ECOLOGIA BALKANICA

International Scientific Research Journal of Ecology

Volume 6, Issue 2
December 2014

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

UNIVERSITY OF PLOVDIV PUBLISHING HOUSE

International Standard Serial Number
Print ISSN 1314-0213; Online ISSN 1313-9940

Aim & Scope

„Ecologia Balkanica" is an international scientific journal, in which original research articles in various fields of Ecology are published, including ecology and conservation of microorganisms, plants, aquatic and terrestrial animals, physiological ecology, behavioural ecology, population ecology, population genetics, community ecology, plant-animal interactions, ecosystem ecology, parasitology, animal evolution, ecological monitoring and bioindication, landscape and urban ecology, conservation ecology, as well as new methodical contributions in ecology. Studies conducted on the Balkans are a priority, but studies conducted in Europe or anywhere else in the World is accepted as well.

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

Peer review process

All articles included in "Ecologia Balkanica" are peer reviewed. Submitted manuscripts are sent to two or three independent peer reviewers, unless they are either out of scope or below threshold for the journal. These manuscripts will generally be reviewed by experts with the aim of reaching a first decision as soon as possible. The journal uses the double anonymity standard for the peer-review process. Reviewers do not have to sign their reports and they do not know who the author(s) of the submitted manuscript are.

We ask all authors to provide the contact details (including e-mail addresses) of at least four potential reviewers of their manuscript. These should be experts in their field of study, who will be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years and should not be members of the same research institution. Members of the Editorial Board of the journal can be nominated. Suggested reviewers will be considered alongside potential reviewers identified by their publication record or recommended by Editorial Board members.

Once an article is accepted, it is published in Ecologia Balkanica immediately online first as a PDF file. The paper will subsequently be published in both full text formatted PDF online (open access) and in the print copy of the journal.

Abstracting & Covarage

- CAB International - CAB Abstracts
- Directory of Open Access Journals (DOAJ)
- GeoBase (Elsevier)
- Index Coupernicus
- Ulrich's Web (ProQuest)
- Zoological Record (Thompson Reuters)
- IMBIOMED
- Reasearch4Life (OARE, AGORA, HINARI)
- BASE (Bielefeld Academic Search Engine)
- EBSCO
- All-Russian Institute of Scientific and Technical Information (VINITI) (Referativnyi zhurnal)
- Socol@r (China)
- Google Scholar Search Engine and many others.

Editorial Board

Editor-In-Chief:
Prof. Iliana Velcheva, PhD
University of Plovdiv, Department of Ecology and Environmental Conservation, BULGARIA

Co-Editor-In-Chief:
Ch. Assist. Prof. Ivelin Mollov, PhD - University of Plovdiv, Department of Ecology, BULGARIA

Secretary:
Ch. Assist. Prof. Vessela Yancheva, PhD - University of Plovdiv, Department of Ecology, BULGARIA

Editorial Board:
Assoc. Prof. Simeon Vasilev, PhD - Chairman of Union of Scientists in Bulgaria - Plovdiv, BULGARIA
Assoc. Prof. Gana Gecheva, PhD - University of Plovdiv, Department of Ecology, BULGARIA
Assoc. Prof. Dilian Georgiev, DSc - University of Plovdiv, Department of Ecology, BULGARIA
Ch. Assist. Prof. Slaveya Petrova, PhD - University of Plovdiv, Department of Ecology, BULGARIA

Associate Editors:
Prof. Peter Genov, DSc - Institute of Zoology, BAS, Sofia, BULGARIA
Prof. Georgi Georgiev, DSc - Forest Research Institute, BAS, Sofia, BULGARIA
Prof. Panos Economidis, Dsc - Aristotle University, Laboratory of Ichthyology, GREECE
Prof. Dan Cogălniceanu, PhD - Ovidius University Constanta, ROMANIA
Prof. Bilal Öztürk. PhD - EGE University, Izmir, TURKEY
Prof. Elena Zheleva, PhD - University of Forestry, Sofia, BULGARIA
Prof. Aziza Sharaby, PhD - National Research Center. Cairo, EGYPT
Assoc. Prof. Željko Tomanović, DSc - Belgrade University, Faculty of Biology, SERBIA
Assoc. Prof. Dimitar Bechev, DSc - University of Plovdiv, Department of Zoology, BULGARIA
Assoc. Prof. Angel Tsekov, PhD - University of Plovdiv, Department of Ecology, BULGARIA
Assoc. Prof. Atanas Arnaudov, PhD - University of Plovdiv, Faculty of Biology, BULGARIA
Assoc. Prof. Vencislava Vancheva, PhD - Agriculture University, Plovdiv, BULGARIA
Assoc. Prof. Jordan Živanović, PhD - Goce Delcev University, Stip, MACEDONIA
Assoc. Prof. Vladimir Pešić, PhD - University of Montenegro, Depart. Biology, MONTENEGRO
Assoc. Prof. Snezana Stavreva-Veselinovska, PhD - Goce Delcev University, MACEDONIA
Assist. Prof. Ignacy Kitowski, PhD - University of Life Sciences in Lublin, POLAND
Assist. Prof. Joanna Czarnecka, PhD - Maria Curie-Sklodowska University, Lublin, POLAND
Assist. Prof. Kerim Çiçek, PhD - Ege University, Bornova/Izmir, TURKEY
Assist. Prof. Marko Sabovljević - University of Belgrade, Institute of Botany and Garden, SERBIA
Dimitra Bobori, PhD - Aristotle University, School of Biology, Thessaloniki, GREECE

Contact Publisher

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

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

Note that there is also an electronic version of the journal – **open access**. You may download the full text version of "Ecologia Balkanica" – Volume 6, Issue 2/2014 from:

http://eb.bio.uni-plovdiv.bg

Partners and collaborators

Index Copernicus (IC) is a world-wide gateway to complex scientific information. This system is aimed at: academic-level researchers, clinical practitioners, information and reprint providers, librarians, journal editors, government agencies.
Contact: Al. Jerozolimskie 146 C, 02-305 Warsaw, Poland.
Tel: +48 22 347 50 77 E-mail: office@IndexCopernicus.com
http://www.indexcopernicus.com/

Elsevier – GEOBASE
GEOBASE is a unique multidisciplinary database supplying bibliographic information and abstracts for development studies, the Earth sciences, ecology, geomechanics, human geography, and oceanography. The database provides current coverage of almost 2,000 international journals, including both peer-reviewed titles and trade publications, and provides archival coverage of several thousand additional journal titles and books. GEOBASE contains over 1.4 million records from 1980 to present, with more than 100,000 citations and abstracts added annually (almost 985 of all records include abstracts).
http://www.elsevier.com

CABI is a not-for-profit international organization that improves people's lives by providing information and applying scientific expertise to solve problems in agriculture and the environment. Our mission and direction is influenced by our member countries who help guide the activities we undertake. These include scientific publishing, development projects and research, and microbial services. 2010 marks 100 years of CABI. Since its beginnings as an entomological committee in 1910, it then developed into a Commonwealth organization before becoming a truly international service in agricultural information, pest identification and biological control in the mid 1980s.
Contact: http://www.cabi.org/

EBSCOhost® databases are the most-used, premium online information resources for tens of thousands of institutions worldwide, representing millions of end-users.
Contact: 10 Estes Street Ipswich, MA 01938 USA
Phone: (978) 356-6500, Toll Free: (800) 653-2726 (USA & Canada)
Fax: (978) 356-6565 E-mail: information@ebscohost.com
http://www.ebscohost.com/

The free scholastic search engine is established by China Educational Publications Import and Export Corporation (CEPIEC). Under the leadership of China Ministry of Education, CEPIEC is the only company dealing with publication ex-im business in the education field. CEPIEC has been serving colleges and universities, research institutions and public libraries in China for more than 20 years, providing them with excellent academic resources from all over the world.
Contact: No 41 Zhong Guan Cun Street, Haidian District, Beijing, P.R.China 100080, Tel: +86 (0)10 6251 4068 E-mail: li_yanxia@cepiec.com.cn
http://www.cepiec.com.cn, http://www.socolar.com/

Directory of Open Access Journals is a service that provides access to quality controlled Open Access Journals. The Directory aims to be comprehensive and cover all open access scientific and scholarly journals that use an appropriate quality control system, and it will not be limited to particular languages or subject areas. The aim of the Directory is to increase the visibility and ease of use of open access scientific and scholarly journals thereby promoting their increased usage and impact.
http://www.doaj.org/

The Journals Impact Factor (JIF) provides a reflecting tools for ranking, categorizing, evaluating, and comparing various International/National Journals having ISSN published by Learning societies Universities and Institutions & Publication house. This factor is used for evaluating the prestige of journals. The evaluation is carried out by considering different the factors like Peer Review, Originality of Content, Technical editing quality, editorial quality and affiliation etc. Thus, the impact factor of a journal is calculated by dividing the number of current year citations to the source items published in that journal during the previous years. http://www.jifactor.org/

Open Access Policy

Ecologia Balkanica provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. Open Access in journal publishing means:
* Direct, barrier-free, online dissemination of scientific results at no charge for readers Authors retain the copyright of their articles,
* Open Access papers may be copied, downloaded, and used for text- and data-mining purposes, provided that such uses are fully attributed on a non-commercial basis,
* High visibility of the work – anyone can read your article at no charge,
* Higher citation rates due to higher visibility,
* Quick publishing as pre-prints published immediately upon acceptance,
* Articles are archived and searched through several major OA repositories

Manuscript submission and peer review process

The following types of manuscripts are accepted: short research notes (up to 4 pages), research articles (up to 10 pages) and review papers (up to 20 pages). Short research notes are shorter submissions of a preliminary nature or those including new records or observed phenomenon etc. Research articles should present significant original research in the various fields of ecology. Review papers should deal with topics of general interest or of contemporary importance, being synthetic rather than comprehensive in emphasis. Authors of Review papers should consult with the Editor before submission.
Submitted manuscripts are sent to two or three independent peer reviewers, unless they are either out of scope or below threshold for the journal. These manuscripts will generally be reviewed by experts with the aim of reaching a first decision as soon as possible. The journal uses the double anonymity standard for the peer-review process. Reviewers do not have to sign their reports and they do not know who the author(s) of the submitted manuscript are.

Once an article is accepted, it is published in Ecologia Balkanica immediately online first as a PDF file. The paper will subsequently be published in both full text formatted PDF online (open access) and in the print copy of the journal. The **rejection rate** of received manuscripts of the journal for 2014 is **13.79%.**

ECOLOGIA BALKANICA – Volume 6, Issue 2 / 2014

CONTENTS

Research Articles

Short notes

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 1-5

Prerequisite for the Development of Ecotourism on the Territory of the Town of Zlatograd and Possible Environmental Issues

Ekaterina Valcheva[1*], *Krasimir Aleksandrov*[2]

1 - Agricultural University, Faculty of Plant Protection and Agroecology,
12 Mendeleev Blvd.,BG-4000 Plovdiv, BULGARIA
2 - Agricultural University, Faculty of Economy,
12 Mendeleev Blvd.,BG-4000 Plovdiv, BULGARIA
* Corresponding author: *katia_valcheva@abv.bg*

Abstract. The basis for the development of ecotourism in Bulgaria is the tourist resource that provides a system of protected areas and historic cultural heritage. One of the key elements of ecotourism are the environmental protection and the creation of real opportunities for economic prosperity and livelihood of communities in settlements near the protected areas. Tourism is considered a priority in the economic development not only in Zlatograd Municipality, but also in Smolyan District. The main prerequisite for the successful development of tourism in the municipality is the realistic assessment of the factors that are relevant to tourism. Object of this study is the town of Zlatograd as an ecotourist destination and the possible environmental issues resulting from the development of tourism.

Keywords: anthropogenic pressures, sustainable management, ecotourism, biodiversity, protected areas.

Introduction

The realization of one of the main directions of the EU Agricultural Policy - Rural Development, provides for the use of different approaches, tools and activities for achieving the goal - development of economic life and the economy in these areas; creating a livelihood and incomes through activities diversification; equalization of social and economic conditions in the regions. One means of achieving this goal is the development of rural tourism (LULCHEVA, 2011).

With its social, economic, political and environmental importance, tourism contributes to strengthening health and recovery of people's work capacity; enriches their knowledge; increases their linguistic culture; creates new jobs; provides income for those employed in it; changes the look of the settlements and the territories in which it is being developed and improves the standard of living of the local population (TONCHEVA, 1994).

It is known that Bulgaria has considerable resources for tourism development. The conditions in the country are suitable for practicing different type of tourism throughout the whole year. The success of tourism is largely related to the state of the environment where it operates. The relationship between tourism and the environment is bilateral. In the last decades we have witnessed quite often the negative impact of the activities, which provide good conditions for tourism, on the environmental components (LAKOV, 2011).

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg
Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

National and nature parks have a leading role in the development of ecotourism, which is a small, but rapidly growing industry, governed by the laws of the market and its regulations. Ecotourism is one of the main priorities for development of the Rhodope Region.

The Rhodope Mountains with their geographical position, climate and numerous mineral springs, natural and historical sights meet all requirements therein to develop ecotourism. The studied area - Zlatograd Municipality, is one of the few significant Rhodope areas which has a huge potential for the development of ecotourism.

Materials and Methods

Study area. Zlatograd Municipality is located about 300 km from the capital Sofia and 70 km from the international resort Pamporovo. Administratively, it is part of Smolyan District and its administrative centre is the town of Zlatograd, the most southern Bulgarian town. Municipality includes nine villages - Startsevo, Dolen, Erma Reka, Alamovtsi, Tsatsatovtsi, Strashimir, Presoka, Kushla, and Fabrika. Zlatograd Municipality has an altitude of 385 to 1118 m. The town of Zlatograd is the lowest settlement in the municipality - 420-550 m.

The terrain is mountainous and it changes suddenly in places - steep slopes alternate with small valleys and highly developed hydrographic network. This explains the fragmentation of the agricultural lands. Their average slope is 10-12°.

The town of Zlatograd is already using its rich potential for tourism. The natural and historical factors are those which give a competitive advantage to Zlatograd Municipality.

The natural and climatic characteristics of the region are a prerequisite for the implementation of a well-organized ecotourism. All villages of the municipality have preserved their local traditions and the beautiful natural environment. Here the tourists could have direct and invaluable contact with nature and local culture.

Climate. The area belongs to the Continental - Mediterranean climatic region, South Bulgarian climatic sub-region, Eastern Rhodopes low mountainous climatic region.

The average annual temperature is 10.8°C, with a maximum in July 20.6°C and a minimum in January - 0.8°C, indicating moderate summers and relatively mild winters. The extreme values of the average maximum and average minimum temperature is 17.1°C and 4.9°C, the average monthly maximum in August is 28.9°C, and the average monthly minimum in January is - 3.9°C.

The average annual rainfall reaches 1000 litres/m^2. The intense rainfall of different length is typical of the Zlatograd Region; they are most often in autumn and when combined with the large catchment basin of the Varbitsa River they are a prerequisite for large floods of the river. The maximum rainfall amounts (in mm) for the period April - October varies from 10.0 for 5 minutes to 46.3 for 60 minutes and 59.7 for more than 60 minutes.

The average annual relative humidity is 75%, with a maximum of 85% in November, 13 are the days with relative humidity equal to or less than 30%, which is indicative of good growth conditions of forest and grass vegetation in the area. The average monthly wind speed is between 0.9 m/sec to 1.2 m/sec, and the average 1.1 m/sec.

Results and Discussion

Analysis of the natural resources in the area

Mineral waters. Part of Zlatograd's natural resources is the geothermal field of hot springs in the Ermorechie. The static reserves are approximately 200.10^6 m^3. The water temperature is 80-100°C, and the dynamic reserves are 30-100 l/sec, average 60 l/sec. The water is with good quality and suitable for drinking; it can be used for treatment.

Biodiversity. The area, in which the town of Zlatograd is located, is characterized by very well preserved biodiversity, and intact habitats for a number of protected plants and animals, which are one of the greatest treasures of the municipality. The number of plants growing in the area is over 200 species. The difference in the habitats is a prerequisite for the presence of plants from almost all ecological groups.

The territory of Zlatograd Municipality is 173,321 km^2. The total area of forests including state owned and private is 139,068 decares. The agricultural lands are 6 times less - 27,363 decares. For the needs of transport have been used 456 decares, and for fund "Settlements" - 4,737 decares. The rivers and the watercourses are 1,642 decares, and the mining territories - 55 decares.

The forests in the region are mixed. The most common tree species on the places facing north are: beech (*Fagus sylvatica*), hornbeam (*Carpinus orientalis*) and durmast (*Quercus petraea),* and on the southern windy slopes - white (*Pinus sylvestris*) and black pine (*Pinus nigra*), sumac *(Cotinus coggygria)*, broom (*Chamaecytisus absinthioides)*, manna ash in not very good condition. On the river alluvial areas are growing - black willow (*Salix nigra*), sallow (*Salix caprea*), elderberry (*Sambucus ebulus*) and rarely hazel (*Corylus avellana*). There are in this region, even though rarely, on area where spruce tree used to grow - Hungarian oak *(Quercus frainetto),* Oriental hornbeam (*Carpinus orientalis*), and in the lower parts - sycamore (*Acer pseudoplatanus*), Norway maple (*Acer platanoides)* and mountain elm.

There are centuries-old trees - oak, hornbeam, sweet chestnut (*Castanea sativa),* maple *(Acer campestre),* white and black pine. The oldest tree of the species domesticated chestnut tree - 278 years old is situated on the territory of the municipality, in the "Balaliyska Reka" area.

There are many shrubs too: wild brier, sumac, red and blue juniper, horsetail, blackberry, and eryngo.

The variety of grass species is wide. There are: wild geranium *(Geranium sylvaticum),* celandine *(Chelidonium majus),* cuckoo-pint (*Arum maculatum*), alpine avens (*Geum montanum*), and many types of ferns.

There are many herbs in the Zlatograd Region - thyme, yarrow, oregano, chicory, wormwood, St. John's wort, wild dog rose, wild mint, sorrel, lemon balm and more.

The fauna in the region is typical of the central highland fauna region species. The exceptional biological diversity here includes: 291 species of birds, 40 species of fish, 39 species of amphibians, 26 species of reptiles (including two tortoise species, 13 species of snakes, 11 species of lizards).

Representatives of the avifauna are: black woodpecker, greater spotted woodpecker, green woodpecker, common chaffinch, siskin, common crossbill, blackbird, chiffchaff, crested tit, coal tit, great tit, jay, nutcracker, hooded crow, tree sparrow, house sparrow, house martin, swallow, black and white stork.

The most common representatives of the ichthyofauna are: pike-perch, carp, barbel, chub, bleak, golden carp, rudd and pike. "Varbitsa" River, "Zlatograd" Dam Lake and "Hasidere" small dam are important to the fishery in the municipality. In 2004 the Hunting and Fishing Association did fish stoking of the dam lake with carp and trout.

The most common representatives of the amphibians are: big water frog, green toad, common frog, and salamander.

Representatives of the reptiles are: green lizard, sand lizard, wall lizard, adder, horned viper, grass snake.

Representatives of the mammals are: wild boar, fox, wolf, brown bear, squirrel, wild rabbit, chamois, pine-marten, marten, otter, weasel, hedgehog, etc.

Part of the wildlife included in **the Red Book of Bulgaria** is: salamander; and in the category endangered species - black and white stork, chamois, pine-marten, and otter.

Culture and traditions. The town of Zlatograd has a very rich cultural heritage, which creates favourable conditions for the development of cultural tourism, as well as many tourist products based on the historical and cultural potential of the region. The architectural reserve of houses from the National Revival period gives a special image of the town. The National Institute of Monuments of Culture has registered in it about 100 monuments of culture. The main features of the houses in the reserve are their white walls around the courtyards, plank-beds, white chimneys, wide oak doors. There are wells in most courtyards and eaves of tiles.

Zlatograd Municipality has got well preserved traditions and distinct Bulgarian values. In the past Zlatograd was called Belovidovo - because of its white houses. Until 1934 its name was Darydere. Zlatograd has been a centre of the Bulgarian education and culture for a long time. The folklore heritage has been preserved by the four municipal cultural clubs - "Prosveta" in the town of Zlatograd, "Progres" in the village of Startsevo, "Orpheus" in the village of Erma Reka and "Iskra" in the village of Dolen.

Zlatograd Municipality has a substantial basis for the development of tourism. Every year new sites are created and the existing ones are modernized and reconstructed. Considerable number of local entrepreneurs is now offering services to tourists in small family hotels and guest houses, and after categorizing them the quality of services has significantly increased.

Summary of the analysis

In terms of sustainable development we are now facing the problems of ecotourism, which is seen as "a responsible journey to natural areas which does not harm the environment and contributes to the well-being of local people".

Ecotourism is carried out in a natural environment and at the same time aims to change it as little as possible (NIKOLOV, 2010). At the same business seeks to support local people and thus creates a scheme of sustainable development with minimal use of resources and co-investment for their conservation. The educational nature of ecotourism is a key element that distinguishes it from the other sections of the nature-oriented tourism and thus makes it recognizable. Education and interpretation of the natural environment (including Environmental education) are important tools in creating a pleasant, meaningful and full ecotourism experience.

Ecotourism attracts people who: wish to interact with the environment in different degrees (different degrees of merging, the feeling to be part of nature); who wish to develop and improve their knowledge, their consciousness, as well as to assess, understand and comprehend ecotourism (NATIONAL STRATEGY FOR SUSTAINABLE TOURISM DEVELOPMENT IN THE REPUBLIC OF BULGARIA 2009-2013).

A few eco trails and bicycle lanes start from Zlatograd and its surroundings and if visitors take these routes they can enjoy the untouched scenery and explore the various historical, cultural and architectural sites of local and national significance.

One of the eco trails passes a protected tree "The centuries-old chestnut" (*Castanea sativa*), 278 years old - the oldest in Smolyan District.

Another eco trail follows the legend of the born two centuries ago in the town of Zlatograd legendary rebel Delyo Voyvoda.

There are many other eco trails which attract tourists:

Eco trail "St. Nedelya". Length of the eco trail - 12 km. Duration - 3 hours. Level difference - about 400 metres. Eco trail signs - blue markings and wooden boards. The trail begins at the Watermill (The Fulling mill) in Zlatograd - the most attractive place of the ethnographic complex. It consists of three traditional systems: wooden wash-tub for scouring (Dyshta), a fulling mill and a watermill (Karadzheyka).

Eco trail "Eco - echo". Length of the eco trail - 10 km. Duration - 2 hours and 30 minutes. Eco trail signs - grey markings and wooden boards. The trail begins at the Courthouse in Zlatograd. It passes a fountain and a shooting ground, a villa area, a recreation area with an ornithological corner in it, "Barchinata", the protected tree "The centuries-old chestnut" (*Castanea sativa*), 278 years old - the oldest in Smolyan District, a recreation area, a ravine, "Dybeto" area (centuries-old oak forest), a fountain and a place for rest, a villa area, hazel-wood, orchards. The trail ends at villa "Beloteks".

Eco trail "Mountain hug". Length - 4 km. Duration of the trail - approx. 2 hours. Marked with signs. Begins at the parking lot of night club "Diana". It goes through a broad-leaved forest. There are many fountains along the trail with crystal clear water as well as recreation areas. The trail leads south to a secluded rock called "Chukata" which you can either climb or go around it. The view from the rock is spectacular and you can see in the north-east Zlatograd.

Eco trail "In the footsteps of Delyu Haidutin". The altitude varies between 250 and 700 metres. The eco trail follows the legend which tells how Delyu Haidutin (Delyu the rebel) reached Kostadin peak where he was hiding from the Turkish troops. The eco trail begins at Zlatograd, the area "The Military Garden" and passes the spring "Bunyov vriz". It continues until an asphalt road, about 200 m. After that you take the mountain trail that goes through virgin forests that have been rarely visited until recently by people. Then you reach the well, built during the Ottoman rule, where the most famous Delyu the rebel used to sit with his fellows to drink from the crystal clear water after heavy and fearsome battles with the enemy. After that the eco trail reaches the area "St. Ilia" (altitude 650 m).

Conclusions and recommendations

Based on this report, we can draw the following conclusions and we can make the following recommendations:

- The favourable geographical location of Zlatograd Municipality and the adjoining tourist area as well as the topography show that the region has potential for ecotourism.
- The bioclimatic characteristics of the area - mild climate, clean air and mountain landscape are a prerequisite for the development of year-round recreation.
- The resources for tourism development in the municipality and the region combine a variety of unique nature, good anthropogenic environment, preserved cultural and historical heritage, traditions and practices typical of the mountain, attractive natural landscapes.
- There is a danger that the local community and users may not fully comprehend the need to protect the forest, as well as the natural and cultural heritage, which would lead to an appreciable reduction of the opportunities for the development of sustainable tourism forms such as ecotourism.
- It is necessary to develop a long-term conception for expanding the possibilities of further usage of the mineral water for treatment, greenhouse heating, etc.
- In our opinion the role of the local authority - municipal administration, municipal council and district administration should be leading in planning and implementation of plans for tourism development.
- Local people should benefit from ecotourism so they can support its expansion and development.
- The natural environment on the territory of Zlatograd Municipality has to be preserved and developed.

References

LAKOV P. 2011. Tourism policy and the development of ecotourism in Bulgaria. – In: *New Knowledge Journal Of Science, Anniversary International Scientific and Applied Conference*, UARD, Bulgaria.

LULCHEVA I. 2011. Rural tourism in the region of Rodopi. - *Economics and Management of Agriculture*, 2: 64-71.

NATIONAL STRATEGY FOR SUSTAINABLE TOURISM DEVELOPMENT IN THE REPUBLIC OF BULGARIA 2009-2013, [www.mi.government.bg/bg/themes]

NIKOLOV N. 2010. *Ecotourism as a non-traditional tourism form*. Bulgarian academy of Sciences, Institute of Geography, Sofia.

TONCHEVA T. 1994. *Ecology and use of nature tourism*. Sofia, NBU, 127 p.

ZHECHEV V., STOILOV D. 1999. Tour of nature and ecotourism, Publishing house "Korekt", Blagoevgrad, 165 p.

Received: 05.12.2014
Accepted: 22.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 7-24

Vegetation Analysis in the Red Sea-Eastern Desert Ecotone at the Area between Safaga and South Qusseir, Egypt

Mohamed G. Sheded[1], Mohamed K. Ahmed[2], Sabah A. Hammad[2*]

1 - Botany Department, Faculty of Science, Aswan University, EGYPT
2 - Botany Department, Faculty of Science, South Valley University at Qena, EGYPT
* Corresponding author: sa_ham2ad@yahoo.com

Abstract. The current work is concerned with the studying the impact of environmental conditions on the vegetation in the arid ecotone located between Red Sea and Eastern Desert from Safaga to south Qusseir. Ninety eight quadrats inside 13 transects were selected to cover the environmental gradient across the ecotone, from the coastal region to the boundary of Eastern Desert. Forty five species were recorded belonged to 24 different families and 38 genera. The perennial species were 38 while the annuals were seven species. *Zygophyllum coccineum* had the highest presence value (89.8%) followed by *Tamarix nilotica* (56.1%) and *Zilla spinosa* (51.02%). Chamaephytes and Hemicryptophytes were the most prevailed life-forms. Chrological analysis exhibited that Saharo-Sindian and Sahro-Sindian with its extension to Sudano-Zambezian elements were the most dominant. TWINSPAN classification technique produced three vegetation groups include nine clusters at the fourth level. These groups identified according to the first and second dominant species as follows: *Convolvulus hystrix - Panicum turgidum, Tamarix aphylla - Limonium pruinosum* and *Nitraria retusa - Tamarix nilotica.* DECORANA results indicated a reasonable segregation among these groups along the ordination axis 1 and 2. Vegetation analysis showed that ecotonal clusters have highest number of species/cluster, high species richness and high species turnover. Therefore, the largest group existed in the ecotone (34 species) while the desert group contained eight species and the coastal group included three species. Among the estimated soil variables in this study, pH, coarse sand, HCO_3^-, SO_4^{2-}, clay and PO_4^{3-} have the highest effect on species distribution. Sodium adsorption ratio (SAR) was the effective factor in detecting the ecotonal species, *Aeluropus lagopoides* and *Limonium pruinosum*.

Keywords: Ecotone, Vegetation Analysis, Red Sea, ordination, Eastern Desert, TWINSPAN.

Introduction

Ecotone is one of the most important subjects in ecological research. It is unstable part at any ecosystem due to its sensitivity to environmental changes (KAMEL, 2003). CLEMENTS (1905) used the term "ecotone" from the combination of two words (eco) *oikos* (home) and *tonos* (tension). Ecotones have received a great attention from ecologists for over 100 years, especially in the context of transition between biomes, geographic vegetation unit and movement of tree lines (FARINA, 2010). According to NAIMAN *et al.* (1988), ecotones may include riparian forests, marginal wet land, littoral lake zones, floodplain lakes, forests areas of significant groundwater-surface water changes and arid lands.

Several researchers investigated the different ecotonal areas around world. For instance, PETERS (2002) studied species dominance at a grassland-shrubland ecotone. HARPER *et al.* (2005) studied the edge effect on forest structure and

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg
Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

composition in fragmented landscapes. TANG & CHUNG (2002) studied rural-urban transition zones in China. TRAUT (2005) studied the role of coastal ecotones by studying the salt marsh/upland transition zone. The arid ecotones took less attention, may due to the poor and scattered vegetation in these region (KARK & RENSBURG, 2006). Diversity at ecotones is dependent on multiple factors, including environmental heterogeneity, spatial mass effect, invasive species spread, animal activities, and hybridization (SENFT, 2009). Ecotones are zones of relatively high vegetation turnover between two relatively homogenous areas (SENFT, 2009). One reputed characteristic of ecotones is that they have higher biological diversity than adjoining areas and thus hold high conservation value (ODUM, 1983; NAIMEN *et al.*, 1988; PETTS, 1990; RISSER, 1995). SHMIDA & WILSON (1985), WOLF (1993) and KERNAGHAN & HARPER (2001) found higher species richness between predetermined altitudinal zones. KIRKMAN *et al.* (1998) and CARTER *et al*. (1994) found higher species richness in wetland/upland boundaries. BROTHERS (1993) found higher species richness at anthopogenic forest edges. Other studies looking at grassland/forest ecotones have found species diversity at ecotones to be intermediate between the two bounded communities (MESZAROS, 1990; TURTON & DUFF, 1992; HARPER, 1995; MEINERS *et al.*, 2000). Ecotones have unique environmental and structural characteristics that may contribute to higher species richness (GOSZ, 1992; RISSER, 1995).

The Red Sea coastal ecotone is a major ecosystem encompassing three countries, Egypt, Sudan and Eretria (about 2200 km). The soil salinity is the main stable limiting factor affects the plant growth. The rain may decrease the soil salinity but quickly the later increases under the effect of high temperature and continued evaporation. The other environmental factors as the physical characteristics of the soil and the compatible ions as K^+ and Ca^{2+} can decrease the effect of the high concentration of sodium chloride and soil sodicity (TAIZ & ZEIGER, 2002).

Several Egyptian researchers studied the vegetation in the Red Sea and Eastern Desert. ZAHRAN (2010) divided the Red Sea coastal lands into two main habitat groups: saline and non-saline. SALAMA & El-NAGGAR (1991) in the wadi system west of Qusseir province showed that two community types were recognized: *Capparis decidua* and *Tamarix nilotica* covering the deltaic areas and the end parts of the wadis. Some members of these communities were halophytes. SHALTOUT *et al.* (2003) studied the phytosociological behavior and size structure of *Nitraria retusa* along the Egyptian Red Sea coast in relation to the prevailing environmental gradients. The vegetation of important wadis that drain into the Red Sea, Wadi Araba, Abu-Ghusun and Gemal, has been investigated under different aspects (EL-SHARKAWY *et al.*, 1982, 1990; SHARAF EL-DIN & SHALTOUT, 1985; DARGIE & EL-DEMERDASH, 1991; ZAREH & FARGALI, 1991; SHEDED, 1992; GALAL, 2011; GALAL & FAHMY, 2012).

Understanding the relationship between the prevailing environmental conditions and the responses of the survived plants can explain how the ecotonal vegetation formed. The aim of the current study is to investigate the impact of soil characteristics on the vegetation structure and species distribution, across the chemo-ecotone between Eastern desert and Red Sea. Chemo-ecotone is (KAMEL, 2003) generally controlled by soil sodicity (Sodium adsorption ratio - SAR).

Material and Methods

Study area

The study area extends along the Red Sea, between latitudes 26° 39′ 47″ N and 25° 43′ 20″ N and altitudes 33° 56′ 28″ E and 34° 32′ 26″ E starting from Safaga till 50 km south Qusseir, which represent a part of the ecotone between Red Sea coast and Eastern Desert (Fig. 1). The study area includes the deltas of 13 drainage basins that represent the main flooding sites in this area.

The selected transects were located at the deltas of wadis (Nuqara, Safaga, Abu Shiqili El-Bahari, Abu Hamra El-Bahari, She'b Goma'a, Quei, Abu Hamra El-Qibli, Hamrawein, Abu Shiqili El-Qibli, Transect10, Esel, Sherm El-Qibli and Um Gheig) which run across the ecotone to discharge into the Red Sea.

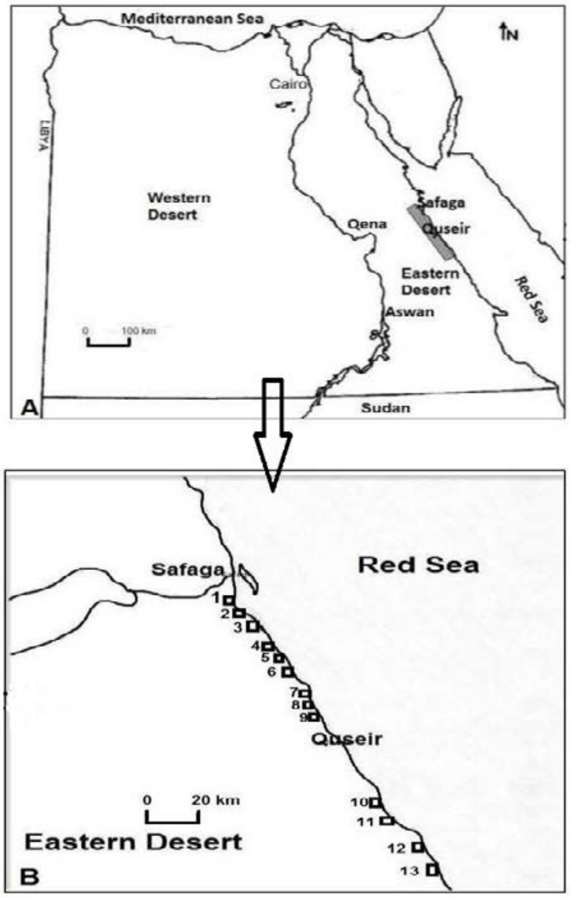


Fig. 1: The transects (1-13) in the study area, a part of the ecotone between Red Sea and Eastern Desert, were selected at the deltas of following wadis: 1. Nuqara; 2. Safaga; 3. Abu Shiqili El-Bahari; 4. Abu Hamra El-Bahari; 5. She'eb Goma'a; 6. Quei; 7. Abu Hamra El-Qibli; 8. Hamrawein; 9. Abu Shiqili El-Qibli; 10. Transect10; 11. Esel; 12. Sherm El-Qibli; 13. Um Gheig.

Geology and Geomorphology

The area from Safaga to south Qusseir represents a part of the Eastern Desert of Egypt at the Red Sea Coast forming a narrow coastal plain boarded from the west by the basement relief. The coastal plain strip of the Red Sea in the study area is occupied by Cenozoic rocks of both Tertiary and Quaternary age. The Tertiary rocks are represented by sandstone, lime-grits, conglomerates, carbonate terrigenous sediments, and gypsum. The Quaternary rocks are composed of wadi and terrace deposits consisting of detritus sand, pebbles, and rare boulders (HUME, 1912, 1934; AKAAD & DARDIR, 1966; SAID, 1990).

Climate

The climate is generally subtropical. Meteorological data during the period 2011-2012 was obtained from El-Qusseir station. The average of mean temperature ranged between 18.43°C in January and 30.81°C in August. The average of maximum temperature was 33.50°C in August and 22.70°C in December, while the average of minimum temperature was 27.09°C in August and 13.89°C in January. The relative humidity (%) ranged between 42.94 in May and 56.06 in December. Wind speed was 2-4 m/s around the year. The rainfall is scanty generally and irregular, yet in the period of study it was rainless.

Transects

Thirteen locations (transects) were selected, perpendicular to the Red Sea coast, depending on the vegetation richness. Transects were named according to the name of the opposite wadi (Table 1). The area of transects and number of stands were dependent on the width of the deltas of different wadis. For example, transect of Wadi Abu Shiqili El-Bahari has length 160m and width 100m while Wadi Sherm El-Qibli (Fig. 2) - 1900m and 300m respectively.

Table 1. Locations of the studied transects along Safaga-Qusseir Road

Location	Latitude	Altitude
Nuqara	26°39'40.50"N	33°56'24.68"E
Safaga	26°37'55.83"N	33°59'15.21"E
Abu Shiqili El-Bahari	26°33'20.69"N	34°02'10.08"E
Abu Hamra El-Bahari	26°24'13.78"N	34°06'37.02"E
She'eb Goma'a	26°22'43.68"N	34°07'55.92"E
Quei	26°20'58.09"N	34°09'00.99"E
Abu Hamra El-Qibli	26°16'40.97"N	34°11'06.78"E
Hamraween	26°15'10.51"N	34°12'01.45"E
Abu shiqili El-Qibli	26°13'48.47"N	34°12'38.78"E
Transect 10	25°53'53.00"N	34°24'34.01"E
Esel	25°51'51.31"N	34°24'42.24"E
Sherm El-Qibli	25°45'49.56"N	34°30'32.51"E
Um Gheig	25°43'23.73"N	34°32'29.55"E

After a reconnaissance survey in 2011, 98 stands were selected in the different transects to represent as much as possible the variation in the vegetation and geo-referenced using GPS techniques. The stands were distributed as possible as to cover all the vegetation depending on the width of wadi delta and the topography of the place. The area of used stand was 625 m^2 (25×25 m).

In each stand, ecological notes, presence or absence of plant species were recorded. The recorded taxa were classified according to the life-form system that proposed by RAUNKIAER (1937) and HASSIB (1951). The presence value of each species was expressed as the number of times a plant species is present in a given number of stands. The number of species within each life-form category was expressed as a percentage of total number of species in the study area. Analysis of phyto-geographical ranges was carried out after ZOHARY (1966, 1972); ABD EL-GHANI (1981, 1985). Taxonomic nomenclature was according to TĂCKHOLM (1974); COPE & HOSNI (1991); BOULOS (1995, 1999, 2000, and 2002) and EL HADIDI & FAYED (1995). Voucher specimens of each species were collected, and identified at the Herbaria of South Valley and Aswan Universities.

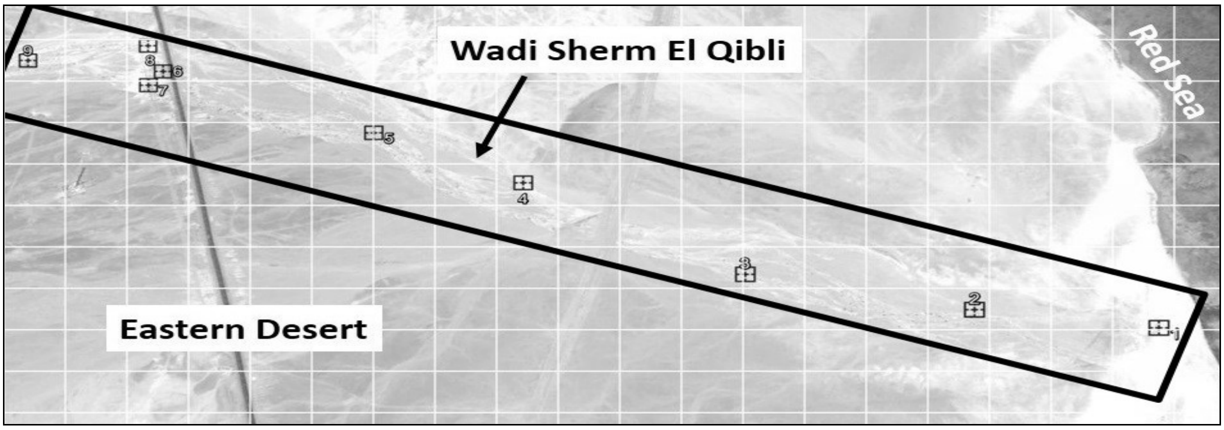


Fig. 2. Transect of Wadi Sherm El-Qibli (1900 m × 300 m) and the distributed quadrats on a gridded map.

Soil samples were collected from the studied stands at 0-50 cm depth. The different fractions of the collected sandy soil samples were separated using the dry sieving method (PIPER, 1950; RYAN *et al.*, 1996). Calcium carbonate was determined after JACKSON (1967). The soil organic matter was determined after SPARKS *et al.* (1996). Soil water content was determined by weighing a fresh sample of the soil and dried it in the oven at 105°C for 24 hours. The soil soluble solutes were extracted by shaking 20 g of soil with 100 ml distilled water for one hour. Filtration through a filter paper was carried to obtain a clear filtrate. Reaction (pH) and electrical conductivity (C, mS cm^{-1}) was estimated in the clear soil filtrate using pH-meter and conductivity meter according to JACKSON (1967). Sodium and potassium were determined by flame photometry according to ALLEN *et al.* (1986). Calcium and magnesium were determined volumetrically by the versene titration method described by UPADHYAY & SHARMA (2005). Chlorides were volumetrically determined as AgCl (KOLTHOFF & STENGER, 1974; HAZEN, 1989). Sulphates wasv estimated by turbidimetry as $BaSO_4$, according to BLACK *et al.* (1965). Phosphates were determined colourimetrically as phospho-molybdate according to WOODS & MELLON (1941). Nitrates were determined spectrophotometrically by the hydrazine reduction method described by KAMPHAKE *et al.* (1967), carbonates and bicarbonates were estimated by titration using the method described by RICHARD (1954).

Two-Way Indicator Species Analysis (TWINSPAN), as a classification technique and Detrended Correspondence Analysis (DECORANA), as an ordination technique, were applied to the presence estimates of the recorded taxa in 98 stands (HILL, 1979a,b). Species richness (α-diversity) of each vegetation cluster was calculated as the average number of species per stand and species turnover (β-diversity) as the ratio between the total species recorded in a certain vegetation cluster and its alpha diversity (PIELOU, 1975; SHALTOUT, 1985). The statistical evolution of vegetation-environmental relationships was done using the linear correlation coefficient (r); the Karl Pearson's Correlation Analysis statistical tool was calculated using SPSS version-20 (PALLANT, 2005).

The relationship between plant species variation and environmental variation was assessed using canonical correspondence analysis (CCA) (TER BRAAK, 1986, 1995). For this analysis, a second samples-by environmental factors data matrix was also constructed; it consisted of 19 environmental factors and 98 quadrats.

Results

The survey resulted in 45 species (38 perennials and 7 annuals) belonged to 38 genera from 24 families (Table 2). Leguminosae was represented by six species and five genera followed by Zygophyllaceae (5 species and 2 genera), Compositae (4 species and 3 genera). Both Boraginaceae and Graminae had three species from 3 different genera. Every family of Caryophyllaceae, Chenopodiaceae, Cruciferae and Resedaceae was represented by two species and 2 genera. Tamaricaeae represented by one genus and 2 species. The other recorded families were represented by only one genus and one species.

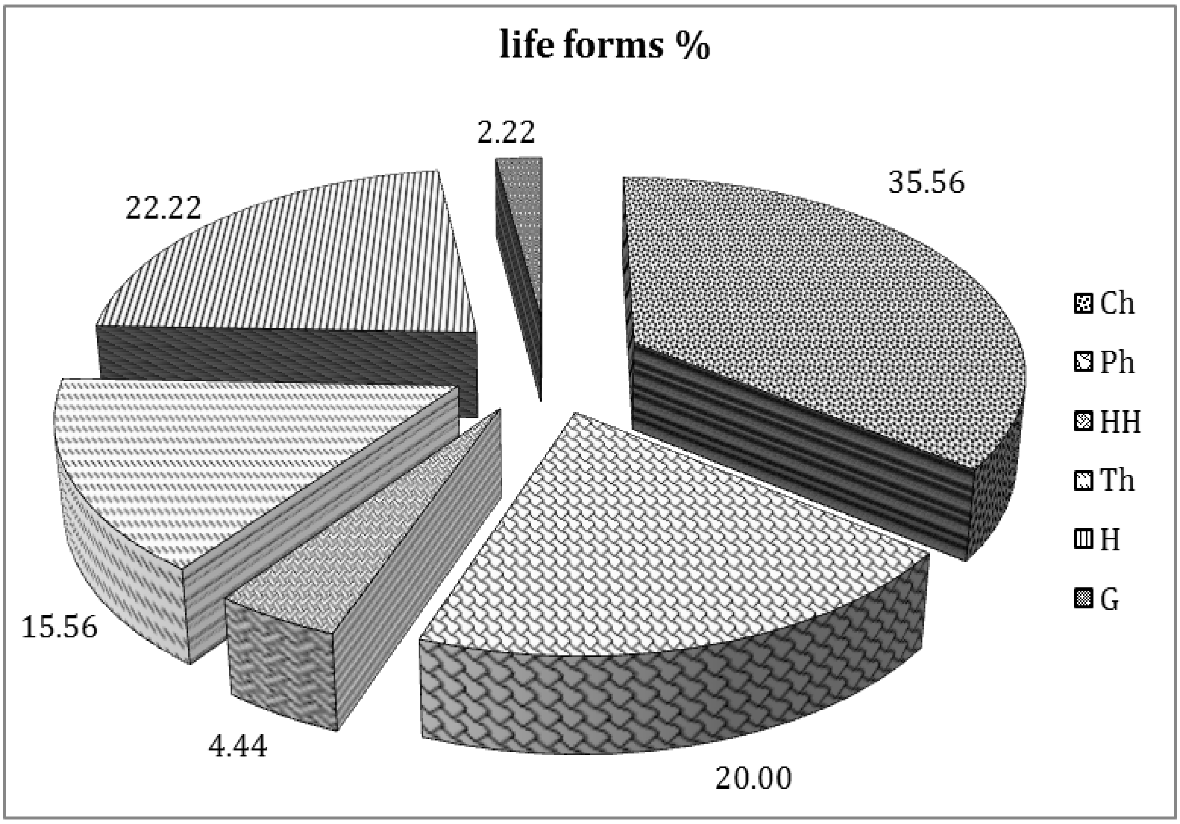


Fig 3. The percent of different plant life forms represented in the study area.

Table 2. Floristic composition, life span (L.S.), life forms (L.F.), chorology and presence value (P%) of the recorded species in the study area.

