determined photometrically.

The pH values of samples were measured by using of pH-meter „Microsyst Labline" MS 2006.

The COD uptake was calculated from the difference between the initial and the final COD concentrations as follows:

$$q = \frac{(C_0 - C_f)}{m} \cdot V$$

where, q is the uptake (mg adsorbate/g adsorbent), C_0 and C_f are the initial and final COD concentrations (mg L^{-1}), m is the adsorbent amount (g) and V is the solution volume (L).

The COD reduction efficiency (RE, %) was estimated by the following equation:

$$RE = \frac{(C_0 - C_f)}{C_0} \cdot 100$$

Kinetic modeling

The following kinetics models were used in this study to model experimental data:

The Lagergren pseudo-first order model was employed due to its simplicity and good fit. This model is most commonly used to describe the adsorption of a solute from a liquid solution. It is based on the assumption that the rate is proportional to the number of free site (EL-NAAS *et al.*, 2010).

The linearized form of this model is given by:

$$lg(q_e - q) = lg\ q_e - \frac{K_{1,ads}t}{2.303} \quad (3)$$

where $K_{1,ads}$ is the kinetic constant of pseudo-first order adsorption (min^{-1}), q_e and q ($mg\ g^{-1}$) are the amounts adsorbed at equilibrium and at time t (min), respectively.

The linear plot of lg (q_e-q) versus t was plotted to evaluate this kinetic model (3) and to determine the rate constant and q_e from the slope and intercept, respectively.

In the pseudo-second order model used, the rate limiting step is the surface adsorption that involves chemisorption, where the adsorbate removal from a solution is due to physicochemical interactions between the two phases. The rate of sorption is proportional to the squire of the number of unoccupied sites (EL-NAAS *et al.*, 2010).

The model is usually represented by its linearized form as follow:

$$\frac{t}{q} = \frac{1}{K_{2,ads}q_e^2} + \frac{1}{q_e}t \quad (4)$$

where $K_{2,ads}$ ($g\ mg^{-1}\ min^{-1}$) is the pseudo-second order rate constant of adsorption.

The q_e and $K_{2,ads}$ parameters are calculated from the slope and intercept of the plot *t/q* versus *t*.

In recent years, Elovich's model has been successfully used to describe the adsorption of pollutants from aqueous solutions (EL-NAAS *et al.*, 2010).

The linearized form of this model is given by the following equation:

$$q = \frac{1}{b} ln\ (ab) + \frac{1}{b} ln\ t \quad (5)$$

where a ($g\ mg^{-1}\ min^{-1}$) is the initial adsorption rate and 1/b ($mg\ g^{-1}$) is a parameter related to the number of sites available for adsorption.

Sorption isotherm modeling

Equilibrium isotherm equations are used to describe experimental sorption data.

The Freundlich isotherm which has been widely used in correlating equilibrium data can be expressed by the following linearized logarithmic form:

$$lg\ q_e = lg\ K_F + \frac{1}{n} lg\ C_e$$

where q_e ($mg\ g^{-1}$) is the amount of COD removed per unit mass of the adsorbent, Ce ($mg\ L^{-1}$) is the residual COD concentration of the aqueous solution, K_F and n are Freundlich constants and measures of adsorption capacity and adsorption intensity, respectively. A higher n value (lower value of 1/n) implies stronger sorbent-pollutant interaction whereas 1/n equal to 1 indicates linear adsorption leading to identical adsorption energies for all sites (FEBRIANTO *et al.*, 2009).

The Langmuir isotherm is based on three assumptions: namely adsorption is limited to monolayer coverage, all surface sites are alike and only can accommodate one adsorbed atom and the ability of a molecule to be adsorbed on a given site is independent of its neighboring sites occupancy (FEBRIANTO *et al.*, 2009).

This isotherm can be described by the following linearized form:

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{1}{q_{max}} . C_e$$

where q_e ($mg\ g^{-1}$) is the equilibrium amount of COD adsorbed, Ce ($mg\ L^{-1}$) is the equilibrium concentration of COD in the solution, q_{max} ($mg\ g^{-1}$) and K_L ($L\ mg^{-1}$) are Langmuir constants representing the maximum monolayer adsorption capacity for the solid phase loading and the energy constant related to the heat of adsorption, respectively.

For the Langmuir isotherm analysis, the separation factor (R_L) value is of special importance:

$$R_L = \frac{1}{1+K_L C_0}$$

where C_0 (mg L^{-1}) is the initial COD concentration in the solution.

FOO & HAMEED, (2010) described four possibilities for the separation factor values, which determine the isotherm type: R_L = 0 (irreversible isotherm), R_L = 1 (linear isotherm), $R_L > 1$ (unfavorable isotherm) and $R_L < 1$ (favorable isotherm).

