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Study on the Changes of Biotic Variables, Their Influence on the Primary Productivity and the Effect of Manuring of Fish Ponds

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Abstract. The experiment was carried out in the period 2004-2006 on seven fish ponds of the Institute for Fishery and Aquaculture in Plovdiv city, Bulgaria with a single area varying from 1.8 to 3.9 daa. Powered fertilization with cattle manure on dose 3000 kg.ha^{-1} was applied. The aim of the experiment was to study the relations between the biotic factors (zooplankton, phytoplankton, zoobenthos, chlorophyll *a*, macrophytes, bacterioplankton, percent of energy utilization by primary productivity (PEU), their influence on the primary productivity and the effect of manuring on the fish ponds. The bigger part of the biotic factor variation was defined by the differences between the monthly samplings compared to the changes between the ponds within the period of investigation (2004/05/06). There was a difference between the first and last months concerning the biotic factors. This great seasonal variability decreases the opportunity for revealing the differences between the manured and the control ponds. The biotic factors as the PP (primary productivity), respiration and PEU in the manured ponds were higher compared to the control ponds during 2005. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds. Increased biotic variable values of the PP, respiration and chlorophyll *a* were found in the manured ponds during 2006. The derived relationships might contribute for enhanced productivity of carp ponds and for improvement of existing management practices in the view of better water quality in fish farming.

Key words: primary productivity, carp fish ponds, biotic environmental factors.

Introduction

Despite the fact that fishponds are not distinguished to support high species diversity, their organisms sustain the main trophic levels. The abiotic environmental factors and biotic components determine the behavior and vital activity of the living organisms in these ponds. The bio-productivity in the fishponds is one of the main biotic characteristics of pond water being a natural source of food in the fishponds. There are a lot of intermediate stages, biological and biochemical processes between the bio-productivity and the final production of the fishpond – the fish (Noriega-Curtis, 1979). The fishpond is a specific artificial aquatic ecosystem, where these interactions take place. Photosynthesis is the basic source for the increasing of oxygen concentration and saturation in the aquatic ecosystems. Its contribution is comparable to the atmospheric aeration. At „bottom-up" manipulations (i.e. manuring) a positive correlation is observed between

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nutrient concentrations on one side and phyto- and zooplankton on the other, which indicates that high nutrient concentrations caused high primary productivity and finally high fish yield (DELINCE, 1992).

The aim of the study was investigation of the relations between the biotic factors, their influence on the primary productivity and the effect of manuring on fish ponds. In this context basic environmental factors and biotic components in manured and control ponds were studied.

Materials and Methods

The institute of Fishery and Aquaculture, Plovdiv is located in the western part of the Upper Thracian valley. The region is characterized by a transitional-continental climate. The study was carried out in the Plovdiv basis during three consecutive years (2004, 2005 and 2006). The ponds are supplied with water from "Maritsa" River by means of "Eni-Ark" irrigation canal. Seven earthen ponds were involved in the experiment, which individual size varies between 1.8 and 3.9 daa (Table 1). According to ZHANG *et al.* (1987) the ponds of this size are among the most productive and easy for management. Their bottom is silty, but the periphery and the shallowest parts of some of them have a strip of 1-2 m width with increased content of sand.

The pond size, their shallowness, prevailing vertical and horizontal homogeneity are part of the preconditions for choosing them for model object.

About 3000 kg.ha^{-1} mineralized manure once in April each year was applied to ponds No 6, 12 and 17. The ponds No 8, 15, 16 and 18 were used as control ponds without manuring. Additionally to the natural food grain forage was given to the fish according to a scheme related to their seasonal growth rate. The periodical examination did not reveal any fish diseases.

The applied polycultural technology (NIKOLOVA *et al.*, 2008a, b) included mixed breeding of 30 individuals of daa one-year old bighead carp (T_1), (*Aristhichthys nobilis* Richardson, 1845), 50 individuals of daa one-year old common carp (K_1), (*Cyprinus carpio* Linnaeus, 1758) and 10 individuals of daa grass carp (one and two-year old) ($A_{1/2}$), (*Ctenopharyngodon idella* Valenciennes, 1844).

The samples were taken from a station localized 1-2 m away from the shore before the outlet device (savak) of each fishpond. The sampling was carried out fortnightly between 8:30 and 11:00 a.m. from May to September (2004, 2006) and from June to September (2005). The final sampling was carried out in the last decade of September. Due to the large number of investigated characteristics some of the samples were taken with one to three days difference. All the samples were taken from the surface layer (0.3-0.5 m depth) according to the Bulgarian and European standards (e.g. EU Water Framework Directive 2000/60/EC).