Species	L.S.	L.F.	Chorology	P%
Amaranthaceae				
Aerva javanica (Burm. f.) Juss. ex Schult.	Per	Ch	SA-SI+S-Z	1.02
Asclepiadaceae				
Leptadenia pyrotechnica (Forssk.) Decne	Per	Ph	S-Z+SA-SI	1.02
Avecinniaceae				
Avicennia marina Forssk.	Per	HH	SA-SI+S-Z	1.02
Boraginaceae				
Arnebia hispidissima Lehm.	Ann	Th	S-Z+SA-SI	2.04
Heliotropium bacciferum Forssk.	Per	Ch	S-Z+SA-SI	3.01
Trichodesma africanum v. *heterotrichum* Bornm.	Per	Ch	IR-TR+SA-SI	7.14
Capparaceae				
Capparis sinaica Veill. in Duh.	Per	Ph	SA-SI+S-Z	2.04
Caryophyllaceae				
Polycarpaea robbairea Kuntze.	Per	H	SA-SI+S-Z	5.1
Polycarpaea repens Forssk.	Per	H	SA-SI+S-Z	9.2
Chenopodiaceae				
Arthrocnemum macrostachyum Moric.	Per	Ch	ME+SA-SI+IR-TR	4.08
Suaeda pruinosa Lange.	Per	Ch	ME+IR-TR	1.02
Cleomaceae				
Cleome droserifolia Forssk.	Per	H	SA-SI+S-Z	5.1
Compositae				
Pulicaria arabica L.	Per	Ch	ME+IR-TR	9.18
Pulicaria incisa Lam.	Per	Ch	S-Z+SA-SI	11.2
Sonchus oleracous L.	Ann	Th	COSM	5.1
Cotula cinerea Delile.	Ann	Th	SA-SI	2.04
Convolvulaceae				
Convolvulus hystrix Vahl.	Per	Ch	S-Z+SA-SI	10.2
Cruciferae				
Morettia philaeana (Delile) DC.	Per	H	SA-SI+S-Z	4.08
Zilla spinosa (L.) Prantl.	Per	Ch	SA-SI	51.02
Leguminosae				
Acacia tortilis (Forssk.) Hayne subsp *raddiana* (Savi) Brenan	Per	Ph	S-Z+SA-SI	2.04
Acacia tortilis (Forssk.) Hayne subsp *tortilis*	Per	Ph	S-Z+SA-SI	18.37
Astragalus vogelii Webb.	Ann	Th	SA-SI+S-Z	16.33
Lotus hebranicus Hochst. ex Brand.	Per	H	SA-SI	29.59
Crotalaria aegyptiaca Benth.	Per	H	SA-SI+S-Z	4.08
Taverniera aegyptiaca Boiss.	Per	Ch	S-Z+SA-SI	6.12
Geraniaceae				
Monsonia nivea v. *intermedia* Tackh. & Boulos.	Per	H	SA-SI+S-Z	2.04
Gramineae				
Aeluropus lagopoides Fresen.	Per	H	SA-SI	5.1
Panicum turgidum Forssk.	Per	G	SA-SI+S-Z+IR-TR+ME	9.18
Phragmites australis (Cav.) Trin. ex Steud.	Per	HH	PAN	13.27
Juncaceae				
Juncus acutus L.	Per	Ch	ME+IR-TR	1.02
Malvaceae				
Malva parviflora L.	Ann	Th	ME+IR-TR+SA-SI	1.02
Nitrariaceae				
Nitraria retusa	Per	Ph	SA-SI+S-Z	43.88
Palmae				
Phoenix dactylifera L.	Per	Ph	S-Z+SA-SI	1.02
Plumbaginaceae				
Limonium pruinosum L.	Per	H	SA-SI	9.18

Species	L.S.	L.F.	Chorology	P%
Resedaceae				
Ochradenus baccatus Delile.	Per	Ph	SA-SI+S-Z	4.08
Reseda pruinosa Delile.	Ann	Th	SA-SI	17.35
Scrophulariaceae				
Kickxia acerbiana (Boiss.) Tackh. &. Boulos.	Per	Ch	SA-SI	4.08
Tamaricaceae				
Tamarix aphylla (L.) H.Karst.	Per	Ph	SA-SI+S-Z+IR-TR+ME	10.2
Tamarix nilotica (Ehrenb.) Bunge.	Per	Ph	SA-SI+S-Z+IR-TR+ME	56.12
Urticaceae				
Forsskaolea tenacissima L.	Per	H	SA-SI+S-Z	9.18
Zygophyllaceae				
Fagonia arabica L.	Per	Ch	SA-SI	4.08
Fagonia indica Burm. F.	Per	Ch	SA-SI+S-Z	4.08
Zygophyllum album L.	Per	Ch	SA-SI+ME+IR-TR+S-Z	23.5
Zygophyllum coccineum L.	Per	Ch	SA-SI+S-Z	89.8
Zygophyllum simplex L.	Ann	Th	S-Z+SA-SI	22.45

Legend: Per, Perennial; Ann, Annual; Ch, Chamaephyte; Ph, Phanerophyte; Th, Therophyte; H, Hemicryptophyte; G, Geophyte; HH, Hydrophytes and Helophytes; PAN, Pantropical; S-Z, Sudano-Zambezian; ME, Mediterranean; IR-TR, Irano-Turanian; SA-SI, Saharo-Sindian; COSM, Cosmpolitan

As shown in Fig. 3, chamaephytes were the most prevailed life form (16 species, 35.56 % of the recorded species). Hemicryptophytes was represented by 10 species (22.22%) while phanerophytes was represented by 9 species forming 20% of recorded species. Therophytes was represented by 15.56% of recorded species (7 species). Geophytes were represented by 2.2% (only one species) while hydrophytes and helophytes were represented by 4.4% of the recorded species (2 species).

Chorological analysis (Fig. 4) showed that the bi-regional species was the most dominant. There was 29 bi-regional species represent 64.44% of the total species. Most of the bi-regional species belong to saharo-sindian/sudano-zambezian. Eight species (17.78%) represented the mono-regional element. They were saharo-sindian. The pluri-regional elements were six species (13.33%) belong to saharo-sindian, sudano-zambezian, irano-turanian and Mediterranean. One species is cosmopolitan element (*Sonchus oleraceous*) and another is pantropical element (*Phragmites australis*).

TWINSPAN classified vegetation into nine clusters at fourth hierarchical level (Fig. 5). The clusters according to the indicator species were as follows: *Convolvulus hystrix* (I), *Astragalus vogelii- Lotus hebranicus* (II), *Acacia tortillis* subspecies *tortilis-Tamarix aphylla- Forsskaolea tenacissima* (III), *Zygophyllum cocineum-Zilla spinosa* (IV), *Limonium pruinosum* (V), *Nitraria retusa-Zygophyllum album* (VI), *Phragmites australis* (VII), *Zygophyllum coccineum* (VIII), *Arthrocnemum macrostachyum* (IX).

The Detrended Correspondance Analysis (DCA) distributed the vegetation on axis 1 to three groups (A, B and C) according to the soil salinity gradient (Fig. 6). The clusters I and II on the left part of axis 1 (group A) are mainly restricted to the desert ecosystem. The ecotonal group (group B) occupied the middle place and contained four clusters (III, IV, V and VII). Group B contained a mixture of species from the two neighbor ecosystems. *Aeluropus lagopoides* and *Zygophyllum album* belong to the coastal ecosystem while *Ochradenus baccatus* and *Reseda pruinosa* belong to desert ecosystem. Finally, group C included three clusters (VI, VIII and IX) occupied the right part of axis 1. It distributed near the seashore as a coastal ecosystem. The familiar halophytic species in the coastal ecosystem are (*Arthrocnemum macrostachyum, Avicennia marina* and *Suaeda pruinosa*). The highest species richness was in of cluster I, III and IV (10.67, 10.57 and 5.75 sp./stand, respectively) while cluster IX have the lowest species richness (2.5 sp./stand). Species turnover (Table 4) was the highest in clusters V, VI, VII (2.79, 2.78, 2.80; respectively).

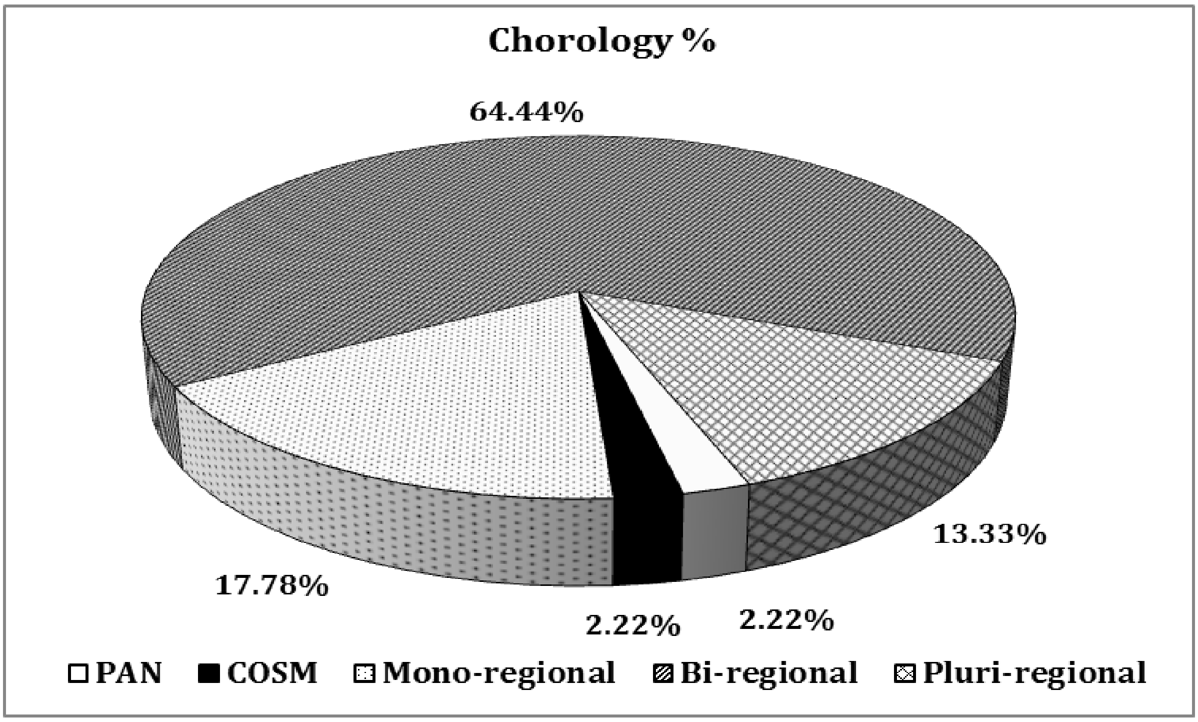


Fig. 4. The chorological affinities of different species as recorded in the study area.

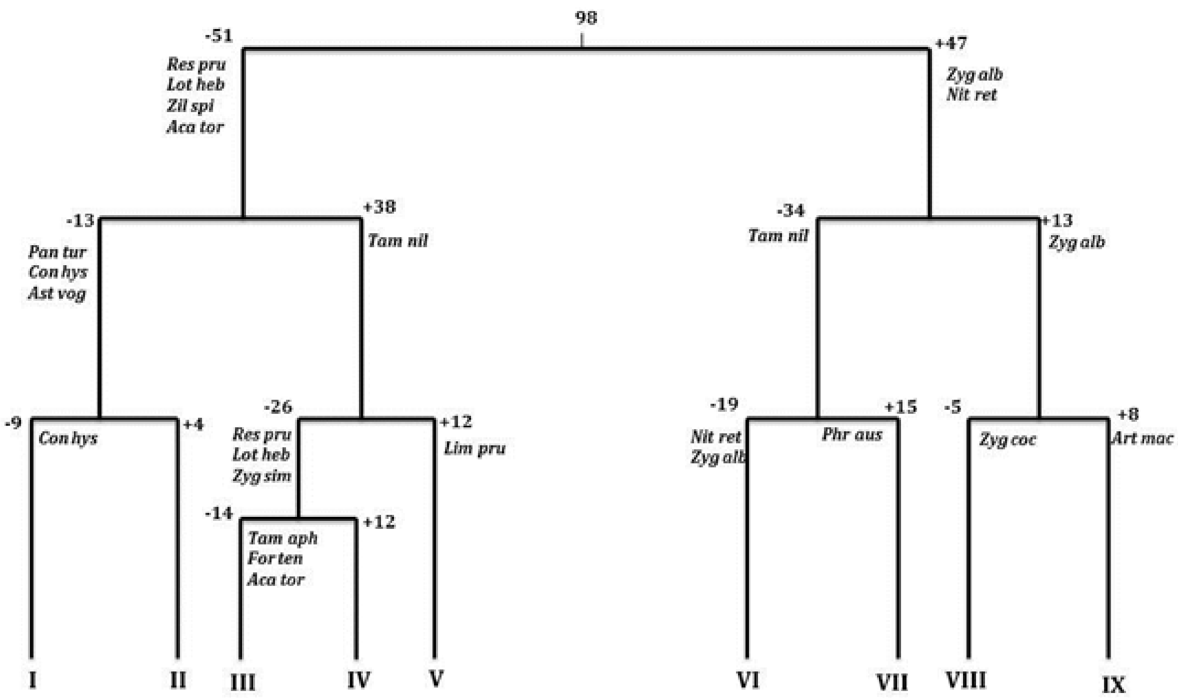


Fig. 5. TWINSPAN dendogram produced 9 clusters at the 4th level of hierarchy.

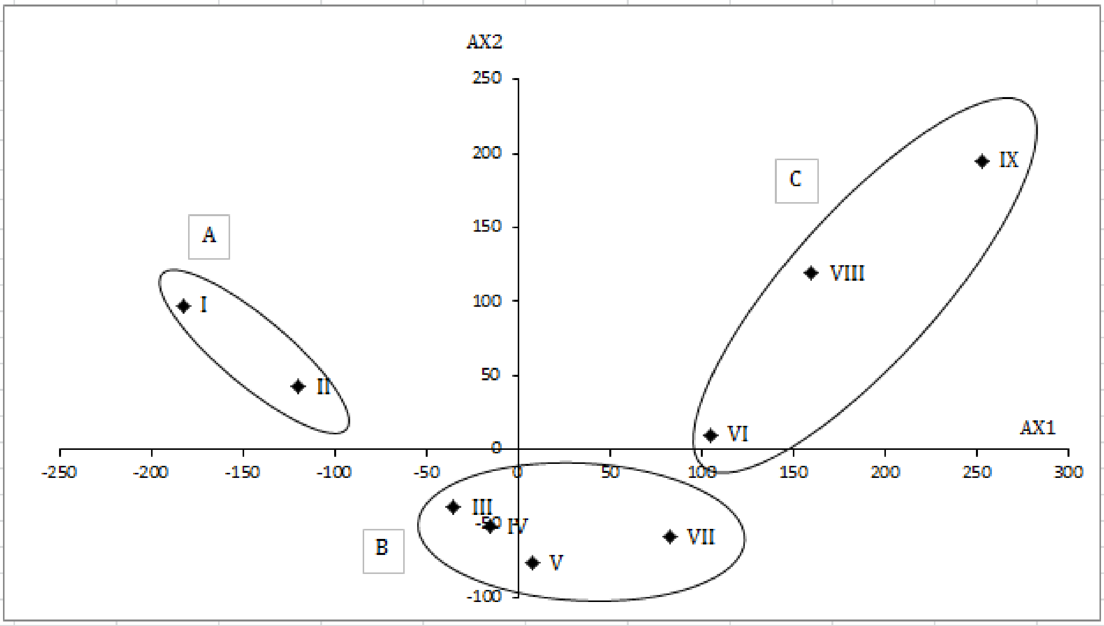


Fig. 6. Relationship between the nine vegetation TWINSPAN clusters of stands and the DCA axes 1 and 2.

Alkalinity, HCO_3^- and SO_4^{-2} correlated significantly positive with axis 1 (Table 3). Coarse sand correlated positively significant with axis 2 (r = 0.849) while clay and PO_4^{3-} correlated significant negatively. Nitrates correlates positively with axis 3. Estimated soil variables for the nine clusters represented in Table 3.

The lowest value of organic matter (2.3%), EC (1.47 mS cm^{-1}), HCO_3^- (0.91 mg g^{-1}), Cl^- (1.61 mg g^{-1}) and Na^+ (0.98 mg g^{-1}) in the soil samples of cluster I. The soil of cluster II had the highest value of fine sand (37.5%) and the lowest values of water content (0.24%), pH (7.64), K^+ (0.77 mg g^{-1}) and Mg^{2+} (0.51 mg g^{-1}). The soil of cluster III had the lowest values of $CaCO_3$ (9.62%), SO_4^{2-} (0.62 mg g^{-1}) and NO_3^- (0.12 mg g^{-1}). Cluster (IV) soil had the highest value of silt (25.67%) and the lowest value of Ca^{2+} (1.17 mg g^{-1}). The soil of cluster (V) had the highest value in EC (47.64 mS cm^{-1}), clay (60.74%), Cl^- (18.25 mg g^{-1}), NO_3^- (0.18 mg g^{-1}), Na^+ (4.48 mg g^{-1}), K^+ (6.51 mg g^{-1}) and Ca^{2+} (6.04 mg g^{-1}) and the lowest values of coarse sand (14.38%) and fine sand (12.99%). The soil of cluster (VII) had the highest value of water content (8.57%), oraganic matter (8.82%) and PO_4^{3-} (2.44 mg g^{-1}). The soil of cluster (VIII) contained the highest percent of coarse sand (48.64%) and the lowest percent of silt (10.41%). Calcium carbonate was 13.45% and estimated Mg^{2+} about 5.45 mg g^{-1}.

Table 3. Correlations coefficients (r) between the estimated soil variables and DCA axes 1, axes 2 and axes 3. * Correlation is significant at p<0.05. ** Correlation is significant at p<0.01.

Soil variables	AX1	AX2	AX3
WC, %	0.484	-0.310	0.619
pH	**0.779***	0.551	-0.570
EC(mS cm^{-1})	0.642	-0.095	0.388
TDS (g L^{-1})	0.528	-0.292	0.261
Coarse Sand, %	0.121	**0.849****	-0.307
Fine Sand, %	0.007	0.388	-0.159
Silt, %	-0.203	-0.521	-0.087
Clay, %	-0.040	**-0.697***	0.343
OM, %	0.640	-0.333	0.424
HCO_3 mg g^{-1}	**0.807****	0.157	-0.194
$CaCO_3$,%	0.424	0.544	0.097
Cl (mg g^{-1})	0.593	-0.090	0.304
SO_4 (mg g^{-1})	**0.672***	0.487	0.045
PO_4 (mg g^{-1})	-0.408	**-0.909****	0.477
NO_3 (mg g^{-1})	-0.410	-0.394	**0.720***
Na (mg g^{-1})	0.646	-0.204	0.485
K (mg g^{-1})	0.416	-0.168	0.519
Ca (mg g^{-1})	0.406	-0.231	0.620
Mg (mg g^{-1})	0.515	-0.055	0.339

The soil of cluster (IX) had the highest value of pH (8.72), HCO_3^- (1.18 mg g^{-1}), SO_4^{2-} (1.45 mg g^{-1}) and the lowest values of both PO_4^{3-} (1.46 mg g^{-1}) and NO_3^- (0.12 mg g^{-1}).

The correlation between the resulted vegetation groups and the soil characteristics is indicated on the ordination diagram (Fig. 7) produced by the canonical correspondence analysis (CCA). The arrows represent the environmental variables and indicate the direction of maximum change of that variable across the diagram. The length of the arrow is proportional to the rate of change.

As shown in Fig. 7, CCA axis 1 correlated positively with SO_4^{2-}, pH, Mg^{2+}, Ca^{2+}, K^+, Na^+, Cl^-, HCO_3^- and OM, while correlated negatively with NO_3^-, PO_4 and coarse sand. CCA axis 2 correlates positively with coarse sand and CO_3 and negatively with PO_4 and clay. Axis 1 clearly represents the salinity gradient. On the other hand, axis 2 correlated strongly and positively with coarse sand. In the same time there is weak positive with fine sand. Axis 2 correlated negatively with clay, silt and phosphate. It is clear that axis 2 is correlated with the soil structure. Group A that represent the desert ecosystem (true xerophytic plants) were affected by soil content of nitrate strongly. Group B in the ecotone affected by the percent of clay and silt in addition to the soil content of phosphate. Finally, group C that represents the coastal ecosystem (halophytic plants) was affected strongly by the in organic osmolytes.

Discussion

It known that ecotone is an area of tension between two interfered ecosystems (CADENASSO *et al.,* 2003a,b). The ecotones may form due to an environmental gradient or competition between the species of the interfered ecosystems. The ecotone can be created by man-made or natural factors, especially the abiotic factor gradients in soils composition, pH, soil salinity, soil mineral content as well as topography (WIENS *et al.,* 1985; VAN DER MAAREL, 1990; KENT *et al.,* 1997; EGGEMEYER & SCHWINNING, 2009). These gradients have specific attributes, creating "hydro-ecotones" or "chemo-ecotones", and so forth (KAMEL, 2003; DE ANGELIS, 2012).

The Red Sea coast represents an ecotone between Red Sea and Eastern desert. ZAHRAN (2010) divided the Red Sea coastal lands into two main habitats: saline (mangrove swamps and the littoral salt marshes) and non-saline (coastal desert plains and coastal mountains). The Red Sea-Eastern Desert ecotone is controlled by soil salinity. Therefore, it identified as chemo-ecotone according the KAMEL (2003).

The current work is carried out to understand the impact of environmental gradient on the species distribution, vegetation structure and to detect the ecotonal area between the coastal halophytic ecosystem and the desert ecosystem.

Forty-five species (38 perennials, 7 annuals) belonging to 38 genera and 24 families were recorded. *Z. coccineum, T. nilotica, Z. spinosa* and *N. retusa* had the highest presence values within the study area. The most frequent life forms are chamaephytes (35.6%). The chorological analysis showed that the Saharo-Sindian chorotypes are the dominant members in the study area. This agreed with the results obtained by HASSAN (1987), SHEDED (1992) and EL-DEMERDASH *et al.* (1994). The ecotone was dominated by Saharo-Sindian chorotype. This reflects the high diversity in the ecotone compared with the desert and coastal ecosystems. This agrees with the result obtained by CARTER *et al.* (1994) and KIRKMAN *et al.* (1998).

The soil texture of clusters I and II (group A) characterized by a high percent of coarse and fine sands compared with the soil of other clusters. The high percentage of coarse sand in the soil decreased the water capacity (ARCHER & SMITH, 1972) and consequently the salinity decreased. This can be considered as a helpful advantage to the growth of xerophytes. Other estimated physical and chemical soil characteristics were lower than that of other clusters.

In addition to that, topography played an important role to disperse the xerophytic plants toward the seacoast especially in the deltas of wadi Sherm El-Qibli, and Abu Shiqili El-Qibli. All the recorded species in

Table 4. Number of stands in each cluster, no.of species/cluster, species richness, species turnover, mean ± standerd deviation of soil variables for stands of the nine vegetation clusters in the study area. The F-value and its probability. *. $P < 0.05$.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	F-value
No of stands	9	4	14	12	12	19	15	5	8	
Sp./cluster	18	8	29	15	10	11	8	5	5	
Species richness	10.67	5.25	10.60	5.75	3.58	3.95	2.86	3.20	2.50	
Species turnover	1.69	1.52	2.74	2.61	2.79	2.78	2.80	1.56	2.00	
WC, %	0.32±0.49	0.24±0.11	0.83±0.86	1.62±1.90	3.25±2.82	3.68±6.15	8.57±10.76	1.18±0.91	3.47±4.98	1.949
pH	7.93±0.50	7.64±0.25	8.20±0.42	8.00±0.40	7.83±0.56	8.20±0.52	7.98±0.55	8.19±0.61	8.72±0.58	1.748
EC, mS cm^{-1}	1.47±1.61	2.44±1.64	2.35±3.74	6.37±13.42	47.64±59.74	32.06±33.56	29.54±36.15	36.23±36.02	24.50±17.23	2.305*
Coarse sand, %	45.94±27.29	38.05±15.37	30.17±24.21	16.04±19.72	14.38±22.26	37.26±25.48	25.44±23.27	48.64±23.98	41.18±32.12	1.851
Fine sand, %	24.03±9.63	37.50±9.59	22.89±14.72	26.21±13.53	12.99±16.14	24.30±15.74	28.64±20.12	30.90±14.53	27.00±15.11	1.053
Silt, %	14.36±11.56	12.50±6.44	17.20±14.30	25.67±13.58	11.90±9.50	14.83±12.32	17.99±8.07	10.41±8.35	12.64±14.16	1.248
Clay, %	15.67±11.93	11.95±9.16	29.75±25.38	32.08±21.12	60.74±37.97	23.61±26.16	27.92±26.57	10.05±15.78	19.18±27.41	2.406*
OM, %	2.30±1.45	2.32±0.41	3.48±1.90	5.66±5.13	8.55±5.88	7.67±5.38	8.82±6.03	7.74±4.71	5.37±2.94	2.173*
HCO_3, mg g^{-1}	0.91±0.24	0.92±0.15	0.97±0.20	1.08±0.27	1.05±0.15	1.00±0.21	1.04±0.27	1.02±0.33	1.18±0.40	1.200
$CaCO_3$, %	11.42±3.62	13.35±0.69	9.62±3.40	11.97±1.89	11.63±3.57	11.58±2.86	12.33±1.23	13.45±1.68	13.40±1.49	1.781
Cl^- , mg g^{-1}	1.61±2.71	1.67±0.31	3.50±5.23	3.24±4.36	18.25±22.5	7.77±6.47	9.02±12.97	12.21±10.91	10.03±4.47	1.954
SO_4^{2-}, mg g^{-1}	0.66±0.56	1.28±0.31	0.62±0.54	0.75±0.66	1.08±0.48	1.05±0.63	1.10±0.53	1.43±0.14	1.45±0.21	2.389*
PO_4^{3-}, mg g^{-1}	1.92±0.33	1.84±0.20	2.29±2.11	2.21±0.48	2.27±0.87	1.81±0.88	2.44±1.00	1.69±0.32	1.46±0.32	0.542
NO_3^-, mg g^{-1}	0.16±0.03	0.16±0.06	0.12±0.09	0.14±0.05	0.18±0.11	0.17±0.05	0.15±0.05	0.13±0.03	0.12±0.05	1.034
Na^+, mg g^{-1}	0.98±0.84	1.25±1.47	1.44±1.78	1.41±2.04	4.48±4.82	3.81±4.35	4.00±4.23	2.79±2.26	3.18±1.45	1.313
K^+, mg g^{-1}	1.69±2.01	0.77±0.89	1.16±1.56	1.12±1.05	6.51±8.39	2.65±5.21	3.97±6.47	1.97±1.52	4.01±5.29	1.133
Ca^{2+}, mg g^{-1}	1.74±2.30	2.58±2.23	1.46±0.95	1.17±1.11	6.04±5.01	4.96±4.56	5.12±5.20	4.84±4.90	2.44±2.23	1.951
Mg^{2+}, mg g^{-1}	0.89±1.05	0.51±0.81	1.01±1.87	0.73±1.24	3.29±2.86	2.88±3.00	3.66±5.36	5.45±8.19	1.15±0.80	1.468

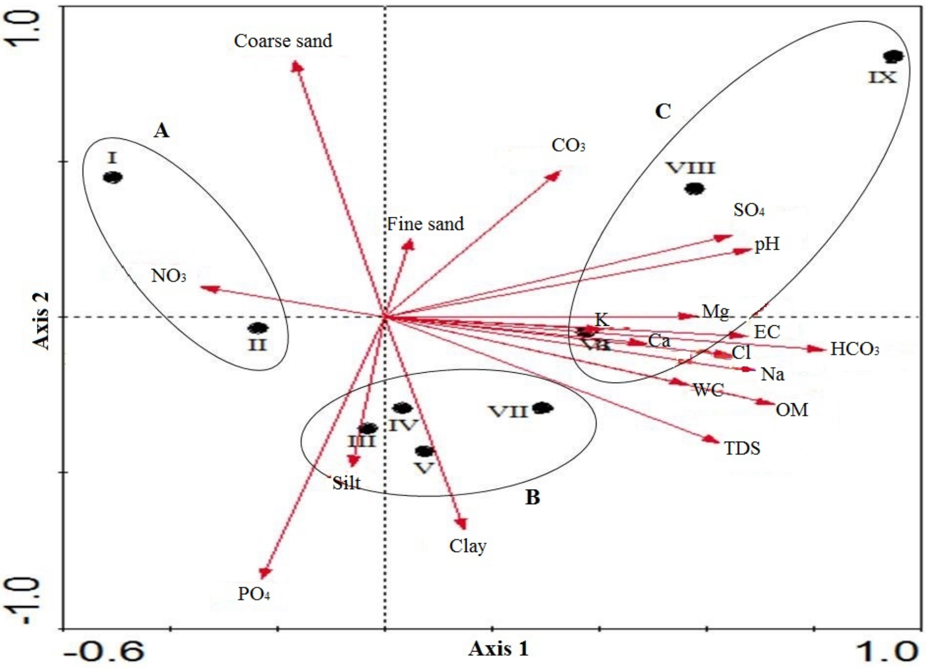


Fig. 7. CCA ordination of the first two axes showing the distribution of the 9 TWINSPAN clusters encircled in three vegetation groups (A, B & C) and different soil variables.

these locations were xerophytic plants. These variations in the elevation lead to form a fragmented ecotone (GOSZ & SHARPE, 1989).

On the other hand, the soil of the coastal ecosystem (group C) was characterized by high content of saline water. The soil texture in group A (desert ecosystem) and group C (coastal ecosystem) was approximately similar. The limiting factor in this case was the salinity and ionic content where the electrical conductivity (EC) increased up to 47.64 mS cm^{-1} in the soil of group B. The maximum EC value in group A reached 2.44 mS cm^{-1}. All the estimated cations and anions were higher in group C. These conditions were encouraging to the growth of halophytes in the coastal zones. In coastal zones as well as inland areas where salt pans exist, sharp ecotones may be maintained between halophytic (salinity-tolerant) and glycophytic (salinity-intolerant) vegetation (BURCHILL & KENKEL, 1991; GROSSHANS & KENKEL, 1997; STERNBERG *et al.*, 2007; TEH *et al.*, 2008; JIANG *et al.*, 2012a,b,c). The computed soil sodicity indicated that the halophytes survived in sodicity range between 2.25 – 3.21 while xerophytes between 0.18 – 1.86 (KAMEL *et al.*, 2013).

The precipitation of silt and clay that carried by rains and torrents from the inland at the ecotonal area (group B) increased the percentage of both in the soil texture. In addition, dissolved potassium and calcium came with rainwaters to the ecotone. The decomposition of plant residues that came with rainwaters increased the organic matter content in the ecotonal zone. The statistical analysis using SPSS showed a significant F value with EC, clay and organic matter. K^+ and Ca^{2+} can decrease the sodium toxicity (TAIZ & ZEIGER, 2002) and lead to enrich the vegetation diversity in the ecotone (TRAUT, 2005). The other chemical and physical soil characteristics were intermediated between the soil characteristics of coastal and desert ecosystems.

These conditions encouraged the species that can tolerate a wide range of drought and salinity (halo-xerophytes) such as *Nitraria retusa, Phragmites australis, Tamarix nilotica, Tamarix aphylla* and *Zygophyllum coccineum* (SAUER, 1965; TÄCKHOLM, 1974; ARONSON *et al.*, 1988). Therefore, they were the most dominant species in the ecotone (group B). The coexistence of both halo-xerophytes with the species of the interfered ecosystems in addition to the ecotonal species enriched the vegetation in the ecotone. There were two ecotonal species, *Limonium pruinosum* (Saharo-Arabian) and *Aeluropus lagopoides*, where survived in narrower range of soil sodicity (SAR) between 1 and 1.5 (KAMEL *et al.,* 2013)**.** The species richness and species turnover in the ecotone (clusters III, IV, V and IV) were higher than that in the desert and coastal ecosystems. This agreed with SHMIDA & WILSON (1985), WOLF (1993), KERNAGHAN & HARPER (2001). CARTER *et al.* (1994) and KIRKMAN *et al.* (1998) found higher species richness in wetland/upland boundaries. BROTHERS (1993) found higher species richness at anthropogenic forest edges. Other studies looking at grassland/forest ecotones have found species diversity at ecotones to be intermediate between the two bounded communities (MESZAROS, 1990; TURTON & DUFF, 1992; HARPER, 1995, LLOYD *et al.,* 2000; MEINERS *et al.,* 2000).

Results obtained by CCA ordination (Fig. 7) showed that the plants of desert ecosystem were affected by the NO_3^- concentration in the soil. It is logical as desert plants depend on the amino acids and soluble proteins more than the other plants (MILE *et al*., 2002). Nitrogen is necessary element in protein synthesis.

On the other hand, the plants in coastal ecosystem were affected by the most of inorganic solutes. This is also may explain their ability to accumulate great amounts of inorganic solutes to readjust their internal osmotic pressure. The distribution of species in saline and marshy habitats relates to salinity in many arid regions has been discussed by several authors as KASSAS (1957), FLOWERS (1975) and MARYAM *et al.* (1995). Also ZAHRAN *et al.* (1996) demonstrate the distribution of some halophytic species is best correlated along a gradient of soil variables as salinity, moisture content, soil texture, organic matter, and calcium carbonate.

The ecotonal plants are characterized by their ability to accumulate inorganic solutes. Therefore, they were affected by the percent of clay which increases the soil field capacity at moderate soil salinity. ANDERSON *et al.* (1990) suggested that the presence and relative abundance of xerophytes may be taken as a measure of the degree of halophytism in a plant community. The high percentage of fine particles, in the soils of ecotone area together with the other factors gives a number of xerophytes a competitive advantage over halophytes, as they are tolerant to salt.

In conclusion, it turns out that although the chemo-ecotone is controlled by soil sodicity, the soil texture fractions played great role in distribution of the vegetation. Although the significant correlation with the anions concentrations in the soil; sodium sodicity was the effective factor in detecting the ecotonal species as *Aeluropus lagopoides* and *Limonium pruinosum* which grew in intermediated range (0.5-2.5) of soil sodicity between the ranges of the coastal and desert ecosystems (KAMEL *et al.,* 2013).

References

ABD EL-GHANIM.M. 1981. *Preliminary studies on the vegetation of Bahariya Oasis-Egypt.* M.Sc. Thesis, Cairo University. 309 p.

ABD EL-GHANI M.M. 1985. *Comparative study of the vegetation of Bahariya and Farafra Oases and the Faiyum region, Egypt.* Ph.D. Thesis, Cairo University. 500 p.

AKAAD S., A. DARDIR. 1966. Geology of the Red Sea coast between Ras Shagara and Mersa Alam, Egypt. - *Geol. Surv. Egypt*, 35: 67–79.

ALLEN S.E., H.M. GRIMSHAW., J.A. PARKINSON., C. QUARMBY. J.D. ROBERTS. 1986. *Methods in plant ecology*. 2nd Edn. Moore, P.D. and S.B. Chapman (Eds.). Blackwell Scientific Publications, Oxford, pp: 411-466.

ANDERSON H., J.P. BAKKER., M. BRONGERS., B. HEYDEMANN. U. IRMLER. 1990. Long-term changes of salt marsh communities by cattle grazing. – *Vegetatio,* 89: 137-148.

ARCHER J.R., P.D. SMITH. 1972. The Relation between bulk density, Available water capacity, and air capacity of soils. - *J. Soil Science*, 23 (4): 475-480.

ARONSON J.A., D. PASTERNAK, A. DANON. 1988. Introduction and first evaluation of 120 halophytes under seawater irrigation. - In: *Arid Lands Today and Tomorrow: Proceedings of an International Research and Development Conference*. Whitehead, E.E., Hutchinson, C.F., Timmerman, B.N., and Varady, R.G.(Eds.), Westview Press, Boulder, CO., pp. 737-746.

BLACK C.A., D.D. EVANS, J.L. WHITE, L.E. ENSMINGER, F.E. CLARK. 1965. *Methods of soil analysis*. Part 1 and 2. Series No.9. American Society of Agronomy. Inc. Publisher, Madison, Wisconsin. U.S.A, 88 p.

BOULOS L. 1995. *Flora of Egypt, Check list*. Al Hadara Publishing, Cairo. Egypt, 283 p.

BOULOS L. 1999. *Flora of Egypt, vol. 1: Azollaceae- Oxalidaceae*. Al Hadara Publishing, Cairo. 419 p.

BOULOS L. 2000. *Flora of Egypt, vol. 2: Geraniaceae-Boraginaceae*. Al Hadara Publishing, Cairo. 352 p.

BOULOS L. 2002. *Flora of Egypt, vol. 3: Verbenaceae- Compositae*. Al Hadara Publishing, Cairo. 373 p.

BROTHERS T.S. 1993. Fragmentation and edge effects in central Indiana old-growth forests. - *Natural Areas Journal,* 13: 268-275.

BURCHILL C.A., N.C. KENKEL. 1991. Vegetation-environment relationships of an inland boreal salt pan. - *Canadian Journal of Botany*, 69(4): 722–732.

CADENASSO M.L., S.T.A. PICKETT, K.C. WEATHERS, S.S. BELL, T.L. BENNING, M. CARREIRO, T.E. DAWSON. 2003a. An interdisciplinary and synthetic approach to ecological boundaries. – *BioScience*, 53: 717-722.

CADENASSO M.L., S.T.A. PICKETT, K.C. WEATHERS, C.G. JONES. 2003b. A frame work for a theory of ecological boundaries. – *BioScience*, 53: 750–758.

CARTER V., P.T. GAMMON, M.K. GARRETT. 1994. Ecotone dynamics and boundary determination in the Great Dismal Swamp. - *Ecological Applications*, 4:189-203.

CLEMENTS F.E. 1905. *Research methods in ecology*. Lincoln University Publishing Company, 334 p.

COPE T.A., H.A. HOSNI. 1991. *A key to Egyptian grasses*. Royal Botanic Gardens, Kew and Cairo University Herbarium. London, 75 p.

DARGIE T.C.D., M.A. El-DEMERDASH. 1991. A quantitative study of vegetation-environment relationships in two Egyptian deserts. - *Journal of Vegetation Science*, 2: 3-10.

DE ANGELIS D. 2012. Self-organizing processes in landscape pattern and resilience: A review. - In: *International Scholarly Research Network*. ISRN Ecology. Article ID 274510, pp. 18.

EGGEMEYER K.D., S. SCHWINNING. 2009. Biogeography of woody encroachment: why is mesquite excluded from shallow soils? – *Ecohydrology*, 2: 81-87.

El-DEMERDASH M.A., A.K. HEGAZY, A.M. ZILAY. 1994. Distribution of the plant communities in Tihamah coastal plains of Jazan region, Saudi Arabia. – *Vegetatio,* 112: 141–151.

EL-HADIDI M.N., A.A. FAYED. 1995. Materials for excursion flora of Egypt (EFE). – *Taeckholmia*, 15: 233 p.

EL-SHARKAWI H.M., F.M. SALAMA, A.A. FAYED. 1982. Vegetation of inland desert wadis in Egypt. III. Wadi Gimal and wadi El-Miyah. - *Feddes Reportorium*, 93: 135-145.

EL-SHARKAWI H.M., F.M. SALAMA, A.A. FAYED. 1990. Vegetation of inland desert wadis in Egypt. X. The wadi system North of Hurghada. - *Feddes Reportorium*.,101: 97-102.

FARINA A. 2010. *Ecology, Cognition and Landscape. Linking Natural and Social Systems. Landscape Series.* Vol.11. Springer Dordrecht Heidelberg-London-New York. 169 p.

FLOWERS T.J. 1975. Halophytes. – In: D.A. Baker and J.L. Hall (eds.), *Ion Transport in Cells and Tissues*. North Holland, Amsterdam, pp. 309-334.

GALAL T.M. 2011. Size structure and dynamics of some woody perennials along elevation gradient in Wadi

Gimal, Red Sea coast of Egypt. – *Ecologia*, 1(1): 56-67.

GALAL T.M., A.G. FAHMY. 2012. Plant diversity and community structure of Wadi Gimal protected area, Red Sea coast of Egypt. - *African Journal of Ecology*, 50 (3): 266-276.

GOSZ J.R., P.J.H. SHARPE. 1989. Broad-scale concepts for interactions of climate, topography and biota at biome transitions. - *Landscape Ecology*, 3(4): 229–243.

GOSZ J.R. 1992. Gradient analysis of ecological change in time and space: Implications for forest management. - *Ecological Applications*, 2(3): 248-261.

GROSSHANS R.E., N.C. KENKEL. 1997. Dynamics of emergent vegetation along natural gradients of water depth and salinity in a prairie marsh: delayed influences of competition, UFS, (Delta Marsh). - *Annual Report*, 32: 83-93.

HARPER K.A. 1995. Effect of expanding clones of *Gaylussacia baccata* (black huckleberry) on species composition in sand plain grassland on Nantucket Island, Massachusetts. - *Bulletin of the Torrey Botanical Club*, 122: 124-133.

HARPER K.A., S.E. MACDONALD, P.J. BURTON, J. CHEN, K.D. BROSOFSKE, S.C. SAUNDERS, E.S. EUSKIRCHEN, D. ROBERTS, M.S. JAITEH, P. ESSEEN. 2005. Edge influence on forest structure and composition in fragmented landscapes. - *Conserv Biol*, 19: 768–782.

HASSAN L.M. 1987. *Studies on the flora of Eastern Desert, Egypt*. Ph.D. thesis, Faculty Sci., Cairo Univ, 515 p.

HASSIB M. 1951. Distribution of plant communities in Egypt. - *Bulletin of Faculty of Science, University of Fouad 1, Cairo, Egypt*, 29: 59-261.

HAZEN A. 1989. On determination of chloride in water. - *American Journal of Chemistry*, 2: 409–425.

HILL M.O. 1979a. *DECORANA: a Fortran program for detrended correspondance analysis and reciprocal averaging*. Cornell Uni., Ithaca, New York, 90 p.

HILL M.O. 1979b. *TWINSPAN: a Fortran program for arranging multivariente data in an ordered two-way table by classification of the individuals and attributes*. Cornell Uni., Ithaca, New York.52 p.

HUME W.F. 1912. Explanatory notes to accompany the Geological map of Egypt, Egypt. Survey Dept. Cairo, pp. 50–60.

HUME W.F. 1934. *Geology of Egypt, Part I, The fundamental Precambrian rocks of Egypt and Sudan, their distribution age and character*. pp. 300–320.

JACKSON M.L. 1967. *Soil chemical analysis*. Prentice-Hall of India, Private limited New Delhi. 498 p.

JIANG J. 2012c. *Mangroves on the move: predictions of storm surge effects on coastal vegetation*. Ph.D. thesis, Department of Biology, University of Miami.147 p.

JIANG J., D.L. DEANGELIS., T.J. SMITH III, S.Y. TEH. H.L. KOH. 2012b. Spatial pattern formation of coastal vegetation in response to external gradients and positive feedbacks affecting soil porewater: a model study. - *Landscape Ecology*, 27(1): 109–119.

JIANG J., D. GAO, D.L. DEANGELIS. 2012a. Towards a theory of ecotone resilience: coastal vegetation on a salinity gradient. - *Theoretical Population Biology*, 82(1): 29-37.

KAMEL M. 2003. Ecotone Classification according to its origin. - *Pakistan Journal of Biological Sciences*, 6 (17): 1553-1563.

KAMEL M., M.G. SHEDED, S.A. HAMMAD. 2013. Chemo-ecotone soil characteristics and their effect on the ionic content and distribution of ecotonal plants. *In Press.*

KAMPHAKE L.J., S.A. HONNAH, J.M. COHEN. 1967. Automated Analysis for Nitrate by Hydrazine reduction. - *Water Research*, 1: 205-216.

KARK S., B.J. Van RENSBURG. 2006. Ecotones: marginal or central areas of transition? - *Israel Journal of Ecology & Evolution*, 52: 29-53.

KASSAS M. 1957. On the ecology of the Red Sea coastal land. - *Journal of Ecology*, 45: 187–203.

KENT M., W.J. GILL, R.E. WEAVER, R.P. ARMITAGE. 1997. Landscape and plant community boundaries in biogeography. - *Prog Phys Geogr*, 21: 315–353.

KERNAGHAN G., K.A. HARPER. 2001. Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. – *Ecography*, 24: 181-188.

KIRKMAN L.K., M.B. DREW, L.T. WEST, E.R. BLOOD. 1998. Ecotone characterization between upland longleaf pine/wiregrass stands and seasonally-ponded isolated wetlands. - *Wetlands*, 18: 346-364.

KOLTHOFF I.M., V.A. STENGER. 1974. *Volumetric Analysis*, 2nd ed. New York: Interscience Publishers.

LIVINGSTON B.E. 1903. The distribution of the upland societies of Kent County, Michigan. - *Botanical Gazette*, 35: 36-55.

LLOYD K.M., A.A.M. MCQUEEN, B.J. LEE, R.C.B. WILSON, S. WALKER, J.B. WILSON. 2000. Evidence on ecotone concepts from switch, environmental and anthropogenic ecotones. - *Journal of Vegetation Science*, 11: 903-910.

MARYAM H., S. ISMAIL, F. ALAA, R. AHMED. 1995. Studies on growth and salt regulation in some halophytes as influenced by edaphic and climatic conditions. - *Pak. J. Bot.*, 27: 151-163.

MEINERS S.J., S.N. HANDEL, S.T.A. PICKETT. 2000. Tree seedling establishment under insect herbivory: edge effects and inter-annual variation. - Plant Ecology, 151: 161-170.

MESZAROS I. 1990. Spatial changes in herb layer in a beech forest/clear-cut area ecotone from northern Hungary. - In: Krahulec F., A.D.Q. Agnew, S. Agnew, J.H. Willems (Eds.). *Spatial processes in plant communities*. SPB Academic. The Hague. pp. 59-69.

MILE O., I. MESZAROS, S.Z. VERSES, G. LAKATOS. 2002. Ecophysiological study on the salt tolerance of a pannonian endemism (*Lepidium crassifolium* (W. et K.)) in inland saline area. - Acts Biol. Szegediensis, 46: 249–250.

NAIMAN U., H. DICAMPS, S. PASTOR, C.A. JOHNSTON. 1988. The potential importance of boundaries to fluvial ecosystems. - *J. North American Benthological Society*, 7: 289-306.

ODUM E.P. 1983. *Basic ecology*. Saunders College Publishing, Philadelphia.

PALLANT J. 2005. *SPSS Survival Manual*. Open Uni. Press, McGraw-Hill Education, 2nd edition, 336 p.

PETERS D.P.C. 2002. Plant species dominance at a grassland–shrubland ecotone: an individual-based gap dynamics model of herbaceous and woody species. - *Ecological Modelling*, 152: 5–32.

PETTS G.E. 1990. The role of ecotones in aquatic landscape management. - In: Naiman R.J. and H. Décamps (Eds.), *The Ecology and management of aquatic-terrestrial ecotones* UNESCO, Paris. pp. 227-261.

PIELOU E.C. 1975. *Ecological diversity*. Willey, Inter Science, New York. 165 p.

PIPER C.S. 1950. *Soil and plant analysis*. Inter Science publishers; New York. 186 p.

RAUNKIAER C. 1937. *The Life Forms of Plants and Statistical Plant Geography*. Clarendon Press, Oxford. 104 p.

RICHARD L.A. 1954. *Diagnosis and improvement of saline and alkali soils. Agricultural Handbook,* no 60. United States Department of Agriculture. 160 p.

RISSER P.R. 1995. The Status of the science examining ecotones. A dynamic aspect of landscape is the area of steep gradients between more homogeneous vegetation associations. - *Bioscience*, 45(5): 318-325.

RYAN J., S. GARABET, K. HARMSON, A. RASHID. 1996. *A soil and plant analysis. Manual adapted for the west Asia and North Africa region*. ICARDA. Aleppo. Syria. 140 p.

SAID R. 1990. *The geology of Egypt*. A. A. Balkema Publishers, Old Post Road, Brookfield, VT 05036, USA. 773 p.

SALAMA F.M., S.M. El-NAGGAR. 1991. Phytosociology of wadi system west of Qusseir province. - Feddes Report, 102: 453-468.

SAUER J. 1965. Geographic reconnaissance of Western Australian seashore vegetation. - Australian Journal of Botany, 13: 39-69.

SENFT A.R. 2009. *Species diversity patterns at ecotones*. MSc Thesis. University of North Carolina at Chapel Hill, Department of Biology. 55 p.

SHALTOUT K.H. 1985. On the diversity of the vegetation in the western Mediterranean coastal region of Egypt. - *Proc. Egypt, Bot. Soc.*, 4: 1355-1376.

SHALTOUT K.H. 2003. The biology of Egyptian woody perennials. 1. *Nitraria retusa* (Forssk.) Asch. *Ass. - Univ. Bull. Environ. Res.*, 6(1): 55–71.

SHARF EL-DIN, A., K.H. SHALTOUT. 1985. On the phytosociology of Wadi Araba in the Eastern Desert of Egypt. - *Proceedings of the Egyptian Botanical Society*, 4: 1311-1325.

SHEDED M.G. 1992. *Environment and vegetation in the South Eastern Desert, Egypt*. Ph.D. thesis, Faculty Sci., Assiut Univ, 180 p.

SHMIDA A., M.V. WILSON. 1985. Biological determinants of species diversity. - *Journal of Biogeography*, 12: 1-20.

SPARKS D.L., A.L. PAGE., P.A. HELMKE, H.R. LOEPPERT, P.N. SOLTANPOUR, M.A. TABTABAI, C.T. JOHANSTON, M.E. SUMNER (eds). 1996. *Methods of soil analyses, part 3: chemical methods*. American Society of Agronomy, Madison, Wisconsin, USA, pp. 1085-1122

STERNBERG L.D.S.L., S.Y. TEH, S.M.L. EWE, F. MIRALLES-WILHELM, D.L. DEANGELIS. 2007. Competition between hardwood hammocks and mangroves. - *Ecosystems*, 10(4): 648–660.

TÄCKHOLM V. 1974. *Student's Flora of Egypt*, 2nd ed. Cairo University Press, Cairo, Egypt. 888 p.

TAIZ L., E. ZEIGER. 2002. *Plant physiology*. 3rd Edition. Sinauer Associates., Sunderland, MA. 690 p.

TANG W.S., H. CHUNG. 2002. Rural–urban transition in China: illegal land use and construction. - *Asia Pac View*, 43: 43–62.

TEH S.Y., D.L. DE ANGELIS, L.D.S.L. STERNBERG, F.R. MIRALLES-WILHELM, T.J. SMITH, H.L. KOH. 2008. A simulation model for projecting changes in salinity concentrations and species dominance in the coastal margin habitats of the Everglades. - *Ecological Modelling*, 213 (2): 245–256.