The Chi-squire error analysis function was used to find out the best fit model to the obtained experimental data (KUSHWAHA *et al.*, 2010). It is given as:

$$CHI^2 = \sum_{i=1}^{n} \frac{(q_{e,i,exp} - q_{e,i,cal})^2}{q_{e,i,exp}}$$

Results and Discussion

Schiff base chitosan-citral characterization

FTIR spectroscopy was used to confirm the structure of the Schiff base chitosan-citral. The FTIR spectra of chitosan (A) and Schiff base (B) samples are shown in Figure 1. Both spectra exhibit the absorption peaks at 1151, 1066, 1026, and 895 cm^{-1}, which can be assigned to the saccharide moiety. Among the bands characteristic to chitosan, in the FTIR spectra of the Schiff base chitosan-citral, a new peak was generated at 1647 cm^{-1}, attributed to C=N vibrations of imines. The figures exhibit a broad band at 3439 cm^{-1}, which corresponds to the stretching vibration of $-NH_2$ and OH bonds. Additionally, the characteristic absorption peak at 1597 cm^{-1} almost disappears, representing a decrease in $-NH_2$ group content, which indicates that the amino groups on chitosan reacted with citral to form a Schiff base chitosan-citral.

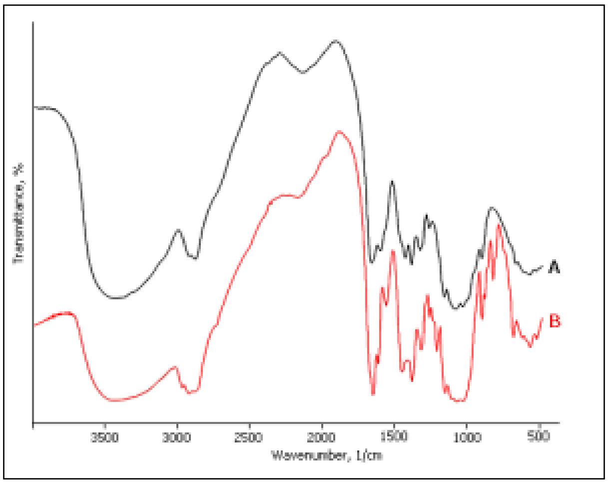

Fig. 1. FTIR – spectra of chitosan (A) and Schiff base chitosan-citral (B).

Effect of pH

The effect of pH was studied in the range of 6.0–9.0, based on the stability of the Schiff base in alkaline medium. The pH value of the solution is an important controlling parameter in the process of adsorption. The effect of pH on COD reduction efficiency in wastewater is shown in Figure 2.

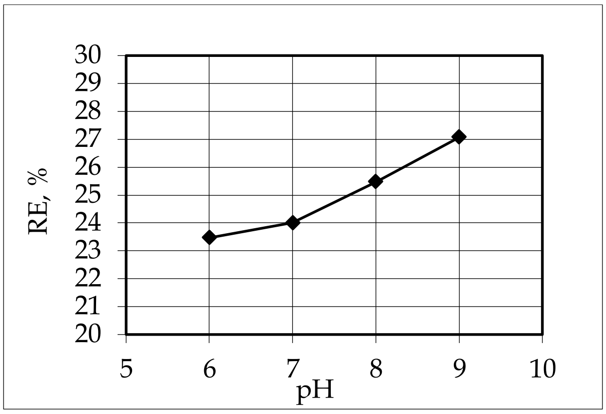

Fig. 2. Effect of pH on COD reduction efficiency (C_0 = 3750 mg L^{-1}, adsorbent dose 10 g L^{-1}, contact time 60 min).

Increasing the pH from 6.0 to 9.0 led to increasing of the COD reduction. The maximum reduction efficiency of 27.5% was observed at pH 9.0, hence this value was used for the rest of the experimental work.

Effect of adsorbent dosage

The adsorbent dosage played an important role in the adsorption process. The results on the adsorbent dosage effect on COD reduction in wastewater are presented in Figure 3. When the adsorbent dose was increased from 5 to 15 g L^{-1} the COD reduction efficiency increased from 18.3 to 28.4%. An increase in the dose beyond 15 g L^{-1} had no significant effect ($p < 0.05$) on COD reduction hence this value was used for the rest of the experiments. It was observed that the percentage removal was found to be increasing with increase in dosage and the results were similar with the investigations made by SARKAR *et al.*, (2006) and DEVI *et al.*, (2012). This could be attributed to a large number of vacant binding sites (as a result of grafting the extra functional groups), which are available for adsorption during the initial stages.