TER BRAAK C. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. - *Journal of Ecology*, 67: 1167-1179.

TER BRAAK C. 1995. *Ordination, in Data Analysis in Community and Landscape Ecology*, Netherlands: Pudoc Wageningen, pp. 91-173.

TRAUT B.H. 2005. The role of coastal ecotones: a case study of the salt marsh/upland transition zone in California. - *Journal of Ecology*, 93: 279–290.

TURTON S.M., G.A. DUFF. 1992. Light environments and floristic composition across an open forest-rainforest boundary in northeastern Queensland. - *Australian Journal of Ecology*, 17: 415-423.

UPADHYAY R.M., N.L. SHARMA. 2005. *Manual of Soil, Plant, Water and Fertilizer Analysis*. New Delhi: Kalyani Publishers.

VAN DER MAAREL E. 1990. Ecotones and ecoclines are different. - *Journal of Vegetation Science*, 1: 135-138.

WIENS J.A., C.S. CRAWFORD, J.R. GOSZ. 1985. Boundary dynamics—a conceptual-framework for studying landscape ecosystems. - *Oikos*, 45: 421–427.

WOLF J.H.D. 1993. Diversity patterns and biomass of epiphytic bryophytes and lichens along an altitudinal gradient in the northern Andes. - *Annals of the Missouri Botanical Garden*, 80: 928-960.

WOODS J.T., M.A. MELLON. 1941. Chlorostannous-Molybdophosphoric blue colour method in sulfuric acid system. In: Jackson M.L. 1958. *Soil Chemical Analysis*. Prentice Hall International. Inc. London, pp. 141-144.

ZAHRAN M.A., K.J. MURPHY, I.A. MASHALY, A.A. KHEDR. 1996. On the ecology of some halophytes and psammophytes in the Mediterranean coast of Egypt. - *Verh Ges. Ökol.*, 25: 133-146.

ZAHRAN M.A. 2010. *Climate – Vegetation. Afro-Asian Mediterranean and Red Sea Coastal Lands. Plant and Vegetation*. Vol. 4. Edited by F. Gilbert. Springer Dordrecht Heidelberg London New York. 324 p.

ZAREH M.M., K.A. FARGALI. 1991. Vegetation of inland desert wadies in Egypt. - *Feddes Repertorium*, 102: 647-656.

ZOHARY M. 1966. *Flora Palaestina*. The Israel Academy of Sciences of Humanities. Goldberg's Press. 364 p.

ZOHARY M. 1972. *Flora Palaestina*. Part II. The Israeal Academy of Science and Humanities, Goldberg's Press. Jerusalem. 489 p.

Received: 19.09.2013
Accepted: 02.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 25-34

Composite Films from Sodium Alginate and High Methoxyl Pectin - Physicochemical Properties and Biodegradation in Soil

*Ayten O. Solak, Svetla M. Dyankova**

Institute of Cryobiology and Food Technologies, 53 Cherni vrah Bul, 1407 Sofia, BULGARIA
* Corresponding author: svetla.diankova@ikht.bg

Abstract. The increased public attention on the waste pollution and the awareness of the hard environmental problems is the reason for the need of new materials which are susceptible to degradation in nature for a short period of time. The biopolymer films and coatings based on renewable natural sources are suitable for obtaining of biodegradable packaging. The newly developed composite films based on sodium alginate and apple high methoxyl pectin were studied for total soluble matter, swelling in water, water vapors transmission rate and biodegradation in soil. The analysis of their behavior in water medium showed a considerably higher rate and degree of dissolution of the pectin monocomponent film compared to the composite and alginate films. The composite alginate-pectin films showed lower water vapors transmission rate even under extreme conditions (38ºC, RH 90 %) compared to the monocomponent films. All investigated films degraded in soil up to 80 days. The good barrier properties to water vapors and the complete biodegradation in soil make the films based on sodium alginate and high methoxyl pectin potential ecological materials for packing and coating of foods and pharmaceutical products.

Key words: composite films, alginate, high methoxyl pectin, solubility, water vapors transmission rate, biodegradation.

Introduction

The rapid technological progress that has been seen during the last decades in its greatest part is owing to the plastics industry. The production and the use of plastic materials are increasing at extremely accelerated rates. For example, in 1950 the output of plastic was 1,5 million tons and in 2008 it reached up to 245 million tons (NOWAK *et al.*, 2011). Around 30% of the manufactured plastic in the world is used as packing. The dramatic increase of the production of synthetic plastics, however, increases their presence in nature after use because of their stability, resistance and the lack of biodegradation. This fact focuses public attention on their accumulation in environment and the merging of problems with pollution which may last for hundreds of years and which would have a disastrous effect on the ecosystem balance (ALBERTSSON *et al.*, 1987). The advanced ecological culture and the awareness of the hard environment problems is the reason for the need of new materials which are susceptible to chemical, biochemical and biological degradation processes as result of which their degradation in nature takes place in a short period of time.

A number of biopolymers have a potential to be used as a base for production of biodegradable materials for packaging and coatings with application in food industry and pharmacy. One of the main advantages of this type of materials is that they are obtained from renewable sources

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

that are widely spread in nature and can be used in native or chemically modified form (SOLAK & DYANKOVA, 2011).

The development of biodegradable polymer coatings and films from natural and renewable sources diminishes the need of synthetic petroleum polymers, eliminates the negative effect on environment, gives a good possibility to meet the changing consumer requirements and market trends for healthy and safe foods, packed with natural materials, which do not pollute the environment (DYANKOVA *et al.,* 2013). In addition to their property to biodegrade they have a number of other advantages, namely – they are biocompatible and bioassimilable, edible, with aesthetic appearance and good barrier and mechanical properties. Besides that this type of materials allows the incorporation of biologically active substances (natural antibacterial and antioxidant components of plant and microbial origin, enzymes, probiotics or other functional ingredients), which gives a possibility for prolongation of the shelf life of the processed foods (KROCHTA *et al.,*1997; MARTÍN-BELLOSO *et al.,* 2009; QUEZADA-GALLO, *et al.,* 2009).

Very often the obtained biopolymer films and coatings are a monohydrocolloid layer of proteins or polysaccharides. In the last several years, however, the technological investigations have been directed towards the development of composite films combining the synergic effects of proteins, polysaccharides and/or lipid components. These materials show combines and/or completely new functional qualities (DA SILVA *et al.,* 2009).

The salts of the alginic acid and the pectin are an example of polysaccharides with good film forming properties, suitable for obtaining of biopolymer films and coatings. Alginates are hydrophilic polysaccharides extracted from various species of brown seaweed *(Phaeophyceae).* They are of interest because of their unique colloid properties and the capacity to form three dimensional networks in the presence of polyvalent cations (for example Ca^{2+}), when a gel or an insoluble polymer is obtained (DRAGET *et al.,* 2006; WILLIAMS, 2009). By chemical structure alginates are unbranched binary copolymers, built of β-D-manuronic acid and its isomer α-L-guluronic acid. There are three types structural elements in the alginate molecules - β (1–4)-D-mannuronate (M-block), α-(1-4)-L-guluronate (G-block), and the third structure contains both monomers in almost equal proportions (MG-block) (*DRAGET et al.,*2006; FANG 2008; WILLIAMS, 2009). From the alginic acid salts the sodium alginate is most often applied in practice.

Pectins are plant heteropolysaccharides whose main chain consists of acidic derivatives of the hexoses (D-galacturonic acid), and the side chains are built of pentoses (D-xylose and L - arabinose) and hexoses (D – glucose and D - galactose). In the main chain the units D-galacturonic acid are linked by α-(1-4) glycosidic bond i.e. it is polygalacturonic acid to which are attached the side chains by β-(1-6) glycosidic bonds. The carboxyl groups of the galacturonic acid are partially esterified with methanol and neutralized with metal or ammonia ions as different pectin substances are obtained – pectins, pectates, pectic acids (DUMITRIU , 2004; PHILIPS & WILLIAMS, 2000). The main characteristic with the greatest influence on the rheological and physicochemical properties of pectin is the degree of esterification (DE) by which is expressed the percentage of the esterified to the total number –COOH groups. Depending on the degree of esterification pectins are divided into two main categories: high methoxyl (HM) and low methoxyl (LM) pectins. The degree of esterification for HM pectins varies from 50 to 100%. LM pectins (DE under 50%) are usually obtained by demethoxylation of the extracted pectins by enzyme processes or by treating with acids or ammonia in alcoholic medium. HM and LM – pectins have a different mechanism of gel forming when dissolved in water (GIGLI *et al.,* 2009; THAKUR *et al.,*1997).

The wide use of alginates and pectins in food industry and medicine is dictated by the lack of toxicity and allergenicity and the unique colloid properties. Both biopolymers are capable to form gels in the presence of divalent cations (FANG *et al.*,2008; BRACCINI & PEREZ, 2001). The alginate and the pectin form synergic mixed gels which lead to structures completely different from those of the pure polymers. They are of particular interest in the creation of biodegradable films with new improved characteristics.

The mechanism of synergic interaction between pectin and alginate has not been fully clarified. The rheological behavior of the mixed solutions and hydrogels has been studied to a great extent (FANG *et al.*,2008; OAKENFULL *et al.*,1990; RAO & COOLEY, 1995), while the investigations in the field of the physicochemical properties of the resulting composite films and their biodegradation have been yet much more weakly examined.

The objective of the present investigation was to analyze newly developed composite biopolymer films based on sodium alginate and high methoxyl pectin in relation to the physicochemical and barrier properties and to determine the period of their biodegradation in soil.

Materials and Methods

Materials. Apple high methoxyl pectin (CpCelso), sodium alginate (Sigma Aldrich), glycerol pa (Merck), calcium chloride pa (Merck).

Obtaining of biopolymer films. The method for obtaining of the films is described in more details in one of our previous articles (DYANKOVA & SOLAK, 2014). Briefly: the film forming solutions (FS) were prepared by mixing of water solutions of sodium alginate (2,5%) and high methoxyl pectin (2,5%) in the following proportions: 100–0% (AG), 75–25% (C_1AG-P), 50–50% (C_2AG-P) and 25–75% (C_3AG-P). As *plasticizer* was used glycerol (0.6g/g polymer), and for the primary cross-linking to each composition was added 0.1 M $CaCl_2$ (1ml/100ml FS). The prepared hydrogel was treated under vacuum for removing of the air bubbles. After that the film forming solutions were poured into petri dishes (0,325 g FS/cm^2) and dried under vacuum (20 kPa, SPT-200 Vacuum Drier) at 35°C. The dry samples were put in 0.3 M solution of $CaCl_2$ for realization of main cross-linking and were washed with distilled water for removing of the excess Ca^{2+}. The films were dried at 25° C and preserved at 50 ± 1% relative humidity before testing.

Analysis of the source pectin - The content of the methoxy-groups, galacturonic acid and the degree of esterification was determined by method described in USP 23 (1995).

Moisture content - by express weight method with infrared dryer (Sartorius Thermo Control YTC 01 L).

Film thickness - The thickness of the film was determined with a digital micrometer with accuracy up to 0.01mm ±5% in five randomly taken sectors of the film.

Water vapors transmission rate (WVTR) determination. The test was carried out according to ISO 2528: (E) (1995) under two specific conditions: temperature 38°C and relative humidity (RH) - 90%; 2) temperature 25° C and RH -75%. Aluminum containers (with dimensions: height, h= 8cm; outer diameter, d= 5.5 cm, inner diameter, d= 5cm) filled with desiccant (anhydrous $CaCl_2$), were covered with samples of the films (diameter 5,5cm), which were attached by a thin layer paraffin wax and on the top was put a plastic ring. The containers were put in a chamber: 1) at 38±1,0°C, RH 90± 2,0% and 2) at 25±1,0°C, RH 75± 2%. The increase in weight was measured at identical time intervals. After the water transfer equilibrated (difference in weight between two consequent measurements was <5%), the water vapor transmission rate (WVTR) was calculated by the formula:

$$WVTR = W/(tA), \quad (1)$$

where W is the increase in weight (mg), t is the duration of the experiment (days) and A is the permeation area (23,75 cm^2). The results are presented as average values of three parallel experiments for a period of 10 days.

Total soluble matter(TSM). TSM was determined according to a method described by RHIM *et al.* (1998). Preliminary weighted samples of the films with equal size (d=2 cm) were put in flasks containing 100 ml distilled water (with sodium azide for inhibition of microbial growth). The flasks were put in a shaking system ("Inkubations-Schüttelschrank BS-4 B.Braun", 100 rpm) at temperature 25°C for 24 hours. The not dissolved particles were separated and dried at temperature 105°C for 24 hours after which they were weighted.

TSM was determined by the formula:

$$TSM=[(Wi-Wf)/Wi]x100, \quad (2)$$

where Wi is the initial weight and Wf is the weight after separation and drying.

Swelling ratio (S) Samples of the films were weighted (Wd) and put in 30 ml distilled water at room temperature for a specific time after which they were taken out, wipe with filter paper and weighted again (Ws). The swelling ratio of the samples was determined by the formula:

$$S= [(Ws -Wd)/ Wd] x100. \quad (3)$$

Biodegradation in soil. The bio-degradability of the films was analyzed using a method previously described in the literature (MARTUCCI *et al.,* 2009). Biopolymer films with dimensions 2 x 3 cm were dried under vacuum (20 kPa, SPT-200 Vacuum Drier) at 65°C for 6 hours and weighted (W_0) with accuracy up to 0.0001 g. For better manipulation the samples were placed in aluminum nets and were put at a depth of 6 cm (aerobic degrading) in soil with given specifications: pH 7,1; NH_4^+ - 6,7 mg/kg; NO_3^- 5,4 mg/kg; K_2O - 21,5 mg/100g ; P_2O_5 - 4,1mg/100g; porosity 78%; total number of microorganisms - $2{,}1.10^8$ CFU/g. The experiment was carried out for a period of 80 days at temperature 18±5°C and soil moisture 48±4 % RH. At a given interval the samples were taken out of the soil, washed with distilled water, dried at 65° C for 6 hours and were weighted again (Wt), in order to calculate the average weight loss by the formula:

$$WL\%=[(W_0-Wt)/W_0]x100. \quad (4)$$

The results are presented as average values of three consequent experiments. Samples of polyethylene films (PE) were used for comparison.

Statistical analysis. Results were analysed by *statistical program Minitab 15.* One-Way ANOVA and Tukey Test were used to determine statistically significant differences ($p<0.05$).

Results and Discussion

Analysis of the source pectin

The quantitative analyses of the samples of the commercial apple pectin showed content of pure pectin - 71,50%. The content of galacturonic acid is 753,0 ± 14,76 mg/g, and of methoxy-groups - 68,50 ± 2,36 mg/g. From the obtained results was calculated the degree of esterification (DE) which is 56,90%. By this index the used pectin is determined as high methoxyl but also with present of a certain percentage free carboxyl groups.

pH of the film forming solutions varies from 2,88 for the pectin to 6,16 for the sodium alginate (AG). The different variants film forming mixtures show pH values in the acidic range: C_1AG-P - 4,25; C_2AG-P - 3,78 and C_3AG-P - 3,54.

Characteristics of the obtained films

The film variants are obtained with different proportions between the two polysaccharides (sodium alginate and pectin) - 1:0, 1:3, 1:1, 3:1, 0:1, glycerol as plasticizer and $CaCl_2$ as a cross-linking agent at two step cross-linking procedure. Visually the resulting films are homogeneous, uninterrupted, without brittle sectors and without presence of air bubbles (Table 1). They are manageable and flexible. The color of the alginate films varies from transparent to slightly opaque. The films with high pectin content are with slight yellowish nuance.

The thickness of the obtained materials depends on the composition, the used technology and on the drying conditions. When pouring of a constant quantity film forming mixture for a given area (0,325 g FS/cm^2), films with thickness from 0,043 to 0,062 mm were obtained (Table 1).

Table 1. Characteristics of the films.

Composition	Appearance	Thickness (mm)	Moisture (%)	WVTR (g x m^{-2}x day^{-1}) T 38°, RH 90%	WVTR (g x m^{-2}x day^{-1}) T 25°C, RH 75%
AG	transparent, flexible	0,054±0,003	11,52±0,30	1080,99±20,56	290,59± 8,90
P	lightly yellowish, flexible	0,062±0,005	14,80±0,20	995,63±15,52	283,87± 10,20
C_1AG-P	lightly opaque, flexible	0,050±0,004	12,82±0,35	337,23±10,80	150,41± 8,60
C_2AG-P	lightly yellowish, flexible	0,048±0,003	14,10±0,40	363,07±11,50	133,24 ± 5,70
C_3AG-P	lightly yellowish, flexible	0.043±0,035	14,20±0,29	460,11±9,08	160,73± 6,20

In this investigation the calcium chloride was used as a gel forming and cross-linking agent. The polyvalent cations like Ca^{2+}, play the role of bridges between the polymer chains of the alginate and build junction zone which form the hydrogel network. For the pectins with DE around 50% (the pectin used by us is with DE 56,90%), the gel forming force depends on a number of factors – pectin concentrations, dissolved sugars and polyols, pH-value of the solution and quantity of the present calcium cation. The degree of cross-linking and the synergy between the two biopolymers have a direct effect on the physicochemical characteristics of the resulting films and particularly on their barrier properties and behavior in water medium. The values of moisture content for the different compositions are relatively close except for the alginate and the pectin films for which a statistically reliable difference is observed ($p<0.05$). When the films are placed in water medium a comparatively fast swelling is observed yet in the first 10 minutes. On Fig. 1 are presented the data for the different film variants for swelling in water as a function of time.

According to the results the monocomponent pectin films are with the highest water absorption capacity – maximum value at the 20th minute after which they decompose. SRIAMORNSAK & KENNEDY (2008) have also observed a higher degree of swelling for the pectin films compared to the alginate. For the alginate and composite films the cross-linking with calcium chloride allows the building of a multitude of binding zones and forming of a polymer network which is more stable. Less water molecules are able to penetrate the sample, and hence the swelling is reduced. This tendency is confirmed by the test for determining of *total soluble matter (TSM).* The applied test is a way to characterize the solubility of the film matrix in water medium. When the samples are in contact with the solvent, water is absorbed which causes breakage of the polymer-polymer bonds, forming of new bonds between the water and the polymer materials, dividing of the polymer, swelling and dissolving at the end. On Fig. 2 are presented the summarized test results. The composite films show a considerably lower solubility compared to the monocomponent pectin film for which the total quantity soluble matter is around 99,13%. Their results are similar to the values obtained for the alginate film. The applied two steps cross-linking with calcium chloride strongly diminishes the solubility in water of the resulting films. The combination of the film forming materials and their synergy, the temperature and the relative humidity of the environment influence the permeability of the resulting films. The tests for water va-

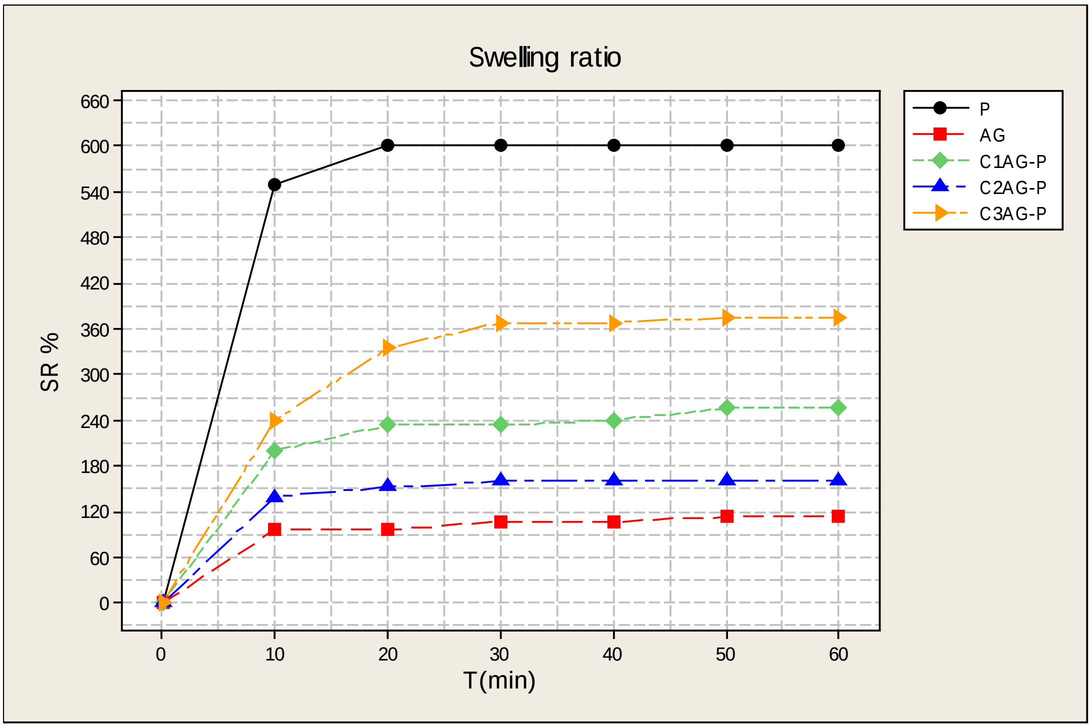


Fig.1. Swelling ratio of films in water

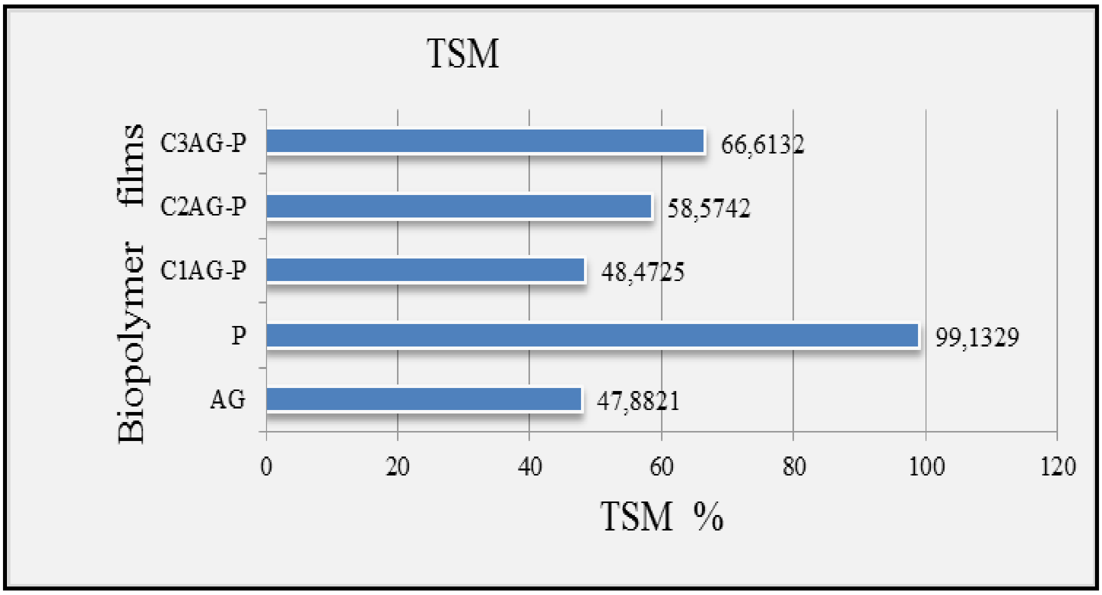


Fig. 2. Total soluble matter (TSM) for the different film variants.

pors permeability (WVP) give an idea of the barrier capacity to water vapors of the biopolymer films. This capacity is an important factor when they are used as packing. All films treated under regime 1 have from 2 to 3,5 times higher permeability to water vapors ($p<0.05$) compared to regime 2 (Table 1), due to the fact that the barrier properties to water vapors diminish with the increasing of the temperature during the WVP measurements (RHIM *et al.*, 2003). The temperature regimes applied during the measurement are above the admissible ranges for preservation of packed foods. These temperature values were selected because one of the objectives was to follow up the behavior of the film under more extreme conditions. The WVTR values for pectin films we obtained are slightly higher than those observed by

LINSHU LIU *et al.* (2007). This is probably due to the difference in the technology of obtaining producing the film and the lower temperature regime in the course of the test. For both regimes (1-38±1˚C, RH 90±2% and 2-25±1˚C, RH 75±2%) the monocomponent polysaccharide films show a higher WVTR than the composite films. This fact can be explained with the generation of continuous physical co-gels when mixing the synergic solutions of the two polysaccharides where the pectin component is dispersed in the gaps of the main alginate gel network. From these mixtures in the process of drying is obtained a more compact and stronger structure which is less permeable to water vapors. The increases in weight as a function of time for both measuring regimes are presented on Fig. 3 and Fig. 4.

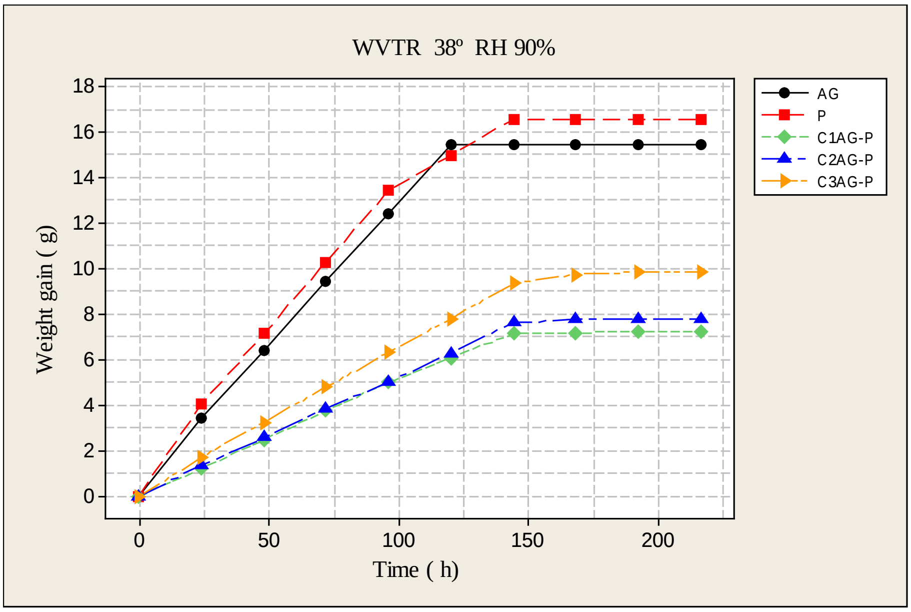


Fig. 3. Increases in weight as a function of time (regime 1).

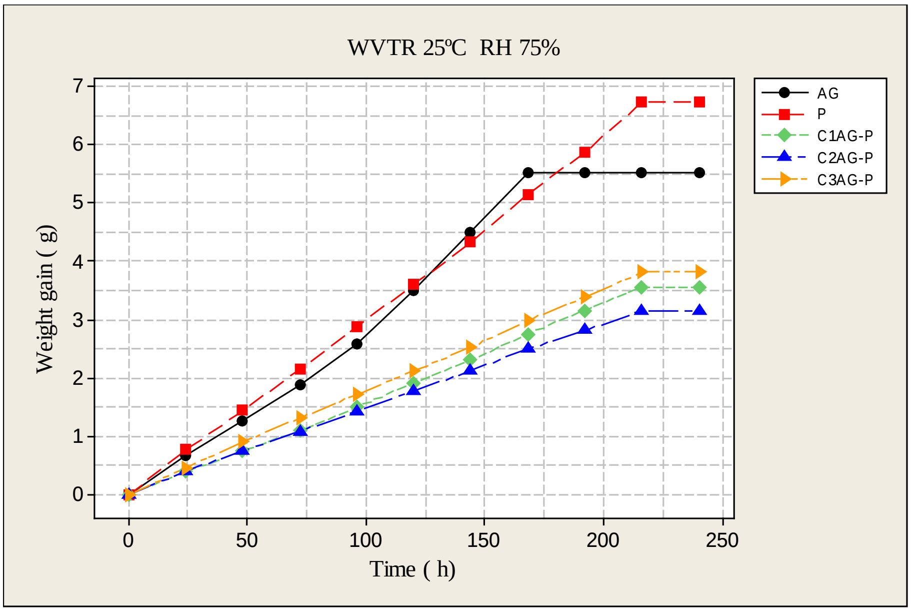
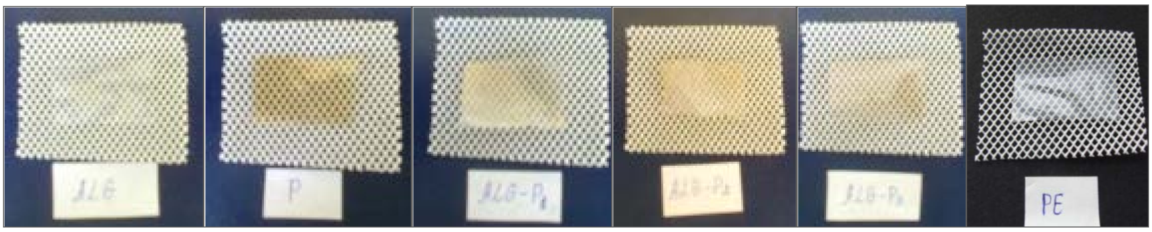


Fig. 4. Increases in weight as a function of time (regime 2).

Biodegradation in soil

The test for biodegradation was carried out by burying of the films in soil in laboratory conditions for 80 days at temperature 18±5ºC. The moisture of the soil was kept around 48±4 %, for preventing the dissolving of the matrix. On Fig. 5 are presented photos of the studied samples before and during the test. The process of degradation begins yet on the second day after burying the films in the soil (Fig 5).

Till the seventh day the loss in weight of the samples is from 30% to 75% with visible changes in the form compared to the initial state (Fig.6). The fastest degradation is observed for the pectin films – on the 9th day the loss in weight is 100%. The alginate films at the end of the period- 80th day, are with 92,13% loss in weight. The composite film C_3AG-P (loss in weight up to 90,09%) degrades faster than the composite films C_1AG-P and C_2AG-P (respectively 76,13 and 77,17%). It has been established that after putting in the soil all films absorb water (to a lower or higher degree) and lose their initial form and structural integrity. After the 30th day a specific smell of decay is observed.



a)

b)

Fig 5. Macroscopic aspect of the films before (a) and after 14 days (b) of burying in soil.

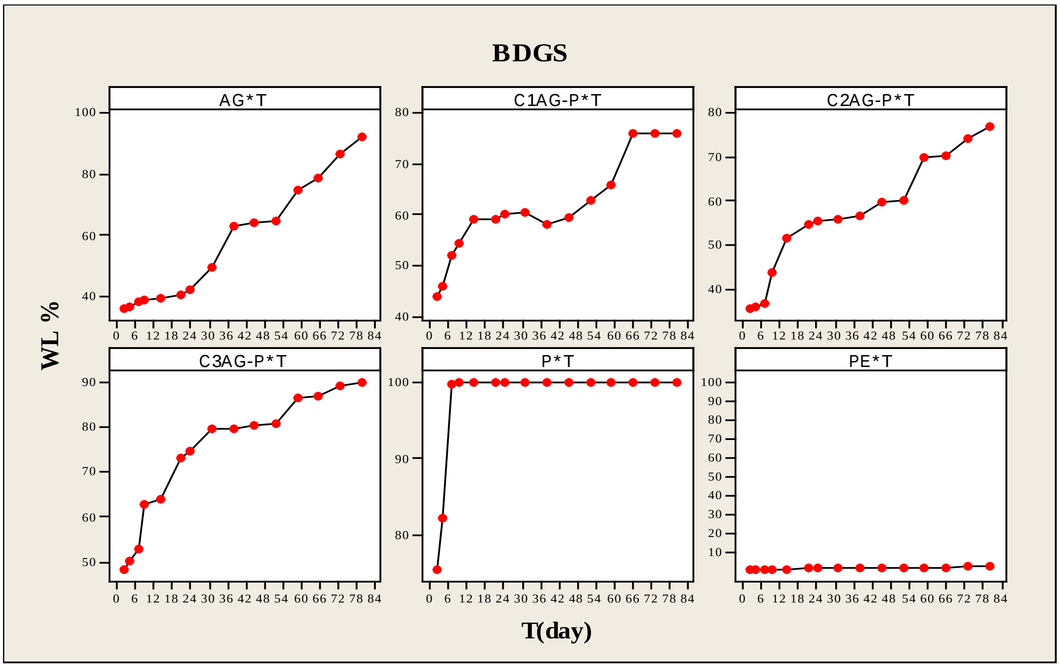


Fig. 6 Biodegradation in soil - weight loss (%) of the films as a function of time

The faster degradation of the pectin films is due to the more weakly cross-linked structure. Besides that the water absorption for the pectin films is 2 to 3 times higher than the other samples (test for swelling rate, Fig. 1). This effect is the cause for the greater susceptibility to microbial attack of the pectin films while the stronger structures of the alginate films make them hardly susceptible to the activity of the microbial hydrolytic enzymes. The situation is similar for the composite films where the degradation rate under the effect of the soil microorganisms is comparable to the alginate ones. The used as a comparison sample polyethylene (PE) remained unchanged.

Conclusion

The use of natural biopolymers with film forming properties gives a good potential to develop new possibilities for utilization of the agricultural raw materials. Alginates and pectins are very suitable plant biopolymers for obtaining of biodegradable (and in particular edible) films and coatings with various applications. The combining of sodium alginate and high methoxyl pectin allows the obtaining well shaped, homogeneous and semi-transparent films. The analysis of their behavior in water medium has shown considerably great differences in the rate and degree of dissolving of the pectin monocomponent film compared to the composite and the alginate films. The composite alginate-pectin films have better barrier properties to water vapors compared to the monocomponent. The test for biodegradation has shown that up to 80 days all studied films degrade in soil in contrast to the samples of synthetic folio which remain unchanged. The films based on sodium alginate and pectin are biodegradable and show low water vapors transmission rate even under extreme conditions which makes them potential materials for packing and coating of foods and pharmaceutical products. Their complete degradation in soil and the fact that they are obtained from renewable sources makes them ecology friendly products whose production turn out to be an investment for environment protection for the future generations.

References

ALBERTSSON A.C., S.O. ANDERSSON, S. KARLSSON. 1987. The mechanism of biodegradation of polyethylene. - *Polymer Degradation and Stability*, 18: 73–87.

BRACCINI I., S. PEREZ. 2001. Molecular basis of Ca2+-induced gelation in alginates and pectins: the egg-box model revisited. - *Biomacromolecules*, 2: 1089–1096.

Da SILVA M.A., A.C. KRAUSE BIERHALZ, T. G. KIECKBUSCH, 2009. Alginate and pectin composite films crosslinked with Ca2+ ions: Effect of the plasticizer concentration. -*Carbohydrate Polymers*, 77: 736–742.

DRAGET K. I., S. T. MOE, G. SKJAK-BRAEK, O. SMIDSROD. 2006. Alginates. In: Stephen A.M., G.O. Phillips, P.A. Williams (Eds.): *Food Polysaccharides and their applications, 2nd ed.,* CRC Press, Taylor and Francis Group, Boca Raton, Fl USA, pp. 290-328.

DUMITRIU S.. 2004. *Polysaccharides: Structural Diversity and Functional Versatility*, 2nd ed., New York, CRC Press.

DYANKOVA, S., A. SOLAK, P. METODIEVA, 2013. Survey of consumer attitudes and preferences preferences regarding the quality, taste characteristics and composition of the meat products. – *Food Processing Industry*, 12:33-36.(In Bulgarian, English abstract).

DYANKOVA S., A. SOLAK. 2014. Preparation and characterization of composite hydrocolloid films from sodium alginate and high methoxyl pectin. - *Bulgarian Chemical Communications,* 46(2): 368-374.

FANG Y., AL-ASSAF S., G.O. PHILLIPS, K.NISHINARI, T.FUNAMI, P.A.WILLIAMS, 2008. Binding behavior of calcium to polyuronates: Comparison of pectin with alginate. - *Carbohydrate Polymers*, 72(2): 334-341.

GIGLI J., C. GARNIER, L. PIAZZA. 2009. Rheological behaviour of low-methoxyl pectin gels over an extended frequency window. - *Food Hydrocolloids*, 23(5): 1406-1412.

INTERNATIONAL STANDARDS ORGANISATION. *1995*. Sheet materialss determination of water vapour transmission rates gravimetric (dish) method; *ISO 2528(E*).

KROCHTA J.M. C., C. MULDER-JOHNSTON. 1997. Edible and biodegradable polymer films: Challenges and opportunities. - *Food Technology,* 51(2): 61-74.

LIU L. S., C. K. LIU, M. L. FISHMAN, K. B. HICKS. 2007. Composite Films from Pectin and Fish Skin Gelatin or Soybean Flour Protein. - *Journal of Agricultural and Food Chemistry*, 55(6): 2349-2355.

MARTÍN-BELLOSO O., M. A. ROJAS-GRAÜ, R. SOLIVA-FORTUNY. 2009. Delivery of Flavor and Active Ingredients Using Edible Films and Coatings, In: Embuscado M. E. and K. C. Huber (Eds.), *Edible Films and Coatings for Food Applications,* Springer Science+Business Media, LLC. 295 p.

MARTUCCI J.F., R.A. RUSECKAITE. 2009. Biodegradation of three-layer laminate films based on gelatin under indoor soil conditions. - *Polymer Degradation and Stability,* 94 (8): 1307–1313.

NOWAK B., J. PAJAK, M. DROZD-BRATKOWICZ, G. RYMARZ. 2011. Microorganisms participating in the biodegradation of modified polyethylene. - *International Biodeterioration and Biodegradation,* 65: 757-767.

OAKENFULL D., A. SCOTT, E. CHAI. 1990. The mechanism of formation of mixed gelsby high methoxyl pectins and alginates. In: G. O. Phillips, D. J. Wedlock, P. A. Williams (Eds.): *Gums and stabilizers for the food industry 5.* IRL Press, Oxfords. 243 p.

PHILLIPS G.O, P.A. WILLIAMS. 2000. *Handbook of Hydrocolloids.* Woodhead Publishing Limited, England, pp. 274-280.

QUEZADA-GALLO J.A. 2009. Delivery of Food Additives and Antimicrobials Using Edible Films and Coatings, In: Embuscado M. E. and K. C. Huber (Eds.): *Edible Films and Coatings for Food Applications.* Springer Science+Business Media, LLC. 315 p.

RAO M. A., H. J. COOLEY. 1995. Rates of structure development during gelation and softening of high-methoxyl pectin-sodium alginate:fructose mixtures. – *Food Hydrocoloids*, 9:229-235.

RHIM J.W., A. GENNADIOS, C.L. WELLER, M.A. HANNA. 1998. Soy protein isolate–dialdehyde starch films. - *Industrial Crops and Production,* 8 (3): 195–203.

RHIM J.W., PARK J.W., S.T. JUNG. and H.J. PARK .2003. Formation and properties of corn zein coated κ-carrageenan films. - *Korean Journal Food Science and Technology*, 29: 1184–1190.

SOLAK A., S. DYANKOVA. 2011. Biopolymer films and coatings with application in the food industry. - *Food Processing Industry*, 3: 39-44. (In Bulgarian, English abstract).

SRIAMORNSAK P., R.A. KENNEDY. 2008. Swelling and diffusion studies of calcium polysaccharide gels intended for film coating. - *International Journal of Pharmaceutics*, 358: 205–213.

THAKUR B, R. SINGH, A. HANDA. 1997. Chemistry and uses of pectin: A review. - *Critical Reviews in Food Science and Nutrition,* 37 (1):47-73.

THE UNITED STATES PHARMACOPEIA, 23-rd ed.1995. *National Formulary 18*, Rockville, United States Pharmacopeial Convention.

WILLIAMS, P. A. 2009. Molecular interactions of plant and algal polysaccharides. - *Structural Chemistry*, 20(2): 299-308.

Received: 04.06.2014
Accepted: 02.12.2014

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 35-41

Physiotypic Plasticity and Survival of Arido-active Euphorbia triaculeata (Euphorbiaceae) in its Natural Habitat

***Turki A. Al-Turki*[1], *Osama H. Sayed*[2], *Yahya S. Masrahi*[2*]**

1 - King Abdul Aziz City for Science and Technology, Riyadh, SAUDI ARABIA
2 - Faculty of Science, Jazan University, Jazan, SAUDI ARABIA
[*] Corresponding author: ymasrahi@gmail.com

Abstract. Leafless stem succulent *Euphorbia triaculeata* Forssk. is abundant in arid regions southwest of the Arabian Peninsula. These regions are characterized by short wet season with erratic rainfall and long dry season with high temperature and high irradiance. Field observations indicated that the plant survives the long dry season and acquires a red stem colour under high irradiance and protracted drought. Our work aimed at investigating survival of this arido-active species in its natural habitat by studying diurnal and seasonal changes in stomatal conductance, cell sap acidity, and chlorophyll fluorescence. Results showed that *E. triaculeata* is obligate crassulacean acid metabolism (CAM) plant. Under protracted drought, low stomatal conductance and dampening of CAM denoted a shift to CAM-idling. Observed stress-induced reduction of Photosystem II activity occurred in concomitance with increased non-photochemical quenching of chlorophyll fluorescence and increased anthocyanin content. These results reflected operation of a photoprotective mechanism involving interplay of non-photochemical energy dissipation via xanthophyll cycle and alleviation of oxidative stress by anthocyanin. It was concluded that *E. triaculeata* survives in its arid habitat by performing obligate CAM, shifting to CAM-idling under protracted drought, increasing non-photochemical excess energy dissipation, and accumulating anthocyanin pigment for its antioxidant attributes.

Key words: chlorophyll fluorescence, Crassulacean Acid Metabolism (CAM), CAM-idling, *Euphorbia triaculeata*, photoprotection.

Introduction

Arid regions southwest of Arabian Peninsula are characterized by high temperature, high irradiance, scarce water, and erratic rainfall (FISHER & MEMBERY, 1998). In these regions, leafless stem succulents rely on large water storage capacity and physiological adaptations including CAM pathway to improves plant water economy (SAYED, 2001a; SAYED, 2001b; LÜTTGE, 2010; MASRAHI *et al*. 2011; MASRAHI *et al*., 2012a). Although several reports indicated that stem succulent *Euphorbia* species exhibit CAM (FEAKINS & SESSIONS, 2010; AL-TURKI *et al*. 2014), scarce information exists on ecophysiology of *E. triaculeata* (MASRAHI, 2004). Our field observations indicated that *E. triaculeata* survives long dry seasons and acquires a red stem colour under protracted drought in arid regions southwest of Saudi Arabia. The present paper aimed at investigating existence of CAM in *E. triaculeata* by studying diurnal and seasonal changes in stomatal conductance and chlorenchyma cell sap acidity. Phase III of CAM takes place under closed stomata and high irradiance, and PSII can become over-energized

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

(NIEWIADOMSKA & BORLAND, 2008; LÜTTGE, 2010). Under such conditions, plants evoke photoprotection by non-radiative excess energy dissipation via xanthophyll cycle (HORTON & RUBAN, 2005; MURCHIE & NIYOGI, 2011). Therefore, our work also involved studying chlorophyll fluorescence quenching, and accumulation of anthocyanin pigment to envisage possible photoprotective mechanisms involved in mitigating harmful effects of high irradiance and drought.

Materials and methods

Study Site: Study site in Jazan Province (17°02′N - 42°54′E, and 125 m.a.s.l.) Southwest of Saudi Arabia is characterized by sand-loam soil, high temperature, high irradiance, scarce water, erratic rainfall, and climate influenced by tropical maritime air mass (MASRAHI, 2012). The dry season is nine-months-long, and the short wet season (June–August) is associated with strong sand storms (MIDDLETON, 1986).

Climatic Conditions. Climatic records (1970-2010) of mean monthly air temperature, and precipitation were obtained curtsey of Ministry of Electricity and Water (Riyadh, Saudi Arabia). During dry season, air temperature and photosynthetic photon flux density (PPFD) were monitored using field digital thermometer (Kestrel 2000, Philadelphia, USA), and PAR/LAI Ceptometer (AcuPar LP-80, Decagon, Pullman, USA), respectively.

Plant Material. Mature *E. triaculeata* Forssk. (Euphorbiaceae) plants were used as plant material.

Measurements. Stomatal conductance was measured using Porometer (AP4, Delta-T, Cambridge, UK). Pulse amplitude modulated chlorophyll fluorescence was measured using fluorometer (FMS2, Hansatech, Norfolk, UK). Measured chlorophyll fluorescence parameters included efficiency of PSII antenna (Fv/Fm), and quantum yield of PSII (ΦPSII) (BAKER, 2008). Non-photochemical quenching of chlorophyll fluorescence (qNP) was calculated using standard fluorescence nomenclature (SAYED, 2003; BAKER, 2008) and the equation:

$$qNP = (Fm-Fm') / (Fm-Fo)$$

where: *Fo* - minimal fluorescence emitted by antenna chlorophyll molecules, *Fm* - maximal fluorescence emitted when PSII traps become closed, and Fm': light-adapted fluorescence maximum.

Oscillation of CAM acidification was studied in chlorenchyma cell sap extracted in the field by grinding a known weight of tissue in distilled water, expressing cell sap through two layers of muslin, and determining cell sap acidity by titration against 0.01N NaOH (OSMOND *et al.*, 1991). Laboratory determination of anthocyanin pigment content involved grinding a known weight of stem chlorenchyma in ice-cold methanolic HCl (0.1% HCl v/v), determining absorbance at 532,653 nm, and calculating anthocyanin content as A532 – 0.24A653 using extinction coefficient 30000 L $mol^{-1}cm^{-1}$ (MURRAY *et al.*, 1991). All experiments were routinely repeated in samples taken from ten different individual plants and standard error was calculated.

Results

Climatic records indicated extreme aridity of the study site reflected by a long dry season, a short wet season (June-August), and total annual rainfall of about 100 mm (Fig. 1). Monitoring daytime air temperature and PPFD during dry season indicated that these parameters can attain high midday values of 43°C, and 2025 μmol $m^{-2}s^{-1}$, respectively (Table 1).

During wet season, nighttime values of stomatal conductance were higher than those recorded during the day (Fig. 2). During dry season, measurements of stomatal conductance indicated that *E. triacultata* exhibited markedly low values of stomatal conductance in the range of 2-4 mmol $m^{-2}s^{-1}$ during both day and night (Fig. 2).

Determination of chlorenchyma cell sap titratable acidity indicated that *E. triaculeata* showed evident diurnal acidity changes during wet season, and that these changes were considerably reduced during dry season (Fig. 3).

Chlorophyll fluorescence measurements during dry season showed reduction in values of Fv/Fm, and ΦPSII compared to those measured during wet season (Fig. 4). This reduction of chlorophyll fluorescence parameters during dry season coincided with increased value of qNP and chlorenchyma anthocyanin pigment content (Table 2).

Table 1. Daytime changes in air temperature, soil temperature, and PPFD at the study site during the dry season in mid-May.

Parameters	Time of Day						
	0600	0800	1000	1200	1400	1600	1800
Air Temperature (°C)	34	36	37	43	42	40	38
PPFD (µmol m^{-2} s^{-1})	500	1500	2000	2025	2250	2125	200

Table 2. Seasonal changes in non-photochemical quenching of chlorophyll fluorescence (qNP), and anthocyanin content of *E. triaculeata* (± se, n =10).

Parameter	Season	
	Wet	Dry
qNP (relative units)	0.02 ± 0.003	0.60 ± 0.005
Anthocyanin (µg g^{-1} dry weight)	150 ± 5.5	850 ± 3.8

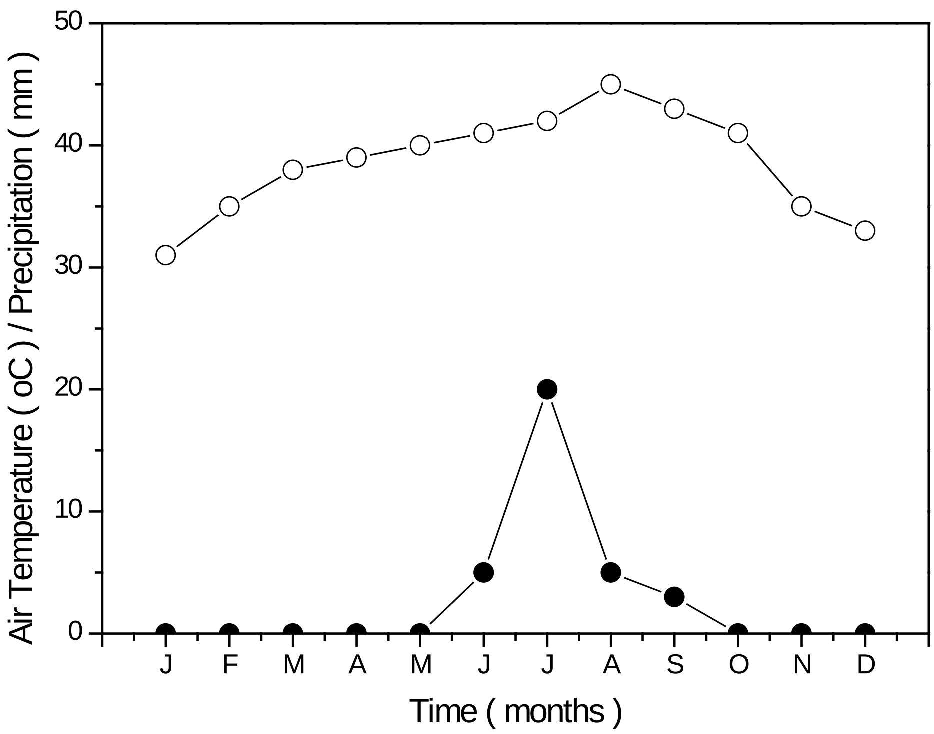


Fig. 1. Climatic records of air temperature and precipitation at the study site.

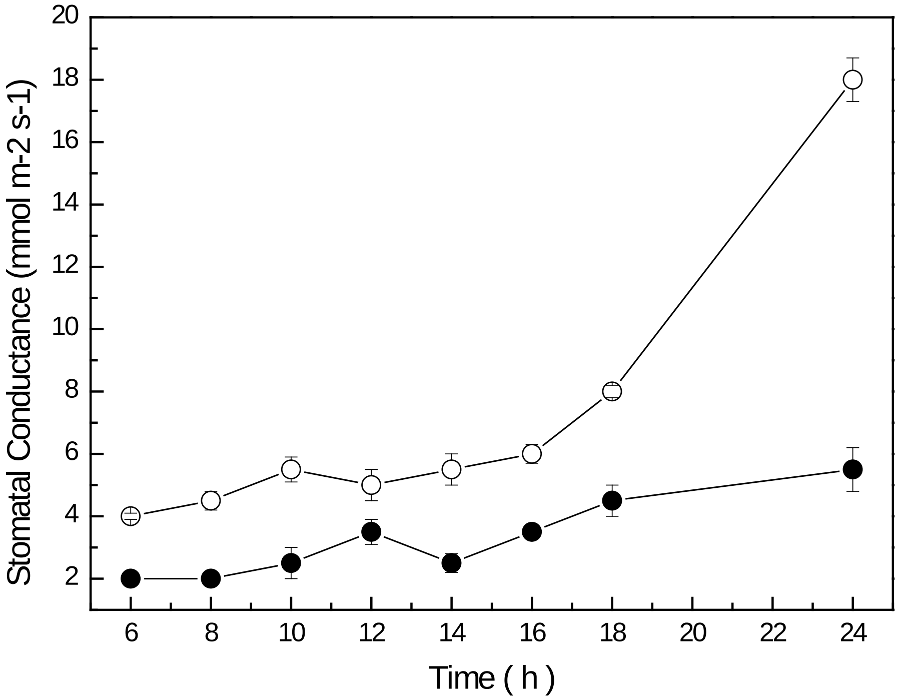


Fig. 2. Diurnal changes in stomatal conductance of *E. triaculeata* during wet season (○) and dry season (●), (± se, n = 10).

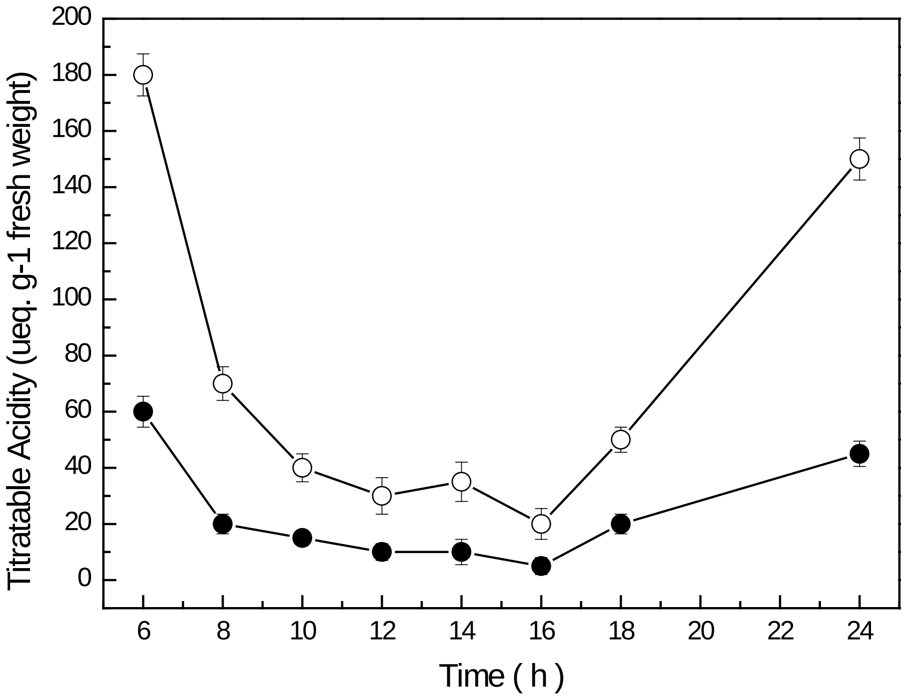


Fig. 3. Diurnal changes in titratable acidity of *E. triaculeata* during wet season (○) and dry season (●), (± se, n = 10).

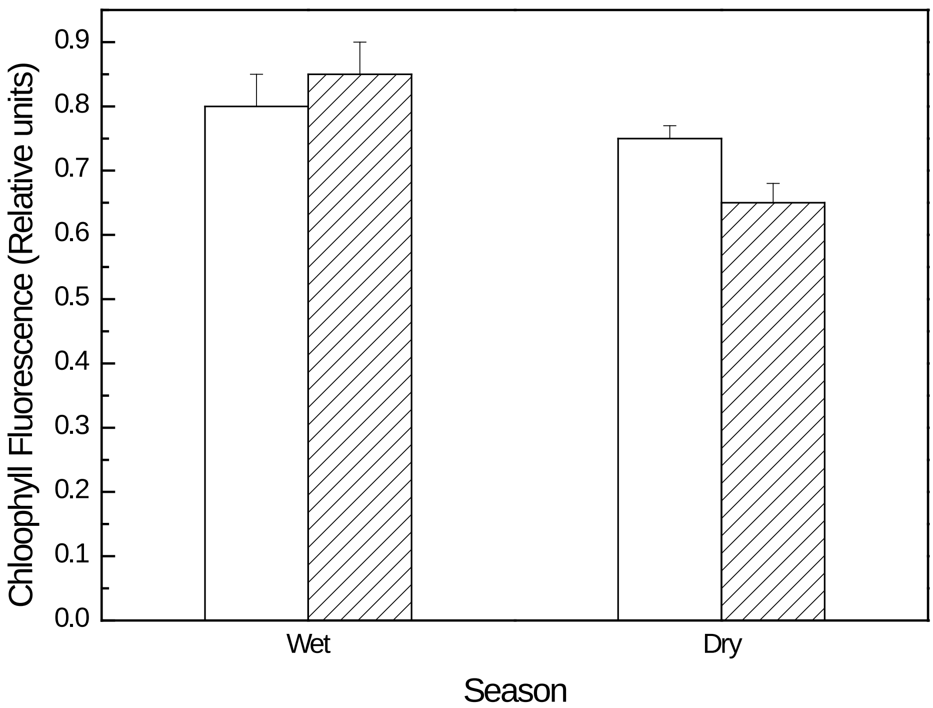


Fig. 4. Seasonal changes in chlorophyll fluorescence parameters F_V/F_m (□) and ΦPSII (■) in *E. triaculaeata*. (± se, n =10).

Discussion

Long duration of dry season and low total annual rainfall are climatic features typical of arid regions (Fig. 1). These climatic features reflect extreme aridity of study site (FISHER & MEMBERY, 1998). Measurements of daytime changes in air temperatures and PPFD during dry season showed high midday values that add to the harshness of the environment (Table 1). Such harsh conditions represent formidable challenges for plant survival in arid regions. In these regions, arido-active stem succulents survive periods of protracted drought by physiological adaptations enabling them to tolerate harsh environmental conditions (SAYED, 2001a,b; MASRAHI *et al.*, 2011; MASRAHI *et al.*, 2012a,b). During wet season, *E. triaculeata* exhibited values of stomatal conductance that were high during the night and low during the day (Fig. 2) a stomatal behavior typical of CAM plants (LÜTTGE, 2010). Determination of chlorenchyma cell sap titratable acidity during both wet and dry seasons revealed diurnal oscillation of acidification-deacidification cycles reflecting operation of obligate CAM (Fig. 3). Obligate CAM has previously been reported in stem succulent *Euphorbia* species (WINTER *et al.*, 2005; AMEH, 2006; FEAKINS & SESSIONS, 2010, AL-TURKI *et al.* 2014). Nocturnal CO_2 uptake and daytime stomatal closure of CAM implies avoidance of gas exchange when environmental conditions favour transpirational water loss and improved plant water economy (LÜTTGE, 2008). However, during dry season, *E. triaculeata* exhibited low values of stomatal conductance during both day and night (Fig. 2) and dampened diurnal oscillation of chlorenchyma cell sap acidity (Fig. 3). These results indicated that *E. triaculeata* shifted from obligate CAM to CAM-idling in response to protracted drought. CAM-idling is a physiotypic modification of CAM that greatly improves plant water economy by day and night stomatal closure, no net CO_2

uptake, and diurnal acidity changes sustained by nocturnal re-fixation of respiratory CO_2 (SAYED 2001b; LÜTTGE, 2010).

Comparison of chlorophyll fluorescence parameters measured in *E. triaculeata* during wet and dry seasons indicated reduction of Fv/Fm and ΦPSII (Fig. 4) denoting reduced efficiency of PSII antenna and PSII quantum yield, respectively (BAKER, 2008). This reduction of PSII activity during dry season coincided with high values of midday air temperature and PPFD (Table 1). Similar reduction of PSII activity manifested by reduction of Fv/Fm and ΦPSII was reported for other CAM plants under stress conditions (MATTOS *et al.*, 1999). This reduction of PSII activity was attributed to over-energized PSII during Phase III of CAM that takes place under closed stomata and high irradiance (NIEWIADOMSKA & BORLAND, 2008). Our observed reduction in PSII activity occurred in concomitance with increased qNP (Table 2) reflecting increased non-photochemical quenching of chlorophyll fluorescence and increased excess energy dissipation as heat (HORTON & RUBAN, 2005). Similar observation of increased qNP during CAM-idling was reported in CAM-performing *Clusia minor* and was attributed to excess energy dissipation as heat via xanthophyll cycle (ADAMS *et al.* 2004; LÜTTGE, 2007). Xanthophyll cycle pigment epoxidation/de-epoxidation is an important element in photoprotection under stress conditions (JAHNS & HOLZWARTH, 2012). Our results also showed marked rise in chlorenchyma anthocyanin content during dry season (Table 2). Anthocyanin is thought to result in alleviation of oxidative stress by processing reactive oxygen species released when PSII becomes over-energized during Phase III of CAM and hence confers photoprotection (GOULD, 2004).

Conclusions

It can be concluded that *E. triaculeata* is obligate CAM plant with ample physiotypic plasticity. This plasticity involves shifting to CAM-idling, operation of photoprotection by non-radiative excess energy dissipation via xanthophyll cycle, and mitigating oxidative stress by increased anthocyanin pigment content. This plasticity is of pivotal importance in alleviating multiple stress during CAM-Idling caused by drought, high irradiance, and high temperature prevailing in the long dry season.

References

ADAMS W.W., C.R. ZARTER, K.E. MUEH, V. AMINARD, B. DEMMING-ADAMS. 2004. Energy dissipation and photo-inhibition: A continuum of photopro-tection. - In: papageorgiou G.C., Govindjee (Eds.): *Chlorophyll Fluores-cence: A Signature of Photosynthesis*. Dordrecht, Springer, pp. 49-64.

AL-TURKI T.A., Y.S. MASRAHI, O.H. SAYED. 2014. Photosynthetic adaptation of *Euphorbia fractiflexa* (Euphorbiaceae) and survival in arid regions of the Arabian Peninsula. - *Journal of Plant Interactions*, 9: 107-111.

BAKER N.R. 2008 Chlorophyll Fluorescence: A probe of photosynthesis *in vivo*. - *Annual Review of Plant Biology*, 59: 89-113.

FEAKINS S.J., A.L. SESSIONS. 2010. Crassulacean acid metabolism influences D/H ratio of leaf wax in succulent plants. - *Organic Geochemistry*, 41: 1269-1276.

FISHER M., D.A. MEMBERY. 1998. Climate. In: GHAZANFAR S.A., M. FISHER (Eds.): *Vegetation of the Arabian Peninsula*. Amsterdam, Kluwer Academic Publishers, pp. 5-38.

GOULD K.S. 2004. Nature's Swiss army knife: The diverse protective roles of anthocyanins in leaves. - *Journal of Biomedicine and Biotechnology*, 5: 314–320.

HORTON P., A. RUBAN. 2005. Molecular design of the photosystem II light-harvesting antenna: photosynthesis and photoprotection. - *Journal of Experimental Botany*, 56: 363-373.

JAHNS P., A.R. HOLZWARTH. 2012. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. - *Biochimica et Biopohysica Acta*, 1817: 182-193.

LÜTTGE U. 2007. Photosynthesis. In: LÜTTGE (Ed.): *Clusia: A Woody Neotropical Genus of Remarkable Plasticity and Diversity*. Heidelberg: Springer-Verlag, pp.135-186.

LÜTTGE U. 2008. Stem CAM in arborescent succulents. - *Trees,* 22: 139-148.

LÜTTGE U. 2010. Ability of crassulacean acid metabolism plants to overcome interacting stresses in tropical environments. - *AoB Plants*, plq005, doi:10.1093/aobpla/plq005

MASRAHI Y.S. 2004. Autecology of *Euphorbia triaculeata* Forssk. M.Sc. thesis. Umm Al-Qura University, Makkah, Saudi Arabia.

MASRAHI Y.S. 2012. *The Illustrated Guide to the Wild Plants of Jazan Region*. Published by Author, Jeddah.

MASRAHI Y.S., M.N. AL-YEMENI, T.A. AL-Turki, O.H. SAYED. 2011. Ecophysiological mechanisms of succulent survival in natural conditions: Photosynthetic carbon Fixation in *Caralluma acutangula* (Decne. Ne.Br.) (Asclepiadaceae). - *Polish Journal of Ecology,* 59: 381-389.

MASRAHI Y.S., M.N. AL-YEMENI, O.H. SAYED. 2012a. Nurse association of the stem succulent *Caralluma acutangula* in its natural habitat. - *Ekologia,* 31: 46-53.

MASRAHI Y.S., T.A. AL-TURKI, O.H. SAYED. 2012b. Crassulacean acid metabolism permutation and survival of *Caralluma* Species (Apocynaceae) in arid habitats. - *Ecologia Balkanica,* 4: 63-71.

MATTOS E.A., B. HERZOG, U. LÜTTGE. 1999. Chlorophyll fluorescence during CAM-phases in *Clusia minor* L. under drought stress. - *Journal of Experimental Botany,* 331:253–261.

MIDDLETON N.J. 1986. Dust storms in the Middle East. – *Journal of Arid Environments,* 10: 83-96.

MURCHIE E.H., K.K. NIYOGI. 2011. Manipulation of photoprotection to improve plant photosynthesis. - *Plant Physiology,* 155:86-92.

MURRY J.R., P. WESLEY, W.P. HACKETT. 1991. Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. - *Plant Physiology,* 97:343-351.

NIEWIADOMSKA E., A.M. BORLAND. 2008. Crassulacean acid metabolism: a cause or consequence of oxidative stress in plants? - *Progress in Botany,* 69: 247–266.

OSMOND C.B., W.W. ADAMS, S.T. SMITH. 1991. Crassulacean Acid Metabolism. In: PEARCY R.W., J. EHLERINGER, H.A. MOONEY, P.W. RUNDEL (Eds.): *Plant Physiological Ecology,* Chapman and Hall, London, pp. 255-280.

SAYED O.H. 2001a. Aridity and plant survival in desert environments. In: Prakash I. (Ed.): *Ecology of Desert Environments*, Jodhpur, Scientific Publishers, pp. 78-103.

SAYED O.H. 2001b. Crassulacean Acid Metabolism 1975-2000: A check list. - *Photosynthetica,* 39: 339-352.

SAYED O.H. 2003. Chlorophyll fluorescence as a tool in cereal crop research. Review. - *Photosynthetica,* 41: 321-330.

WINTER K., J. ARANDA, J.A.M HOLTUM. 2005. Carbon isotope composition and water-use efficiency in plants with crassulacean acid metabolism. - *Functional Plant Biology,* 32: 381-388.

Received: 11.06.2014
Accepted: 02.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 43-50

Occurrence and Spatial Distribution of Brachionus Species: A Bioindicator of Eutrophication in Bhoj Wetland, Bhopal

Najeeb Ahmad Bhat[1], Rajni Raina[2], Ashwani Wanganeo[1]*

1 - Department of Environmental Sciences and Limnology, Barkatullah University, Bhopal (M.P), 462026, INDIA
2 - Department of Zoology, Govt. Science and Commerce College Benazir, Bhopal (MP), 462026, INDIA
* Corresponding author: najahbhat@gmail.com

Abstract. The aim of this study conducted during 2008-2009 was to explore the impact of water quality on *Brachionus* species in littoral zone of Bhoj wetland as they are particularly sensitive to changes in water quality. Since *Brachionus* species are usually considered to be useful indicators of water quality their community structure not only allows estimates of the level of pollution but also indicate the trend of general conditions over time. In the present investigation seven species viz., *Brachionus caudatus*, *Br. falcatus*, *Br. calyciflorus*, *Br. angularis*, *Br. forficula*, *Br. quadridentata* and *Br. urceus* were recorded in the wetland.

Keywords: *Brachionus* spp., bioindicator, trophic status, Bhoj wetland.

Introduction

Zooplankton grazing on phytoplankton can transfer more than 50% of carbon fixed by primary production to higher trophic levels (HART *et al.*, 2000; LAWS *et al.*, 1988; SCAVIA, 1980). Zooplanktons are microscopic organisms which do not have the power of locomotion and move at the mercy of the water movements. The organization of biological communities in aquatic ecosystems is closely dependent on the variations of physical and chemical conditions linked to natural and anthropogenic factors (POURRIOT & MEYBECK, 1995). The zooplankton communities, very sensitive to environmental modifications, are important indicators for evaluating the ecological status of these ecosystems (MAGADZA, 1994). The presence and the relative predominance of various copepod species have been used to characterize the eutrophication level of aquatic ecosystems (PARK & MARSHALL, 2000; BONECKER *et al.*, 2001). Herbivorous zooplankton is recognized as the main agent for the top-down control of phytoplankton, and the grazing pressure exerted by cladocerans and copepods on algae and cyanobacteria is sometimes an important controlling factor of harmful algal blooms (BOON *et al.*, 1994).

Rotifers occur almost everywhere and constitute an important group of zooplankton community in aquatic ecosystems of the world. The abundance of rotifers is more or less governed by the interaction of a number of physical, chemical and biological properties of ambient waters. Rotifers, especially *Brachionus*, constitute an important link in the food chain of inland waters. They are considered preferred food for many fish

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

larvae (GUERGUESS, 1993). The rotifer communities of Bhoj wetland – an important man made Ramsar site of central India has great biogenic, ecological and socio-economic importance. It is under severe environmental stress on account of anthropogenic pressure. Attempts are being initiated for its management and biodiversity conservation.

Materials and Methods

Bhoj wetland (located in the Bhopal city, the state capital of Madhya Pradesh, India (latitude 23° 12′-23° 16′ N and longitude 77°18′–77° 23′ E), is the backbone of Bhopal having profound economic and irrigational importance. It has an area of 31 km^2 and a catchment area of 361 km^2. The various physico-chemical characteristics such as temperature, pH, conductivity, TDS, D.O, Free CO2, total alkalinity, Hardness (total, calcium and magnesium), Chloride, nitrate and phosphate were analysed using the standard APHA (2000) and ADONI (1985) methods.

For the quantitative analysis of zooplankton, 10 liters of surface water samples were collected with minimal disturbance and filtered through a No. 25 bolting silk cloth net of mesh size 63 µm and concentrated to 100 ml and were preserved by adding 2ml of 4% formalin simultaneously. The quantitative analysis of zooplankton was carried in Sedgwick-Rafter cell. Identification of the samples was performed following the works of EDMONDSON (1959) and ADONI (1985). The results have been expressed as $ind.l^{-1}$ (WANGANEO & WANGANEO, 2006).

$$\text{Number of zooplankton “n”} = \frac{\text{C x 1000 mm}^2}{\text{A x D x E}}$$

where C = Number of organisms recorded; A = Area of field of microscope; D = Depth of field (SRC depth) in mm; E = Number of fields counted.

$$\frac{\text{Number of zooplankton}}{1} = \frac{n \text{ x } Vol. of\ concentrat}{(ml)}$$

Results

Physico-chemical characteristics

The physico-chemical parameters of water in upper basin of Bhoj wetland have been given in the Table 1. The atmospheric temperature ranged from 21.63 ^oC to 39.83 ^oC. Water temperature varied between 18.13°C to 27.13°C depending on the seasonal atmospheric temperature.The pH value ranged between 7.62 to 8.61units indicating its alkaline nature. Electrical conductivity values ranged from 231.11 to 413.75µS/cm at 25°C. Total dissolved solids fluctuated from 148.89 mg/l to 256.67mg/l. Dissolved oxygen content of water samples varied between 3.96 mg/l and7.78 mg/l. The total alkalinity value fluctuated between 48.11 mg/l and 128.75 mg /l. Increase in alkalinity values is related to the decrease in the water level. The value of total hardness fluctuated from 74.89mg/l to 146.25mg/l. In the wetland the average values of calcium hardness in waters varied from 60.40 mg/l to 102.23 mg/l. On the other hand, minimum magnesium hardness was noted to be 3.12 mg/l as against maximum value of 10.70 mg/l. The chloride concentrations in the wetland waters ranged between 23.9 mg/l and 54.5 mg/l. The nitrate nitrogen content varied aberrantly throughout the study period. Phosphorus, the most vital nutrient effecting productivity of natural water, fluctuated between 0.13 mg/l to 0.69 mg/l.

Study conducted on the zooplankton group revealed dominance of seven *Brachionus* spp., among the rotifer group. Each species viz., *Br. caudatus*, *Br. falcatus*, *Br. calyciflorus*, *Br. angularis, Br. forficula, Br. quadridentata* and *Br. urceus* contributed a percentage of 46%, 18%, 15%,14%, 5% and 1% respectively (Fig. 1).

Zooplankton

Seven species of genus *Brachionus* identified from the eight different sites of the Bhoj wetland revealed that site I (Kamla park) recorded relatively maximum population density (1030 Ind. l^{-1}) followed by site VII near Prempura Ghat (1000 $Ind.l^{-1}$). Site II (Gandhi medical college) recorded least density (60 $Ind.1^{-1}$) Table 2 and Fig. 2.

Table 1. Physico-chemical parameters on annual mean basis

Parameter	Unit	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
AT	°C	29.33	34.67	37.44	39.83	30.56	34.89	29.67	29.44	29.67	27.78	23.78	21.63
WT	°C	18.56	26.33	24.44	24.44	27.13	20.44	22.22	25.44	24.11	22.00	20.67	18.13
pH	Units	8.20	8.19	8.61	8.59	7.87	7.79	7.62	7.82	8.09	8.12	8.22	8.50
TDS	$mg1^{-1}$	148.89	168.89	172.22	166.67	177.78	256.67	167.78	176.67	205.56	196.67	172.22	195.00
EC	µS/cm	231.11	245.56	265.56	251.11	267.78	286.67	257.78	264.44	307.78	305.56	288.89	413.75
DO	$mg1^{-1}$	6.09	6.21	7.13	7.78	7.60	6.83	7.24	6.47	6.79	5.29	7.04	3.96
T. Alk	$mg1^{-1}$	80.44	93.11	48.11	100.22	84.44	81.33	79.78	75.78	79.67	89.33	86.44	128.75
T. Ha	$mg1^{-1}$	84.89	97.11	94.56	98.11	74.89	86.22	83.11	83.33	97.56	98.67	114.44	146.25
Ca . Ha	$mg1^{-1}$	70.89	79.20	74.14	69.44	62.04	60.40	63.22	63.00	70.21	70.43	76.01	102.23
Mg. Ha	$mg1^{-1}$	3.40	4.08	4.95	6.96	3.12	6.27	4.83	4.94	6.64	6.86	9.34	10.70
Chloride	$mg1^{-1}$	23.9	25.1	33.7	34.3	32.3	33.2	29.6	31.8	35.5	44.8	47.2	54.5
Nitrate	$mg1^{-1}$	0.48	0.39	0.50	0.62	0.57	0.75	0.52	0.47	0.59	0.56	0.48	0.45
T. Phos	$mg1^{-1}$	0.17	0.18	0.18	0.27	0.24	0.26	0.27	0.25	0.29	0.33	0.13	0.69

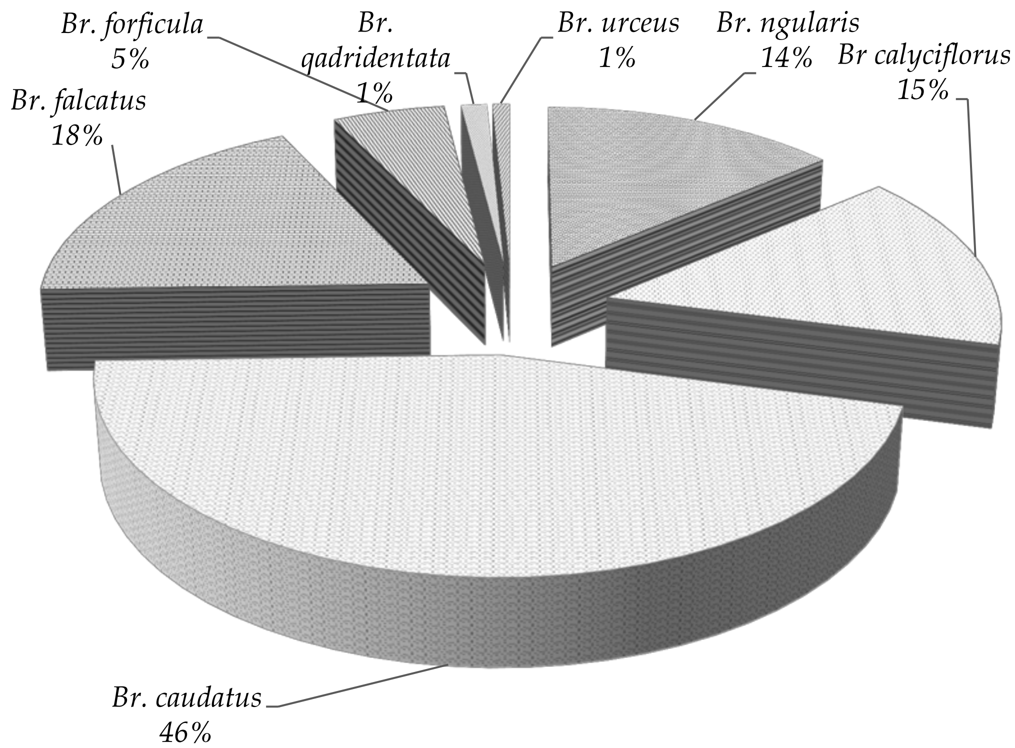


Fig. 1. Percent contribution of different species of *Brachionus* species.

On the basis of site average values, *Brachionus caudatus* contributed maximum density of 284 Ind. 1^{-1} while *Brachionus urceus* was represented by a least density of 5 Ind. 1^{-1}.

Brachionus caudatus recorded the highest population density throughout the study period except at sites II, VII, VIII respectively (10 Ind. 1^{-1}, 180 Ind. 1^{-1} and 80 Ind. 1^{-1})**.** At sites II (20 Ind.1^{-1}) and VIII (150 Ind.1^{-1}) it was *Brachionus calyciflorus* and at site VII *Brachionus angularis* (360 Ind.1^{-1}) was the dominant species.

In the present investigation of Bhoj wetland, on an average basis, *Brachionus caudatus* (189 Ind.1^{-1}) was recorded the maximum represented species density in the study period while *Brachionus urceus* (3.0 Ind.1^{-1}) was recorded least represented species (Table 3).

On an overall monthly basis, highest numerical density (990 Ind. 1^{-1}) was documenting in the month of July while lowest (20 Ind.1^{-1}) was recorded in the month of August. On monthly basis among species, highest population density (620 Ind. 1^{-1}) was represented by *Brachionus caudatus* in the month of December while same species (500 Ind.1^{-1}) were also registered in the month of July (Table 3 and Fig. 3).

The similarity between stations depending on different *Brachionus* species, the results of the Bray-Curtis cluster analysis are shown in Fig. 4. During the period of study highest similarity were observed between sites V and VI. These were linked together at level 1 (first joint). Further, second highest similarity was observed between sites III and VIII hence linked together at level 2. All the species have similar distributional patterns at these two pair of sites. However, most dissimilar community composition was found at site II with rest of the sites (Fig. 4).

Table 2. Quantitative enumeration of *Brachionus* Species on the basis of site variation

Species	I	II	III	IV	V	VI	VII	VIII	$\bar{X}$	SD ±
Br. angularis	20	0	20	30	110	60	360	90	**86**	117
Br. calyciflorus	70	20	40	80	20	160	220	150	**95**	74
Br. caudatus	520	10	80	310	570	520	180	80	**284**	228
Br. falcatus	380	10	30	90	130	180	60	30	**114**	122
Br. forficula	40	10	20	20	10	20	140	0	**33**	45
Br. quadridentata	0	10	10	10	0	0	20	10	**8**	7
Br. urceus	0	0	0	0	0	0	20	20	**5**	9
Total	**1030**	**60**	**200**	**540**	**840**	**940**	**1000**	**380**		

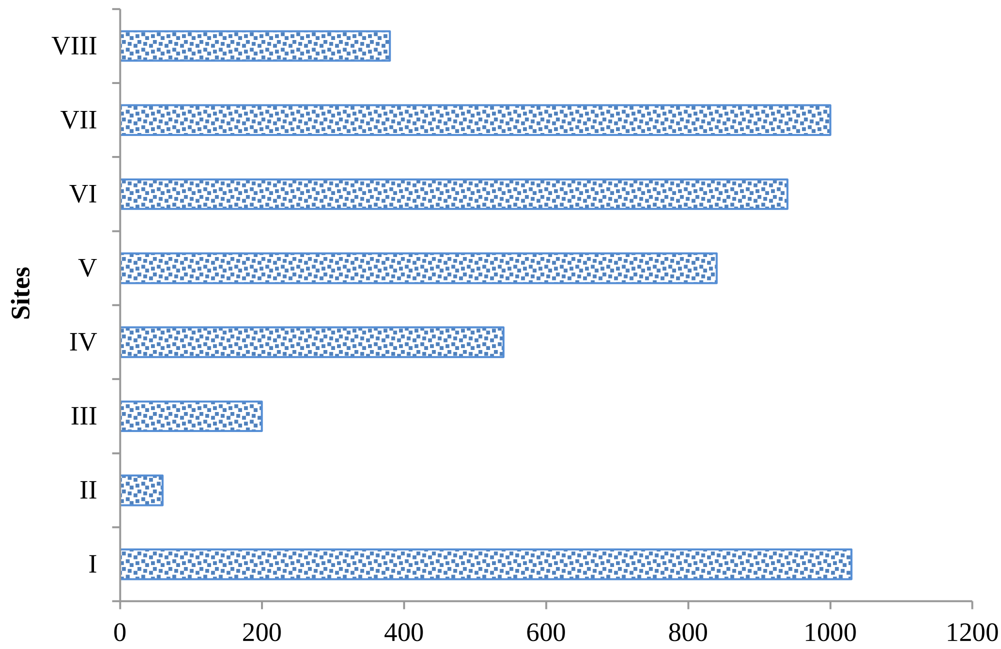


Fig. 2. Variation of different sites.

Table 3. Quantitative enumeration of *Brachionus* species (Ind. 1-1) during 2008-2009.

Species	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Average
Br. angularis	20	10	20	10	20	-	-	20	-	10	170	410	**58**
Br. calyciflorus	50	50	120	80	50	100	20	50	10	20	90	120	**63**
Br. caudatus	50	10	100	80	130	500	-	20	10	440	620	310	**189**
Br. falcatus	30	10	250	140	60	360	-	20	10	20	-	10	**76**
Br. forficula	40	-	30	40	70	30	-	30	20	-	-	-	**22**
Br. quadridentata	-	-	30	-	10	-	-	-	-	-	-	20	**5.0**
Br. urceus	-	-	20	-	20	-	-	-	-	-	-	-	**3.0**
Total	190	80	570	350	360	**990**	20	140	50	490	**880**	**870**	**416**

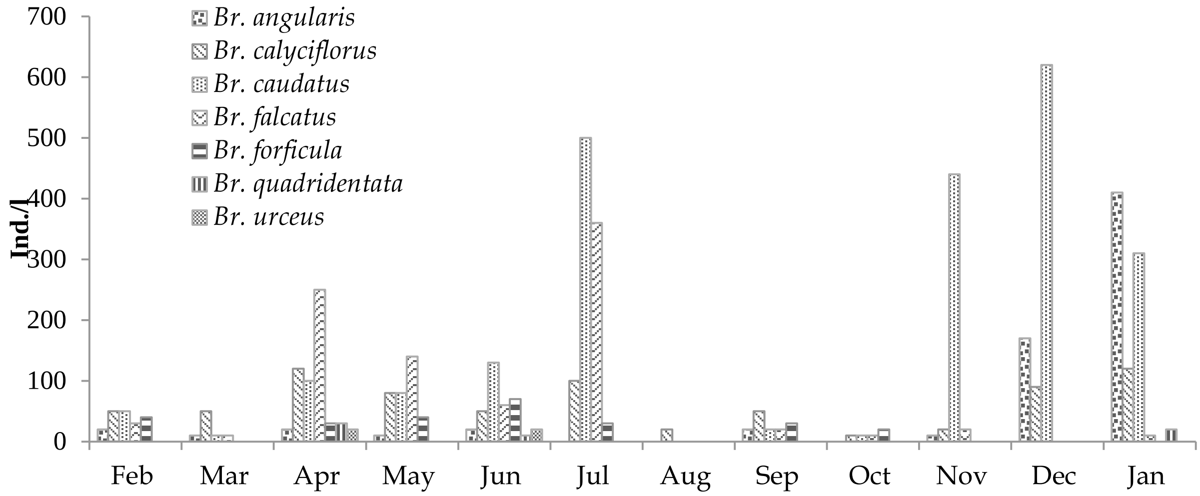


Fig. 3. Quantitative enumeration of *Brachionus* species (Ind. 1-1) during 2008-2009.

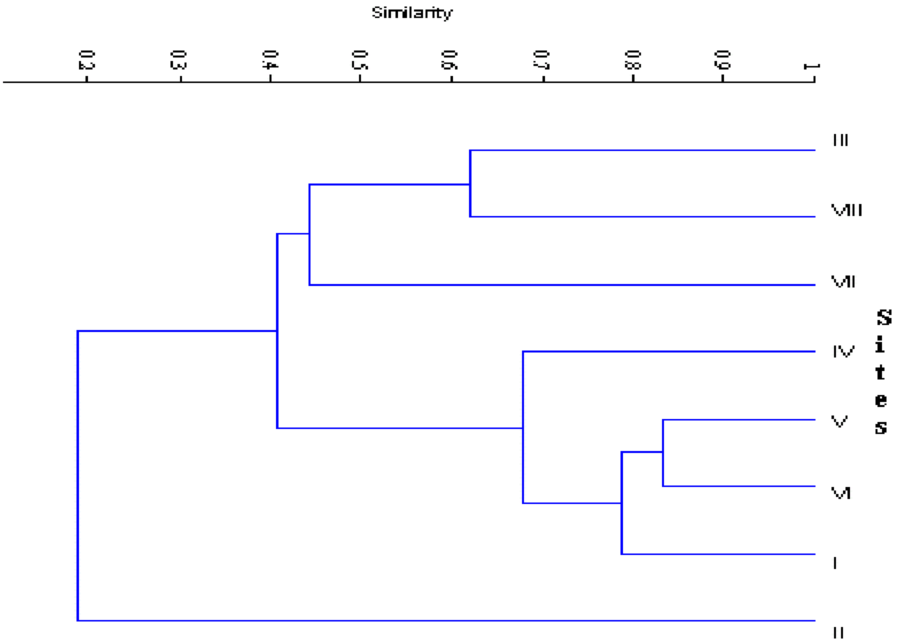


Fig. 4. Bray-Curtis cluster analysis.

Discussion

Brachionus formed the dominant and diversified genus among the rotifers throughout the study period. These species are found extensively in eutrophic waters (BERZINS & PEJLER, 1989; SAMPAIO *et al*., 2002). *Brachionus* species has cosmopolitan distribution in India and during the present study; it was represented by seven species viz., *Brachionus angularis, Br. calyciflorus, Br. caudatus, Br. falcatus, Br. forficula, Br. quadridentata* and *Br. urceus.* HUTCHINSON (1967) observed that *Brachionus* species are very common in temperate and tropical waters, having alkaline pH. MAGEED (2008) and UZMA (2009) stated that presence of more than five species of *Brachionus* reflects eutrophication of water bodies.

KAUSHIK & SAXENA (1995) have also reported abundance of *Brachionus* in various water bodies of central India. An abundance of *Brachionus* in tropical region has been registered and various species of this genus dominate plankton community in warmer part of peninsular India (FERNANDO, 1980; HILLBRITCH-ILKOWSKA, 1983; GULATI, 1990; ERBERN *et al*., 2002; YILDIZ *et al*., 2007). In the present investigation nutrients like phosphate and nitrate were in higher concentration due to decreased water level by evaporation and more organic load due to anthropogenic activities. These nutrients have been chiefly responsible for an increase in organic production particularly in the form of dense macrophytic growth and the overall deterioration of water quality. The deterioration of water quality and other associated problems as a result of racing eutrophication have reduced the recreational and aesthetic appeal of the wetland, besides other economic benefits.

Keratella with *Brachionus* is indicative of nutrient rich status of the water body (BERZINS & PEJLER, 1987). High concentration of rotiferan *Brachionus* species in the waterbody may be due the alkaline nature of water. According to DHANPATHI (2000) many species of rotifers are having preference for more alkaline water. The species like *Brachionus* build higher population during period when alkalinity is high.

Brachionides (*Brachionus* spp.) were the most dominant genera in the present study. Abundance of such species is considered as biological indicator for eutrophy (NOGUEIRA, 2001) and SLADECEK (1983) also suggests that this genus is the index of eutrophic water. MULANI *et al.,* 2009) reported *Brachionus* spp. to be present in typical tropical conditions while SAMPAIO *et al.* (2002) reported *Brachionus* spp. to be indicator of eutrophication.

Among the 7 species of genus, *Brachionus caudatus* was the most dominant, which is supported by the observations of Nene (1985) observed its highest abundance in Masunda lake. PATHAK & MUDGAL (2002) recorded this spp. as one of the dominant rotifer member in Virla reservoir (M.P).

Brachionus calyciflorus in particular is considered to be a good indicator of eutrophication (SAMPAIO *et al.,* 2002). Further, as per SAMPAIO *et al.,* (2002); DULIC *et al.,* (2006) and SOUSA *et al*., (2008) *Brachionus* genus is renowned to tolerate polluted waters.

However, quantitatively *Brachionus* was the main and significantly abundant genera interms of abundance and periodicity. This was in accordance with observations of GEORGE (1961); JAYA (1994); HIWARE & JADHAV (1998).

The sustainable domination structure and high species diversity in Bhoj wetland indicate a higher ecological status of the wetland, although high densities of Rotiferan *Brachionus* species can indicate the rising fertility of the wetland. The present studies indicate the bio diverse nature of rotifers of Bhoj wetland waters during the study period in which physico-chemical and environmental factors play a crucial role in determining the diversity and density of *Brachionus*.

Acknowledgements

The author deem their heartfelt gratitude to Prof. Ashwani Wanganeo Head, Department of Environmental Sciences and Limnology, Barkatullah University Bhopal for providing necessary facilities and valuable time during manuscript preparation for his help.

References

ADONI A.D. 1985. *Work book of limnology*. Pratibha Publication, Sagar, (M.P.) India.

APHA 2000. *Standard methods for the examination of the water and waste water. 21th edition.* American Public Health Association. *Washington Aquaculture Engineering*, 19 p.

BERZINS B., B. PEJLER. 1987. Rotifer occurrence in relation to pH. - *Hydrobiologia*, 182: 171-182.

BERZINS B., B. PEJLER. 1989. Rotifer occurrence in relation to temperature. - *Hydrobiologia*, 175: 223-231.

BOON P.I., S.E. BUNN, J.D. GREEN, R.J. SHIEL. 1994. Consumption of cyanobacteria by freshwater zooplankton: Implications for the success of 'top-down' control of cyanobacterial blooms in Australia. - *Australian Journal of Marine and Freshwater Research*, 45: 875–887.

BONECKER C.C., F.A. LANSAC-TÔHA, L.F.M. VELHO, D.C. ROSSA. 2001. The temporal distribution pattern of copepods in Corumbá Reservoir, State of Goiás, Brazil. -*Hydrobiologia*, 453/454: 375–384.

DHANAPATHI M. 2000. *Taxonomic notes on the Rotifers from India*. IAAB Publication, Hyderabad. 175 p.

DULIC Z., V. MITROVIC-TUTUNDZIC, Z. MARKOVIC, I. ZIVIC. 2006. Monitoring water quality using zooplankton organisms as bioindicators at the Dubica fish farm, Serbia. - *Archive of Biological Science*, 58: 245-248.

EDMONDSON W.T. 1959. *Fresh water biology*, 2nd edition. John Willey and Sons. New York, 127-169.

ERBERN R., J. LAJTNER, G. KLOBUEAR, I. MAGUIRE, A. LUCIA, A. STAMBUK. 2002. Zooplankton reflecting the trophic state of the dam reservoir Modrac (Bosnia and Herzegovina). – In: *International conference on limnology of shallow lakes.* Balantonfured, Hungary. 51p.

FERNANDO C.H. 1980. The freshwater zooplankton of Sri Lanka with a discussion of topical freshwater zooplankton composition. - *Hydrobiologia*, 65: 85-129.

GEORGE M.G. 1961. Observation on rotifer from shallow ponds in Delhi. - *Current Science*, 30: 247-250.

GUERGUESS S.K. 1993. Distribution of some rotifers in the Egyptian inland waters. - *Bulletin of National Institute of Oceanography and Fisheries, Egypt*, 19: 249-275.

GULATI R.D. 1990. Zooplankton structure in the Loosdrecht lakes in relation to trophic status and recent restoration measures. - *Hydrobiologia,* 191: 173-188.

HART D., L. STONE, T. BERMAN. 2000. Seasonal dynamics of the Lake Kinneret food web: the importance of the microbial loop. - *Limnology and Oceanography*, 45: 350–361.

HILLBRITCH-ILKOWSKA A. 1983. Response of planktonic rotifers to the eutrophication process and to the autumnal shift of blooms in Lake Biwa, Japan. I. Changes in abundance and composition of rotifers. - *Japanese Journal of Limnology*, 44 (2): 93-106.

HIWARE C.J., B.V. JADHAV. 1998. Studies on the microzootic fauna of Salim Ali Lake at Aurangabad, Maharashtra, India. - *Journal of Aquatic Biology,* 13(1&2): 18-21.

HUTCHINSON G.E. 1967. *A treatise on limnology, Vol. II, Introduction to lake biology and the limnoplankton.* John Wiley and Sons, Inc, New York. London Sydeny, 115 p.

JAYA D.M. 1994. Seasonal variation and population density of rotifers in three lakes of Hyderabad. - *Journal of Aquatic Biology*, 9(1 & 2): 41-44.

KAUSHIK S., D.N. SAKSENA. 1995. Trophic status and rotifer fauna of certain water bodies in central India. - *Journal Environmental Biology,* 16(1&2): 285-291.

LAWS E.A., BIENFANG P.K., ZIEMANN D.A., CONQUEST L.D. 1988. Phytoplankton population dynamics and the fate of production during the spring bloom in Auke Bay, Alaska. - *Limnology and Oceanography*, 33: 57–65.

MAGADZA C.H.D. 1994. Evaluation of eutrophication control in Lake Chivero, Zimbabwe, by multivariate analysis of zooplankton. - *Hydrobiologia*, 272: 277–292.

MAGEED A. 2008. Distribution and long-term historical changes of zooplankton assemblages in Lake Manzala (south Mediterranean Sea, Egypt). - *Egyptian Journal of Aquatic Research,* 33(1): 183-192.

MAY R.M. 1986. How many species are there? - *Nature*, 324: 514-515.

MULANI S.K., M.B. MULE, S.U. PATIL. 2009. Studies on water quality and zooplankton community of the Panchgangariver in Kolhapur city. - *Journal of Environmental Biology,* 30: 455-459.

NENE V.N. 1985. *Ecological studies of Masunda lake Thane.* M.Sc. Thesis submitted to University of Mumbai, 156p.

NOGUEIRA M.G. 2001. Zooplankton composition dominance and abundance as indicators environmental compartmentalization in Jurumirim reservoir (Paranapanema river), Sao Paulo, Brazil. - *Hydrobiologia,* 455: 1-18.

PARK G.S., H.G. MARSHALL. 2000. Estuarine relationships between zooplankton community structure and trophic gradients. - *Journal of Plankton Research*, 22: 121–135.

PATHAK S.K., L.K. MUDGAL. 2002. A preliminary survey of Virla reservoir of Khargone (Madhya Pradesh) India. - *Indian Journal Environmental and Ecoplanning,* 6 (2): 297-300.

POURRIOT R., M. MEYBECK. 1995. Zonation physique, chimique et écologique dans les lacs. - In: R. Pourriot & M. Meybeck (eds.), *Limnologie générale.* Masson. Collection d'Ecologie. 404–410p.

SAMPAIO E., O. ROCHA, T. MATSUMURA, J. TUNDISI. 2002. Composition and abundance of zooplankton in the limnetic zone of seven reservoirs of the Paranapanema River, Brazil. - *Brazilian Journal of Biology*, 62: 525-545.

SCAVIA D. 1980. An ecological model of Lake Ontario. - *Ecological Modelling*, 8: 49–78.

SLADECEK V. 1983. Rotifer as indicators of water quality. - *Hydrobiologia*, 100: 169-201.

SOUSA W., J.L. ATTAYDE, E.S. ROCHA, E.M.E. ANNA. 2008. The response of zooplankton assemblages to variations in the water quality of four man-made lakes in semi-arid northeastern Brazilian. - *Journal of Plankton Research*, 30: 699-708.

UZMA A. 2009. *Studies on plankton communities of some eutrophic water bodies of Aligarh.* M.Sc. Thesis, Fisheries and Aquaculture Unit, Department of Zoology, Aligarh Muslim University (AMU), Aligarh, India.

WANGANEO A., R. WANGANEO. 2006. Variation in zooplankton population in two morphologically dissimilar rural lakes of Kashmir Himalayas. - *Proceedings of National Academy of Science, India*, 76(B)III: 222-239.

YILDIZ S., A. ALTINDAG, M. ERGÖNÜL. 2007. Seasonal fluctuations in the zooplankton composition of a eutrophic lake: Lake Marmara (Manisa, Turkey). - *Turkish Journal of Zoology,* 31: 121-126.

Received: 11.06.2014
Accepted: 02.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 51-55

Allelopathic Effects of Shoot and Root Extracts From Three Alien and Native Chenopodiaceae Species on Lettuce Seed Germination

Yamina Bouchikh-Boucif*[*1,3], Abderahmane Labani[2], Kheloufi Benabdeli[3], Slimane Bouhelouane[4]

1 -National Institute of Agronomic Research (INRAA), ALGERIA
2 - Department of Biology, University of Moulay Taher, Saida, ALGERIA
3 - Department of biology, Science of nature and Life Faculty, University of Mascara, ALGERIA
4 - Haut-commissariat au développement de la steppe, Saida, ALGERIA
* Corresponding author: *yaminabouchikh@yahoo.fr*

Abstract. One basic method of improving rangelands in the country is the use of native as well as exotic species of adaptable plants. Some species of Atriplex, like *Atriplex canescens* and *Atriplex nummularia* has been introduced in many thousands hectares of rangelands since more than 20 years, it feeds some debates on the algerian scientific community, so that's why it is important to know the impact and necessary to consider its effects on native species. In the current study the effect of chemical competition of *Atriplex canescens* and *Atriplex nummularia* comparing to native *Atriplex halimus* by observing the effect of aqueous extracts of leaves, stems and roots of the three chenopod species assayed at 0.06, 0.63, 1.55, 3.12 and 6.25 g /l on the germination of lettuce seed test. Seed germination was significantly inhibited by shoot alien species extracts especially *A.nummularia* at concentrations ranging from 1.55 to 6.26 g/l with decrease rate of 20% in the lettuce seed tests indicating the presence of allelopathic substances, in 0,06 the germination increased to more than 10% comparing to the water irrigated seeds. An opposed effect than the expected had been found because *Atriplex canescens* had a less allelopathic effect than our native plant *Atriplex halimus*.