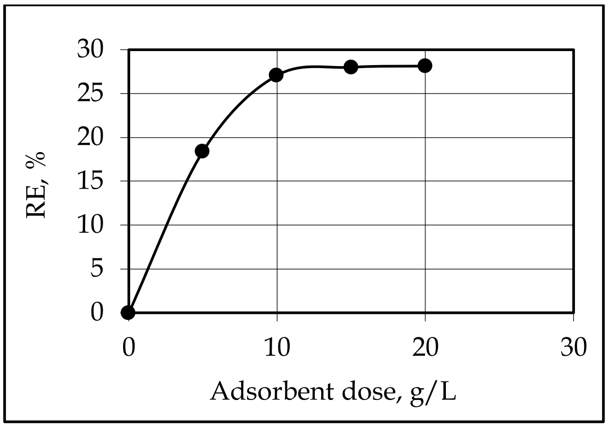

Fig. 3. Effect of adsorbent dose on COD reduction efficiency (C_0 = 3750 mg L^{-1}, pH 9.0, contact time 60 min).

Effect of contact time and kinetic modeling

Equilibrium time is another important operational parameter for the adsorption process effectiveness. The effect of contact time on COD uptake is presented in Figure 4 (by data pints). The COD reduction kinetics data indicated that after 180 min the equilibrium was reached and the equilibrium COD uptake was found to be 88.7 mg g^{-1}. The established highest COD reduction efficiency was 35.3%.

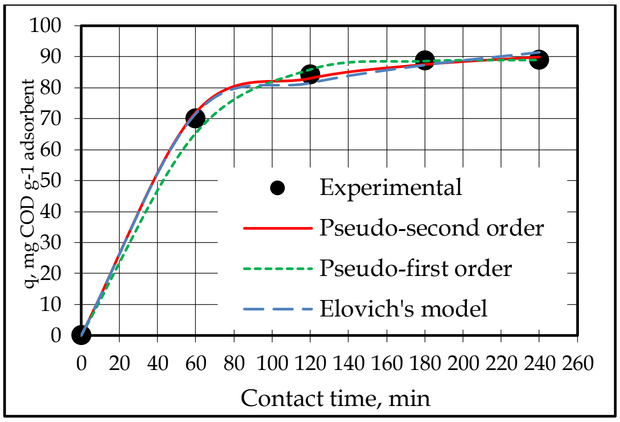

Fig. 4. Kinetics of COD uptake and fits of used kinetic models (C_0 = 3750 mg L^{-1}, pH 9.0, adsorbent dose 15 g L^{-1}).

Different kinetics models have been used to investigate the sorption mechanism and potential rate controlling steps, which are helpful for selecting optimum operating conditions for the full-scale batch process (FEBRIANTO *et al.*, 2009). The pseudo-first order, pseudo-second order and Elovich's models were employed in this study to modeling the experimental data. Results from linear regression analysis are presented in Figures 5, 6 and 7, and the kinetic parameters calculated for the used models are shown in Table 1.

Data from Table 1 for coefficients of determination (R^2) and the CHI-squire error proved that the pseudo-second order model provided the best fit for the kinetic data. Therefore, probably the rate limiting step is the surface adsorption that involves chemisorption, where the absorbate removal from a solution is due to physicochemical interactions between the two phases (EL-NAAS *et al.*, 2010). PATHAK *et al.* (2016) also reported that the second-order kinetic model was the best choice to describe the adsorption of pollutants from dairy wastewater by using of rice husk as adsorbent.

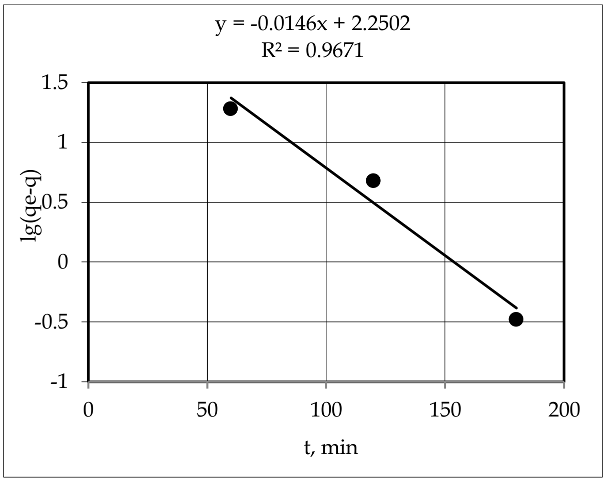

Fig. 5. Plot of lg(q_e – q) vs. t according to the pseudo-first order model (C_0 = 3750 mg L^{-1}, pH 9.0, adsorbent dose 15 g L^{-1}).