Key words: *Atriplex halimus, Atriplex canescens, Atriplex nummularia*, allelopathy, germination, rangeland.

Introduction

In Algerian highlands, rangelands extend over more than 20 million ha. The majority of these rangelands, 7,5 millions ha in 1995 were considered in poor to fair conditions (BENSOUIAH, 2003). Several authors have recognized the decline in productivity of rangelands by the decrease of some important range plant species and increase in unpalatable ones over the last few decades (LE HOUEROU, 1968; AIDOUD, 1989, LE HOUEROU, 1991; LE HOUEROU, 1992, AIDOUD, 1996; BEDRANI, 2001; NEJRAOUI, 2003; NEJRAOUI, 2006).

One basic method of improving these rangelands in the country is the plantation of native as well as exotic species of palatable plants (Fig. 1). Some species of Atriplex, *Atriplex canescens* and *Atriplex nummularia* has been introduced in many thousands hectares of rangelands since more than 20 years, it feeds some debates on the north African scientific community (BENABADJI & BOUAZZA, 2002; BENABADJI *et al.*, 2004; BENABDELI, 2008; BENARADJ, 2010) so that's why it is important to know the impact and necessary to consider its effects on our native species.

Algerian ecologists pulled alarm to denounce the negative impact due to the introduction of imported atriplexes because their unsociability, beyond the ecological approach, we preferred a biochemical methodology because of semiochemical or more precisely allelopathic effect of some plants giving the ability of a plant to inhibit or promote germination and growth of plants of the same or different species in the same space Allelopathic plants interfere with nearby plants by dispersing chemicals into the soil that may inhibit neighboring plant growth, nutrient uptake or germination (ABHILASHA *et al.*, 2008) typical allelopathic inhibitory effects result from the action of groups of allelochemicals that collectively interfere in various physiological processes altering the growth patterns of plants (KIL & SHIM, 2006). That action of allelochemicals can affect the respiration, photosynthesis, enzyme activity, water relations, stomatal opening, hormone levels, mineral availability, cell division and elongation, structure and permeability of cell membranes and walls (CHOU, 1999; REIGOSE *et al.*, 1999), through these actions, allelopathic substances may play a role in shaping plant community structure in semi-arid and arid lands (JEFFERSON & PENNACCHIO, 2003). Our work consists at first in the study of the allelopathic activity of native Atriplex like *A.halimus* and indigenous species as *A. canescens*, *A. nummularia* on germination.

Fig. 1. Plantation of *Atriplex canscens* in Ouest Algeria rangeland, Ain Skhouna (Saida).

Fig. 2. Degraded rangeland, Maamoura, Saida.

Materials and Methods

1-Extraction procedure: fresh plant material of *Atriplex halimus*, *Atriplex canescens* and *Atriplex nummularia* were collected from the rangelands of Saida situated in East Algeria (Fig. 2), after washing with distilled sterilized water and dried, plants were separated into root, stem and leaves, extracts were obtained by soaking 0.06, 0.63, 1.55, 3.12, 6.25 grams of crushed plant materials in 1L of distilled sterilized water for 24 hours and the, filtered , the extract were stored at 4 °C when not used, however (JEFFERSON & PENNACCHIO, 2003). The extracts were generally used within a week.

2- Lettuce seed germination bioassay: Seeds were surface sterilized with a 5% aqueous solution of sodium hypochlorite for 2 min, rinsed five times with distilled sterilized water and dried between two sterilized paper towels. Twenty uniform seeds of lettuce were germinated in sterilized petri-dishes lined with two layers of Whatman No. 1 filter paper and moistened with 5 mL of solution concentration in treatment, distilled water was used in control, each treatment had five replicates, incubated at 25±2°C in growth chamber of culture, seeds were considered germinated when the radicle extended through the seed coat. The germinated seeds were counted for determining the germination rate.

Results and Discussion

The results of our study show that the leaves of all three chenopod species produce allelopathic compounds (Fig. 3). These compounds inhibited seed germination in the lettuce seed test; our findings are consistent with those reported elsewhere for other species in a variety of plant families (CHIAPUSIO *et al*., 1997; MACIAS *et al*., 1999; ESCUDERO *et al*., 2000; MACIAS *et al*., 2000).

The degree of inhibition was largely dependent on the concentration of the extracts being tested and to the species from which they were derived, the inhibition of seed germination were accentuated on the approximately all the treatment with a decrease level mostly on root and leaf solution extract followed by stem solution extract on *A. nummularia,* the root solution extract of *A. halimus* had the most inhibitory effect comparing to the other organs of this plant, the same finding was observed with *A. canescens* (Fig. 4, Fig. 5).

We can note that *Atriplex nummularia* had the most allelopathic effect with a maximum reduction level of 30% comparing to the germination control in the biggest concentrations but also promote the germination by removing the dormancy with a germination rate exceeding the control treatment on the lowest treatment of 0,06 g/l of all organs. We can also say that *A. canescens*, the most introduced forage plant in Algeria had no more effect than *A. halimus* the native one, the inhibitory effect on germination is also depending on the salinity level of the plant, because *A. halimus* contain more salinity on his leaf then the other species.

Conclusions

The results from this study strongly suggest that allelopathy in exotic Atriplex may be a possible mechanism controlling the timing of the other plant germination and seedling establishment, after our preliminary research we can plan to study the allelopathic effect of our three atriplex on germination and seedling growth of our most important economically and ecologically perenéen plants as *Stipa tenacissima*, *Lygeum spartum* and *Artemisia herba alba*.

References

ABHILASHA D., N. QUINTANA, J. JOSHI. 2008. Do allelopathic compounds in invasive *Solidago Canadensis* restrain the native European flora. - *J. Ecol.*, 96: 993-1001.

AIDOUD A. 1989. *Contribution à l'étude des écosystèmes pâturés des hautes plaines Algéro-oranaises. Fonctionnement, évaluation, et évolution des ressources végétales.* [Contribution of study of grazy Algerian-oran high plains ecosystems, operation, evaluation and evolution of plant resources]. Thèse doctorat, USTHB, Alger, 240 p.

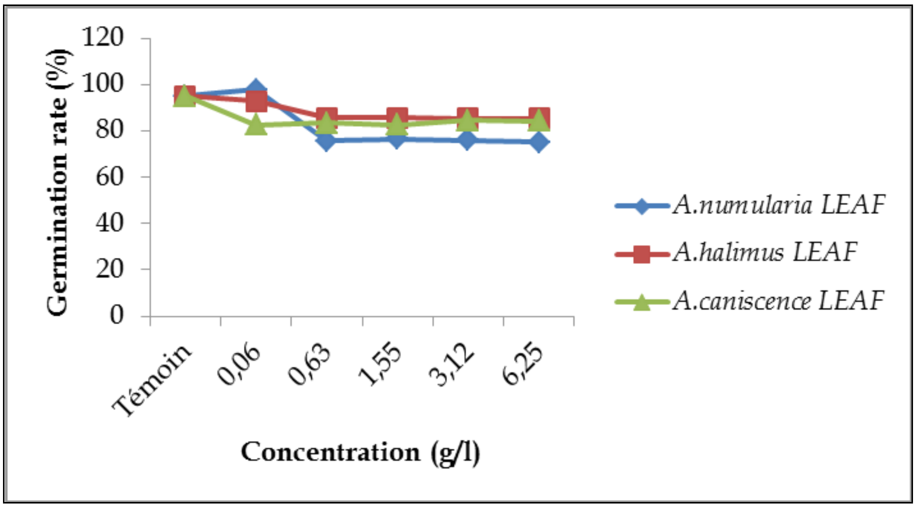


Fig. 3. Germination rate of lettuce seed under difference aqueous extracts concentration from the leaves of the three species.

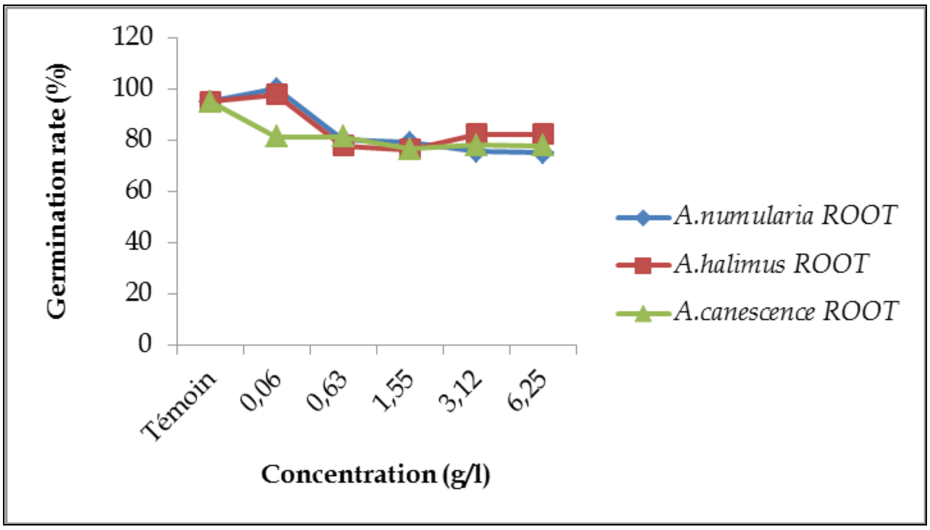


Fig. 4. Germination rate of lettuce seed under difference aqueous extract concentration from the roots of the three species.

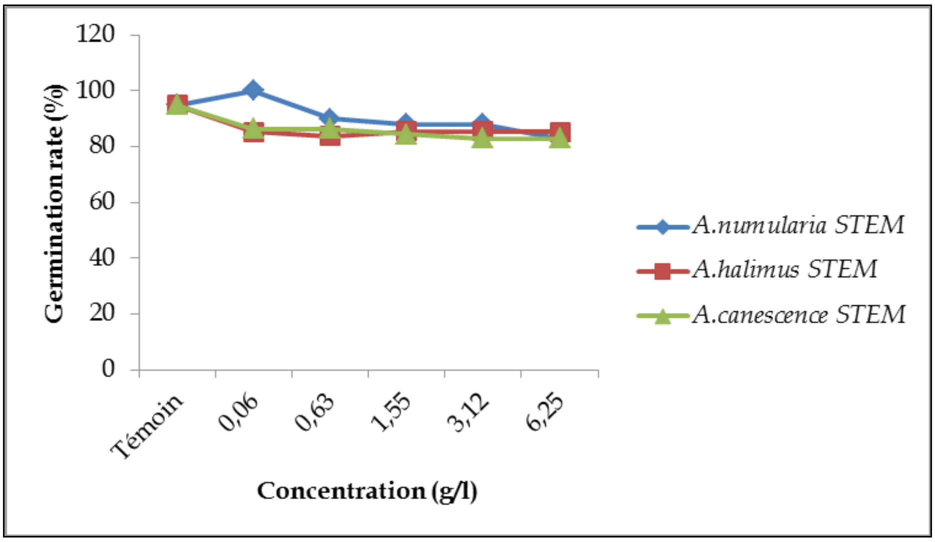


Fig. 5. Germination rate of lettuce seed under difference aqueous extract concentration from the stems of the three species.

AIDOUD A. 1996. La régression de l'alfa *(Stipa tenacissima* L.), graminée pérenne, un indicateur de désertification des steppes algériennes. - *Sécheresse,* 7: 187-193.

BEDRANI S. 2001. *Les contraintes au développement des zones steppiques et la mise en valeur par les concessions*. [Constraints of development of steppe area and valorization by accession]. Ministère de l'agriculture. Alger.

BENABADJI N., M. BOUAZZA. 2002. Contribution à l'étude du cortège floristique de la steppe au Sud d'El Aricha (Oranie, Algérie). - *Sci. Tech.*, Special Edition: 11-19.

BENABADJI N., M. BOUAZZA. M. MERZOUK. S. GHEZLAOUI. 2004. Aspects phytoécologiques des Atriplexaies au Nord de Tlemcen (Oranie, Algérie). - *Rev. Sci et Tech*, 22: 62-79.

BENABDELI K., A. BENGUERAI. H. YEROU. 2008. L'utilisation de l'espace steppique comme terrain de parcours entre identification, potentialités, utilisation et contraintes socio-écologiques en Algérie. - *Revue de l'écologie-environnement,* 04-nov: 54-67.

BENARADJ A., K. MEDERBAL. K. BENABDELI. 2010. Remontée biologique du parcours steppique à *Lygeum spartum* après une durée de Mise en défens dans la steppe sud-oranaise de Naâma (cas de la station de Touadjeur). – *Mediterranea*, 21: 10-48.

BENSOUIAH R. 2003. Du Nord au Sud: le recours à l'environnement, le retour des paysans?, - In: *Communication présentée aux 15èmes Journées de la Société d'Ecologie Humaine Marseille*,. Décembre 2003, pp. 11-12.

CHIAPUSIO G., M. SANCHEZ, M. REIGOSA, L. GONZALEZ, F. PELLISSIER. 1997. Do germination indices adequately reflect allelochemical effects on the germination process? - *Journal of Chemical Ecology,* 23: 2445–2453.

CHOU C.H. 1999. Roles of allelopathy in plant biodiversity and sustainable agriculture. - *Crit. Rev. Plant Sci.*,18: 609-636.

ESCUDERO A., M. ALBERT, J. PITTA, J. PEREZ-GARCIA. 2000. Inhibitory effects of *Artemesia herba-alba* on the germination of the gypsophyte *Helianthemum squamatum*. - *Plant Ecology*, 148: 71–80.

JEFFERSON L., M. PANNACIO. 2003. Allelopathic effects of foliage extracts from four chenopodiaceae species on seed germination. - *J. Arid Env.*, 55: 275-285.

KIL J.H., K.C. SHIM. 2006. Allelopathic effects of *Tagetes minuta* L. and *Eupatorium rugosum rugosum* Houtt. Aqueous extracts on seedling growth of some plants. - *Allelopathy J.*, 18: 315-322.

LE HOUEROU H.N. 1968. La désertisation du Sahara septentrional et des steppes limitrophes (Libye, Tunisie, Algérie). - *C.-R. duColloque de Hammamet, Programme Biologique International, section Conservation Terrestre* et *Annales Algériennes de Géographie*, 6: 2-27.

LE HOUEROU H.N. 1991. La Méditerranée en l'an 2050: impacts respectifs d'une éventuelle évolution climatique et de la démographie sur la végétation. Les écosystèmes et l'utilisation des terres: étude prospective. - *La Météorologie,* VI1 séries, 36: 4-37.

LE HOUEROU H.N. 1992. Climatic change and desertification. - *Impact of Science on Society,* 766: 183-201.

MACIAS F.A., R.M. OLIVA, R.M. VARELA, A. TORRES, J.M.G. MOLLINILLO. 1999. Allelochemicals from sunflower leaves. – *Phytochemistry*, 52: 613–621.

NEDJRAOUI D. 2003. *Les mécanismes de suivi de la désertification en Algérie proposition d'un dispositif national de surveillance écologique à long terme.* Doc. OSS, 37 p.

NEDJRAOUI D. 2006. La recherche scientifique, un moyen de lutte contre la désertification. – In: *Com. Conf. Intern.* Université des Nations Unies, Alger, Déc. 2006.

REIGOSE M.J., A. SANCHEZ-MOREIRAS, L. GONZALEZ. 1999. Ecophysiological approach in allelopathy. - *Crit. Rev. Plant Sci.,* 18: 577-608.

Received: 12.09.2014
Accepted: 02.12.2014

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 57-65

Growth of Populus and Salix Species under Compost Leachate Irrigation

Tooba Abedi[1*], Shamim Moghaddami[1], Ebrahim Lashkar Bolouki[2]

1 - Academic Center for Education, Culture and Research Rasht, I.R. IRAN
2 - Guilan Research Center of Agriculture and Natural Resources, Rasht, I.R. IRAN
* Corresponding author: tooba.abedi@yahoo.com

Abstract. According to the known broad variation in remediation capacity, three plant species were used in the experiment: two fast growing poplar's clones - *Populus deltoides*, *Populus euramericana,* and willows *Salix alba*. *Populus* and *Salix* cuttings were collected from the nursery of the *Populus* Research Center of Safrabasteh in the eastern part of Guilan province at north of Iran. The *Populus* clones were chosen because of their high biomass production capacity and willow- because it is native in Iran. The highest diameter growth rate was exhibited for all three plant species by the 1:1 treatment with an average of 0.26, 0.22 and 0.16 cm in eight months period for *P. euroamericana*, *P. deltoides* and *S. alba*, respectively. Over a period of eight months a higher growth rate of height was observed in (P) and (1:1) treatment for *S. alba* (33.70 and 15.77 cm, respectively) and in (C) treatment for *P. deltoides* (16.51 cm). *P. deltoides* and *S. alba* produced significantly ($p<0.05$) smaller aboveground biomass in (P) treatment compared to all species. *P. deltoides* exhibited greater mean aboveground biomass in the (1:1) treatment compared to other species. There were significant differences ($p<0.05$) in the growth of roots between *P. deltoides*, *P. euramericana* and *S. alba* in all of the treatments.

Key words: Phytoremediation, growth parameters, fast growing, Compost leachate.

Introduction

An important principle of phytoremediation is to match the proper plant species and subspecies to the contaminated site and planned applications. Consideration must be given to soil, microclimate, region, and pests and diseases, as well as the contaminant or mix of contaminants to be cleaned up. Fast-growing tree species have been suggested as appropriate plants for phytoremediation of contaminated water and land because they possess a number of beneficial attributes (JUSTIN *et al.*, 2010). As the species of many fast-growing, short-rotation trees, such as *Salix* sp. and *Populus* sp., are genetically diverse, the opportunity exists to select genotypes resistant to high salt or metal concentrations, or for high or low metal uptake (JUSTIN *et al.*, 2010). Attractive motives for the further research and application of phytoremediation systems are additional ecological benefits such as carbon sequestration, erosion control, reduced pollution, and improved landscape appearance with a high degree of public acceptability (ZALESNY & BAUER, 2007). Additional features that have contributed to the success of such uses include ease of rooting, quick establishment, fast growth, and elevated rates of photosynthesis and water usage (ZALESNY *et al.*, 2008).

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

The production of renewable energy sources, also in the form of biomass, has been increasingly proposed in Iran. Providing sufficient plant nutrients (artificial fertilizers) for their optimal growth is of essential importance. At the same time, fertilizers represent an important production cost. Their substitution with waste sources could be a promising option with regards to the reduction of production costs and the simultaneous reduction of spending on the treatment of waste sources like landfill leachate, wastewater from compost production, sludge, etc. (JUSTIN *et al.*, 2010; HOLM & HEINSOO, 2013).

This paper describes a pot experiment with the aim of obtaining data on the response with respect to biomass accumulation two fast-growing poplar clones (*Populus deltoides* and *Populus euramericana*) and a native willows (*Salix alba*) to different concentrations of compost leachate.

Many research papers can be found which investigate the reuse of landfill leachate from old or closed municipal landfill sites with low leachate strength. There is a lack of data on the reuse of high-strength leachate such as wastewater from the production of open windrow compost from green waste and organic municipal wastes, which is the subject of this study.

Materials and Methods

The experiment was performed at the green house of *Populus* Research Center of Safrabasteh, located in the eastern part of Gilan province at north of Iran (37º 19´N, 49º 57´E) during the 2013 growing season. According to the known broad variation in remediation capacity, three plant species were used in the experiment: two fast growing poplar's clones *Populus deltoides*, *Populus euramericana* and willows *Salix alba*. *Populus* and *Salix* cuttings were collected from the nursery of the *Populus* Research Center of Safrabasteh in the eastern part of Guilan province at north of Iran. The *Populus* clones were chosen because of their high biomass production capacity and willow, because it is native in Iran.

At the beginning of the growing season, in mid-March 2013, 20 cm long cuttings were processed from young, 1-year old seedlings of *Populus* and *Salix* trees. Cuttings were planted in pots filled with loamy-sand soil of the vicinity of the area with 40 cm depth. The upper 5 cm of each cutting was left above the substrate. The initial substrate used in the experiment was analysed in the laboratory. Substrate analyses, physical characteristics and the analytical methods applied are listed in Table 1.

Table 1. Soil analyses and physical characteristics of the substrate used in the experiment

Component	Unit	Amount
pH		8.31
EC	mS/cm^{-1}	0.128
C_{org}	%	0.08
N_{tot}	%	0.01
P	mg kg^{-1}	0.69
K	mg kg^{-1}	57.60
Ca	mg kg^{-1}	400
Mg	mg kg^{-1}	24
Soil texture		Loamy sand
sand	%	86
silt	%	5
clay	%	9

Compost leachate was taken from a collection reservoir where leachate from open composting of organic municipal wastes and various gardening and plant wastes had been collected. The site is Compost Plant of Municipal Waste Management of Rasht, North of Iran (37º 10´N, 49º 34´E). Its chemical analyses were performed in the Laboratory of Guilan Department of Environment (Rasht, North of Iran) using approved International standards.

The composition of leachate used in the experiment is presented in Table 2. The leachate was dark brown in color and had a putrid odor. The leachates were stored in a 20 L plastic tank and mixed with tap water to reach the specified degree of dilution. Before each container was filled, chemical analysis of leachate was performed (Table 2).

Table 2. Composition of pure compost leachate

Parameter	Unit	Amount
pH		5.22
EC	mS/cm^{-1}	1.26
N_{tot}	mgL^{-1}	21.384
NO_2	mgL^{-1}	0.08
NO_3	mgL^{-1}	21.3
SO_4	mgL^{-1}	7101
PO_4-P	mgL^{-1}	22.11
Na	mgL^{-1}	310
K	mgL^{-1}	250
Ca	mgL^{-1}	152
Mg	mgL^{-1}	1103
Pb	mgL^{-1}	0.27
Ni	mgL^{-1}	0.342
Cd	mgL^{-1}	0.0047
Cr	mgL^{-1}	Trace
COD	mgL^{-1}	260500
BOD	mgL^{-1}	130000
TSS	mgL^{-1}	3060.6
Turbidity	mgL^{-1}	12500

During the first 8 weeks, the plants were irrigated daily with tap water via hand irrigation. Ten plants of each species were used in the experiment. The experiment started at the mid of May 2013 when three treatments were applied to plants: (C) tap water (control), (P) pure leachate and (1:1) one unit (by volume) of leachate mixed with one units of tap water. The experiment lasted until the beginning of December 2013. The pots containing the plants were placed randomly on the experimental field under a transparent roof to protect them against rain. The plants were irrigated with the respective water mixtures to the water holding capacity of the substrate in the pot (0.5 L per pot) in the first weeks of the experiment. With the growth of the plants, the amount of water, added in a daily irrigation event was adjusted to the plant´s demands. Pure leachate was the leachate without dilution. The tap water for treatment (C) and for the preparation of the water mixtures was used from the public drinking water supply.

The growth of the trees was monitored bi-monthly by diameter and height measurements. Diameter was measured from the sprout-out of the principal shoot and height was measured from the sprout-out of the principal shoot to the base of the apical bud.

Mean rates of growth in diameter and height for each treatment were calculated of the growth phase (bi-monthly duration of the experiment including 8 months) of the individual lines (Fig. 1, 2 and 3).

Plants were divided into two parts: aboveground and root system. Plant roots were separated carefully from the substrate and washed with distilled water. After roots and stems were oven-dried at 60°C for 48 h, the biomass of aboveground and root were measured (NAVARROA *et al*., 2014).

The experimental layout was a completely randomized design containing three plant species with five replicates of each treatment. The data were analyzed using the SPSS 16.0 statistical package. Statistical differences between the treatments of each plant species and the statistical difference between the plant species for each treatment were determined by analysis of variance. Results were considered significant at $p<0.05$. The rate of growth of the trees was shown on graphs of diameter and height against time.

Results

High amounts of mass load of elements are added to plants in the pots. Mass loads of N, P and K were much higher in leachate treatments than soil, but Ca mass load was lower compare to soil. The higher ion concentration of the leachate treatment was also reflected in the higher electrical conductivity (1.26 mS/cm) compared to the soil (0.128 mS/cm). Heavy metals were low in collected leachate.

The highest diameter growth rate was exhibited for all three plant species by the (1:1) treatment with an average of 0.26, 0.22 and 0.16 cm in eight months period for *P. euroamericana*, *P. deltoides* and *S. alba*, respectively (Fig. 1). Over a period of eight months, the higher growth rate of height was observed in (P) and (1:1) treatment for *S. alba* (33.70 and 15.77 cm, respectively) and in (C) treatment for *P. deltoides* (16.51 cm) (Fig. 2). The plants growth stopped in the sixth month for all three species in the (P) treatment. All of the *P. deltoides* and *S. alba* in this treatment died at the end of growth period.

The diameter and height of tree species were significant differences in all treatments ($p<0.05$) (Table 3).

P. deltoides and *S. alba* produced significantly ($p<0.05$) smaller aboveground biomass in (P) treatment compared to all species. *P. deltoides* exhibited greater mean aboveground biomass in the (1:1) treatment compared to other species (Table 4). *P. euramericana* exhibited significantly ($p<0.05$) greater mean aboveground biomass in the (P) and (C) treatments compared to the other species (Table 4).

The results of the total aboveground biomass were separated into two groups: the (C) and (1:1) treatments with greater total aboveground dry mass and the (P) treatment with significantly ($p<0.05$) smaller total aboveground dry mass for all three plant species (Table 4). *P. deltoides* exhibited the greatest mean total aboveground dry mass accumulation in all of the treatments (Fig 3).

The pattern of root dry mass accumulation across treatments was similar to the aboveground mass accumulation, with the greatest mean root dry mass developed in the 1:1 treatment for *S. alba* . *P. deltoids* and *P. euramericana* exhibited greatest mean root dry mass in C treatment. The total values of root *S. alba* dry mass were higher than the *P. deltoides* root dry mass in the 1:1 treatment (Fig. 4). There were significant differences ($p<0.05$) in the growth of roots between *P. deltoides* , *P. euramericana* and *S. alba* in all of the treatments (Fig. 4). *P. deltoides* and *S. alba* exhibited the greatest mean total aboveground dry mass accumulation in all of the treatments (Fig. 4). *P. euramericana* was the plant species with the smallest amounts of root dry mass accumulation (Fig. 4).

Table 3. Probability values from analysis of variance testing of treatment and period on diameter and height trait of three species irrigated with compost leachate during 8 months.

Trait	Source of variation	
	Treatment	Period
Diameter	<0.0001	0.001
Height	<0.0001	<0.0001

Table 4 . Dry mass (g) of trees components for species and treatment.

Species	Treatment	Biomass component (g)	
		Aboveground dry mass	Root dry mass
	P	0	0
P. deltoides	1:1	20.42±4.14	1.14±0.90
	C	15.86±11.38	1.05±0.67
	P	1.42±0	0.44±0
P. euramericana	1:1	0.77±0	0.45±0
	C	29.57±11.85	1.53±0.19
	P	0	0
S. alba	1:1	6.70±2.97	1.44±0.47
	C	13.34±4.59	0.78±0.32

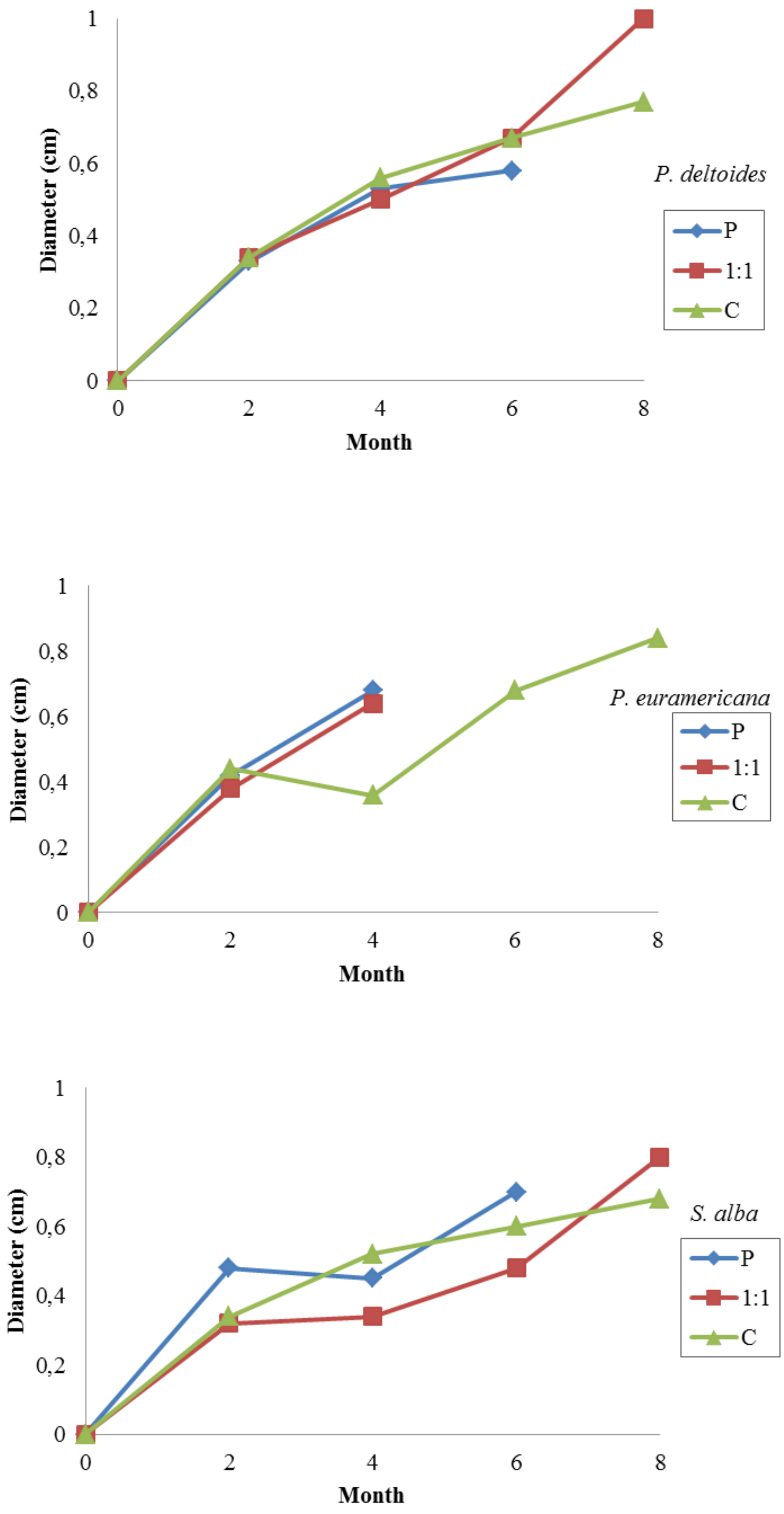


Fig. 1. Mean growth in diameter (cm) of *Populus deltoides*, *Populus euramericana* and *Salix alba* with three concentration of compost leachate.

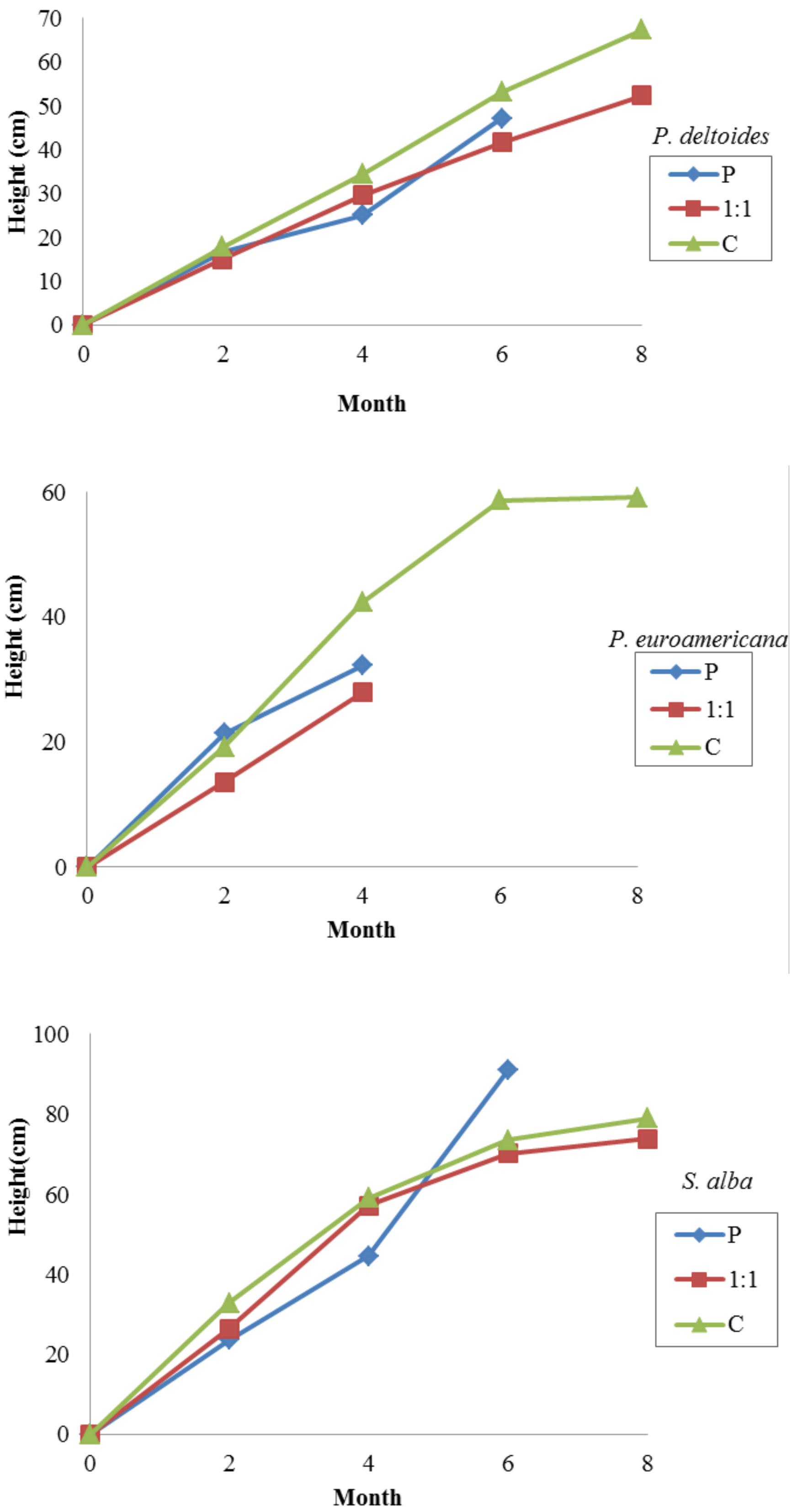


Fig. 2. Mean growth in height (cm) of *Populus deltoides*, *Populus euramericana* and *Salix alba* with three concentration of compost leachate.

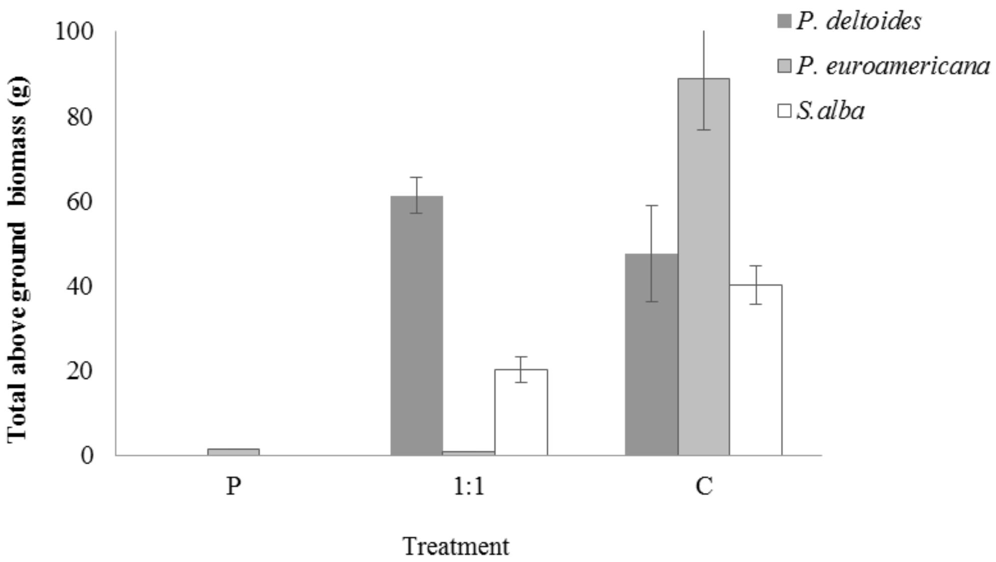


Fig. 3. Comparison of total above ground biomass of three species after 8 months irrigation with pure leachate (P), concentration mixture of leachate (1:1) and tap water (C).

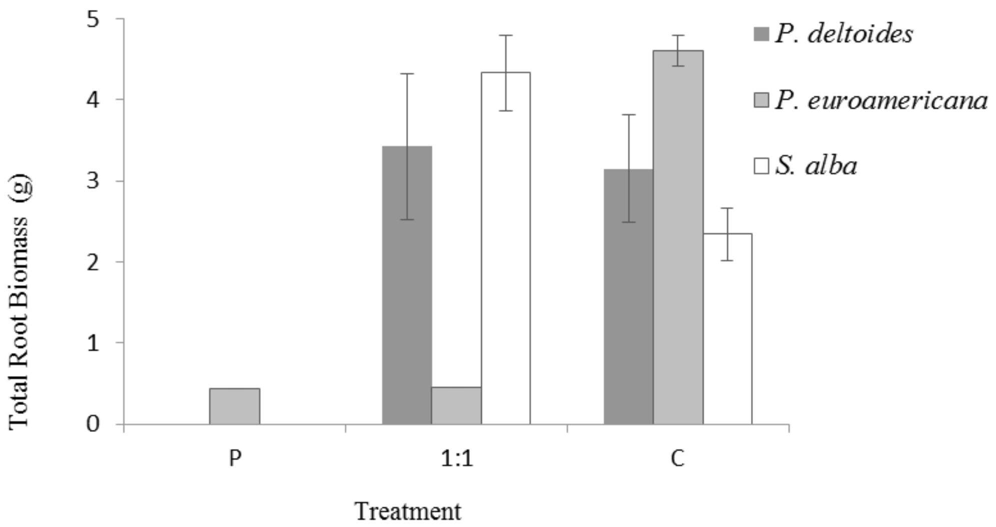


Fig. 4. Comparison of total root biomass of three species after 8 months irrigation with pure leachate (P), concentration mixture of leachate (1:1) and tap water (C).

Discussion

Several studies report positive effects of leachate irrigation on tree growth, showing its fertilizing potential. ZALESNY & BAUER (2007) found that *Salix* clones S287 and S566 exhibited responses favoring leachate irrigation over water. JUSTIN *et al*. (2010) detected the use of landfill leachate treatments resulted a considerably increased aboveground biomass compared to the control tap water treatment. They also found that the growth and biomass accumulation in compost wastewater treatments was reduced compared to tap water and landfill leachate treatments. The use of leachate in (1:1) treatment in our study showed much growth rate and biomass accumulation in tree species.

The concentrations and amounts of wastewater that could be used depend on the constituents of the wastewater and soil, as well as the nutrient demands of the genotypes tested (ZALESNY & BAUER, 2007).

There were statistically significant differences between the aboveground and root biomass in all treatment for all three species. For *P. deltoides* and *S. alba* the higher elemental concentration in the (P) treatment was toxic, indicating that the (P) treatment already had too high concentration of salts and other elements in the water mixture and was toxic to the development of the species.

The compost leachate was a by-product of composting of organic matter, having a low pH (5.22) which is a sign of unfinished degradation processes of raw organic matter due to the inadequate oxygen levels. (P) treatment showed visual signs of stress, with less erect foliage for all three plant species and progressive loss of their lower leaves that leads to damage and died of *P. deltoides* and *S. alba* seedlings. This can be attributed to the combination of high elemental concentrations, imbalance of nutrients, low pH due to the presence of volatile fatty acids and the high salinity of the irrigation leachate. Saline treatments increased root Mg concentration in both aboveground and root biomass (NAVARROA *et al*., 2014).

The application rates of nitrogen were also high in compost leachate. In general agricultural practice it is an acknowledged fact that additional nitrogen is applied to overcome specific toxicity problems and stimulate vegetative growth (AYERS & WESTCOT, 1994).

Reports on significant phytotoxic effect of compost wastewater (addressed as compost liquor) leached from aerobically digested green waste in opened windrow compost piles can be found also from JUSTIN *et al*. (2010) study. The compost wastewater in their measurements had high concentration of BOD_5, ammoniac nitrogen, electrical conductivity and low pH, similar to concentrations in our study. The combined effect of all present components in untreated compost wastewater contributes to phytotoxicity and addresses a need of sufficient pre-treatment, its use only after finished composting process, high dilution, or low mass load of compost wastewater before its use as a plant fertilizer. In addition to the parameters considered, the high concentrations of sulfate (402 mg SO_4/L) in compost wastewater, reported to produce a negative effect on plant growth in water-saturated root zone, should also be underlined (KADLEC & WALLACE, 2009).

However, the negative influence of sulfide toxicity, after the transformation of sulfate in anaerobic environment, on the nutrient uptake of wetland plants was presented. Similar water-saturated environment in the bottom parts of our pots was most probably created also in all compost leachate treatments (7101 mg SO_4/L in our study), where the growth stopped and the water use was significantly reduced, which additionally contributed also to the accumulation of salts.

In addition to ion toxicity, ion imbalances and their interactions (such as Ca^{2+}–Na^+ and Na^+–K^+) can also influence plant growth and survival (JUSTIN *et al*., 2010).

FUNG *et al*. (1998) reported that high levels of salt (1.0% NaCl) rapidly reduced the growth of *Populus* and had an immediate effect on predawn leaf water potential, photosynthesis and stomatal resistance. *Populus* has been reported to be sensitive to salt.

It is obvious that pure compost leachate could be treated by land application. However, transferring the experiment to the field would enable leaching of the excess water from the root zone, and the washing-out of salts by precipitation to the lower soil layers and thus better survival with the same amounts of pure compost leachate as used in the pot experiment. The development of above ground biomass is important from the wastewater consumption and phytoremediation point of view (JUSTIN *et al*., 2010). Contaminants may be either sequestered and/or degraded in the leaves and other tissues or be volatilized through leaf stomata and transpired into the atmosphere. In the 1:1 treatment the greatest above ground biomass was developed by *P. deltoides*, while the generated above ground biomass of *S. alba* and *P. euramericana* was respectively after that. *P. deltoides* developed greater aboveground biomass compared to *P. euramericana* and *S. alba*, and this was statistically significant.

ZALESNY & BAUER (2007) selected fast-growing *Populus* and *Salix* clones and their genomic groups after irrigation with landfill leachate during one growing season. In their experiment *Populus* exhibited the greatest diameter and dry mass, and *Salix* exhibited the greatest height and dry mass of root. In our experiment, *S. alba* exhibited the lowest above ground biomass. *P. euramericana* exhibited the greatest diameter growth and grew in all treatment and no high variation was observed. The species survived in pure leachate despite of other two species. The species has succulent and healthy seedlings than two other ones. It was less sensitive to high salinity and acidity than *P. deltoides* and *S. alba*, it indicates the probability of a higher capacity for accumulation of elements like heavy metals.

It is reported, that the greatest phytoremediation levels are not necessary connected with the highest biomass yield (GREGER & LANDBERG, 1999; KLANG-WESTIN & ERIKSSON, 2003; ZALESNY & BAUER, 2007), and that a higher concentration of metals and nutrients is accumulated in the bark than in the wood tissue (PULFORD & DICKINSON, 2005; DIMITRIOU *et al*., 2006; ADLER *et al*., 2008). This should be tested in future elemental analysis of the plant material.

References

ADLER A., DIMITRIOU I., ARONSSON P., VERWIJST T., WEIH M., 2008. Wood fuel quality of two *Salix viminalis* stands fertilised with sludge, ash and sludge–ash mixtures. - *Biomass and Bioenergy*, 32 (10): 914–925.

AYERS R.S., WESTCOT D.W. 1994. *Water Quality for Agriculture*. FAO Irrigation and Drainage, Paper 29, Rev. 1, Italy.

DIMITRIOU I., ARONSSON P., WEIH M. 2006. Stress tolerance of five willows clones after irrigation with different amounts of landfill leachate. - *Bioresource Technology,* 97: 150–157.

GREGER M., LANDBERG, T. 1999. Use of willow in phytoextraction. - *International Journal of Phytoremediation*, 1: 115–123.

HOLM B., HEINSOO K. 2013. Influence of Composted Sewage Sludge on the Wood Yield of Willow Short Rotation Coppice. An Estonian Case Study. - *Environment Protection Engineering*, 39 (1): 17-32.

JUSTIN M.Z., N. PAJK, V. ZUPANC, M. ZUPANC. 2010. Phytoremediation of landfill leachate and compost wastewater by irrigation of *Populus* and *Salix*: Biomass and growth response. - *Waste Management,* 30: 1032–1042.

KADLEC R.H., S.D. WALLACE. 2009. *Treatment Wetlands*, Second ed. CRC Press, Taylor and Francis Group, Boca Raton, pp. 416, 417.

KLANG-WESTIN E., J. ERIKSSON. 2003. Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. - *Plant Soil*, 249: 127–137.

NAVARROA J. M., O. PÉREZ-TORNEROA, A. MORTEB. 2014. Alleviation of salt stress in citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the rootstock salt tolerance. - *Journal of Plant Physiology*,171: 76– 85.

PULFORD I.D., N.M. DICKINSON. 2005. Phytoremediation technologies using trees. - In: Prassad, M.N.V., Naidu, R. (Eds.), *Trace Elements in the Environment*. CRC Press, New York, USA, pp. 375–395.

ZALESNY A. J., R. S. JR. ZALESNY, A. H. WIESE, B. SEXTON, R. B. HALL. 2008. Sodium and chloride accumulation in leaf, woody, and root tissue of *Populus* after irrigation with landfill leachate. - *Environmental Pollution,* 155: 72-80.

ZALESNY R.S. JR., O.E. BAUER. 2007. Selecting and Utilizing *Populus* and *Salix* for Landfill Covers: Implications for Leachate Irrigation. - *International Journal of Phytoremediation*, 9: 497–511.

Received: 29.09.2014
Accepted: 02.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 67-74

Rare and Endangered Geophyte Plant Species in Serpentine of Kosovo

Naim Berisha, Fadil Millaku*, Elez Krasniqi, Bekim Gashi

Faculty of Mathematics and Natural Sciences, Department of Biology, University of Pristina, KOSOVO
* Corresponding author: fadil.millaku@uni-pr.edu

Abstract. Our study documents information on rarity, geographical distribution, taxonomy and conservation status of 11 geophyte species in serpentine soils of Kosovo, already included in the Red Book of Vascular Flora of Kosovo. Kosovo's serpentine vegetation represents a diversity that yet has not been sufficiently explored. Large serpentine complexes are found in the northern Kosovo but also southern part of the country is rich in serpentines, therefore in endemics. Serpentine rocks and soils are characterized by low level of principal plant nutrients (N, P, K, Ca) and exceptionally high levels of Mg and Fe. Serpentines play particular importance for flora of the country due to their richness in endemic plant species. The following 11 plant species have been studied: *Aristolochia merxmuelleri*, *Colchicum hungaricum*, *Crocus flavus*, *Crocus kosaninii*, *Epimedium alpinum*, *Gentiana punctata*, *Gladiolus illyricus*, *Lilium albanicum*, *Paeonia peregrina*, *Tulipa gesneriana* and *Tulipa kosovarica*. Five out of eleven studied geophytes fall within Critically Endangered IUCN based threat category and five out of eleven are local endemics. *Aristolochia merxmuelleri* and *Tulipa kosovarica* are steno-endemic plant species that are found exclusively in serpentine soils. Information in our database should prove to be valuable to efforts in ecology, floristics, biosystematics, conservation and land management.

Key words: Kosovo, rare, serpentine, IUCN, geophyte.

Introduction

Serpentine soils deriving from ultramafic rocks release toxic heavy metals into the environment (HERATH *et al.*, 2014). That is why plants growing in these soils experience reduced growth due to phytotoxicity of these metals. Serpentine soils are often shallow, rocky and vulnerable to drought. As a result of extreme physical and chemical conditions, serpentine soils support a high proportion of endemic plant species that are adapted to their harsh environment (KAZAKOU *et al.*, 2008; VICIC *et al.*, 2014). Serpentine soils itself represent a suitable model system for studying plant adaptation, speciation as well as species interactions (ANACKER, 2014). In the other hand geophyte plant species bear their perennating buds below the surface of the soil, being thus far more resistant to drought and the stressful environment of the serpentine soils. It is well known that Mediterranean basin has remarkable plant diversity (COWLING *et al.*, 1996) and in particular the presence of geophytes in large numbers in this area have long been noted (RAUNKIAER, 1934). Serpentine soils cover minor surfaces in the global level, comprising less than 1% of the earth's surface (COLEMAN & JOVE, 1992). Large

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg
Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

areas of Balkan Peninsula are covered by serpentine substrate, more than any other European part. More than 300 endemic taxa (species and subspecies) occur on serpentine in the Balkans (STEVANOVIC *et al.*, 2003). In the territory of Kosovo there have been found serpentine rocks (Fig. 1) which are very rich, from the aspects of flora and vegetation (REXHEPI, 1985; KRASNIQI & MILLAKU, 2007). Serpetine soils flora and vegetation in Kosovo has been investigated by many researchers so far, such as: BLECIC & KRASNIQI (1971), KRASNIQI (1972, 1987), HUNDOZI (1987), REXHEPI (1979, 1982a,b, 1997, 2000), REXHEPI & MILLAKU (1996), KRASNIQI & MILLAKU (2004), MILLAKU *et al.* (2007, 2011), PRODANOVIC *et al.* (2012). Plant taxa that inhabit serpentine soils and rocks are known as serpentinophytes. Additionally, according to VICIC *et al.* (2014) serpentine plant species have developed variety of physiological adaptations, one of them being selective heavy metal, Ca and Mg uptake and its translocation to the leaves. As described in variety of studies, serpentine soils have properties that are highly disadvantageous for most plants. As a crucial factor on plant survival on serpentine soils is considered the tolerance on heavy metals, especially to Ni. Also another known strategy of plants to cope with high levels of heavy metals is hyper accumulation (KAZAKOU *et al.*, 2008). There are however still some questions unclear to this date in regard to serpentine endemics and the existing competition level in serpentine soils (MOORE & ELMENDORF, 2011). Traits at a species level indicate however that endemics are typically slow-growing stress tolerators, rather than fast growing competitive dominants (ANACKER & HARRISON, 2012; FERNANDEZ-GOING *et al.*, 2012), in addition the stress tolerance traits of endemic species are consistent with a tradeoff of competitive abilities for serpentine tolerance traits, by which drought adaptation may come at the cost of fast growth rate (GRIME *et al.*, 2008). So far, variety of serpentine endemism studies has revealed much about the nature of plant endemism in general. In particular, serpentine research has highlighted the role of geology as a major environmental determinant of endemism through direct effects on topography and soil properties and indirect effects on habitat availability, degree of spatial isolation as well as microclimate (ANACKER, 2014).

Fig. 1. Map showing distribution of serpentine massifs on Kosovo.

Materials and Methods

On the basis of material collected during the field work on the serpentines of Kosovo (2011-2013), we selected a group of plants significant to these sites that were additionally geophytes and rare plant species. Identification of collected plants was made according to Flora of Serbia (JOSIFOVIĆ, 1972, 1973; SARIĆ & DIKLIĆ, 1986), Flora of Albania (VANGJELI *et al.*, 2000), Flora Europaea (TUTIN *et al.*, 1964, 1967, 1976) while the nomenclature used was according to the databases of the Plant List (theplantlist.org).

For each species, while in the field we have filled up information forms with all the necessary data for assessment of threat category. In the same time we have taken plant samples and mount them in herbaria, based on standard measures (*all of them are recorded, labeled and placed in the Herbaria of* *the Faculty of Natural Sciences, University of Prishtina*). We have taken photographs of the investigated plant species, recorded the GPS coordinates, counted the number of mature individuals, the main threats were recorded, we have measured the Area of Occupancy (AOO), the habitat type was noted, the geological – pedological composition, the altitude, as well as habitat degradation scale. Extent of Occurrence (EOO) was calculated later on, based on the compiled maps from the UTM coordinates. In order to assess the threat category of each species, we have been based in an explicit spatial approach, allowing insertion of certain obscurities in the entry data (IUCN 2012, MACE *et al.*, 2008).

Based on possessed data, we have given a preliminary assessment, according to IUCN Red List and Criteria. Finally, the threat category has been given based on the software-program RAMAS - Red List Professional (AKÇAKAYA & ROOT, 2007). RAMAS Red List implements the IUCN criteria for classifying species into threat categories but in the meantime it allows for explicit incorporation of uncertainties in the input data. In other words, input data, such as the number of mature individuals, can be specified either as a number, or as a range of numbers, or a range of numbers plus a best estimate (MILLAKU *et al.*, 2013).

Results and Discussion

From 11 geophyte plant species that have been analyzed, we have the following results in regard to their IUCN based threat category in Kosovo: *Aristolochia merxmuelleri* Greuter & E.Mayer, *Colchicium hungaricum* Janka, *Crocus flavus* Watson., *Tulipa kosovarica* Shuka, L. Tan, K. & Krasniqi, E., and *Tulipa gesneriana* L. all belong to the "Critically Endangered (CR)" threat category (Fig. 2). This is mainly as a result of low number of mature individuals found in the corresponding habitats. We have two geophyte plant species categorized as "Endangered (EN)": *Crocus kosanini* Pulevic and *Fritillaria messanensis* subsp. *gracilis* (Ebel) Rix. their area of occupancy was less than 300 km^2 (Fig. 3). Other two geophyte plant species: *Epimedium alpinum* L. and *Paeonia peregrina* Mill. have been categorized as "Vulnerable (VU)" due to the fact that a decrease in the number of mature individuals has been noticed as a result of human settlements and activities (Fig. 4). While two last geophyte species that we have investigated: *Gentiana punctata* L and *Lilium albanicum* Griseb. have been categorized as "Least Concern (LC)" due to the fact that they are abundant at the given moment, though they can be threatened in the near future (Fig. 5).

As serpentine soils derive from ultramaphic rocks formed from hydrative and metamorphic transformation of rocks from the earth's mantle (CHIARUCCI *et al.*, 2007), they do give rise to an unusual and sparse plant associations that are tolerant to extreme soil conditions, including lack of essential plant nutrients (as nitrogen, potassium and phosphorous) (FRAZELL *et. al*, 2009). There are plenty of known examples of serpentine soils and distinctive ecologic plant communities associated with them (ALEXANDER *et al.*, 2007).

In our study, we have analyzed eleven geophyte plant species that are found in serpentine soils, with few exceptions being found also in calcareous or silicate substrate (Table 1) and tried to correlate their scarcity, their life form and therefore their threat status to their habitat soils. As known, plants growing in these soils must tolerate calcium deficiency, drought, poor-quality soils, exposure to heavy metals and full sun. Geophytes in the other hand have their underground storage organs, chiefly for storing water and energy (carbohydrates) enabling them to survive in these extreme environment conditions. Out of 11 studied geophyte plant species five are Balkan endemics and one is stenoendemic (*Tulipa kosovarica* Shuka, L. Tan, K. & Krasniqi, E.). While, regarding their conservation status, from these eleven investigated geophytes we have five "Critically Endangered (CR)", two "Endangered (EN)" geophyte plant species, two "Vulnerable (VU)" geophyte plant species and two geophyte plant species that have been categorized as "Least Concern (LC)" due to the fact that they are widespread and we have abundant data on their situation.

Table 1. Species distribution and correlating data.

Pop. code	Species	Locality(ies) name	Altitude (m)	Geographical substrate	Ecological conditions of species habitat
001	*Colchicum hungaricum* Janka	Gadime, Guranë	650 up to 700	Serpentine, Calcareous	Limited dispersal area and very low number of mature individuals.
002	*Crocus flavus* Watson	Guranë	692	Serpentine	Very low number of mature individuals.
003	*Aristolochia merxmuelleri* Greuter & E.Mayer	Mirushe	470	Serpentine	One population with limited dispersal area.
004	*Tulipa gesneriana* L.	Krevenik	583	Serpentine	One population with limited dispersal area.
005	*Tulipa kosovarica* Shuka L, Tan K, Krasniqi E.	Mirushe, Llapushnik, Guriq	650 up to 900	Serpentine	Three populations with very low no. of mature individuals.
006	*Crocus kosaninii* Pulevic	Kaçanik, Brezovice, Blinaje, Guranë	600 up to 2000	Serpentine	Extreme limited dispersal areal in four populations.
007	*Fritillaria messanensis subsp. gracilis* Rix.	Lumëbardh i Pz., Vrellë, Syne, Rusoli, Maja e Vjellakut, Gryka e Rugovës	440 up to 1900	Serpentine, Calcareous	Each of 3 bigger populations in Kosovo is characterized with low no. of mature individuals.
008	*Lilium albanicum* Griseb.	34 localities in Albanian Alps of Kosovo and Sharri Mountains	684 up to 2479	Serpentine, Calcareous	Despite of distribution, its populations are always characterized with low. no. of mature individuals.
009	*Gentiana punctata* L.	31 localities in Albanian Alps of Kosovo & Sharri Mt.	1000 up to 2445	Serpentine, Silicate	Species populations are widely distributet. Stable.
010	*Paeonia peregrina* Mill.	Gllarevë, Gërmi, Gazimestan	570 up to 765	Serpentine, Calcareous	Species is characterized with stable populations though their habitats are subject of human activity.
011	*Epimedium alpinum* L.	Bokat e Morinës	500 up to 1100	Serpentine	Limited dispersal area.

Fig. 2 – Critically endangered (**CR**) plant species. **1**. *Colchicum hungaricum* Janka., **2**. *Crocus flavus* Watson, **3**. *Aristolochia merxmuelleri* Greuter & E. Mayer, **4**. *Tulipa gesneriana* L., **5**. *Tulipa kosovarica* Shuka L, Tan K, Krasniqi E.

Fig. 3 - Endangered (**EN**) plant species. **1**. *Crocus kosaninii* Pulevic., **2.** *Fritillaria messanensis subsp. gracilis* Rix.

Fig. 4 - Vulnerable (**VU**) plant species. **1**. *Paeonia peregrina* Mill. **2**. *Epimedium alpinum* L.

Fig. 5 – Least Concern (**LC**) plant species. **1**. *Lilium albanicum* Griseb., **2**. *Gentiana punctata* L.

References

AKÇAKAYA H.R., W.T. ROOT. 2007. *RAMAS red list: spatial and temporal data analysis for threatened species classification under uncertainity* (version 3.0 Professional). Applied Biomathematics, Setauket, New York, USA.

ALEXANDER E., R. COLEMAN, T. KEELER-WOLF, S. HARRISON. 2007. *Serpentine Geoecology of Western North America: Geology, Soils, and Vegetation*, New York: Oxford University Press. ISBN 0-19-516508-X.

ANACKER B.L., S.P. HARRISON. 2012. Climate and the evolution of serpentine endemism in California. - *Evolutionary Ecology*, 26: 1011–1023.

ANACKER B.L. 2014. The nature of serpentine endemism. - *American Journal of Botany*, Online first. doi: 10.3732/ajb.1300349

BLECIC V., F. KRASNIQI. 1971. Zajednica endemicnog sibljaka forzicije i krtusca

(*Polygalo forsythietum europaeae*) u jugozapadnoj Srbiji. - *Glas. Repub. Zav. Zas. Prirod. Muz.*, Titograd, 4: 35-40.

CHIARUCCI A., A.J.M. BAKER. 2007. Advances in the ecology of serpentine soils. - *Plant Soil,* 293: 1–2. doi:10.1007/s11104-007-9268-7

COLEMAN R., C. JOVE. 1992. Geologic origin of serpentinites. - In: Baker A.J.M., J. Proctor, R.D. Reeves (Eds.).*The vegetation of ultramafic (serpentine) soils.* Intercept Ltd, Andover, U.K. pp. 1-18.

COWLING R.M., P.W. RUNDEL, B.B. LAMONT, M.K. ARROYO, M. ARIANOUTSOU. 1996. Plant diversity in mediterranean-climate regions. - *Trends in Ecology and Evolution*, 11: 362-366.

FERNANDEZ-GOING B.M., B.L. ANACKER, S.P. HARRISON. 2012. Temporal variability in California grasslands: Soil type and species functional traits mediate response to precipitation. - *Ecology*, 93: 2104–2114.

FRAZELL J., R. ELKINS, A. TOBY O'GEN, R. REYNOLDS, N. GENTRY. 2009. *Trees and shrubs for Northern California Serpentine Landscapes. ANR 8400 Publication*, University of California, US.

GRIME J.P., J.D. FRIDLEY, A.P. ASKEW, K. THOMPSON, J.G. HODGSON, C.R. BENNETT. 2008. Longterm resistance to simulated climate change in an infertile grassland. - *Proceedings of the National Academy of Sciences*, 105: 10028-10032.

HERATH I., P. KUMARATHILAKA, A. NAVARATNE, N. RAJAKARUNA, M. VITHANAGE. 2014. Immobilization and phytotoxicity reduction of heavy metals in serpentine soil using biochar. - *Journal of Soils and Sediments*, Online first. doi: 10.1007/s11368-014-0967-4

HUNDOZI B. 1987. Salvio-Scorzoneretum villosae. - *Buletini i punimeve shkencore*, FSHMN. 10: 132-138.

IUCN. 2012. *Guidelines for Application of IUCN Red List Criteria at Regional and National Levels*: Version 4.0. Gland, Switzerland and Cambridge, UK: IUCN. iii + 41p.

KAZAKOU E., P.G. DIMITRAKOPOULOS, A.J.M. BAKER, R.D. REEVES, A.Y. TROUMBIS. 2008. Hypotheses, mechanisms and trade-offs of tolerance and adaptation to serpentine soils: from species to ecosystem level. - *Biological Reviews*, 83: 495-508 doi: 10.1111/j.1469-185X.2008.00051.x

KRASNIQI E., F. MILLAKU. 2004. *Aster albanicus* Deg., përhapja në Shqipëri e Kosovë, vitaliteti ekologjik dhe vendëndodhje të reja në territorin e Kosovës. - *Studime Biologjike*, 9: 120-125.

KRASNIQI E., F. MILLAKU. 2007. The association Hyperico-Euphorbietum glabriflorae Rexhepi 1978 in the serpentine terrains of Drenica Mountain. - *Hacquetia*, 6(2): 183-193.

KRASNIQI F. 1972. *Sumska vegetacija brdskog regiona Kosova.* Zajednica Naucnih Ustanova Kosova. Studije. 27. Pristina, 133 p.

KRASNIQI F. 1987. Endemics in flora of Kosovo and the problem of their protection. ANU BiH. Posebna izdanja. - *Odjeljenje Prirodnih i Matematicnih Nauka*. Sarajevo, 14: 119-124.

MACE G.M., N.J. COLLAR, K.J. GATSON, C. HILTON-TAYLOR, H.R. AKÇAKAYA, N. LEADER-WILLIAMS, E.J. MILNER-GULLAND, S.N. STUART. 2008. Quantification of extinction risk: IUCN's system for classifying threatened species. - *Conservation Biology,* doi: 1523-1739.2008.01044.x

MILLAKU F., F. REXHEPI, E. KRASNIQI, Q. PAJAZITAJ, XH. MALA, N. BERISHA. 2013. *Libri i Kuq i Florës Vaskulare të Republikës së Kosovës* – MMPH, Prishtinë. 5 p.

MILLAKU F., P. HEISELMAYER, F. REXHEPI, E. KRASNIQI, C. EICHBERGER, A. HAZIRI. 2007. Endemic, stenoendemic and relict plants in Serpentines of Kosova. - *Sauteria,* 16: 149-162.

MILLAKU F., E. KRASNIQI, F. REXHEPI. 2011. The association *Stipeto-Convolvuletum Compacti* Ass. nova in Kosovo. - *Hacquetia*, 10(2): 137-147.

MOORE K.A., S.C. ELMENDORF. 2011. Plant competition and facilitation in systems with strong environmental gradients. - In S.P. Harrison, N. Rajakaruna (Eds.), Serpentine: *The evolution and ecology of a model system*, University of California Press, Berkeley, California, USA. pp. 223–236.

PRODANOVIC D., Z. KRIVOSEJ, L. AMIDZIC. 2012. Ecological features of steppe flora on the Ibar valley serpentine, Northern Kosovo. - *Natura Montenegrina*, 11(3): 405-424.

RAUNKIAER C. 1934. *The life forms of plants.* Oxford, Clarendon Press.

REXHEPI F., F. MILLAKU. 1996. *Gjinia potentilla* L. në Ballkan. - *Buletini i FSHMN'së*, 11: 161-168.

REXHEPI F. 1979. Kontribut për njohjen e florës së serpentineve në Kosovë. - *Bioteknika,* 1(2): 53-70.

REXHEPI F. 1982a. Vegjetacioni i Kosovës dikur dhe sot. - *Përparimi,* 2:229-259.

REXHEPI F. 1982b. Vegjetacioni i Kosovës dikur dhe sot. - *Përparimi,* 3:383-422.

REXHEPI F. 1985. Mbrojtja e florës dhe vegjetacionit në serpentinet e Kosovës. - *Natyra e Kosovës,* 5: 61-72.

REXHEPI F. 1985. Mbrojtja e florës dhe vegjetacionit në serpentinet e Kosovës. - *Natyra e Kosovës,* 5: 61-72.

REXHEPI F. 1997. Mediterranean, Submediterranean and Illyric floristic elements in the Kosovo's flora (Yugoslavia). - *Bocconea*, 5(2): 451-456.

REXHEPI F. 2000. *Bimët endemike të Kosovës.* UP, Prishtinë. 150 p.

SARIC M., N. DIKLIC. 1986. *Flora SR Srbije X,* SANU, Beograd, Yugoslavia.

STEVANOVIC V., K. TAN, G. ITRAOU. 2003. Distribution of the endemic Balkan flora on serpentine I. - obligate serpentine endemics. - *Plant Systematic and Evolution*, 242: 149-170.

THE PLANT LIST. 2013. Version 1. Available at: [www.theplantlist.org] (accessed 20th of September).

VICIC D., M. STOILJKOVIC, N. BOJAT, M. SABOVLJEVIC, B. STEVANOVIC. 2014. Physiological tolerance mechanisms of serpentine toleran plants from Serbia. - *Revue d'Ecologie (La Terre et la Vie),* 69: 185-195.

JOSIFOVIC M., L. STJEPANOVIC, M. JANKOVIC, B. GAJIC, M. KOJIC, N. DIKLIC. 1972. *Flora SR Srbije III,* SANU, Beograd, Yugoslavia.

JOSIFOVIC M., L. STJEPANOVIC, M. JANKOVIC, B. GAJIC, M. KOJIC, N. DIKLIC. 1973. *Flora SR Srbije IV,* SANU, Beograd, Yugoslavia.

Received: 25.09.2014
Accepted: 18.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 75-81

Distribution of Kotschy`s Gecko Mediodactylus kotschyi (Steindachner, 1870) (Reptilia: Gekkonidae) in South-West Bulgaria

Alexander Pulev*, Lyuben Domozetski, Lidia Sakelarieva

South-West University "Neofit Rilski", Faculty of Mathematics and Natural Sciences,
Department of Geography, Ecology and Environmental Protection, 66 "Ivan Mihailov" Str.,
2700 Blagoevgrad, BULGARIA
* Corresponding author: spu@abv.bg

Abstract. The current paper presents the contemporary distribution of *Mediodactylus kotschyi* in south-west part of Bulgaria. The valleys of Struma River (to the south of the city of Blagoevgrad), and Mesta River (to the south of the town of Gotse Delchev), as well as the sides of the surrounding mountains up to the altitude of 650 m have been surveyed for the presence of the species. A great number of new localities (55) have been registered, and most of them (48) are situated in anthropogenized habitats. For the first time the subspecies *Mediodactylus kotschyi bibroni* has been found in natural (4 localities) and seminatural habitats (3 localities). The presence of Kotschy`s Gecko in the city of Blagoevgrad - the northern frontier of its range in the Struma River valley, has been confirmed. The highest locality of Kotschy`s Gecko, known till now all over Bulgaria, has been registered (the village of Lyubovka, 606 m above sea level).

Key words: *Mediodactylus kotschyi*, distribution, Struma River valley, Mesta River valley, Bulgaria.

Introduction

Three subspecies of Kotschy`s Gecko *Mediodactylus kotschyi* (Steindachner, 1870) have been found in Bulgaria – *Mediodactylus kotschyi danilewskii* (Strauch, 1887), *Mediodactylus kotschyi rumelicus* (Müller, 1940), and *Mediodactylus kotschyi bibroni* (Beutler & Gruber, 1977). Their distribution in the country is allopatric and south-west Bulgaria (the Struma River and Mesta River valleys) is inhabited by *M. k. bibroni*. The distribution of this subspecies is a matter of a certain zoogeographical interest since part of the northern frontier of its range passes through Bulgaria. *M. k. bibroni* is a Balkan endemic. Its native range spreads in south-west Bulgaria, most of Greece (including some of the Aegean islands), Albania and Macedonia. The subspecies has been introduced to south-east Italy (Apulia and Basilicata) (RÖSLER, 2000; BÖHME *et al.*, 2009).

For the first time in the Bulgarian part of the Struma River valley the Kotschy`s Gecko have been found in the town of Petrich (BURESCH & ZONKOW, 1933). GEISSLER & BRÜHL (1980) mentioned the town of Sandanski as a locality for the species. In an unpublished research DOBREV (1986) reported several localities – the village of Kulata, the village of Levunovo, the town of Melnik, the town of Kresna, the village of Gorna Breznitsa, and the building of Kresna inn (in the middle of Kresna gorge). The town of Melnik was published as a locality by MOELLER (1990), the village

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg
Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

of Kulata – by BESHKOV (1993), the village of Gorna Breznitsa – by RÖSLER (2000), and Kresna inn – by BESHKOV & NANEV (2002). STOJANOV *et al.* (2011) summarized the data and marked on a map the distribution of Kotschy`s Gecko in Bulgaria without reporting the specific localities (one of the mapped localities in the Struma River valley is a new one – the village of Katuntsi, B. NAUMOV, 2013, Sofia, pers. comm.). By the beginning of 2013 it was assumed that the building of Kresna inn was the northernmost point of distribution of Kotschy`s Gecko in the Struma River valley (DOBREV, 1986; PETROV & BESHKOV, 2001; BESHKOV & NANEV, 2002; STOJANOV *et al.*, 2011). PULEV & SAKELARIEVA (2013) reported two other localities – the city of Blagoevgrad and the village of Cherniche.

The subspecies belonging of Kotschy`s Gecko specimens along the Struma River valley was identified by DOBREV (1986) and RÖSLER (2000) and later confirmed by BESHKOV & NANEV (2002), and by STOJANOV *et al.* (2011).

BESHKOV & NANEV (2002) registered the species in the Mesta River valley (the town of Gotse Delchev) without subspecies determination. STOJANOV *et al.* (2011) determined the specimens, found in Gotse Delchev, as belonging to *M. k. bibroni*. RÖSLER (2000) supposed the occurrence of this subspecies in the Bulgarian part of the Mesta River valley. The locality in the town of Gotse Delchev was considered to be the highest one in Bulgaria – up to the altitude of 550 m (BESHKOV & NANEV, 2002; STOJANOV *et al.*, 2011).

The occurrence of Kotschy`s Gecko in Bulgaria is mostly synanthropic. Populations in natural habitats were described only for two of the subspecies – *M. k. danilewskii* (at some places on the Black sea coast and under the entrance of the Orlova chuka cave, Ruse region), and *M. k. rumelicus* (on the hills of the city of Plovdiv) (STOJANOV *et al.*, 2011). There are no data in the literature so far that *M. k. bibroni* occurs in natural habitats in the territory of Bulgaria.

The aim of the study is to establish the distribution of *Mediodactylus kotschyi* in south-west Bulgaria.

Material and Methods

The research was carried out in the period from 2001 to 2014 with high intensity after 2009. The studied area is situated in south-west Bulgaria. The climate is Continental Mediterranean, characterized with dry summer and mild winter - the average January temperature is above 0°C in the areas with altitudes less than 700 m (VELEV, 2002). The studied area includes the valleys of Struma River (to the south of the city of Blagoevgrad), and Mesta River (to the south of the town of Gotse Delchev), as well as the sides of the surrounding mountains up to 650 m above sea level.

Sixty one settlements in the Struma River valley and 9 – in the Mesta River valley, situated at different altitudes, have been researched. The Kotschy`s Gecko has been registered mainly in the night through inspection of the buildings. Reliable data have been obtained also from local people. The species has been discovered accidentally during the day or at nightfall in natural habitats when searching for other species.

Results and Discussion

Totally 55 new localities in south-west Bulgaria have been registered (41 in settlements, 7 at single buildings, 4 in natural, and 3 in seminatural habitats).

The Kotschy`s Gecko has been found in several villages in the Struma River valley: Chuchuligovo, Marino pole, General Todorov, Rupite (alt. 50-100 m); Marikostinovo, Novo Konomladi, Novo Delchevo, Ribnik, Damyanitsa, Lebnitsa, Struma, Valkovo, Drakata, Mikrevo (alt. 100-150 m); Karnalovo, Starchevo, Drangovo, Kapatovo, Kromidovo, Hotovo, Leshnitsa, Dolna Gradeshnitsa, Kamenitsa, Gorna Krushitsa, Slivnitsa, Strumyani (alt. 150-200 m); Strumeshnitsa, Sklave, Vranya (alt. 200-250 m); Kalimantsi, Zornitsa, Harsovo (alt. 250-300 m); Samuilovo, Polenitsa, Ilindentsi, Poleto (alt. 300-350 m); Petrovo (alt. 400-450 m); Klyuch (alt. 450-500 m); Lyubovka (alt. 600-650 m) (Fig. 1). The species has also been registered on the walls of single buildings at Rupite area (between the church complex – N41°27'32" E23°15'52", alt. 87 m and the pumping station – N41°27'45" E23°15'41", alt. 99 m), the building of Peyo Yavorov railway station (Fig. 2), linesman's lodge at the railway crossing on the road to the village of Oshtava, and the railway station building of the town of Simitli (Fig. 1). The species has been searched for but not found in the following villages: Topolnitsa, Parvomay, Kavrakirovo, Mihnevo, Spatovo, Laskarevo, Vinogradi, Lozenitsa, Ladarevo, Zheleznitsa, Strumsko, Pokrovnik, Belasitsa, Kolarovo, Gabrene, Krupnik, Yanovo, Kamena, Yavornitsa, Skrat, Bogoroditsa, Rozhen, Vlahi, Stara Kresna.

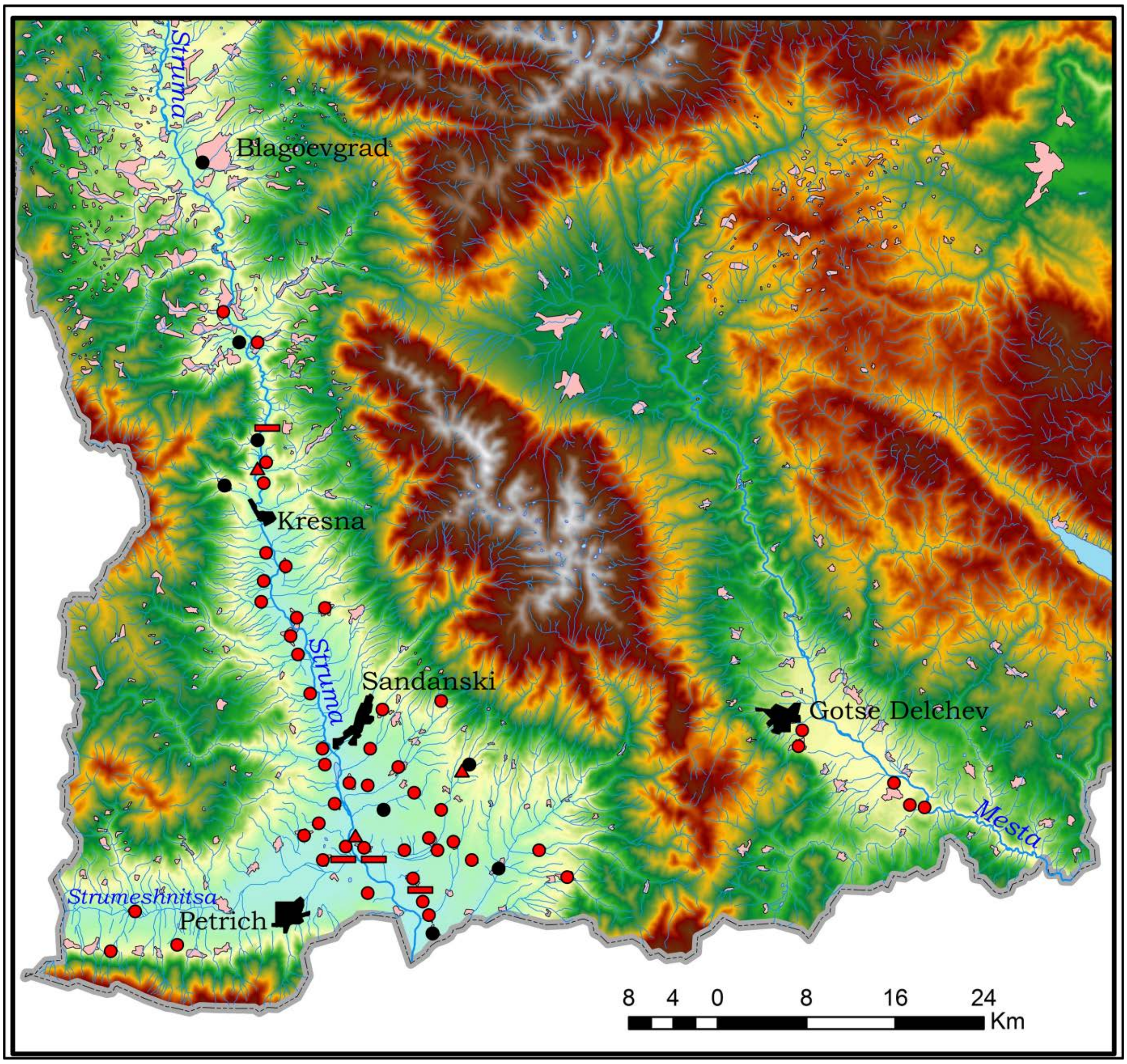


Fig. 1. Distribution of *Mediodactylus kotschyi* in south-west Bulgaria. Legend: Towns in black and black circles – known localities; red circles – new anthropogenic localities; red rectangles – new localities in natural habitats; red triangles – new localities in seminatural habitats.

From the results of the study it could be concluded that the Kotschy`s Gecko is widely distributed in the settlements situated at altitudes up to 300 m in the Struma River valley. It is rare to find it at higher altitudes. However, the species was registered in a large barn, situated on a southwestern-facing slope in the village of Lyubovka, at the altitude of 606 m during the day (N41°34'39" E23°21'51", 5 ad., 24.08.2013). This is the highest locality of Kotschy`s Gecko known till now all over

Bulgaria. It occurrence there could be explained with the suitable microclimatic conditions on the southwestern-facing slopes of Pirin mountain.

The presence of Kotschy`s Gecko in the industrial area of the city of Blagoevgrad (PULEV & SAKELARIEVA, 2013) has been confirmed. Several specimens (1 ad., 07.05.2013; 1 ad., 3 juv., 05.09.2013; 1 ad., 17.09.2013) were found at the city exit toward the village of Pokrovnik, N42°00′24″ E23°05′02″, alt. 356 m. Some new localities were registered – on the walls of buildings close to the railway crossing in the industrial area: N42°00′36″ E23°05′17″, alt. 361 m, 12 ad., 05.09.2013, and 1 juv., 06.10.2013, at air temperature of 14°C; on the walls of the linesman's lodge at the railway crossing toward the industrial area (N42°00′41″ E23°05′20″, alt. 365 m, 1 ad., 19.10.2013) (Fig. 3); on the walls of the linesman's lodge at the railway crossing toward Gramada residential district (N42°00′54″ E23°04′55″, alt. 364 m, 1 ad., 05.09.2013; 3 juv., 19.10.2013); and on the walls of buidings in the east part of the industrial area (N42°00′21″ E23°05′34″, alt. 367 m, many specimens, S. MARKOVSKA, 2013, Blagoevgrad, pers. comm.) (Fig. 1). The occurrence of Kotschy`s Gecko at some other places, situated to the north of Kresna inn – the village of Cherniche railway station (PULEV & SAKELARIEVA, 2013), the town of Simitli railway station and the village of Poleto, indicates that the northern frontier of its range in the Struma River valley is not the middle of Kresna gorge as DOBREV (1986), PETROV & BESHKOV (2001), BESHKOV & NANEV (2002) and STOJANOV *et al.* (2011) assumed.

The Kotschy`s Gecko has been found not only in anthropogenic, but also in several natural habitats in the Struma River valley. Some specimens were registered at the foot of the southern slope of Kozhuh volcanic hill, on stones around the ruins of the ancient city of Heraclea Sintica (N41°26′59″ E23°15′52″, alt. 104 m, several specimens, S. IVANOV, 2014, Petrich, pers. comm., and N41°27′03″ E23°15′57″, alt. 116 m; 2 ad., 10.08.2011; 1 ad., 24.08.2012). Five adults were observed at the foot of sand scarps between the villages of Marino Pole and Marikostinovo (N41°25′20″ E23°20′49″, alt. 167 m) on 02.05.2013.

Fig. 2. *Mediodactylus kotschyi*, adult, Peyo Yavorov railway station, 06.09.2010, 15 h.

Fig. 3. *Mediodactylus kotschyi*, female, Blagoevgrad, 19.10.2013, 20 h.

Other two adults were found on stony ground south of the village of General Todorov, close to the railway (N41°26′53″ E23°17′26″, alt. 87 m) on 18.08.2013. Some specimens were registered in Kresna gorge - on the rocks opposite Kresna inn, beside the railway (N41°47′01″ E23°09′23″, alt. 245 m; 2 ad., 13.05.2012 and 3 ad., 26.06.2012) (Fig. 1).

The natural habitats, where specimens of Kotschy`s Gecko have been found, are situated up to the altitude of 245 m, and all of them are pretty close to anthropogenic habitats (the most remote one is at a distance of 650 m). It is not clear whether the natural populations are autochthonous or have derived from the neighbouring synanthropic populations.

Probably the number of natural populations of Kotschy`s Gecko in the Struma River valley are much more than the registered so far. It is difficult to observe the species because of its nighttime activity, and most likely, because of the lower number of these populations. But the range of the species is larger in anthropogenic habitats than in natural ones. All natural populations have been found in or to the south of Kresna gorge, where the driest and the warmest region in Bulgaria is with the longest period of vegetation (230 days) and the highest temperature sum during this period – up to 4400°C (VELEV, 2002).

Three other localities have been found in seminatural habitats during the study (Fig. 1): the railway embankments 500 m to the north of Peyo Yavorov railway station (N41°45′15″ E23°09′10″, alt. 204 m, 1 ad., 19.07.2011), the railway embankments 700 m to the north-west of the village of General Todorov (N41°27′37″ E23°16′17″, alt. 98 m, 2 ad., 12.06.2014) and the ruins of "St. Nikola" church near the town of Melnik (N41°31′14″ E23°23′33″, alt. 478 m, 4 ad., egg-shells, 22.09.2013) (Fig. 4). The church has not been used for more than 100 years and only its eastern wall, where the specimens and the clutches were found, has remained till nowadays.

Fig. 4. *Mediodactylus kotschyi*, adult, "St. Nikola" church near Melnik, 22.09.2013, 16 h.

The first specimen of Kotschy`s Gecko, found in the Mesta River valley, was caught by G. Manolev on 01.08.1990 and sent to the Institute of Zoology, Bulgarian Academy of Sciences (G. MANOLEV, 1990, Blagoevgrad, pers. comm.). The specimen (ad.) was caught on the brick wall of a single inhabitable building, southeast of the town of Gotse Delchev (N41°34′01″ E23°45′03″, alt. 514 m). The main locality in the Mesta River valley is the town of Gotse Delchev, where Kotschy`s Gecko is widely distributed at the altitude from 520 to 570 m (Fig. 5). The species was also registered near the town in the village of Musomishta on 07.09.2013. 21 specimens (7 ad., 4 subad., 10 juv.) were observed on the walls of two houses in the center of the village (N41°33′12″ E23°44′47″, alt. 542 m) for about 30 minutes (Fig. 1).

Kotschy`s Gecko has been found at some other localities in the Mesta River valley: on the walls of old houses in the northern part of the town of Hadzhidimovo (N41°31′17″ E23°51′19″, alt. 468 m); on the walls of a single building (villa), situated at 1100 m south-east of the town of Hadzhidimovo, by the road (N41°30′22″ E23°52′15″, alt. 499 m) and on the walls of "St. George" chapel near the Matnitsa River mouth (N41°30′20″ E23°53′10″, alt. 502 m) (K. MILUSHEVA, 2014, Hadzhidimovo, pers. comm.) (Fig. 1).

The species has been repeatedly searched for in other settlements in the Mesta River valley (the villages of Koprivlen, Novo Lyaski, Blatska, Hvostyane, Dabnitsa, Garmen), but has not been found yet. It has not been registered in the center part of the town of Hadzhidimovo as well. It is possible for the populations of Kotschy`s Gecko in the towns of Gotse Delchev (and its surroundings) and Hadzhidimovo (and its surroundings) to be isolated one from another as well as from the populations in northern Greece.

STOJANOV *et al.* (2011) draws attention to the absence of the species at many places in Bulgaria despite the good living conditions there and this have been confirmed during the present study. *M. k. bibroni* is a Mediterranean faunistic element, and the temperature is a limiting factor for its distribution. However, it has not been found in many places with suitable climatic conditions in south-west Bulgaria. Its (present) distribution in the Struma River and Mesta River valleys could be explained by the influence of temperature on the one hand and by its accidental spread by human activities on the other hand.

Fig. 5. *Mediodactylus kotschyi*, female, Gotse Delchev, 07.09.2012, 21 h.

Probably this spread is largely due to the railway infrastructure and the transportation of goods and loads. Finding Kotschy`s Gecko at some railway stations and linesman's lodges (Cherniche, Simitli, Blagoevgrad) is a proof of this statement. At the same time, the species is absent or is sparsely distributed in the surrounding areas. The lack of railway infrastructure could explain the low species abundance in the Mesta River and the Strumeshnitsa River (right tributary of Struma River) valleys.

According to STOJANOV *et al.* (2011) the species could easily be found at lighted places where it preys in the night. However, it is not always (so) easy to observe Kotschy`s Gecko in the settlements. In some of them (the villages of Samuilovo, Klyuch, Poleto and Musomishta, and the city of Blagoevgrad) it cannot be found in numerous suitable buildings but can be found in others with many specimens. Probably Kotschy`s Gecko is rarer in the periphery of its range because of the limiting effect of climate and the altitude or due to its recent colonization. Local people not always pay attention to its existence despite the high density of its populations.

The populations of Kotschy`s Gecko are usually with high density in the settlements. BESHKOV (1993) reported that about 70 specimens were recorded in the village of Kulata for 3 hours. In the present study, the highest numbers have been recorded in the villages of Mikrevo, Strumyani and Novo Delchevo where up to 25 – 30 specimens have been observed simultaneously on the walls of a single house.

The high number of anthropogenic localities and the large abundance of Kotschy`s Gecko in most of them are due to several factors. The first factor is the heating of buildings in the winter, which facilitates the survival during this unsuitable season. Kotschy`s Gecko has not been found in desolate buildings out of settlements (the ruins of "St. Nikola" church are not included). Probably spending the winter in buildings, which are not heated, is not possible. However, the species has been recorded in desolate buildings within the settlements (in the entire studied area), where possibly it periodically migrates from the adjacent habitable buildings. The second factor is the suitable microhabitats in the buildings. Kotschy`s Gecko escapes from predators climbing on the walls, finds crevices in walls well lit by the sun or finds spaces for laying eggs under the roof-tiles. The third factor is the lighting in the evening, and in the night. It gives Kotschy`s Gecko a certain advantage as it facilitates finding food and sustaining the body temperature.

References

BESHKOV V. 1993. [Amphibia and Reptilia]. - In: Sakalian M. (Ed.): *National strategy for conservation of biodiversity. Basic reports. Vol. I.* Sofia, United States Agency for International Development, pp. 567-584. (In Bulgarian).

BESHKOV V., K. NANEV. 2002. [*Amphibians and Reptiles in Bulgaria*]. Pensoft, Sofia-Moscow, 120 p. (In Bulgarian).

BÖHME W., P. LYMBERAKIS. R. AJTIC. A. DISI. Y. WERNER. V. TOK et al. 2009. Mediodactylus kotschyi. - In*: IUCN 2014. The IUCN Red List of Threatened*

Species. Version 2014.2. Available at:[www.iucnredlist.org]. Accessed: 29.09.2014.

BURESCH IW., J. ZONKOW. 1933. Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel. I Teil: Schildkrötten (Testudinata) und Eidechsen (Sauria). - *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien,* 6: 150-207. (In Bulgarian, German summary).

DOBREV D. 1986. [*Studies on the distribution and taxonomy of Kotschy`s Gecko (Tenuidactylus kotschyi Steindachner, 1870) in Bulgaria*]. Unpublished Master Thesis, Sofia University "St. Kliment Ohridski", 37 p. (In Bulgarian).

GEISSLER L., A. BRÜHL. 1980. Zur Herpetofauna Südwestbulgariens 2. - *Aquarium-Terrarium*, Leipzig, 27(7): 246-247.

MOELLER T. 1990. Zur Herpetofauna im Süden Bulgariens. - *Aquarium-Terrarium*, Leipzig, 43(7): 431-434.

PETROV B., V. BESHKOV. 2001. Amphibians (Amphibia) and the Reptiles (Reptilia) in Kresna Gorge (SW Bulgaria). - In: Beron P. (Ed.): *Biodiversity of Kresna Gorge (SW Bulgaria).* Sofia, National Museum of Natural History & Institute of Zoology, BAS, pp. 297-303. (In Bulgarian, English summary).

PULEV A., L. SAKELARIEVA. 2013. Herpetofauna in the city of Blagoevgrad, south-western Bulgaria. - *Biodiscovery*, 7: 3; DOI: 10.7750/BioDiscovery.2013.7.3.

RÖSLER H. 2000. Zur Taxonomie und Verbreitung von Cyrtopodion kotschyi (Steindachner, 1870) in Bulgarien (Sauria: Gekkonidae). - *Gekkota*, 2: 3-19.

STOJANOV A., N. TZANKOV. B. NAUMOV. 2011. *Die Amphibien und Reptilien Bulgariens*. Frankfurt am Main, Chimaira, 588 p.

VELEV S. 2002. [Climatic regioning]. - In: Kopralev I. (Ed.): *Geography of Bulgaria. Physical geography. Socio-economic geography.* Sofia, Bulgarian Academy of Sciences, Publishing House "ForCom", pp. 155-156. (In Bulgarian).

Received: 01.10.2014
Accepted: 22.12.2014

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 83-92

Species Diversity and Distribution of Amphibians and Reptiles in Nature Park "Sinite Kamani" in Stara Planina Mt. (Bulgaria)

Stanimira R. Deleva[1], Ivelin A. Mollov[2*], Valentina M. Fidanova[1], Alexander K. Mechev[3]

1 - Sterna Consult Ltd., Tsarevo Str. 10-A Plovdiv, BULGARIA
2 – University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tzar Assen Str., 4000, Plovdiv, BULGARIA
3 - NGO „Green Balkans", 1 Scopie Str., 4000, Plovdiv, BULGARIA
* Corresponding author: mollov_i@yahoo.com

Abstract. The current study presents briefly the species composition and distribution of the amphibians and reptiles in the Nature Park "Sinite Kamani" in Stara Planina Mnt. Bulgaria, based on a 2×2 km UTM grid. Between 2012 and 2014, we identified total 20 species (7 amphibians and 13 reptiles). We documented three new amphibian species for the region (*Hyla arborea, Rana dalmatina* and *Rana graeca*, which is discovered for the area for the first time) and three species of reptiles (*Testudo hermanni, Ablepharus kitaibelii* and *Lacerta trilienata*). The contemporary conservation status for each species is presented and conservation threats and problems, specific for the park are discussed.

Key words: herpetofauna, "Sinite Kamani", Nature Park, UTM, NATURA2000, Bulgaria.

Introduction

Nature Park "Sinite kamani" is situated on the south slope of Slivenska Mountain, which is a part of Eastern Balkan Mountain. It occupies 127.3 km^2 and its borders reach the Balkan ridge to the north, Asenovska River and Asenovets Dam to the west, Bozhurska River to the east and the urbanized area of Sliven with contiguous arable lands to the south. The relief is predominantly low-mountain and only highest parts lie in the middle mountain belt. The altitude varies from 290 m in the south part to 1181 m around Bulgarka Peak, which is the highest in Eastern Balkan Mountain (KUMPULA *et al.*, 2006). The whole territory of the park is also included in NATURA 2000 – site code "BG0000164", site name "Sinite Kamani" (EEA, 2012).

An imperative for adequate management of protected areas is detailed and up-to-date knowledge of the area's biodiversity. Studies on species diversity, distribution, and ecology of the herpetofauna in protected areas are amongst the priorities at the European community level (POPGEORGIEV *et al.*, 2010).

The herpetofauna of Nature Park "Sinite kamani" is extremely poorly studied and the available data on the occurrence and distribution of the amphibians and reptiles in the park are rather selective in nature and scares. Until now based on the published literary data there are 4 species of

© Ecologia Balkanica

amphibians in the park and 10 reptile species (see Appendices 1 & 2).

The aim of the current study is to present the contemporary species composition of the amphibians and reptiles in NP "Sinite kamani", to map their distribution on a 2×2 km UTM grid and to present their conservation status and discus the specific conservation threats and problems for the herpetofauna in the park.

Material and Methods

Study area. The park is situated in the area with transitional continental climate, while only the highest part is with typical mountain climate. Mean annual temperature in Sliven is 12.4ºC, during the coldest month (January) is 1.2ºC while the hottest (July) is 23.2ºC. At the top of the mountain around "Sinite kamani" resort mean annual temperature is 7.7ºC, the hottest month has 17.4ºC and the coldest -2ºC. Annual amount of precipitation rises from 587 mm in Sliven to 830 at the top of the mountain. The soils in the lower part of the park are luvisols while in the upper they are replaced by cabmisols. There are a lot of places, predominantly with luvisols, where the soils are eroded. The vegetation in the lower part is represented by oak forests, which were strongly influenced by human activity in the past and partially replaced by shrubs of *Carpinus orientalis, Quercus pubescens* and *Fraxinus ornis.* Upward the mountain is the area of hornbeam-oak belt communities – forest of *Carpinus betulus, Quercus dalechampii* and *Fagus silvatica* (KUMPULA *et al.*, 2006).

Field studies. The field studies were conducted in June-October 2012; April-September 2013 and April-June 2014. Amphibians and reptiles were determined visually using the field guides of ARNOLD & OVENDEN (2002) and BISERKOV *et al.* (2007). For each recorded species are given valid common and Latin name following SPEYBROECK *et al.* (2010). Some individuals are identified by their sounds, their eggs or larvae and skin sheds. Route method was used in accordance with the methodology of the Executive Environment Agency and water. The route of the route was established selectively in the most appropriate locations with suitable habitats for amphibians and reptiles. Each locality was masked using GPS device "Garmin" (manufacturer specified accuracy ± 5 m).

Mapping. For the purposes of mapping we used a standard 10×10 km UTM grid, which was divided into a 2×2 km grid for more detailed representation (Fig. 1). The territory of the study site encompasses 53 2×2 km squares, 13 complete and 40 partial.

Results and Discussion

We recorded 20 species of amphibians and reptiles, or 43% of recognized species in Bulgaria (Table 1). Their UTM distribution is presented in Appendices 3 and 4. We detected the presence of seven amphibian species, one from order Caudata and six from order Anura, corresponding to 4% and 24% (28% totally) of the total Bulgarian batrachofaunal diversity, respectively (TZANKOV & POPGEORGIEV, 2014). Reptiles were represented by 13 species – two from order Testudines, six from suborder Sauria, and five from suborder Serpentes, respectively 5,4%, 16,22%, and 13,51% (35,13% totally) of the total herpetofaunal diversity of Bulgaria (SPEYBROECK *et al.*, 2010). For comparison in another NATURA2000 site – SPA "Besaparski ridove" with similar total area POPGEORGIEV *et al.* (2010), reported 24 species (9 amphibians and 15 reptiles).