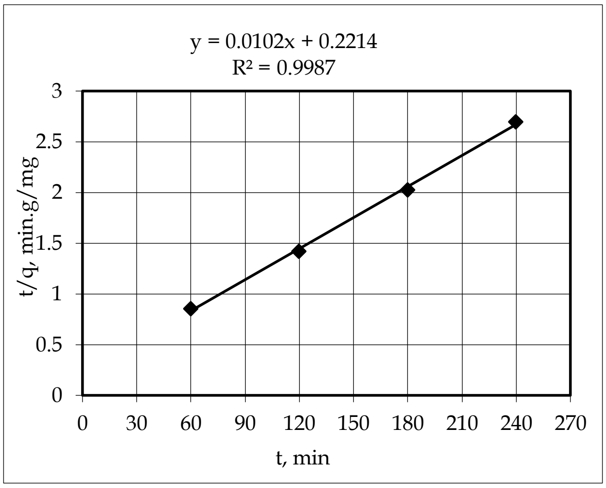

Fig. 6. Plot of t/q vs. t according to the pseudo-second order model (C_0 = 3750 mg L^{-1}, pH 9.0, adsorbent dose 15 g L^{-1}).

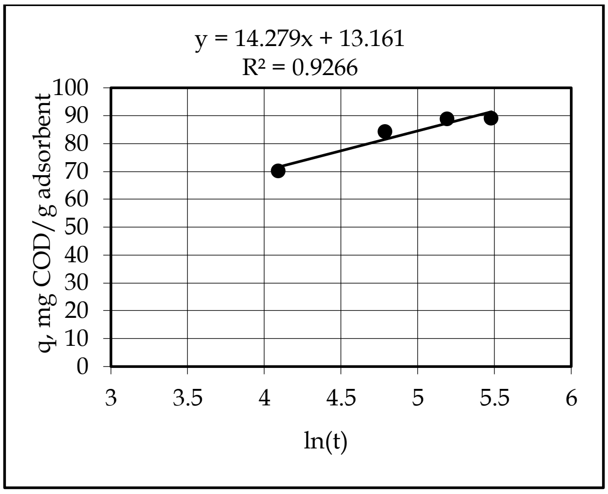

Fig. 7. Plot of q vs. Ln(t) according to the Elovich's model (C_0 = 3750 mg L^{-1}, pH 9.0, adsorbent dose 15 g L^{-1}).

Sorption isotherm modeling

The experimental data for equilibrium adsorption were fitted to the Freundlich and Langmuir isotherm models as presented in Figures 8 and 9. Isotherm model parameters calculated for the used models are shown in Table 2. The results from the adsorption isotherm study indicated that the experimental data were the best fit by the Langmuir model with higher coefficient of determination and lower Chi-squire error in comparison to the Freundlich model. A higher n value (lower value of 1/n) obtained for Freundlich model implies stronger sorbent-pollutant interaction, whereas calculated values of the Langmuir separation factor were R_L < 1.0, which corresponded to the favorable isotherm type. Similar results were obtained by PATHAK *et al.* (2016).

Table 1. Kinetic parameters for the adsorption of COD on the Schiff base chitosan-citral

Pseudo-first order model				Pseudo-second order model				Elovich's model			
q_e, mg g^{-1}	$K_{1,ads}$, min^{-1}	R^2	CHI^2	q_e, mg g^{-1}	$K_{2, ads}$, g (mg min)$^{-1}$	R^2	CHI^2	a, mg (g min)$^{-1}$	1/b, mg g^{-1}	R^2	CHI^2
177.9	0.034	0.967	0.34	98.0	4.7×10^{-4}	0.999	0.09	0.176	14.3	0.927	0.21

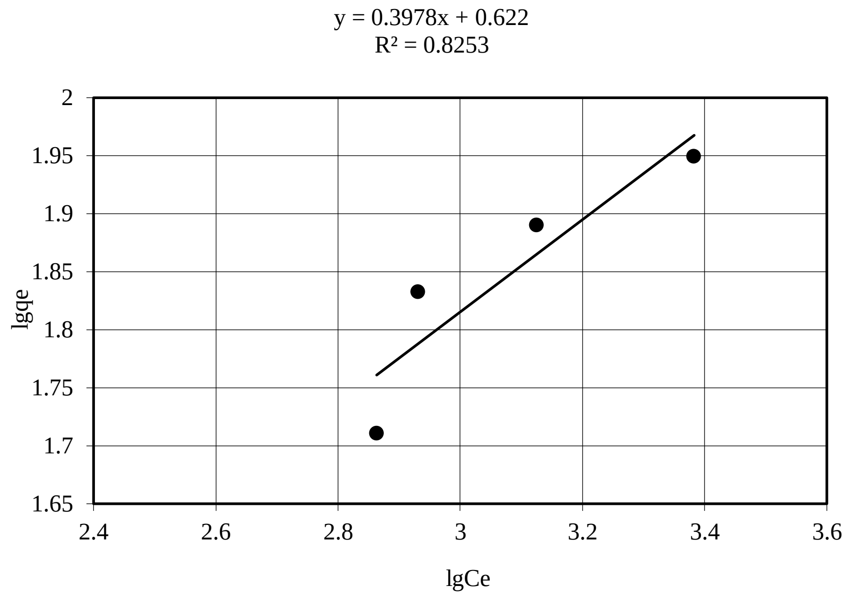

Fig. 8. Freundlich plot for COD reduction using Schiff base chitosan-citral adsorbent (40.0 °C, pH 9.0, adsorbent dose 15 g L^{-1}, contact time 240 min).