We managed to record all of the previously reported species for which we found published locality data for NP "Sinite kamani". There are few more species previously recorded in the territory of the park by Spiridonov (1986) and Daskalova (2001), but they were never officially published. During this study we documented three new amphibian species (*Hyla arborea, Rana dalmatina* and *Rana graeca*) and three new reptilian species (*Testudo hermanni, Ablepharus kitaibelii* and *Lacerta trilienata*).

The Greek Stream Frog (*Rana graeca*) is discovered in the area for the first time and this is the species' most northeastern locality ever recorded in Bulgaria. The locality is situated at N42° 44.736' E26° 26.655' (818 m a.s.l.) from an ecotone area of woody-shrub/grass vegetation, approximately 900 m south from Ichera Village, 24.5.2014 (Appendix 5-C).

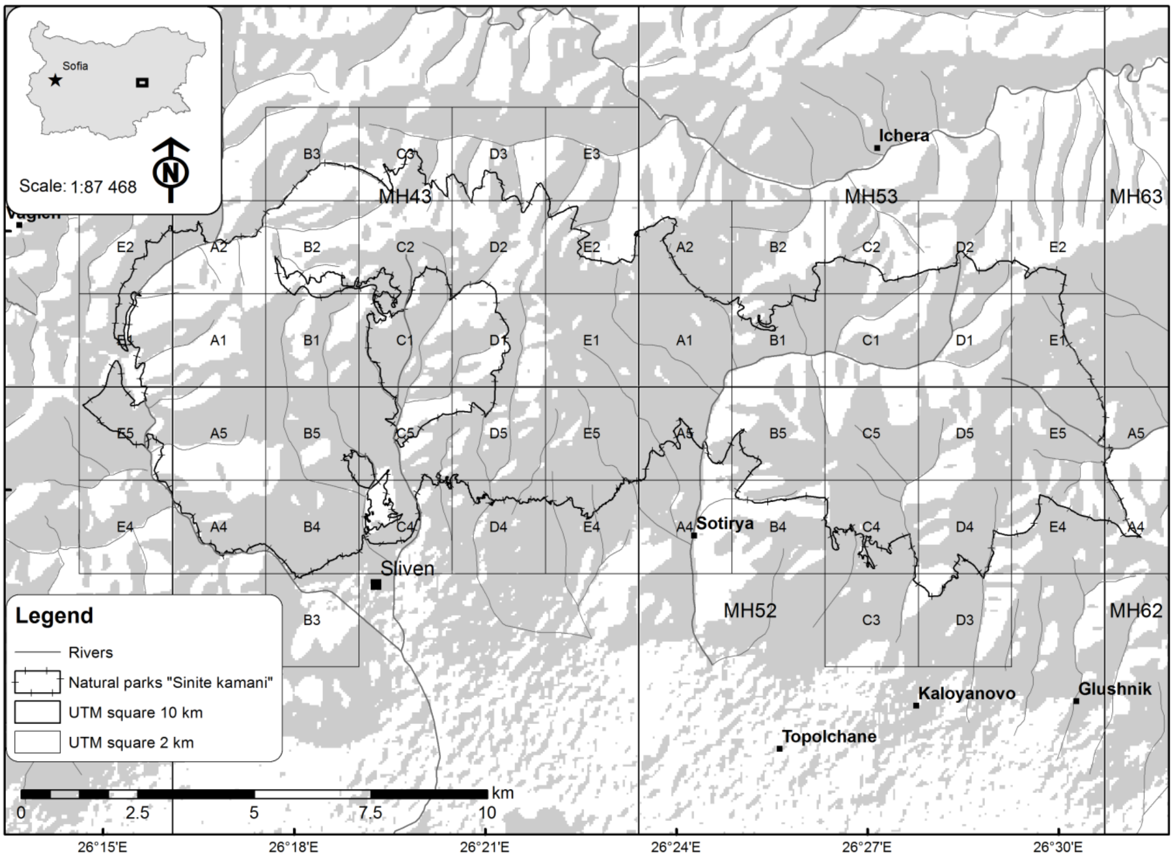


Fig. 1. Map of NP "Sinite kamani" with 2×2 km UTM grid.

Bufo bufo, B. viridis and *Rana graeca* were recorded with only one specimen from a single square each. The most common species was *Pelophylax ridibundus*, which is one the most common amphibians in the area and in Bulgaria (BISERKOV *et al.,* 2007). The other commonly met species was *Salamandra, salamandra*, registered mainly in the ridge area (Appendix 5-A). The lowest locality is 390 meters a.s.l. (at Asenevets Dam) and the highest - 953 m a.s.l. (near the cave Futula).

From the reptiles the most common reptile species in the Nature Park is the Green Lizard (*Lacerta viridis*) (Appendix 6-D) and the Snake-eyed Skink (*Ablepharus kitaibelii*). The two tortoise species (*Testudo graeca* and *T. hermanni*) are registered mainly in the south parts of the park, where suitable habitats of deciduous forests with shrubs are still available with almost equal number of localities. From the snakes the Caspian Whip Snake (*Dolichopis caspius)* was recorded near the area "Selishteto"; the Smooth Snake was recorded in the "Karandilla" area and the Grass Snake (*Natrix natrix*) and Nose-horned Viper (*Vipera ammodytes*) were recorded from three localities each, mainly from the south part of the park.

Conservation problems and threats

All registered species (except *Rana dalmatina* and *Natrix natrix*) are protected by the Bulgarian legislation and all recorded species have international conservation status (Table 1). High vulnerability and with the highest conservation status are the two species of tortises.

The dependence of the amphibians on the availability of water in the area for breeding makes them particularly vulnerable to this factor. Of great importance for the maintenance of the popu-

Table 1. Species diversity of amphibians and reptiles recorded in NP "Sinite kamani". *Legend: L* – number of localities; *N* - number of 2×2 km UTM squares in which species was detected. Details on abbreviations for the conservation status are provided below the table.

Species	L	N	Conservation status					
			BPA	RDB	92/43	BERN	IUCN	CITES
Salamandra salamandra (L., 1758)	4	4	III	-	-	III	LC	-
Bufo bufo L., 1758	1	1	III	-	-	III	LC	-
Bufo viridis (Laur., 1768)	1	1	III	-	IV	II	LC	-
Hyla arborea (L., 1758)	2	2	III	-	IV	II	LC	-
Rana dalmatina Bonaparte, 1838	2	2	-	-	IV	II	LC	-
Rana graeca Boul., 1891	1	1	III	-	IV	III	LC	-
Pelophylax ridibundus (Pall., 1771)	3	3	IV	-	-	III	LC	-
Testudo graeca L., 1758	11	8	II,III	EN	II,IV	II	V	II
Testudo hermanni Gmel., 1789	14	4	II,III	EN	II,IV	II	NT	II
Ablepharus kitaibelii Bibron et Bory, 1833	11	7	III	-	IV	II	LC	-
Anguis fragilis L., 1758	5	4	III	-	-	III	-	-
Lacerta viridis (Laur., 1768)	14	8	III	-	IV	II	LC	-
Lacerta trilineata Bedriaga, 1886	1	1	III	-	IV	II	LC	-
Podarcis tauricus (Pall., 1814)	5	2	III	-	IV	II	LC	-
Podarcis muralis (Laur., 1768)	5	3	III	-	IV	II	LC	-
Coronella austriaca Laur., 1768	2	2	III	-	IV	II	-	-
Dolichophis caspius (Gmel., 1789)	6	5	III	-	IV	II	-	-
Zamenis longissimus (Laur., 1768)	7	4	III	-	IV	II	LC	-
Natrix natrix (L., 1758)	3	3	-	-	-	III	LR/lc	-
Vipera ammodytes (L., 1758)	3	3	III	-	IV	II	LC	-

BPA – Biodiversity Protection Act of Bulgaria (State Gazette, 2002). Annexes: II – Species whose conservation requires the designation of special areas for habitat protection; III – Species protected statewide; IV – Species under protection and regulated use.
RDB – Red Data Book of Bulgaria (2011). EN – Endangered species.
92/43 – Council Directive 92/43/EEC (CD92/43, 1992). Annexes: II – Species whose conservation requires the designation of special areas of conservation; IV – Species in need of strict protection.
BERN – Bern Convention on the Conservation of European Wildlife and Natural Habitats (Bern, 1979). Appendices: II – Strictly protected fauna species; III – Protected fauna species
IUCN – International Union for Conservation of Nature, RedList (IUCN, 2014). V – vulnerable; NT - near threatened; LR/lc – Lower Risk/least concern; LC - least concern
CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1975). Appendix II - Species not necessarily now threatened with extinction but that may become so unless trade is closely controlled.

lations of amphibians is the presence of standing water (dams, lakes, ditches, water tanks, etc.). Maintenance of existing and creation of new ones is crucial. Negative impact for the populations of some species of frogs and tailed amphibians is the development of road infrastructure.

Major threat to populations of reptiles is the changes or destruction of their preferred habitats (for example forests dominated by oak and replacing them with conifers). Problem, which should be given particular attention, is the illegal collection of tortoises for sale or for consumption. Also the direct killing and collection by poachers and tourists: snakes, tortoises, lizards. As a result of poaching and collecting the reptilian populations deline drastically.

Another threat is fires and arson. The most severe losses have the slow-moving invertebrates, reptiles and amphibians.

Grazing cattle in the forests and overgrazing in farmland is another threat for the amphibians and reptiles. The negative effect of grazing is due mainly to goats. Goat grazing directly affects the undergrowth, the grass vegetation and prevents and stops natural regeneration of forests.

Disturbance of wildlife is a factor with moderate effect. Disturbance caused by tourism, is able to compromise the reproductive process of a number of species, mainly reptiles in rocky habitats.

Recommendations

For preserving the populations of amphibians and reptile in the Nature Park we recommend the following:

- Conducting future studies on population dynamics and distribution of all recorded species.
- Creating small artificial ditches with water and maintenance of existing old ones to attract amphibians and facilitate their reproduction.
- Fencing of roads in the regions where tangent to the area and tunneling for unimpeded passage of reptiles and amphibians.
- Insufficient environmental awareness is often at the root of destructive attitude towards nature, which in turn leads to a reduction of populations, destruction of animals and plants and other priority species. Manifestation of this attitude are illegal hunting and fishing, dumping of waste outside designated areas, industrial pollution and construction waste, and fetched and irrational use of renewable natural resources. Conducting educational seminars and preparation of educational materials for the locals, would play a positive effect.
- Planning and implementation of effective control on the field and the application of strict penalties for offenders are required, both in domestic and in international environmental law.

Acknowledgements

The authors would like to express their sincerest gratitude to Dr. Georgi Popgeorgiev for the prepared UTM map of the studied region and to the Directorate of NP "Sinite Kamani" for the given data and help during the study.

This research is conducted under the project "Habitat recovery and biodiversity conservation in "Sinite Kamani" Nature Park, financed by the Operation Programme Environment of the Ministry of Environment and Waters.

References

ARNOLD N., D. OVENDEN. 2002. *A field guide to the Reptiles and Amphibians of Britain and Europe.* Harper Collins Publishers, 288 p.

Bern. 1979. Convention on the Conservation of European Wildlife and Natural Habitats, Bern, 19.9.1979, Available at: [http://conventions.coe.int/Treaty/en/Treaties/Html/104.htm]

BESHKOV V. 1966. [Studies on the systematics and the distribution of the slow worm (*Anguis fragilis* L.) in Bulgaria]. – *Izvestia na Zoologicheskia institute s muzei,* 21: 185-200 (In Bulgarian).

BISERKOV C., B. NAUMOV, N. TSANKOV, A. STOYANOV, B. PETROV, D. DOBREV, P. STOEV. 2007. [*Key to Amphibians and Reptiles in Bulgaria.*] Sofia, Green Balkans, 196 p. (In Bulgarian).

BURESH I., I. TSONKOV. 1932. [Distribution of the venomous snakes (Viperidae) in Bulgaria and the Balkan Peninsula]. – *Trudove na Balgarskoto Prirodoizpitatelno Obshtestvo*, 15-16: 189-206 (In Bulgarian).

BURESH I., I. TSONKOV. 1933. Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel. I Teil: Schildkrötten (Testudinata) und Eidechsen (Sauria). - *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien,* 6: 150–207 (In Bulgarian).

BURESH I., I. TSONKOV. 1934. Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel. II Teil: Schlangen (Serpentes). - *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien,* 7: 106–188 (In Bulgarian).

BURESH I., I. TSONKOV. 1941. Untersuchungen über die Verbreitung der Reptilien und Amphibien in

Bulgarien und auf der Balkanhalbinsel. III Teil: Froschlurche (Amphibia, Caudata). - *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien,* 14: 171-237 (In Bulgarian).

BURESH I., I. TSONKOV. 1942. Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel. IV Teil: Froschlurche (Amphibia, Salientia). - *Mitteilungen aus den Königlichen naturwissenschaftlichen Instituten in Sofia – Bulgarien,* 15: 68–145 (In Bulgarian).

DRENSKI P. 1955. [Composition and distribution of ticks (Ixodoidea) in Bulgaria]. - *Proceedings of the Institute of Zoology with Museum,* 4-5: 109-166 (In Bulgarian).

CD92/43. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, Available at: [eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:01992L0043-20070101:EN:NOT].

CITES. 1975. Convention on International Trade in Endangered Species of Wild Fauna and Flora, July 1 1975, Available at: [http://www.cites.org]

European Environment Agency (EEA). 2012. Natura2000 Network Viewer. [http://natura2000.eea.europa.eu/Natura2000/SDF.aspx?site=BG0000164]

EUZET L., C. COMBES, G. BATCHVAROV. 1974. Sur un Noveau Polystomatidae Européen Parasite de L'Amphibien Bufo viridis Laur. - *Vie Milieu,* 24(1): 129-140.

IUCN. 2014. Red List of Threatened Species. Version 2014.3., Available at: [http://www.iucnredlist.org]

KOVACHEV V. 1905. [A contribution to the study of the amphibians and reptiles I Bulgaria]. – *Sbornik za narodni umotvorenia, nauka i knijnina,* 21: 1–13 (In Bulgarian).

KOVACHEV V. 1912. [*The Herpetological Fauna of Bulgaria (Reptiles and Amphibians)*]. Publishing house "Hristo G. Danov", Plovdiv, 90p. (In Bulgarian).

KOVACHEV V. 1917. [Amphibians and reptiles in the captured territories in 1912 and adjacent areas]. – *Journal of BAS,* 15: 175-178.

KOVATSCHEFF V. 1903. Beiträge zur Kenntnis der Reptilien- und Amphibienfauna Bulgarien. - *Verhandlungen der k.k.zoologisch-botanischen Gesellschaft in Wien,* Jahrg. 1903: 171-173.

KUMPULA T., S. NEDKOV, M. NIKOLOVA. 2006. Land Cover Classification of Sinite Kamani Natural Park Using Aster Terra Staellite Image. - In: *Proceedings of the First International Conference on Cartography and GIS,* Borovets, Bulgaria, January 25th-28th, 2006, University of Sofia.

LEHRS P. 1931. Westasiatische Elemente in der Herpeto-Fauna Europas. - *Annales Musei Nationalis Hungarici, Budapest,* 27(1930/1931): 279-284.

POPGEORGIEV G., N. TZANKOV, Y. KORNILEV, B. NAUMOV, A. STOJANOV. 2010. Species Diversity of Amphibians and Reptiles in the Special Protected Area "Besaparski Ridove", Southern Bulgaria. - *Biotechnology & Biotechnological Equipment,* 24(Special Edition): 661-666.

Red Data Book of Republic of Bulgaria. 2011. Animals, Vol. 2. Available at: [http://e-ecodb.bas.bg/rdb/bg/]

SPEYBROECK J. 2005. Herpetological trip in southern Bulgaria - May 2005 [Online]. Available at: [www.hylawerkgroep.be/jeroen/index.php?id=9].

SPEYBROECK J., W. BEUKEMA, P.-A. CROCHET. 2010. A tentative species list of the European herpetofauna (Amphibia and Reptilia) - an update. – *Zootaxa,* 2492: 1-27.

State Gazette. 2002. Biodiversity Protection Act of Bulgaria, Nr 77 of August 9, 2002.

TZANKOV N. D., G. S. POPGEORGIEV. 2014. Conservation and declines of Amphibians in Bulgaria. - In: Heatwole, H., J. W. Wilkinson (Eds.) *Amphibian Biology, Part 4: Status of Conservation and Decline of Amphibians: Eastern Hemisphere: Southern Europe &*

Turkey. Pelagic Publishing, vol. 11: 131-139.

UNDZHIAN E. 2000. [*Studies on the vertebrates in the valley of "Lomovete" and the territory of nature park "Russenski Lom", district Russe and Razgrad, III. Amphibians, IV. Reptiles*]. Publ. NP "Russenski Lom", Russe, 88 p. (In Bulgarian).

Received: 19.07.2014
Accepted: 22.12.2014

APPENDIX 1

Amphibian distribution in Nature Park "Sinite Kamani" and its surroundings with UTM codes (10x10 km), according to the available literature data

Salamandra salamandra - "In the mountain around Sliven" - **MH42** (KOVATSCHEFF, 1903; BURESH & TSONKOV, 1941), "Sliven" - **MH42** - (KOVACHEV, 1912), "The vicinity of Byala Town" - **MH33** (УНДЖИЯН, 2000); *Bufo bufo* - "In the mountain near Sliven, under Kutelka Peak" - MH43 (BURESH & TSONKOV, 1942), "near Sliven" - **MH42** (SPEYBROECK, 2005); *Bufo viridis* - ""Slivenski mineralni bani" near Sliven" - **MH42** (EUZET *et al.*, 1974); *Pelophylax ridibundus* - "near Sliven" - **MH42** (SPEYBROECK, 2005).

APPENDIX 2

Reptile distribution in Nature Park "Sinite Kamani" and its surroundings with UTM codes (10x10 km), according to the available literature data

Testudo graeca - "The vicinities of Sliven" - **MH42** (UNDZHIAN, 2000); *Anguis fragilis* - „Ablanovo, Sliven District" - **MH42** (KOVACHEV, 1912; BURESH & TSONKOV, 1933), „Byala village, Sliven District" - **MH33** (BURESH & TSONKOV, 1933), "Stara Planina Mnt. near Sliven" – **MH42** (BESHKOV, 1966); *Lacerta viridis* - "Sliven" - **MH42** (KOVACHEV, 1912; LEHRS, 1931; BURESH & TSONKOV, 1933; DRENSKI, 1955; SPEYBROECK, 2005), „Byala village, Sliven District" - **MH33** (BURESH & TSONKOV, 1933); *Podarcis tauricus* - "Slivenskite bani" - **MH42** (KOVACHEV, 1912; BURESH & TSONKOV, 1933); *Podarcis muralis* - "near Sliven" - **MH42** (SPEYBROECK, 2005); *Dolichophis caspius* - "Sliven" - **MH42** (Kovachev, 1905; BURESH & TSONKOV, 1934); *Zamennis longissimus* - "Sliven" - (KOVACHEV, 1905; 1912; BURESH & TSONKOV, 1934), "Sotirya" - **MH52** (KOVACHEV, 1917); *Coronella austriaca* - "Sliven" - **MH42** - (KOVACHEV, 1912; BURESH & TSONKOV, 1934), „Ablanovo, Sliven District" - **MH42** (KOVACHEV, 1912; BURESH & TSONKOV, 1934), „Byala village, Sliven District" - MH33 (BURESH & TSONKOV, 1934); *Natrix natrix* - "Sliven vicinities" - **MH42** (BURESH & TSONKOV, 1934); *Vipera ammodytes* - "Sliven" - **MH42** (KOVACHEV, 1905; 1912; BURESH & TSONKOV, 1932).

APPENDIX 3

Amphibian distribution in Nature Park "Sinite Kamani" with UTM codes (2x2 km), based on the recorded localities in the current study

Salamandra salamandra - MH41-E5, MH42-D5, MH43-A1, MH53-C1; *Bufo bufo* – MH53-C2; *Bufo viridis* – MH52-A5; *Hyla arborea* - MH41-E5, MH53-C2; *Rana gaeca* – MH53-C2; *Rana dalmatina* - MH41-E5, MH42-A5; *Pelophylax ridibundus*[1] - MH41-E5, MH42-C5, MH42-E5 and others.

APPENDIX 4

Reptile distribution in Nature Park "Sinite Kamani" with UTM codes (2x2 km), based on the recorded localities in the current study

Ablepharus kitaibelii - MH41-E5, MH42-A5, MH42-B5, MH42-C5, MH43-B1, MH43-D1, MH52-D4; *Anguis fragilis* - MH42-A5, MH43-E1, MH43-E2, MH43-D1; *Coronella austriaca* - MH52-C4, MH53-A2; *Dolichophis caspius* - MH41-E5, MH42-B5, MH42-C5, MH42-D5, MH42-E4; *Lacerta trilineata* – MH42-C4; *Lacerta trilineata* - MH41-E5, MH42-D5, MH42-B5, MH42-C4, MH42-C5, MH42-E4, MH43-B1, MH53-B2; *Natrix natrix* - MH42-C5, MH43-E1, MH52-E4; *Podarcis muralis* - MH41-E5, MH42-D5, MH43-D1, MH52-D4; *Podarcis tauricus* - MH42-A5, MH42-B5; *Testudo graeca* - MH41-E5, MH42-A5, MH42-C4, MH42-C5, MH42-D5, MH42-E4, MH42-E5, MH52-D4; *Testudo hermanni* - MH42-D4, MH42-E4, MH42-E5, MH52-D4; *Zamennis longissimus* - MH42-C5, MH42-E4, MH43-D1, MH53-A1; *Vipera ammodytes* - MH42-C5, MH42-D4, MH52-A5.

---

[1] *Pelophylax ridibundus* is abundant and frequently met in many aquatic habitats throughout the territory of the Nature Park, so most of its localities were not recorded.

APPENDIX 5
Amphibians from Nature Park "Sinite Kamani" (photographs)

A – Fire salamander (*Salamandra salamandra)* from "Lokvata" area. Photo: A. Mechev.

B - Marsh Frog (*Pelophylax ridibundus*) from the southern parts of the park. Photo: A. Mechev

C - Greek Stream Frog (*Rana graeca*) from ecotone area - woody-shrub/grass vegetation, aprox. 900 m south from Ichera Village (818 m a.s.l.), 24.5.2014. Photo: A. Mechev

D – The southern parts ot "Sinite Kamani" Nature Park. Photo: S. Deleva.

APPENDIX 6
Reptiles from Nature Park "Sinite Kamani" (photographs)

A – Spur-thighed Tortoise (*Testudo graeca*) from "Sinite Kamani" Nature Park. Photo: S. Deleva

B – Slow Worm (*Anguis fragilis*) from"Sinite Kamani" Nature Park. Photo: B. Borisov.

C - Eastern Green Lizard (*Lacerta viridis*) from"Sinite Kamani" Nature Park. Photo: B. Borisov

D - Aesculapian Snake (*Zammenis longissimus*) from"Sinite Kamani" Nature Park. Photo: S. Deleva

E – Grass Snake (*Natrix natrix*) from"Sinite Kamani" Nature Park. Photo: S. Deleva.

F – Nose-horned Viper (*Vipera ammodytes*) from"Sinite Kamani" Nature Park. Photo: S. Deleva

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 93-100

Effect of Individual and Combined Treatment with Azadirachtin and Spodoptera littoralis Multicapsid Nucleopolyhedrovirus (SpliMNPV, Baculoviridae) on the Egyptian Cotton Leafworm Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae)

El-Sayed H. Shaurub*, Afaf Abd El-Meguid, Nahla M. Abd El-Aziz

Department of Entomology, Faculty of Science, Cairo University, Giza, EGYPT
* Corresponding author: sayedshaurub@yahoo.com

Abstract. The tetranortriterpenoid, azadirachtin, and the entomopathogenic virus, nucleopolyhedrovirus, are used as safe and new control measures for combating agricultural insect pests instead of the use of synthetic insecticides. They can be mixed together as an integrated pest management strategy. Thus, the current investigation was designed to determine the mortality, duration and weight gain of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae, and the yield of *Spodoptera littoralis* multicapsid nucleopolyhedrovirus (SpliMNPV) (Baculoviridae) when the fourth larval instars were treated individually with the LC_{50} of azadirachtin and of SpliMNPV, and in combination with each other using the LC_{25}, compared to non-treated larvae (control). The results obtained showed that combined treatment significantly enhanced the larval mortality by about 58.40 %, *i.e.* potentiation. Both individual and combined treatment significantly decreased the larval weight gain, whereas the larval duration was significantly increased, with the highest change in case of combined treatment. Azadirachtin–NPV mixture significantly decreased the viral yield (number of polyhedral inclusion bodies/g fresh larval body weight) by about 36.05 % compared to the individual treatment with the NPV. It can be concluded that although azadirachtin enhanced the pathogenicity (% larval kill) of SpliMNPV to *S. littoralis*, azadirachtin–SpliMNPV mixture is unlikely to be useful for the mass production of this viral isolate. Thus, these laboratory observations require validation in field studies under commercial growing conditions.

Key words: *Spodoptera littoralis*, azadirachtin, duration, weight gain, mortality, nucleopolyhedroviruses, viral yield.

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is highly polyphagous infesting about 87 host plants including important field crops and various fruits and ornamental trees (TIESSEN, 2012).

Conventional pesticides, particularly organophosphorus and pyrethyroid insecticides, are generally used for combating agricultural insect pests, but their indiscriminate usage may cause environmental pollution, toxic residual effect, development of pest resistance, negative impacts on non-target organisms and adverse effects on human and animal health (SUNDARARAJ, 1997). These problems forced to search for new control measures especially from plant sources, as plant-derived molecules are eco-friendly, bio-degradable, target specific and development of resistance by pests against them has not been reported so far (GAUTAM *et al.*, 2013). Today about 200 plants with insecticidal activities are known (SINGH *et al.*, 2001). Among these plants, the neem tree

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

(*Azadirachta indica* A. Juss) (Meliaceae) has received a worldwide attention, where the active ingredient isolated from its seeds is a tetranortriterpenoid compound called azadirachtin, which acts as a potent insect growth regulator (REMBOLD *et al*., 1982; KUBO & KLOCKE, 1982). The insecticidal activity of azadirachtin to nearly 550 insect species, including lepidopteran ones, was also reported (ABD EL-GHANY *et al*., 2012; WONDAFRASH *et al*., 2012; RADWAN & EL-SHIEKH, 2012; HUMMEL *et al*., 2012).

On the other hand, nucleopolyhedrovirus (NPV) (Baculoviridae) is a natural microbial pathogen of Lepidoptera, which is an ideal tool in integrated pest management programs, highly specific to its host insects, safe to the environment, humans, other plants and natural enemies (YANG *et al*., 2012). However, the narrow host range, slow speed of kill, large dose requirement and resistance development are some of the problems which have limited its use. These concerns have opened new arenas for the improvement of NPV formulations (MOSCARDI, 1999). In this context, the effect of NPV has been enhanced by combining with adjuvants like neem seed extracts (KUMAR *et al*., 2008; BAJWA & AHMAD, 2012; WAKIL *et al*., 2012; ZAMORA-AVILÉS *et al*., 2013). Accordingly, there is a need to evaluate the species specific NPV either individually or in combination with synergists to understand its compatibility and efficiency.

Therefore, the objective of the present study is to elucidate the effect of azadirachtin and *Spodoptera littoralis* multicapsid nucleopolyhedrovirus (SpliMNPV), either alone or in combination with each other, on mortality, duration and weight gain of *S. littoralis* fourth larval instars, and on the yield of this viral isolate. The data may be helpful in formulating some control strategy for this pest.

Material and Methods

Insects. A susceptible strain of *S. littoralis* was established from egg masses obtained from Faculty of Agriculture, Cairo University, Egypt. Newly hatched larvae were transferred into plastic boxes (18×12×10 cm) containing about 0.5 cm thick semi-synthetic diet on which larvae were fed (SHOREY & HALE, 1965). The pupae were sexed and placed in sterilized containers (18×15×30 cm) for adult emergence. The containers were lined with tissue paper as an ovipositing stratum. Adult moths were fed on 10% sucrose solution. Insects were reared under laboratory conditions of 25–27ºC, 65–70% RH and 12h: 12h (L: D) photoperiod.

SpliMNPV and azadirachtin. The viral isolate SpliMNPV (Baculoviridae) was provided by Entomovirology Laboratory (Faculty of Agriculture, Cairo University, Egypt) in the form of a suspension in sterile distilled water, with a stock concentration of 3.4×10^{10} polyhedral inclusion bodies (PIB)/ml. This suspension was stored at -20ºC till use. Azadirachtin (96% purity) was obtained as a powder from Carl Roth GmbH + Co. Kg., and a stock solution (200 ppm) was prepared in sterile distilled water and stored at 4ºC till use (KUMAR *et al*., 2008; WAKIL *et al*., 2012; ZAMORA-AVILÉS *et al*., 2013).

Purification of occlusion bodies (polyhedra) of SpliMNPV. Occlusion bodies were purified following the methods of HUNTER-FUJITA *et al*. (1998). Infected *S. littoralis* cadavers were collected and homogenized in 0.05M Tris, pH 7.5-7.8, 0.1 % sodium dodecyl sulfate (SDS) (2 ml buffer/g larva). The homogenate was filtered twice through a piece of muslin and cotton wool to eliminate the insect fragments. Filtrate was clarified three times by centrifuging at 5000 rpm for 3 min, using a Beckman J2-21 MIE centrifuge and a 20 JA rotor. The supernatant containing the polyhedra was centrifuged at 3000 rpm for 10 min under cooling (4ºC). The pellets of semi-purified polyhedra were re-suspended in Tris-SDS and centrifuged at 4000 rpm for 10 min under cooling. Finally, the pellets of the purified polyhedra were re-suspended in sterile distilled water (1 ml H_2O/g viral precipitate), counted using a bacterial counting chamber under phase contrast microscopy at × 400, and stored at -20ºC until required.

Mortality, development and growth of larvae, and viral yield. A preliminary experiment was carried out to estimate the LC_{25} and LC_{50} values of SpliMNPV and azadirachtin against newly molted *S. littoralis* fourth larval instars, following the technique of treatment of the surface of semi - synthetic diet (ZAMORA-AVILÉS *et al.*, 2013), with minor modifications. Five viral concentrations ($3.4×10^9$, $3.4×10^8$, $3.4×10^7$, $3.4×10^6$ and $3.4×10^5$ PIB/ml) and six serial concentrations of azadirachtin (50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm) were prepared. One ml of each concentration was poured onto the surface of the same semi - synthetic diet used for rearing, thoroughly swirled and air-dried. The treated diet was offered for 24 h to 4 h-previously starved fourth larval instars. Then, the larvae were fed on fresh untreated diet until pupation or death. The control experiment consisted of larvae fed on the same diet treated with distilled water only. Each concentration was repeated three times of 50 larvae each. The number of dead larvae was recorded. All the experiments were incubated at 25ºC. The same above procedures were also followed in case of studying the combined effect of SpliMNPV and azadirachtin, using a mixture of NPV and azadirachtin (LC_{25}: LC_{25}) (v/v).

The duration and weight gain of larvae treated alone with the LC_{50} of azadirachtin and of SpliMNPV, or in combination with the LC_{25} of azadirachtin and of SpliMNPV were recorded. In each treatment, larvae were individually weighed, SpliMNPV was purified from each larva and the number of PIB was counted following the technique described above. The concentration of PIB was expressed as the number of PIB/g fresh larval body weight. The experiment was repeated three times.

Statistical analysis. The percentage of mortality of treated larvae was corrected against that of non - treated ones (control) using Abbott's formula (ABBOTT, 1925) as follows: Corrected mortality (%) = (% Observed mortality - % Control mortality/100 - % Control mortality) 100. The corrected mortality was then subjected to propit analysis (FINNEY, 1971), from which the LC_{25} and LC_{50} were estimated. The correlation coefficient "*r* - value" was also determined for the dose – mortality relationship.

The interaction between azadirachtin and the virus, in relation to larval mortality, was differentiated according to the co-toxicity factor (MANSOUR *et al.*, 1966) as follows: Co - toxicity factor (%) = (%Observed mortality - %Expected mortality/%Expected mortality) 100, where a positive factor of 20 or more is considered as potentiation, a negative factor of 20 or more is considered as antagonism, while intermediate values (-20 and +20) indicate additive effect.

The toxicity bioassay was determined with POLO–PC software (LEORA SOFTWARE, 1987). Data were analyzed by one–way analysis of variance (ANOVA) using Costal Statistical Software (Cohort Software, Berkeley). When the ANOVA statistics were significant (at $P<0.05$), the means were compared by Duncan's multiple range test (SAS INSTITUTE INC., 2008).

Results

The bioassay test (Table 1) revealed that the mortality of the fourth larval instars of *S. littoralis* increased progressively with the increase of the viral concentrations (r = 0.995), with LC_{25} and LC_{50} values of 10.10×10^6 and 8.43×10^8 PIB/ml distilled water, respectively. The same pattern was also attained for the larval treatment with azadirachtin (r = 0.850), with LC_{25} and LC_{50} values of 9.95 and 23.15 ppm, respectively.

The results demonstrated (Fig. 1) show that the larval mortality (79.20 ± 3.85%) obtained due to the combined treatment with the LC_{25} of SpliMNPV and of azadirachtin was significantly enhanced ($P<0.05$) to about 36.05 % of that recorded as a result of treatment with the LC_{50} of the virus alone (50.23 ± 2.95%). Thus, the LC_{25} of SpliMNPV combined with that of azadirachtin became approximately ≥ the LC_{79}, instead of being theoretically equal to the LC_{50}. The estimated co - toxicity factor was 58.40%.

The weight gain of larvae treated alone or in combination with SpliMNPV and azadirachtin was significantly reduced ($P<0.05$) compared to the control. Combined treatment reduced the larval weight by about 53.70 and 19.35% compared to the single treatment with the virus and azadirachtin, respectively (Table 2).

In the present study, single and combined treatment of *S. littoralis* larvae with azadirachtin and NPV significantly increased (P<0.05) the larval duration compared to the control, with the highest magnitude in case of combined treatment (Table 2).

Combining azadirachtin with SpliMNPV resulted in a significant decrease (P<0.05) in the viral yield by about 36.05% compared to the infection with the virus alone. The decrease in the viral yield was concomitant with the decrease in the larval weight gain (Table 2).

Table 1. Susceptibility of *Spodoptera littoralis* fourth larval instars to the entomopathogenic virus SpliMNPV and azadirachtin.

Treatment	LC_{50}	95% Fiducial limits		LC_{25}	95% Fiducial limits		Slope	χ^2 (d.f.)
		Lower	Upper		Lower	Upper		
SpliMNPV	8.43×10^8 (PIB/ml)	4.22×10^8 (PIB/ml)	2.53×10^9 (PIB/ml)	10.10×10^6 (PIB/ml)	5.05×10^6 (PIB/ml)	3.03×10^7 (PIB/ml)	0.3510	$\chi^2_{(3)} = 3.45$
Azadirachtin	23.15 (ppm)	9.26 (ppm)	45.84 (ppm)	9.95 (ppm)	4.70 (ppm)	13.02 (ppm)	0.8906	$\chi^2_{(4)} = 5.23$

Data are mens of three replicates of 50 larvae each (Total n = 150 larvae).

Table 2. Weight gain and duration of *Spodoptera littoralis* fourth larval instars treated alone with the entomopathogenic virus SpliMNPV and azadirachtin and in combination with each other.

Treatment	Larval weight gain ± SE (mg)	Larval duration ± SE (days)	Viral yield ± SE (PIB/ml)
Control	0.93 ± 0.10[a]	10.35 ± 0.03[a]	-
SpliMNPV	0.54 ± 0.09[b]	12.45 ± 0.04[b]	$3.44\times10^5 \pm 0.60\times10^5$[a]
Azadirachtin	0.31 ± 0.02[c]	15.48 ± 0.34[c]	-
Azadirachtin+ SpliMNPV	0.25 ± 0.01[d]	18.24 ± 0.25[d]	$2.20\times10^5 \pm 0.80\times10^5$[b]

Data are mens of three replicates of 50 larvae each. Figures followed by different letters were statistically significant (P<0.05) using one - way analysis of varianve (ANOVA). PIB: Polyhedral inclusion bodies.

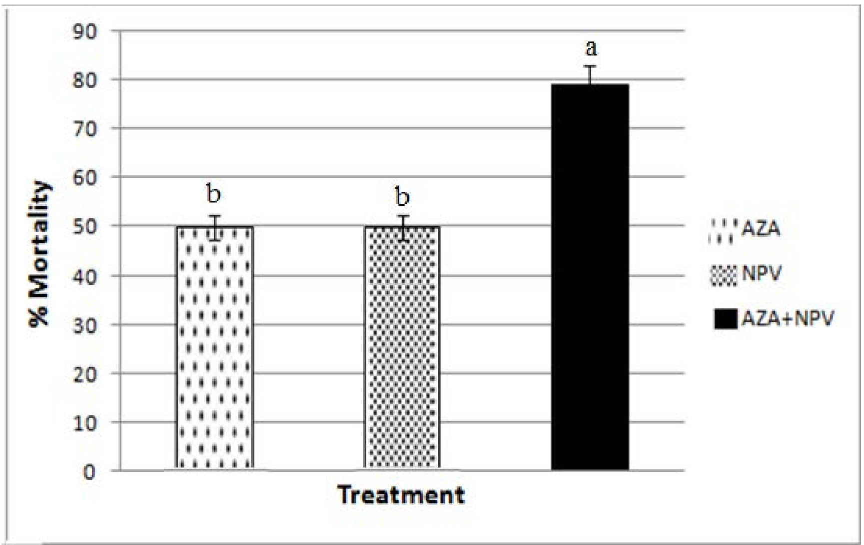


Fig. 1. Mortality of *Spodoptera littoralis* fourth larval instars treated alone with the LC_{50} of the entomopathogenic virus SpliMNPV (8.43×10^8 PIB/ml) and the LC_{50} of azadirachtin (23.15 ppm), and the combined treatment with the LC_{25} of SpliMNPV (10.10×10^6 PIB/ml) and the LC_{25} of azadirachtin (9.95 ppm). Graph bar represents the standard error (SE) of the mean of three replicates; 50 larvae each. Columns with different letters are significantly different from each other (P< 0.05) using one-way analysis of variance (ANOVA). AZA: Azadirachtin; NPV: SpliMNPV; AZA + NPV: Azadirachtin combined with SpliMNPV.

Discussion

The present study may help in better management of *S. littoralis* larvae in future using SpliMNPV and azadirachtin. The LC_{50} value of SpliMNPV against *S. littoralis* larvae (8.43× 10^8 PIB/ml reached) in the present investigation was considered higher than that reported for other NPVs against several lepidopteran species (MANGOLER, 1974; JAMES & ARTHUR, 1992). KRISHNAYYA *et al.* (2000) reported that the LC_{50} value of ingested azadirachtin against the third larval instars of *Spodoptera litura* was 110.20 ppm. Thus, based on the LC_{50} level, it appears that the fourth larval instars of *S. littoralis* fed on azadirachtin in the current study, with LC_{50} of 23.15 ppm, were about five times as susceptible as the third larval instars of *S. litura*.

The co-toxicity factor (58.40%) attained in this study indicates that interaction between azadirachtin and SpliMNPV was potentiation, based on the formula suggested by MANSOUR *et al*. (1966). Similarly, enhancement of the virulence of NPV after combination with azadirachtin has been reported by other authors (KOPPENHÖFER & KAYA, 2000; KUMAR *et al*., 2008; WAKIL *et al*. 2012; ZAMORA-AVILÉS *et al.* 2013). The potentiation of the infectivity of NPV after combination with azadirachtin may be attributed to the damage of the mid gut secretory cells and the peritrophic membrane, with a concomitant decrease in the activities of the digestive enzymes, thereby facilitating the easy penetration of the active viral bodies and proliferation for subsequent pathogenic effects (KUMAR, 1998; BAJWA & AHMAD, 2012; ZAMORA-AVILÉS *et al*., 2013).

The decrease in larval weight gain of *S. littoralis* treated either alone or in combination with azadirachtin and SpliMNPV is in accordance with the results obtained for the larvae of *S. littoralis* (ABD EL-GHANY *et al*., 2012), *Spodoptera frugiperda* (ZAMORA-AVILÉS *et al*., 2013) and *Helicoverpa armigera* (WONDAFRASH *et al*., 2012; WAKIL *et al*., 2012) fed on azadirachtin and NPV alone or in combination with each other. SCHMUTTERER (1988) reported that azadirachtin and azadirachtin containing neem seed extracts acts as anti - feedant to insects. This may be due to the direct action of azadirachtin on the centers that control feeding and metabolism (BARNBY & KLOCKE, 1987). KUMAR *et al*. (2008) attributed the decrease in larval weight gain in *H. armigera* treated alone or in combination with NPV and azadirachtin to low food consumption and utilization in terms of the efficiency of conversion of the ingested and digested food into biomass. Moreover, it has been concluded that reduced growth, as a consequence of sub-lethal treatments, may be the result of diversion of host energy from metabolism and growth to combat or support the pathogen (ROTHMAN & MYERS, 1996) or hormonal changes induced by the pathogen (PARK *et al*., 1993). Reduced larval weight gain is important from the practical standpoint because it might negatively impact pest population dynamics in the subsequent generations (ZAMORA-AVILÉS *et al*., 2013).

In contrast to the results obtained for the larval weight gain, treatment with NPV or azadirachtin, either separately or in combination with each other, decreased the larval duration. These results are in consistence with those attained for *H. armigera* (KUMAR *et al*., 2008) and *S. frugiperda* (ZAMORA-AVILÉS *et al*., 2013) treated alone or in combination with azadirachtin and NPV. The molting delay which results from treatment with azadirachtin was attributed to the inhibition of ecdysteroid production (MALCZEWSKA *et al*., 1988). On the other hand, baculovirus blocks molting and interferes with normal development as it causes weak expression of the ecdysteroid UDP - glucosyltransferase gene which encodes the enzyme ecdysteroid UDP - glucosyltransferase. This enzyme catalyzes the transfer of glucose from UDP-glucose to ecdysteroids which are insect molting hormone (REILLY, 1995). Moreover, failure of pupation, as a result of viral infection, was due to maintenance of high level of circulating juvenile hormone (SUBRAMANYAM & RAMAKRISHNAN, 1981).

SpliMNPV yield decreased due to combining with azadirachtin. Similar results were also reported by other authors (COOK *et al*., 1996; ZAMORA-AVILÉS *et al*., 2013). These results can be interpreted by the suggestion of SHAPIRO *et al*. (1986) who suggested that NPV growth and yield are highly dependent on cell growth, thus NPV yield depends on the rate of larval weight gain during infection rather than on the initial or final weight.

Conclusions

It appears that azadirachtin is a valuable component for the formulation of SpliMNPV, by mixing at the ratio of LC_{25}: LC_{25}, especially in inundative release programmes, as it synergized the viral pathogenicity, in terms of larval mortality. Nevertheless, this mixture is unlikely to be useful for the mass production of this viral isolate. Thus, future studies on field efficacy under commercial growing conditions are required to demonstrate that the observed laboratory potentiation of azadirachtin–SpliMNPV mixture will be translated to improved control of *S. littoralis* in the field.

Acknowledgements

We are thankful to Prof. Dr. Said Abu El-Ela, Entomovirology Laboratory, Faculty of Agriculture, Cairo University, for providing us with the entomovirus. Sincere thanks are also due to the colleagues in Faculty of Agriculture, Cairo University, for offering us with the stock culture of the experimental insect.

References

ABBOTT W.S. 1925. A method of computing the effectiveness of an insecticide. - *Journal of Economic Entomology*, 18: 265-267.

ABD EL-GHANY M.A., M. FARAG, H. YOUSEF, M.H.M. AHMED, S. EL-BADRAWY, A.A.H. EL-RAHMAN. 2012. Insecticidal activity of *Melia azedarach* L. triterpenoids against *Spodoptera littoralis* (Boisd.). - *Journal of American Sciences*, 8: 661-667.

BAJWA A.A., A. AHMAD. 2012. Potential applications of Neem based products as biopesticides. - *Health*, 3: 116-120.

BARNBY M.A., J.A. KLOCKE. 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fabr.) (Noctuidae). - *Journal of Insect Physiology*, 32: 69-75.

COOK S.P., R.E. WEBB, K.W. THORPE. 1996. Potential enhancement of the gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus with the triterpene azadirachtin. – *Environmental Entomology*, 25: 1209-1214.

FINNEY D.J. 1971. *Probit Analysis*, third ed. London. Cambridge University Press. 333 p.

GAUTAM K., P. KUMAR, S. POONIA. 2013. Larvicidal and GC – MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. - *Journal of Vector Borne Diseases*, 50: 171 – 178.

HUMMEL H.E., D. F HEIN, H. SCHMUTTERER. 2012. The coming of age of azadirachtin and related tetranortriterpenoids. - *Journal of Pesticides*, 5: 82-87.

HUNTER-FUJITA F.R., P.F. ENTWISTLE, H.F. EVANS, N.E. CROOK. 1998. *Insect Viruses and Pest Management*. New York. John Wiley and Sons. 632 p.

JAMES R.F., R.R. ARTHUR. 1992. Virulence and multigeneration passage of a nuclear polyhedrosis virus selected for an increased rate of vertical transmission. - *Biological Control*, 2: 171-175.

KOPPENHÖFER A.M., H.K. KAYA. 2000. Interaction of a nuclear polyhedrovirus with azadirachtin and imidacloprid. - *Journal of Invertebrate Pathology*, 75: 84-86.

KRISHNAYYA P.V., M.S. SUNDER, P.A. RAO. 2000. Evaluation of lethal concentrations of azadirachtin based proprietary formulation for larvicidal and ovicidal effects of *Spodoptera litura* (Fabr.). - *Journal of Entomological Research*, 24: 147-150.

KUBO I., J.A. KLOCKE. 1982. Short communication: Azadirachtin, insect ecdysis inhibitor. - *Agricultural and Biological Chemistry*, 46: 1951-1953.

KUMAR N.S. 1998. Combined Effect of Nuclear Polyhedrosis Virus and Azadirachtin on the Feeding, Growth, Development, Biochemical and Histopathological Changes of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). (Ph. D. thesis). Bharathiar University, India.

KUMAR N.S., K. MURUGAN, W. ZHANG. 2008. Additive interaction of *Helicoverpa armigera* nucleopolyhedrovirus and azadirachtin. - *BioControl*, 53: 869-880.

LEORA SOFTWARE. 1987. *POLO – PC: A User's Guideline to Probit or Logit 786 Analysis*. LeOra Software.

MANGOLER A. 1974. Bioassay of nuclear polyhedrosisvirus of the gypsy moth, *Prothetria dispar*. - *Journal of Invertebrate Pathology*, 23: 190-196.

MALCZEWSKA M., D.B. GELMAN, B. CYMBOROWSKI. 1988. Effects of azadirachtin on development, juvenile hormone and ecdysteroid titres in chilled *Galleria mellonella* larvae. - *Journal of Insect Physiology*, 34: 725-732.

MANSOUR N.A., M.E ELDEFRAWI., A. TAPPOZADA, M. ZIED. 1966. Toxicological studies on the Egyptian cotton leafworm *Prodenia litura* F. VII. Potentiation and antagonism of órganophosphorus and carbamates. - *Journal of Economic Entomology*, 59: 307-311.

MOSCARDI F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. - *Annual Review of Entomology*, 44: 257-289.

PARK E.J., J.P. BURAND, C.M. YIN. 1993. The effect of baculovirus infection on ecdysteroid titer in gypsy moth larvae (*Lymantria dispar*). - *Journal of Insect Physiology*, 39: 791-796.

RADWAN O.A., Y.W.A. EL-SHIEKH. 2012. Degradation of neem oil 90% EC (Azadirachtin) under storage conditions and its insecticidal activity against cotton leafworm, *Spodoptera littoralis*. - *Researcher*, 4: 77-83.

REILLY D.R. 1995. Baculovirus-encoded ecdysteroid UDP-glucosyltransferases. - *Insect Biochemistry and Molecular Biology*, 25: 541-550.

REMBOLD H., G.K. SHARMA, T. GZOPPELT, H. SCHMUTTERER. 1982. Azadirachtin: A potent insect growth regulator of plant origin. – *Journal of Applied Entomology*, 93: 12-17.

ROTHMAN L.D., J.H. MYERS. 1996. Debilitating effect of viral diseases on host Lepidoptera. - *Journal of Invertebrate Pathology*, 67: 1-10.

SAS INSTITUTE INC. 2008. *NC SAS / STAT® 9.2 User's Guide*. Cary, SAS Institute Inc.

SCHMUTTERER H. 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. - *Journal of Insect Physiology*, 34: 713-719.