Table 2. Isotherm model parameters for the adsorption of COD on the Schiff base chitosan-citral

Langmuir model				Freundlich model			
q_{max}, mg g^{-1}	K_L, L mg^{-1}	R^2	CHI^2	K_F	n	R^2	CHI^2
119.0	1.27x10^{-3}	0.968	1.32	4.19	2.5	0.825	1.82

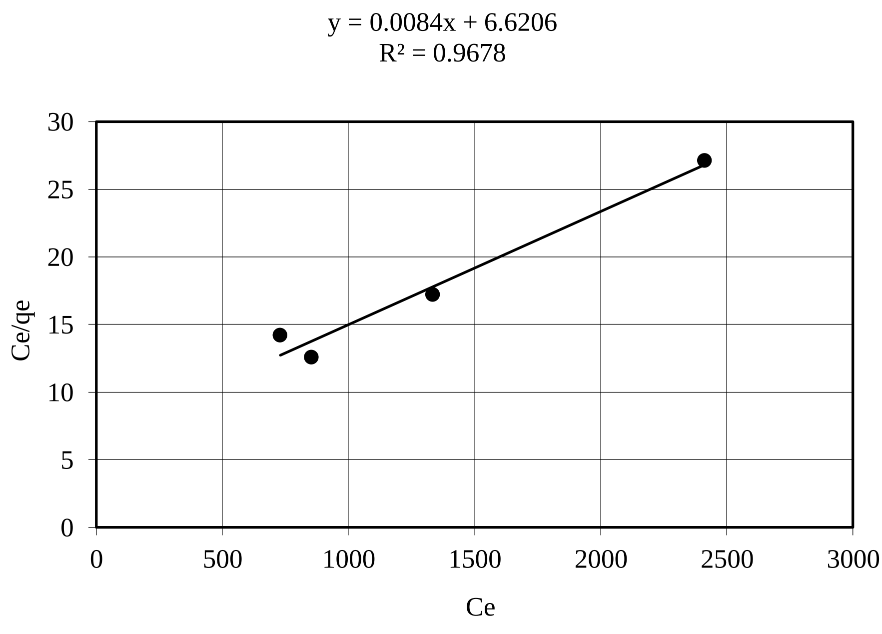

Fig. 9. Langmuir plot for COD reduction using Schiff base chitosan-citral adsorbent (40.0 °C, pH 9.0, adsorbent dose 15 g L^{-1}, contact time 240 min).

The results for COD reduction efficiency in application of chitosan and Schiff base chitosan-citral as adsorbents at different initial COD concentrations (Co, mg L^{-1}) are presented in Figure 10. The enhancement of adsorption efficiency between 23.2 and 29.8% in dependence of initial COD concentration of dairy wastewater was observed by adsorption with Schiff base chitosan-citral adsorbent compared to chitosan. This is probably due to increases of adsorption sites resulting from chitosan modification (KYZAS & BIKIARIS, 2015).

At lower pH, the amino groups in chitosan are protonated and make it positively charged and since particles in the effluent are negatively charged, the electrostatic interaction will be strong DEVI *et al.*, (2012). Chitosan is a very attractive adsorbent by allowing the molecules to bind negatively charged surface via ionic or hydrogen bonding or electrostatic interaction. When pH is increased, surface charge of chitosan is decreased so the charge neutralization becomes less important. The probable mechanism for wastewater treatment using Schiff base chitosan-citral could be explained with modifying process by grafting reactions. The addition of extra functional groups to chitosan increases the number of adsorption sites and consequently the adsorption capacity. The mechanism of bridging probably becomes the major mechanism of interaction between adsorbent and pollutants.

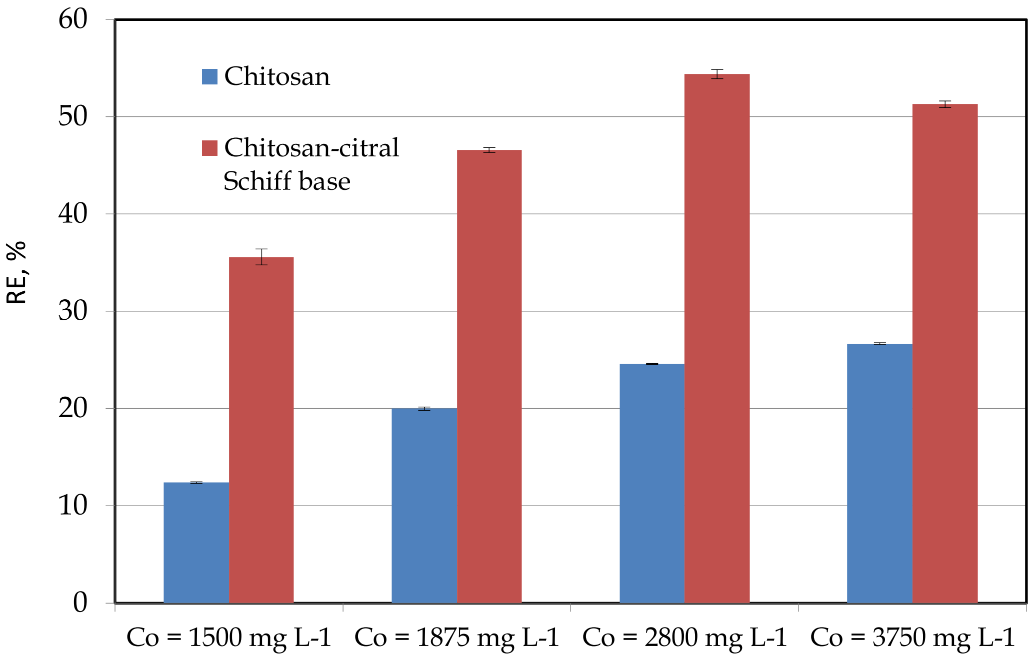

Fig. 10. COD reduction efficiency in application of chitosan and Schiff base chitosan-citral as adsorbents (40.0 °C, pH 9.0, adsorbent dose 15 g L^{-1}, contact time 240 min).

Conclusions

For the first time, a wastewater treatment study was carried out using Schiff base chitosan-citral. Enhancement of the adsorption efficiency up to 29.8% in dependence of initial COD concentration of dairy wastewater was observed by adsorption with Schiff base chitosan-citral adsorbent, compared to chitosan at the same experimental conditions. The major advantage of the Schiff base chitosan-citral as adsorbent material is the possibility of its application at alkaline medium. The results from this study indicated that Schiff base chitosan-citral could be applied for physicochemical pre-treatment by adsorption of dairy wastewater before biological unit of the wastewater treatment plant to reduce the load. Chemical oxygen demand (COD) of the investigated wastewater was found to be on pH, adsorbent dosage and contact time dependent. The maximum percentage reduction of COD concentration of 35.3% was observed at 40.0 °C, pH 9.0, adsorbent dose 15 g L^{-1}, contact time 180 min, and agitation speed 100 rpm. It was found that the Langmuir isotherm fitted well the equilibrium data of COD uptake (R^2 = 0.968), whereas the kinetic data were best fitted by the pseudo-second order model (R^2 = 0.999).

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Received: 09.05.2017
Accepted: 26.06.2017

ECOLOGIA BALKANICA

2017, Vol. 9, Issue 1 June 2017 pp. 103-104

Short note

Liposcelis meridionalis (Rosen, 1911) (Psocoptera: Liposcelididae) – New to the Bulgarian Fauna

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Abstract. The first record of *Liposcelis meridionalis* (Rosen, 1911) (Psocoptera: Liposcelididae) in Bulgaria was reported: Bulgaria, East Rhodopes Mts., South of village of Stremtsi, N41° 42' 56.16'' E25° 24' 29.81'', 500 m a.s.l.

Keywords: barklice, distribution, Bulgaria.

The species *Liposcelis meridionalis* (Rosen, 1911) (Psocoptera: Liposcelididae) has never been previously recorded in Bulgaria, but was collected from neighboring Greece (LIENHARD, 1998; LIENHARD & SMITHERS, 2002). In this short note I report the first find of *L. meridionalis* in Bulgaria.

Material examined: Bulgaria, East Rhodopes Mts., South of Stremtsi Village, mixed forest with *Pinus silvestris* and various broad leaf tree and bush species, in a dead trunk of *Betula alba* laying on the ground at the forest edge, 28.10.2016, 4 ad. (3 in ethanol, 1 mounted on microscope slide with glycerin, Fig. 1 and 2), N41° 42' 56.16'' E25° 24' 29.81'', 500 m a.s.l., associated Psocoptera: *Lepinotus reticulatus* Enderlein, 1905, D. Georgiev leg., det., coll.