SHAPIRO M., J.L. ROBERTSON, R.A. BELL. 1986. Quantitative and qualitative differences in gypsy moth nuclear polyhedrosis virus produced in different-aged larvae. - *Journal of Economic Entomology*, 79: 1174-1177.

SHOREY H.H., R.L. HALE. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. - *Journal of Economic Entomology*, 58: 522-524.

SINGH R., B. SINGH, R.A. VERMA. 2001. Efficiency of different indigenous plant products as grain protectant against *Callosobruchus chinensis* L. on pea. - *Indian Journal of Entomology*, 63: 179-181.

SUBRAMANYAM B., N. RAMAKRISHNAN. 1981. Influence of a baculovirus infection on molting and food consumption by *Spodoptera litura*. - *Journal of Invertebrate Pathology*, 38: 161-168.

SUNDARARAJ R. 1997. Evaluation of neem seed oil and some insecticides against the babul whitefly *Acaudaleyrodes rachipora* (Singh) (Aleyrodidae: Homoptera) on *Acacia senegal* seedlings. - *Pestology*, 21: 34-37.

TIESSEN S. 2012. Indiana's most unwanted invasive plant pests, Indiana Co-operative Agricultural Pest Surveys Program (CAPS), Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval). [http://www.extension.entm.purdue.edu/CAPS/pestInfo/egyptLeafworm.htm]. Accessed: 2.7. 2012.

WAKIL W., M.U. GHAZANFAR, F. NASIR, M. ABDUL QAYYUM, M. TAHIR. 2012. Insecticidal efficacy of *Azadirachta indica*, nucleopolyhedrovirus and chlorantraniliprole singly or combined against field populations of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). - *Chilean Journal of Agricultural Research*, 72: 53-61.

WONDAFRASH M., E. GETU, G. TEREFE. 2012. Neem, *Azadirachta indica* (A. Juss) extracts negatively influenced growth and development of African bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). - *Academic Journal of Entomology*, 5: 22-27.

YANG M.M., M.L. LI, Y. ZHANG, Y.Z. WANG, L.J. QU, Q.H. WANG, J.Y. DING. 2012. Baculoviruses and insect pests control in China. - *African Journal of Microbiology Research*, 6: 214-218.

ZAMORA-AVILÉS N., J. ALONSO-VARGAS, S. PINEDA, J. ISAAC-FIGUEROA, P. LOBIT, M. MARTÍNEZ-CASTILLO. 2013. Effects of a nucleopolyhedrovirus in mixtures with azadirachtin on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae and viral occlusion body production. - *Biocontrol Science andTechnology*, 23: 521-534.

Received: 04.12.2014
Accepted: 22.12.2014

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 101-103

Short note

On the Gastropod Species Diversity of the "Izgoryaloto Gyune" Nature Reserve (Western Rhodopes, Bulgaria)

Dilian G. Georgiev*

Department of Ecology and Environmental Conservation, University of Plovdiv,
24 Tzar Assen Str., BG-4000 Plovdiv, BULGARIA
* Corresponding author: diliangeorgiev@abv.bg

Abstract. In the "Izgoryaloto Gyune" reserve (Bulgaria, West Rhodopes) there are a total of 24 known species of snails after present study. Two species (*Pomatias elegans* and *Cecilioides janii*) which occurrence in the West Rhodopes was considered as doubtful were registered on the territory of this protected area. Thirteen species were of conservation status: eleven species of all registered are included in the IUCN Red List for 2011 and two in the Bulgarian Biodiversity Act.

Key Words: malacofauna, protected area, Bulgaria.

Introduction

The malacofauna of the Bulgarian nature reserves is not well known. There is only one report of the species *Pomatias rivularis* (Eichwald, 1829) found in the "Izgoryaloto Gyune" Reserve in the paper of IRIKOV & MOLLOV (2006). Authors summarize all the literature available and represent a lot of new information on the terrestrial gastropod fauna in the West Rhodopes where the reserve is situated. It is placed on the slopes of the mountain along the Vacha River near Krichim town. The main habitats of the reserve are broad leaf forests on calcareous terrains.

In this short communication I report some finds of terrestrial and freshwater snail species registered during a project study on the biodiversity of the reserve in a relation to its future management plan.

Material and Methods

The material was collected on 06.09.2013. The snails were collected by hand or a sieve from various localities in the reserve in broad leaf forest dominated mainly by *Carpinus orientalis*, *Quercus* sp., and *Fagus sylvatica*. The localities coordinates were derived by a GPS.

Species were determined mainly by DAMJANOV & LIKHAREV (1975), WELTER-SCHULTES (2012), and a reference shell collection.

The conservation statute is according CUTTELOD *et al.* (2011), WELTER-SCHULTES (2012), and the Bulgarian Biodiversity Act (State Gazette, 2002).

Results

Class Gastropoda

Family Hydrobiidae

1. *Grossuana* cf. *angeltsekovi* Glöer & Georgiev, 2009: found in a shallow karst spring, N42.02281 E24.46898.

Family Pomatiasidae

2. *Pomatias rivularis* (Eichwald, 1829): Literature data: IRIKOV & MOLLOV (2006),

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

UTM-grid 10x10 km KG85: „28.VIII.2004, leg. D. Georgiev". Not found during present study.

3. *Pomatias elegans* (Müller, 1774): Literature data: IRIKOV & MOLLOV (2006) consider that this species is not known for sure to occur in the West Rhodopes: "This species was reported for the Rhodopes Mountain in general by DAMJANOV & LIKHAREV (1975), and later it was cited by HUBENOV (In: DELCHEV *et al.*, 1993). Unfortunately, so far there are no documented data for the actual presence of this species in the Western Rhodopes, so we assume that this is an invalid taxon for this part of the mountain". Present study proves that *P. elegans* is part of the land malacofauna of the West Rhodopes. The species was registered in great numbers the following localities: N42.03336 E24.47000, N42.03321 E24.46963, N42.03166 E24.46860, N42.02913 E24.47189, N42.02281 E24.46898.

Family Aciculidae

4. *Platyla similis* (Reinhardt, 1880): Locality N42.02256 E24.46946.

Family Vertiginidae

5. *Vertigo* sp.: Locality N42.02256 E24.46946.

6. *Truncatellina claustralis* (Gredler, 1856): Locality N42.02283 E24.47037, N42.02256 E24.46946.

7. *Truncatellina cylindrica* (J. Ferussac, 1807): Locality N42.03327 E24.47071.

Family Orculidae

8. *Pagodulina subdola* (Gredler, 1856): Locality N42.02256 E24.46946, N42.02281 E24.46898.

Family Valloniidae

9. *Acanthinula aculeata* (Müller, 1774): Locality N42.02256 E24.46946.

Family Enidae

10. *Chondrula microtragus* (Rossmässler, 1839): Locality N42.03327 E24.47071, N42.02685 E24.47028.

11. *Zebrina detrita* (Müller, 1774): Locality N42.03356 E24.47053, N42.03327 E24.47071, N42.03336 E24.47000, N42.03321 E24.46963, N42.02913 E24.47189, N42.02685 E24.47028, N42.02283 E24.47037.

Family Clausiliidae

12. *Alinda biplicata* (Montagu, 1803): Locality N42.03166 E24.46860.

Family Ferussaciidae

13. *Cecilioides janii* (De Betta & Martinati, 1855): Literature data: IRIKOV & MOLLOV (2006) considered that this species does not occur in the West Rhodopes: "This species is reported by DAMJANOV & LIKHAREV (1975) for the north part of the Rhodopes Mountain. As a result of our study and because of the absence of any other literary sources we think that this is an invalid taxon for the Rhodopes Mountain."

The species was found during present study. Locality coordinates N42.02283 E24.47037.

Family Euconulidae

14. *Euconulus fulvus* (Müller, 1774): Locality N42.02283 E24.47037, N42.02256 E24.46946.

Family Zonitidae

15. *Oxychilus glaber* (Westerlund, 1881): Locality N42.03166 E24.46860, N42.02283 E24.47037, N42.02281 E24.46898

16. *Vitrea neglecta* Damjanov & Pinter, 1969: Locality N42.03336 E24.47000.

17. *Vitrea contracta* (Westerlund, 1871): Locality N42.03321 E24.46963, N42.02256 E24.46946.

Family Helicidae

18. *Cattania rumelica* (Rossmässler, 1838): Locality N42.03336 E24.47000, N42.03321 E24.46963, N42.03166 E24.46860, N42.02283 E24.47037, N42.02256 E24.46946, N42.02281 E24.46898.

19. *Cepaea vindobonensis* (Ferussac, 1821): Locality N42.03321 E24.46963.

20. *Helix albescens* Rossmässler, 1839: Locality N42.03356 E24.47053, N42.03327 E24.47071, N42.02913 E24.47189, N42.02685 E24.47028, N42.02256 E24.46946.

21. *Helix lucorum* Linnaeus, 1758: Locality N42.02283 E24.47037.

Family Helicodontidae

22. *Lindholmiola girva* (Frivaldsky, 1835): Locality N42.03321 E24.46963, N42.03166 E24.46860.

Family Hygromiidae

23. *Xerolenta obvia* (Menke, 1828): Locality N42.03356 E24.47053, N42.03327 E24.47071, N42.02685 E24.47028.

24. *Euomphalia strigella* (Draparnaud, 1801) Locality N42.03336 E24.47000, N42.03321 E24.46963, N42.02913 E24.47189.

Conservation status of the species. In The IUCN Red List for 2011 in the category LC were included 11 species from all registered in the reserve: *Grossuana angeltsekovi*, *Platyla similis*, *Acanthinula aculeata*, *Chondrula microtragus*, *Cattania rumelica*, *Cepaea vindobonensis*, *Helix albescens*, *Helix lucorum*, *Lindholmiola girva*, *Xerolenta obvia*, *Euomphalia strigella* (CUTTELOD *et al*., 2011).

In the Bulgarian Biodiversity act (State Gazette, 2002), Annex 4 there were two species included: *Helix lucorum* and *Helix pomatia*.

Acknowledgements

The study was finances by project "Developing management plans for Chervenata Stena and Izgoryaloto Gyune" reserves coordinated by the Regional Environmental Inspection – Plovdiv and NGO "Prizma Nishava".

References

CUTTELOD A., SEDDON M., NEUBERT E. 2011. European Red List of Non-marine Molluscs. *IUCN Global Species Programme, IUCN Regional Office for Europe, IUCN Species Survival Commission*, 98 p.

IRIKOV A., MOLLOV I. 2006. Terrestrial gastropods (Mollusca: Gastropoda) of the Western Rhodopes (Bulgaria). - In: Beron P. (ed). *Biodiversity of Bulgaria. 3. Biodiversity of Western Rhodopes (Bulgaria and Greece) I.* Pensoft & Nat. Mus. Natur. Hist., Sofia, pp. 753-832.

State Gazette. 2002. *Biodiversity Protection Act of Bulgaria,* Nr 77 of August 9, 2002.

WELTER-SCHULTES F. 2012. European non-marine molluscs, a guide for species identification. *Planet Poster Editions*, Göttingen, 674 p.

Received: 20.10.2014
Accepted: 22.12.2014

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 105-107

Short note

Data on the Wintering of the Rook, Corvus frugilegus Linnaeus, 1758 (Aves: Corvidae) in Plovdiv, Bulgaria

*Dobromir Dobrev**, *Vladimir Dobrev, Georgi Gerdzhikov*

Bulgarian Society for the Protection of Birds, Leonardo da Vinci 5, 4000 Plovdiv, BULGARIA
*Corresponding author: dobromir.dobrev@bspb.org, dobromir.dobrev1@gmail.com

Abstract: In the current study we report a short summary on the wintering of the Rook in the town of Plovdiv. During our field study we estimated that no less than 29 706 ± 8722 Rooks are roosting in Plovdiv. Our findings revealed that the number of wintering individuals of this species has increased in a ten years period by 35 times in comparison to a previous study and reached a maximum in February when 59 280 Rooks were counted. Further investigation on that matter is needed so that the wintering pattern of the Rooks could be revealed entirely.

Key words: Rook, *Corvus frugilegus*, wintering, Plovdiv, Bulgaria.

The Rook is a widespread resident species across Europe where its population has been estimated at more than 10 000 000 of pairs. The Rook has an extremely large range. Although a decline in species population was registered in several countries, its European population is stable or slightly increasing BIRDLIFE INTERNATIONAL (2014). On the other hand the overall population trend appears to be decreasing, but the decline is not believed to be sufficiently rapid to approach the thresholds for "Vulnerable species" under the population trend criterion of IUCN and currently the species is listed as least concern. In Bulgaria the population is estimated to be stable with 15 000 – 25 000 breeding pairs VASILEV (2007). The Rook is unevenly distributed across the country, mainly concentrated in Northern Bulgaria, Tundzha river basin and Sofia district. The population development of the species in Bulgaria has been studied well and numerous data on its distribution is available. In the period following 2000 decrease in colonies has been observed because of the devastation of the riverside forests and forest-shelter belts VASILEV (2007). However, usually in September the Rook is roosting in parks in the bigger settlements, like Sofia, where some 10 000 individuals were registered to overwinter NANKINOV (1982A). At the same time between 80 000 and 100 000 individuals were found to be wintering in the northeastern part of Bulgaria VASILEV, NANKINOV (2003). Another study carried out in the mid-90 in southern Bulgaria revealed a large congregation of roosting rooks in the town of Plovdiv – 1700 individuals were counted on a roosting site in a city park KASHEROV (1995). The recent study aimed at defining the numbers of wintering rooks in the town of Plovdiv. The census was carried out between November 2006 and March 2007 in Plovdiv as the rooks were counted

 Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

from minimum of two stationary viewpoints BIBBY ET AL. (1999). Observations were conducted between 16:00 and 19:00 at CET once per month in the duration of the study period from naturally elevated features of the landscape (town hills), so that there was always a clear visibility to the surroundings of the town. Binoculars Nikon 10x50 and Swarovski scope 60x were used for the count and identification of the birds. Species composition, numbers and meteorological conditions were registered as well.

In the studied period six counts were conducted altogether. Except for January 2007 when two counts were done, rooks were counted once per month. The maximum number of rooks was established in February when 59 280 individuals were counted, while the minimum number was established in March – only 5481 individuals. Our results revealed that the number of wintering rooks in Plovdiv has increased immensely in comparison to the previous study KASHEROV (1995). Wintering rooks in Plovdiv equal to 1 to 6 % of the wintering population of the species in Bulgaria KOSTADINOVA, GRAMATIKOV (2007). The mean number of birds during the study period was 29 706 ± 8722. Baring in mind the total number of the birds, this is the largest known roosting congregation of rooks wintering in a settlement in Bulgaria. More, although the species is listed as resident to Bulgaria and most of Europe BIRDLIFE INTERNATIONAL (2014) individuals from neighboring countries could be wintering in the inner part of Bulgaria as well NANKINOV, DOBRININA (2002). Another contradiction considering the numbers is the large increase of the wintering rooks in only 10 years period (35 times), while an overall decrease of the population in Bulgaria after 2000 was registered VASILEV (2007). The higher numbers could be due to the juvenile birds as the species often occurs with much higher numbers after the breeding season PATEFF (1950) and/or to birds coming from Ukraine and Russia NANKINOV, DOBRININA (2002). In the current study the initial number of birds in the late November was 7542. Afterwards rooks' numbers gradually increased to a maximum of 59 280 individuals in February and then in only 20 days the numbers went to a minimum – 5481 birds in Mid-March. Our findings confirm the results of the previous study of the pattern of presence of the Rooks with peak numbers in February and decrease in March VASILEV, NANKINOV (2003), although the current study consisted of only 6 counts in comparison to the 15 counts from the previous one KASHEROV (1995). This could be explained by the worse weather conditions in the coldest months of the year and the snow coverage in northern latitudes when rooks are pushed to find food resources in the lowlands and at lesser latitudes. Additionally, inner parts of the towns have specific micro climate and could shelter species form the harsh weather conditions KASHEROV (1995). Rooks were flying mainly from two directions onward their roosts - western and eastern as the birds were almost equally distributed between these two directions. Quite often their flocks were mixed with the European jackdaw (Corvus monedula Linnaeus, 1758) which sometimes consisted of up to 50% of the flock. As the previous study has reported when the weather conditions were mild the birds were flying higher and arriving sometimes when completely dark at the roosting sites and the opposite scenario happened when the weather conditions were worse. Rooks were mainly roosting at Lauta Park and the surrounding space. Our observations seem to confirm that there is dependence between the duration of the day and the time of arrival of the rooks at the roosting site – in longer days the rooks were arriving later in time than in the short winter days when the light intensity was decreased SWINGLAND (1976); KASHEROV (1995). Although the breeding biology and distribution of the Rook in Bulgaria is well studied a little is known considering species wintering pattern. Wintering congregation of the Rook are known from other sites in Bulgaria VASILEV, NANKINOV (2003); (authors unpublished data), but until recently there is no detailed data about the pattern of wintering presence of the species at the roosting sites in the settlements and their surroundings. Due to the flock structure of the Rook our study could have even underestimated the number of the birds as more than 85 000 birds have been established in a stranded count (authors' unpublished data). If it appears that the whole breeding population congregates at a very few wintering and roosting sites, then it is of vital importance such sites to be identified and a number of measures to be taken in order to ensure suitable protection status of the species during wintering congregations.

Acknowledgments: We thank an anonymous review for the useful notes on the final version of the manuscript.

References

BIBBY C., M. JONES, S. MARSDEN. 1999. *Expedition Field Techniques. Bird Surveys - Royal Geographical Society,* London.

BIRDLIFE INTERNATIONAL. 2014. *Species factsheet: Corvus frugilegus.* Available at: [http://www.birdlife.org on 21/10/2014].

KASHEROV P. 1995. *Breeding biology, numbers dynamics and behaviour of the rook (Corvus frugilegus L.) in the wintering roosts in Plovdiv.* BCs Thesis, Faculty of biology, Plovdiv University. 13 p.

KOSTADINOVA I., GRAMATIKOV, M. 2007. *Important bird areas and NATURA 2000 in Bulgaria.* Bulgarian society for the protection of birds, Conservation series, Book 11, S. BSPB. 639 p. (In Bulgarian).

NANKINOV D. 1982. Birds of Sofia city. – *Orn. Inf. Bull.*, 12. 386 p. (In Bulgarian).

NANKINOV D., I. DOBRININA. 2002. *On the origin of rooks, wintering on the Balkans.* – BLBRDC 1988 – 1999, M., pp. 221–230.

PATEFF P. 1950. [*Birds in Bulgaria*]. S., BAS. 364 p. (In Bulgarian).

SWINGLAND I.R. 1976. The influence of light intensity on the roosting times of the rook (Corvus frugilegus). - *Animal behaviour,* 24(1): 154–158.

THE IUCN RED LIST OF THREATENED SPECIES. Version 2014.2. [www.iucnredlist.org]. Downloaded on 21 October 2014.

VASILEV V. 2007. The Rook. - In: Iankov, P. (Ed.): *Atlas of the breeding birds in Bulgaria.* BSPB Conservation Series, Book 10. – Bulgarian Society for the Protection of Birds, Sofia. (In Bulgarian).

VASILEV V., D. NANKINOV. 2003. Distribution of the rook *Corvus frugilegus* L. during winter months in northeastern of Bulgaria. – *SB. Scientific papers of the Shumen University "Ep. K. Preslavski", Biology*, 32–35 (In Bulgarian).

Received: 24.10.2014
Accepted: 22.12.2014

© Ecologia Balkanica

ECOLOGIA BALKANICA

2014, Vol. 6, Issue 2 December 2014 pp. 109-112

Short note

Level of Synanthropy of the Amphibians and Reptiles from the City Of Plovdiv (Bulgaria)

Ivelin A. Mollov*

University of Plovdiv, Faculty of Biology, Department of Ecology and Environmental Conservation, 24 Tzar Assen Str., 4000, Plovdiv, BULGARIA

* Corresponding author: mollov_i@yahoo.com

Abstract. The current study determines the level of synanthropy of the amphibians and reptiles in the city of Plovdiv, based on Nuorteva's Index of synanthropy, with slight modification, proposed here for the first time.

Key words: Amphibia, Reptilia, Synanthropy Index, Plovdiv, Bulgaria

In the conditions of the urban environment, some species undergo a process of synanthropization, i.e. adaptations and new mechanisms of regulation at population level to the new environment are formed (VERSHININ, 1987). According to the classification given by KLAUSNITZER (1987) there are four ecological groups of animals in subordination to their level of synanthropy: *hemerophobes* – species, which avoid urban environment; *hemerodiaphores* – species, which existence doesn't depend on the anthropogenic transformation of the landscape; *hemerophiles* – species, which prefer habitats made by humans and *synanthropes* – species, which are directly connected with habitats made by man and their existence depend on the human activity. Synanthropes on the other hand are *obligate* and *facultative*. Obligate synanthropes are species that occur in a (micro) climatic zone in anthropogenic conditions only in urban areas, usually within the human settlements and they do not or rarely occur elsewhere in nature. Facultative (optional) synanthropes are species found in urban areas and human settlements, where they find optimal conditions for existence, while they can form natural populations in natural biotopes.

Based on species distribution along the urban gradient and field observations an attempt for classification of the amphibians and reptiles in Plovdiv was made in previous studies (MOLLOV, 2005; 2011). That classification showed that from amphibians there are no species that can be classified as synanthropic. One species (*B. viridis*) was considered "hemerophilic", because it occurs mainly in urban and suburban areas of the city and has high ecological plasticity that allows it to occur in a variety of habitats (polytopic species). Two species (*H. arborea* and *P. ridibundus*) were also polytopic, but they were found in all three urban zones (urban, suburban and rural) and that's why they were considered "hemerodiaphoric", as *P. syriacus* was also added to this category. Two species (*B. bufo* and *R. dalmatina*) were

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

registered only in the suburban and rural areas, only in few habitat types (stenotopic), and they were classified as "hemerofobic" (MOLLOV, 2011). Only one reptile species (*M. kotschyi*) showed the characteristics of a typical synanthrope - polytopic species inhabiting a wide range of anthropogenically created urban habitats, some of which are unsuitable for habitation for all other reptiles. One species (*P. tauricus*) was classified as "hemerophilic", although it is found in all three areas, the numbers in the central urban part was significantly higher. Four species (*L. viridis, E. orbicularis, N. natrix* and *D. caspius*) were registered in all three zones of the city (with the exception of *N. natrix,* which is absent from the urban center) and were polytopic, occurring in a wide range of urban habitats (except *D. caspius,* which has a special preference for the habitats and is stenotopic species), which makes them "hemerodiaforic". Two species (*L. trilineata* and *N. tessellata*) were absent from the central zone and occur in several urban habitats (stenotopic species) and were classified as "hemerophobic".

Later PULEV & SAKELARIEVA (2013) made similar classification, based on the same criteria, for the amphibians and reptiles of Blagoevgrad (South-West Bulgaria). However, no quantification criteria was used in these classifications.

One known techniques for numerical expression of the level of synanthropy of a species is the Index of synanthropy (SI) proposed by NUORTEVA (1963), allowing more precise classification on a species level, whish even divides obligate and facultative synanthropes:

$$SI = \frac{2a+b-2c}{2},$$

where: a – percentage (%) of the individuals in the urbanized area (human settlements), b – percentage (%) of the individuals in the agricultural areas, c – percentage (%) of the individuals in biotope, little affected by anthropogenic influence.

The Index of Synanthropy has values from +100 to –100, where:

+100 – Full preference to densely populated urban areas and human settlements;

+75 – Clear preference to urban areas and human settlements;

+50 – Slight preference to urban areas;

0 – Indifferent to urban areas and human settlements;

-25 – Preference to non-populated areas;

-50 – Avoidance of urban areas and human settlements;

-75 – Clear avoidance of urban areas and human settlements;

-100 – Complete absence in urban areas.

This index has been successfully applied to determine the level of synanthropy of dipterous insects (FORATTINI *et al.*, 1993; VIANNA *et al.*, 1998; MARÍ & JIMÉNEZ-PEYDRÓ, 2011), spiders (VALESOVA-ZDARKOVA, 1966; SACHER, 1983), birds (NUORTEVA, 1971) and others. The Index may have different values for the same group of animals in different cities at different altitudes and in different latitude. However, in the proposed by Nuorteva index, some difficulties in determining the so-called "urbanized area", "agricultural areas" and "biotope, little affected by anthropogenic influence" occur and each author interpreted them differently and used the Index with some qualifications and modifications. For example, in the case of Plovdiv, the Plovdiv hills may be characterized as "biotope, little affected by anthropogenic influence", but at the same time they are in the city center, which is an urban area. Maritsa River passes through the entire length of the city and passes through urban, agricultural and less affected by anthropogenic impact areas. More so the given definitions are also not clear about the size and the boundaries of these areas. Therefore, we propose some changes to the explanations of the Nuorteva's formula, based on the concept of the urban gradient (MCDONNELL & PICKETT, 1990; MCDONNELL *et al.,* 1997). We offer the formula to be used for the three urban zone, along the urban gradient. The "urban" zone (the central urban parts) to be used, instead of "urbanized area" (marked "a" in the formula); the "suburban" zone (suburbs) to be used, instead of "agricultural areas" (marked as "b" in the formula) and the "rural" zone can be used instead of "biotope, little affected by anthropogenic impact" (marked as "c" in formula). In our opinion, the proposed explanations do not change the meaning of the Index of synanthropy, but bring more clarity in the definition of the three zones. Depending of the peculiarities of each city, the three zones from the urban gradient, in most cases are more easily identifiable for each city and more precisely defined. For this reason, their use in the formulation of Nuorteva's formula will be much more efficient, without changing the meaning of the Index of synanthropy.

For the identified amphibians and reptiles in Plovdiv (MOLLOV, 2011), for the first time in the current study, the Index of synanthropy was calculated and the species were categorized into ecological groups, depending on their level of synanthropy (Table 1), as proposed by KLAUSNITZER (1987). The Index of Synanthropy confirmed our previous classification of the species in four environmental groups with slight changes. We recommend using Nuorteva's index (with our proposed modification) as a measure of classification of species according to their level of synanthropy (based on Klausnitzer's classification), with the following values: SI = 100 - obligate synantropes; SI = 90 ÷ 99 - facultative synantropes; SI = 51 ÷ 89 - hemerophiles; SI = -50 ÷ 50 – hemerodiafores; SI = -100 ÷ -49 - hemerophobes. We believe that this proposed scale can be successfully applied to other groups of animals as different groups are likely to require some modifications, depending on their environmental peculiarities and characteristics. Our hypothesis can be tested with the conduct of other future similar studies with different organisms in an urban environment.

References

FORATTINI O.P., I. KAKITANI, E. MASSAD, D. MARUCCI. 1993. Studies on mosquitoes (Diptera: Culicidae) and anthropic environment. A - Survey of resting adults and synanthropic behaviour in South-Eastern, Brazil. - *Revista de Saúde Pública*, 27(6): 398-411.

KLAUSNITZER B. 1987. *Ökologie der Großstadtfauna,* Stuttgart/New York: Fischer, 225 p.

MARÍ P.B., R. JIMÉNEZ-PEYDRÓ. 2011. Differences in mosquito (Diptera: Culicidae) biodiversity across varying climates and land-use categories in Eastern Spain. - *Entomologica Fennica,* 22: 190-198.

MCDONNELL M.J., S.T.A. PICKETT, P. GROFFMAN, P. BOHLEN, R.V. POUYAT, W.C. ZIPPERER, R.W. PARMELLE, M.M. CARREIRO, K. MEDLEY. 1997. Ecosystem processes along an urban-to-rural gradient. – *Urban Ecosystems,* 1: 21-36.

MCDONNELL M.J., S.T.A. PICKETT. 1990. Ecosystem structure and function along urban-rural gradients: an unexploited opportunity for ecology. – *Ecology,* 71(4): 1232-1237.

MOLLOV I. 2005. A study on the amphibians (Amphibia) and reptiles (Reptilia) in three urban protected areas in the town of Plovdiv (South Bulgaria). – *Scientific Studies of the University of Plovdiv - Biology, Animalia,* 41: 79-94.

MOLLOV I. 2011. Habitat distribution of the amphibians and reptiles in the city of Plovdiv. - *Biharean Biologist,* 5(1): 25-31

NUORTEVA P. 1963. Synanthropy of blowflies (Diptera, Calliphoridae) in Finland. - *Annales Entomologici Fennici,* 29: 1-49.

NUORTEVA P. 1971. The synanthropy of birds as an expression of the ecological cycle discorder caused by urbanization. - *Annales Entomologici Fennici*, 8: 547-553.

PULEV A., L. SAKELARIEVA. 2013. Herpetofauna in the city of Blagoevgrad, SW Bulgaria. - *BioDiscovery,* 7(3): 1-6.

SACHER P. 1983. Spinnen (Araneae) an und in Gebäuden. — Versuch einer Analyse der synanthropen Spinnenfauna in der DDR. - *Entomologische Nachrichten, Berlin,* 27: 97-204.

VALESOVA-ZDARKOVA E. 1966. Synanthrope Spinnen in der Tschechoslowakei. - *Senckenbergiana Biologica*, 47: 73-75.

VERSHININ V. 1987. [Adaptive peculiarities of the Pool frog populations in the environment of the big city] - *Ecologiya,* 1: 46-50 (In Russian).

VIANNA E.E.S., J.G.W. BRUM, P.B. RIBEIRO, M.E.A. BERNE, P. SILVEIRAJR. 1998. Synanthropy of Calliphoridae (Diptera) in Pelotas, Rio Grande Do Sul State, Brazil. – *Brazilian Journal of Veterinary Parasitology*, 7(2): 141-147.

Received: 11.11.2014
Accepted: 22.12.2014

Table 1. Level of synanthropy of the amphibians and the reptiles in the city of Plovdiv, based on SI values.

Description	Scale SI	Ecological group	Species and SI value
Full preference	+100	**Synanthropes**	*Mediodactylus kotschyi* (93.72)
to densely populated		(obligate)	
urban areas and settlements		(facultative)	
			Bufo viridis (89.82)
Clear preference	+75		*Podarcis tauricus* (89.63)
to urban areas and		**Hemerophyles**	
human settlements			
Slight preference	+50		
to urban and			
human settlements			
			Hyla arborea (34.85)
			Emys orbicularis (32.14)
	+25		
			Dolichophis caspius (7.14)
Indifference to	0		
urban areas		**Hemerodiaphores**	*Pelophylax ridibundus* (-3.68)
Slight avoidance	-25		*Pelobates syriacus* (-25.00)
of urban areas			
			Natrix natrix (-38.89)
			Lacerta viridis (-49.12)
Avoidance of	-50		*Bufo bufo* (-50.00)
urban areas and			*Natrix tessellata* (-50.00)
human settlements			
Clear avoidance of	-75	**Hemerophobes**	
urban areas and			
human settlements			
Complete absence			*Rana dalmatina* (-100)
from urban areas	-100		*Lacerta trilineata* (-100)

ECOLOGIA BALKANICA
2014, Vol. 6, Issue 2 December 2014 pp. 113-117

Short note

Effect of Some Environmental Factors on the Toxicity of Azadirachtin to the Egyptian Cotton Leafworm Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae)

El-Sayed H. Shaurub, Afaf Abd El-Meguid, Nahla M. Abd El-Aziz*

Department of Entomology, Faculty of Science, Cairo University, Giza, EGYPT
* Corresponding author: sayedshaurub@yahoo.com

Abstract. Although the bio-degradation of azadirachtin was well studied under storage or environmental conditions, its toxicity was not characterized so far under the impact of environmental conditions. Therefore, the present study aims to elucidate the toxicity of azadirachtin, at the level of LC_{50}, to the fourth larval instars of *Spodoptera littoralis* (Boisduval) under the stress of temperature, ultraviolet radiation (365 nm) (UV-A) and of artificial sunlight. The increase in post-treatment temperatures insignificantly affected azadirachtin toxicity. Likewise, insignificant change was also attained for the increase in the exposure periods of both UV and sunlight, except for exposure to sunlight without UV filter starting from 10 h, where the toxicity of azadirachtin was significantly declined. It can be concluded that azadirachtin toxicity will last efficiently against *S. littoralis* larvae under the stress of the environmental factors prevailing during the season of cotton cultivation in Egypt.

Key words: Azadirachtin, *Spodoptera littoralis*, sunlight, temperature, toxicity, ultraviolet.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) is the major cotton pest in Egypt. The use of synthetic pesticides during the last half century has often been careless and indiscriminate which resulted in malicious effects on the environment and leads to "ecological backlash" (SUNDARARAJ, 1997). Concern about this has led to a surge of research into alternative pest control approaches. One of the efforts is the development of botanical insecticides as a novel and safer strategy. Azadirachtin is one of the most promising natural compounds of plant origin. It is a tetranortriterpenoid isolated from the seeds of the neem tree, *Azadirachta indica* A. Juss (Meliaceae) acting as an insect growth regulator (REMBOLD *et al.*, 1982), and displayed insecticidal activity to nearly 550 insect species including lepidopteran ones (DEBASHRI & TAMAL, 2012; HUMMEL *et al.*, 2012). Moreover, azadirachtin seems to be selective, non-mutagenic, readily degradable, with low toxicity to non-target and beneficial organisms and causes minimal disruption to ecosystem (SUNDARAM, 1996). Recently, RAIZADA *et al.* (2001) proved that azadirachtin did not produce any signs of toxicity, mortality, and change in tissue weight, pathogenicity and serum blood parameters to mammals.

Most studies dealt with the bio-degradation of azadirachtin under storage or environmental conditions (SCHMUTTERER, 1988; SUNDARAM, 1996; RAM *et al.*, 2001; RADWAN & EL-SHIEKH, 2012). However, there are no studies dealt directly with its toxicity under the influence of environmental factors. Therefore, the

© Ecologia Balkanica
http://eb.bio.uni-plovdiv.bg

present study aims to explore the toxicity of azadirachtin to *S. littoralis* fourth larval instars under the stress of temperature, ultraviolet radiation (UV-A) and artificial sunlight.

Material and Methods

Insects. A susceptible strain of *S. littoralis* was established from egg masses obtained from Faculty of Agriculture, Cairo University, Egypt. Newly hatched larvae were transferred into plastic boxes (18×12×10 cm) containing about 0.5 cm thick semi-synthetic diet on which larvae was fed (SHOREY & HALE, 1965). The pupae were sexed and placed in sterilized containers (18× 15×30 cm) for adult emergence. The containers were lined with tissue paper as an ovipositing stratum. Adult moths were fed 10% sugar solution. Insects were reared under laboratory conditions of 25-27°C, 65-70% RH and 12h: 12 h (L: D) photoperiod.

Azadirachtin. Azadirachtin (96% purity) was obtained as a powder from Carl Roth GmbH + Co. Kg. A stock solution (200 ppm) was prepared in sterile distilled water and stored at 4°C till use.

Bioassay. A preliminary experiment was carried out to determine the toxicity of azadirachtin against the fourth larval instars of *S. littoralis*. Six serial concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm) of azadirachtin-acetone solution were prepared. One ml of each concentration was poured onto the surface of the same semi-synthetic diet used for rearing, thoroughly swirled and air-dried. The treated diet was offered for 24 h to newly-molted fourth larval instars, pre-starved for 4 h. Then, the larvae were fed on fresh non-treated diet until pupation or death. The control experiment consisted of larvae fed on semi-synthetic diet treated with distilled water only. Each concentration was repeated three times of 50 larvae each (Total n = 150 larvae). All the experiments were incubated at 25°C. The percentage of mortality was recorded.

Temperature. Five groups (50 larvae each) of newly molted fourth larval instars of *S. littoralis* were fed on semi-synthetic diet of a surface area of 500 mm^2 treated with one ml of 23.15 ppm azadirachtin-acetone solution (LC_{50}). These groups were kept respectively at five controlled temperature regimes (15, 20, 25, 30 and 35°C). The control experiments consisted of larvae fed on a diet treated with acetone only and kept also at the same tested temperatures. Larval mortality was observed until pupation or death for determining the toxicity of azadirachtin. Each experiment was replicated three times.

UV radiation. Sunlight-UV was simulated using a series of Vilber-Lourmat T-15 LN fluorescent lamps, which primarily radiate energy in the UV-A (315-400 nm) and UV-B range (280-315 nm), with a peak at 365 nm (IGNOFFO *et al.*, 1997). These lamps were held into a heat-controlled chamber (20°C). Eight glass Petri dishes containing one ml of the LC_{50} of azadirachtin-acetone solution each were respectively irradiated for 2, 5 and 10 min, and for 1, 3, 5, 10 and 24 h. Azadirachtin solutions were set at a vertical distance of 30 cm from T-15 LN UV lamps. Another eight dishes, each containing one ml of the LC_{50} of azadirachtin-acetone solution covered with aluminum foil were used as the shielded samples. These dishes were then exposed to the same previous exposure periods. Azadirachtin-acetone solution, which was not exposed to UV and the non-azadirachtin sample (non-treated control) were also run. After exposure to UV radiation, all azadirachtin samples (non-shielded exposed, shielded exposed, non-exposed and non-treated control samples) were then dispersed onto the surface of the semi-synthetic diet for determining their toxicity against newly molted fourth larval instars of *S. littoralis*. Each experiment was repeated three times of 50 larvae each.

Sunlight radiation. The sunlight radiation experiments were carried out using a sunlight simulator (ELDONET), which measures solar or artificial radiation in three biological important spectral bands: UV-B (280-315 nm), UV-A (315-400 nm) and PAR (photosynthetic active radiation, 400-700 nm). Azadirachtin-acetone solutions, at the level of LC_{50}, were exposed to sunlight for 1, 3, 5, 7, 10 and 12 h. One ml was used for each exposure period. The samples were surrounded by ice to avoid the increase of the temperature absorbed by the sample solution, and set at a vertical distance of 30 cm from the light source. Non-exposed and non-treated samples were prepared. After exposure to sunlight, all azadirachtin-acetone samples were dispersed onto the semi-synthetic diet surface for determining their toxicity against newly molted fourth larval instars of *S. littoralis*. Each experiment was repeated three times of 50 larvae each. The same previously described procedures were also carried out, except for the use of UV filter to eliminate UV radiation.

Statistical analysis. The percentage of mortality of treated larvae was corrected against that of non - treated ones (control) using Abbott's formula (ABBOTT, 1925) as follows: Corrected mortality (%) = (% Observed mortality - % Control mortality/100 - % Control mortality) 100. The corrected mortality was then subjected to propit analysis (FINNEY, 1971), from which the LC_{50} was estimated. The toxicity bioassay was determined with POLO–PC software (LEORA SOFTWARE, 1987).

Data were analyzed by one–way analysis of variance (ANOVA) using Costal Statistical Software (Cohort Software, Berkeley). When the ANOVA statistics were significant (at $p<0.05$), the means were compared by Duncan's multiple range test (SAS INSTITUTE INC., 2008).

Results and Discussion

The tested temperatures insignificantly affected the toxicity of azadirachtin against *S. littoralis* larvae, where the percentage of mortality remained approximately around the 50% (> 44 to ≤ 50%) (Table 1). Whereas, it was found that heat storage at 72 ± 2°C for 3 days decreased the insecticidal activity of commercial neem oil against *S. littoralis* larvae (RADWAN & EL-SHIEKH, 2012). They speculated that as the commercial neem formulations contain not only azadirachtin, but also other minor potential bioactive liminoids, the insecticidal effect of the preparation is more complex than that of the pure azadirachtin. Moreover, heat storage reduced significantly azadirachtin bioactivity against *Spodoptera litura* larvae compared to the refrigerated storage (RAM *et al.*, 2001).

Table 1. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different temperature regimes.

Temperature °C	% Mortality ± SE
15	48.01 ± 4.2[a]
20	49.44 ± 3.9[a]
25	44.50 ± 4.2[a]
30	47.21 ± 5.7[a]
35	49.92 ± 2.8[a]

Each experiment was repeated three times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

The present results indicate that the toxicity of azadirachtin against *S. littoralis* larvae was insignificantly affected by exposure to UV radiation (UV-A) up to 24 h in both shielded and non-shielded samples, where the percentage of larval mortality was around 50% (Table 2). In comparison, at least 200 h of exposure to UV radiation was necessary to significantly reduce the biological activity of azadirachtin (BARNBY *et al.*, 1989).

Table 3 shows that exposure of azadirachtin to sunlight up to 12 h using a UV filter insignificantly affected its toxicity to *S. littoralis* fourth larval instars. The same pattern was also attained up to 7 h of exposure without using a UV filter, whereas toxicity of the same samples exposed to 10 and 12 h was significantly decreased by about 11.2 and 17.8 %, respectively. These results are more or less similar to the findings of JOHNSON *et al.* (2003) which indicated that the biological activity of azadirachtin had been retained even after 30 days of exposure to sunlight when some stabilizers were added. Also, SCHMUTTERER (1988) reported that the effect of azadirachtin-containing extracts normally lasts about 4-8 days under field conditions.

Table 2. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different exposure periods of ultraviolet radiation (UV-A) (365 nm)

Exposure time	% Mortality ± SE	
	Non-shielded samples	Shielded samples
0 min	48.8 ± 8.6[a]	50.0 ± 3.7[a]
2 min	49.4 ± 6.7[a]	50.0 ± 3.4[a]
5 min	48.8 ± 8.6[a]	50.0 ± 2.9[a]
10 min	48.3 ± 7.1[a]	49.4 ± 3.0[a]
1 h	50.0 ± 9.3[a]	50.0 ± 4.1[a]
3 h	48.9 ± 6.1[a]	47.0 ± 3.9[a]
5 h	50.0 ± 5.3[a]	48.9 ± 2.4[a]
10 h	47.7 ± 4.0[a]	50.0 ± 2.9[a]
24 h	48.3 ± 2.5[a]	50.0 ± 3.7[a]

Each experiment was repeated 3 times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

Table 3. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different exposure periods of artificial sunlight

Exposure time (h)	% Mortality ± SE	
	Without UV filter	With UV filter
0	50.0 ± 4.7[a]	50.0 ± 3.7[a]
1	50.0 ± 5.1[a]	50.0 ± 2.6[a]
3	48.9 ± 4.9[a]	50.0 ± 1.1[a]
5	48.8 ± 3.6[a]	50.0 ± 2.1[a]
7	47.7 ± 2.3[a]	50.0 ± 3.6[a]
10	44.4 ± 1.7[b]	50.0 ± 1.7[a]
12	41.1 ± 3.7[b]	49.4 ± 1.3[a]

Each experiment was repeated 3 times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

Conclusions

The present study evaluated the toxicity of azadirachtin under the stress of some environmental factors which are comparable to those prevailing during the season of cotton cultivation in Egypt. The findings the toxicity of azadirachtin was not affected up to 35°C and up to 24 h of exposure to UV in both shielded and non-shielded samples indicate that its toxicity against *S. littoralis* larvae will last effective under the temperature prevailing during the season of cotton cultivation in Egypt (27-35°C), and at any time of the photophase.

Acknowledgements

The authors are so grateful to Prof. Dr. Tarek El-Tayeb, National Institute of Laser Sciences, Cairo University, for his help to operate the sunlight simulator during the experimental work.

References

ABBOTT W.S. 1925. A method of computing the effectiveness of an insecticide. - *Journal of Economic Entomology*, 18: 265-267.

BARNBY M.A., R.B. YAMASAKI, J.A. KLOCKE. 1989. Biological activity of azadirachtin: Three derivatives, and their ultraviolet radiation degradation products against tobacco budworm (Lepidoptera: Noctuidae) larvae. - *Journal of Economic Entomology*, 82: 58-63.

DEBASHRI, M. & M. TAMAL. 2012. A review on efficiency of *Azadirachta indica* A. Juss based biopesticides: An Indian perspective. - *Research Journal of Recent Sciences*, 1: 94-99.

FINNEY D. J. 1971. *Probit Analysis*, third ed. London. Cambridge University Press. 333 p.

HUMMEL H.E., D. F HEIN, H. SCHMUTTERER. 2012. The coming of age of azadirachtin and related tetranortriterpenoids. - *Journal of Pesticides*, 5: 82-87.

IGNOFFO C.M., C. GARCIA, S.G. SAATHOFF. 1997. Sunlight stability and rain-fastness of formulations of baculovirus *Heliothis*. - *Environmental Entomology*, 26: 1470-1474.

JOHNSON S., P. DUREIA, S. DHINGRA. 2003. Photostabilizers for azadirachtin A (a neem-based pesticide). - *Journal of Environmental Science and Health*, 38: 451- 462.

LEORA SOFTWARE. 1987. *POLO – PC: A User's Guideline to Probit or Logit 786 Analysis*. LeOra Software.

RADWAN O.A., Y.W.A. EL-SHIEKH. 2012. Degradation of neem oil 90% EC (Azadirachtin) under storage conditions and its insecticidal activity against cotton leafworm, *Spodoptera littoralis*. - *Researcher*, 4: 77-83.

RAIZADA R.B., M.K. SRIVASTAVA, R.A. KAUSHAL, R.P. SINGH. 2001. Azadirachtin, a neem biopesticide: Subsequent toxicity assessment in rats. - *Food and Chemical Toxicity*, 39: 473 – 483.

RAM N., B. S. PAMAR, R. NIWAS. 2001. Degradation and bioactivity of azadirachtin A and dihydroazadirachtin A-based neem (*Azadirachta indica* A. Juss) formulations. - *Pesticide Research Journal*, 13: 66-73.

REMBOLD H., G. K SHARMA, C. GZOPPELT, H. SCHMUTTERER. 1982. Azadirachtin: A potent insect growth regulator of plant origin. - *Journal of Applied Entomology*, 93: 12-17.

SAS INSTITUTE INC. 2008. *SAS / STAT® 9.2 User's Guide*. Cary, NC. SAS Institute Inc.

SCHMUTTERER H. 1988. Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. - *Journal of Insect Physiology*, 34: 713-719.

SHOREY H. H., R.L. HALE. 1965. Mass rearing of the larvae of nine noctuid species on a simple artificial medium. - *Journal of Economic Entomology*, 58: 522-524.

SUNDARAM K.M.S. 1996. Azadirachtin biopesticide: A review of studies conducted on its analytical chemistry, environmental behaviour and biological effects. - *Journal of Environmental Science and Health*, 31: 913-948.

SUNDARARAJ R. 1997. Evaluation of neem seed oil and some insecticides against the babul whitefly *Acaudaleyrodes rachipora* (Singh) (Aleyrodidae: Homoptera) on *Acacia senegal* seedlings. - *Pestology*, 21: 34-37.

Received: 04.12.2014
Accepted: 22.12.2014

LILYANA DIMITROVA YURUKOVA (1949 – 2014)

It is with great sadness that on August 22th, 2014 we lost Prof. Dr. Lilyana Yurukova.

Dr. Yurukova was a professor at the Institute of biodiversity and ecosystem research (ex Institute of Botany), Bulgarian Academy of Sciences, Sofia, and has been an active and valuable member of the biological scientific community in Bulgaria. She will be greatly missed by family, friends, and colleagues.

Lilyana Yurukova was born 27 May 1949 in Sofia, Bulgaria. She received Ph.D. in 1986 in the field of Ecology and Ecosystem Protection. In 1972 she became a chemist at the Institute of Botany, in 1977 a leader of the Analytical laboratory, in 1987 a research fellow, in 1996 associate professor and in 2012 professor. She also held teaching position at Plovdiv University from 1994.

Prof. Yurukova was an editor of Section Environmental Contamination - Ecosystems in Quintessence, USA. She had been an active member in several professional groups including Bulgarian Phytocoenological Society, INTECOL (International Association for Ecology), EURASAP (European Association for the Science of Air Pollution), Bulgarian branch of IHSS (International Humic Substances Society) and IAD (International Association for Danube Research).

She was regular reviewer of articles in 11 international journals (Atmospheric Pollution, Bulletin of Environmental Contamination and Toxicology, Environmental Monitoring and Assessment, Water, Soil and Air Pollution, Science of the Total Environment, etc.).

Her commitment and contribution to the European moss survey (UNECE ICP Vegetation) was acknowledged.

Prof. Yurukova was an organizer of schools on biomonitoring in 2 colleges and 1 university in Northeastern Greece in the period 2009-2012.

She published numerous journal articles, book chapters and reports, being co-author and author of 170 publications. During the last 15 years Prof. Yurukova has worked on 23 projects. She supervised 11 M.Sc. and 5 Ph.D. students.

Prof. Yurukova' work on the biomonitoring was distinguished by enormous breadth and scope. She has made basic contributions to understanding the pollution monitoring with mosses, lichenized fungi, macromycetes, vascular plants, animal tissues and organs, as well as to ecotoxicological effects of heavy metals and toxic elements in edible mushrooms and bee products.

As her Ph.D. student, colleague and friend I would like to note that she was an exceptional teacher and devoted researcher. Lilyana Yurukova was one of the most courageous, inspiring and stimulating people ever.

GANA M. GECHEVA