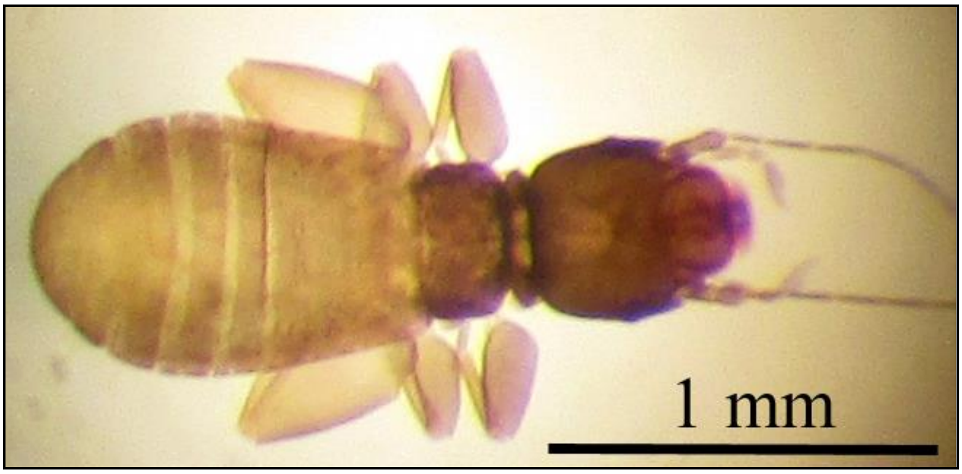

Fig. 1. Female (slide-mounted) of *Liposcelis meridionalis* from E Rhodopes, general view.

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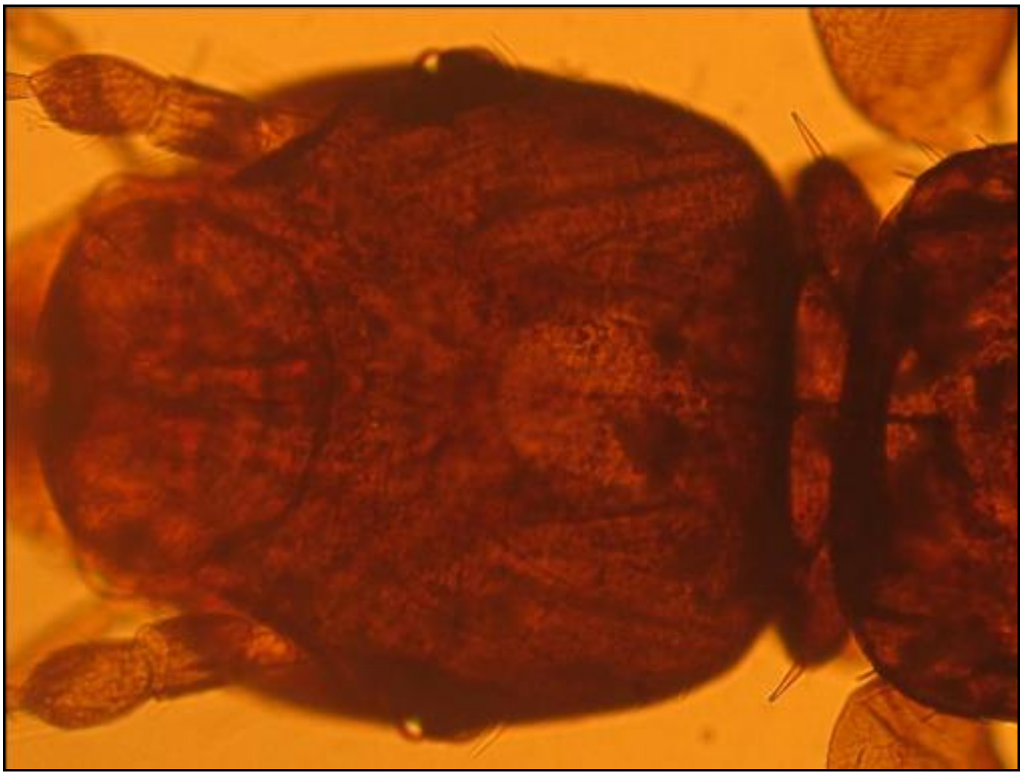

Fig. 2. Same specimen of *Liposcelis meridionalis* - close view of the head, the pronotum and the anterior part of the mesonotum (light microscope, 40x).

Acknowledgements. I would like to express my gratitude to Dr Charles Lienhard (Natural History Museum of Geneva) for the very useful discussion concerning the *Liposcelis meridionalis* identification.

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Received: 27.01.2017
Accepted: 19.02.2017

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. 9, iss. 1, 2017 of Ecologia Balkanica.

Alexi Popov - National Museum of Natural History, BAS (BULGARIA)
Anelia Stojanova – Plovdiv University, Faculty of Biology, Department of Zoology (BULGARIA)
Charles Lienhard - Natural History Museum of Geneva (SWITZERLAND)
Elif ÖztetİK - Anadolu University (TURKEY)
Feza Can Cengiz - Mustafa Kemal University (TURKEY)
Gana Gecheva - Plovdiv University, Faculty of Biology, Department of Ecology and EC (BULGARIA)
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Joanna Czarnecka - Maria Curie-Sklodowska University in Lublin (POLAND)
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Mitko Subchev - Institute on Biodiversity and Ecosystem Research, BAS (BULGARIA)
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The manuscripts must be prepared 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. All abbreviations and acronyms should be defined at first mention. To facilitate reader comprehension, abbreviations should be used sparingly.

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The manuscripts should conform to the following format:
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The standard order of sections should be: Abstract, Key words, Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusions (optional), Acknowledgements (optional) and References.

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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. **Avoid presentation of the same information as text and/or figure and/or table.**
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In the *Acknowledgements* 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. No fold-outs are accepted. Tables must be numbered consecutively. **They should be placed within the text at the desired position by the author(s).** An explanatory caption, located on the top of the table, should be provided.

Example:

Table 1. Shannon-Wiener indexes in the burned ($\mathbf{H}_{burned}$) and control ($\mathbf{H}_{control}$) 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 (16 × 24 cm) which is the limit for all illustrations. Magnification should be shown by scale bars. Colour illustrations are accepted, but will appear only in the electronic version of the journal (PDF). The illustrations in the hardcopy printed version will be greyscale. All illustrations must be sharp, of high quality with at least 300 dpi. The following formats are acceptable: JPEG, GIF, TIFF, EPS. Figures must be numbered consecutively and should be provided with an explanatory legend below them. *When the figures are presenting maps of the studied area, we recommend using some kind of GIS software for the preparation of the maps, or use of other indicative or topographical maps.*

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Fig. 1. Indicative map of the study area.

All tables and figures must be referred to in the text!

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Literature citations in the text should indicate the author's surname in SMALL CAPITALS (for more information on SMALL CAPITALS and how to make them in Word) with the year of publication in parentheses, e.g. CARLIN (1992); BROOKS & CARLIN (1992); SHAPIRO *et al.* (1968). Citations in brackets should be divided with semicolons and the author's name and the year of publication with comma (*example:* CARLIN, 1992; BROOKS & CARLIN, 1992; SHAPIRO *et al.,* 1968). If there are more than two authors, only the first should be named, followed by "*et al.*" in *italic*. References at the end of the paper should be listed in alphabetical order by the first author's family name and chronologically. If there is more than one work by the same author or team of authors in the same year, a, b, etc. is added to the year both in the text and in the list of references. Each citation in the text must be accompanied by a full reference in the list of references and vice versa. **<u>Please provide DOI numbers for all electronic resources (journal articles, online articles, books), if they have DOI, when cited in the references list!</u>**

Examples:

A journal article:

AUTHOR A. 1990. Title of the article. - *Full title of the journal*, 56(3): 35-105.

AUTHOR A., B. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(2): 35-105.

AUTHOR A., B. AUTHOR, C. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(1): 35-105.

A book:

AUTHOR A. 2000. *Title of the book.* Vol. I. Place of publication. Publishing house.

Proceedings or book chapter:

AUTHOR A., B. AUTHOR 1990. Title of the contribution. - In: Author A. (Ed.): *Title of the book or proceedings.* Place of publication. Publishing house, pp. 235-265.

Software:

STATSOFT INC. 2004. *STATISTICA (Data analysis software system)*, Vers. 7. Computer software. Available at: [http://www.statsoft.com].

Website:

FAUNA EUROPAEA. 2007. *Invertebrates. Fauna Europaea.* Vers. 1.1. Available at: [http://www.faunaeur.org]. Accessed: 12.10.2009.

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it is recommended to be used. If there is not such a summary, the author's names must be transcribed and the title of the paper must be translated into English and put in square brackets. If the name of the journal is also not in Latin letters it also should be transcribed (not translated). This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian, English summary).

Example:

ANGELOV P. 1960. Communications entomologiques. I. Recherches sur la nourriture de certaines espèces de grenouilles. – *Godishnik na muzeite v grad Plovdiv*, 3: 333-337. (In Bulgarian, Russian and French summary).

KOROVIN V. 2004. [Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural]. Ekaterinburg, Published by Ural University, 57 p. (In Russian).

Names of persons who provided unpublished information should be cited as follows: "(ANDERSSON, 2005, Stockholm, pers. comm.)".

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Small capitals and italic letters. The Latin genus and species names must be cited completely once in the text and should be typed in *italic*. Family names of the authors in the citations and the publications listed in the reference list must be in SMALL CAPITALS, but never for collectors, preparators, acknowledgements, etc.

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Mean values should always be accompanied by some measure of variation. If the goal is to describe variation among individuals that contribute to the mean standard deviation (SD) must be used. When the aim is to illustrate the precision of the mean standard errors (SE) should be given. The last paragraph of Materials and Methods section should briefly present the significance test used. Quote when possible the used software. Real p values must be quoted both at significance or non-

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The authors of articles that are based on experiments that caused injuries or death of animals should explain and justify the grounds of the study and state that the scientific results of the study is at least in trade-off with the sufferings caused. In the Materials and Methods section of the manuscript, the authors should detail as precisely the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not invasive. When alternative non-harming techniques exist, but were not used, the manuscripts may not be considered for publication.

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