We studied the following biotic factors: PP, zooplankton, phytoplankton, zoobenthos, chlorophyll *a*, macrophytes, bacterioplankton, PEU (Table 2). The total solar radiation in MJ m^{-2} was recorded by pyranometer type M 80 M manufactured in the former Soviet Union. The PP (g.O_2.m^{-2}.24 h^{-1}) was determined by a light/dark bottle technique in its oxygen modification (VOLLENWEIDER *et al.*, 1969).

Table 1. Schedule of the ponds included in the experiments 2004, 2005 and 2006 year (pond number and area in daa is shown in the brackets).

Year	Variants of breeding	
	Manured ponds	Control ponds
2004	6 (3.8) 17 (2.6)	8 (3.8) 16 (2.7) 15 (3.1) 18 (1.8)
2005	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)
2006	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)

Table 2. The methods applied for studying biotic variables in the current study.

Zooplankton	The zooplankton numbers were calculated by the method of DIMOV (1959). The biomass quantity was obtained by the volume-weight method of PRIKRYL (1980).
Phytoplankton	The phytoplankton was qualitatively determined, quantitatively counted and biovolume calculated afterwards (LAUGASTE, 1974).
Zoobenthos	The zoobenthos was first dried on filter paper and then weighted in order to obtain the biomass.
Chlorophyll *a*	The chlorophyll *a* was extracted and measured after ISO 10260 (1992).
Macrophytes	The macrophyte cover was visually estimated as percentage of total aquatic area.
Bacterioplankton	The bacterioplankton was microscopically determined by direct counting after RAZOUMOV (1932) in its contemporary modification of NAUMOVA (1999), and biomass after STRASKRABOVA *et al.* (1999).
PEU,%	The Percent of solar Energy Utilized by phytoplankton gross primary production was calculated as ratio of released oxygen converted into joules after ABAKOUMOV (1983) to measured solar energy multiplied by 100.

Firstly, the pond water was taken and homogenized in a 10 l plastic bucket and then the bottles were filled with water. Three pairs of light dark and initial bottles were used for each sample. The determination of the exposure period of the bottles was calculated as the light part of the day was separated in 5 equal time intervals. The bottles were exposed in 0.1, 0.3 and 0.5 m depth layers for a period including the second and third part of the above mentioned five time intervals. Within this period about 55-60% of total daily production was synthesized according to VOLLENWEIDER *et al.* (1969). The exposure depths depended on the measured Secchi transparency (S) and they usually were in the range 0.25.S - 3.S approximately.

Due to the high productivity of fishponds we frequently had to shorten the exposure time. In fact, the exposure time took one hour in most of the cases. In order to avoid the problem with oversaturation of water sample with oxygen and bubbles appearance in the bottles an original author's methodology was developed. The water sample was transferred to an empty plastic bottle double bigger volume than the sample. The bottle was pressed by hand until the liquid reached the bottleneck. Then the bottle was tightly closed and vigorously shaken. The elastic bottle walls tried to return to its normal position by creating reduced pressure insight the bottle. This drove the excessive dissolved oxygen to convert into a gaseous phase. Finally, the oxygen concentration in the water was lower than the saturation value under the instant atmospheric pressure and there were no bubbles released in the bottles during the exposure. The obtained productivity values were calculated for 1 m^2.

The diverse characteristics of fishponds presented by a great number of measurements allowed applying statistical methods. The application of statistical package Canoco for Windows 4.55 (TER BRAAK & SMILAUER, 2002) provides the opportunity to generalize the relative power and interactions of the whole multitude of factors in the presented study by means of principal component analysis.

Results and Discussion

Principal Component Analysis (PCA) – biotic factors (2004)

Spatial changes between the ponds. There was no separation between the manured and the control (without manure added) ponds concerning the biotic factors within 2004 (Fig. 1). The control ponds were more than the manured ponds and this probably increased the heterogeneity. Thus, according to the analysis performed there was no clear difference due to the applied manuring presented by all measured biotic factors.

The first main axis explains 23.1% of the variability in the biotic factors. This is the biggest part from the general spatial variation of the analyzed biotic variables. The changes in the zooplankton number and the zooplankton biomass together with the changes in the benthos biomass were the main contributors for the first main axis formation. The first and the second axes explain cumulatively 44.7% (Table 3) from the total spatial variation. Concerning the second axis, the gross primary productivity (GPP), percentage of the solar energy utilization (PEU), respiration and the GPP/respiration ratio were the main factors explaining the bigger part of the variation. The explained cumulative spatial variation significantly increases with the adding of the third axis (60.0%) and the fourth axis (73.5%). The last two axes add considerably lower percentage of the explained variability compared to the first two axes.

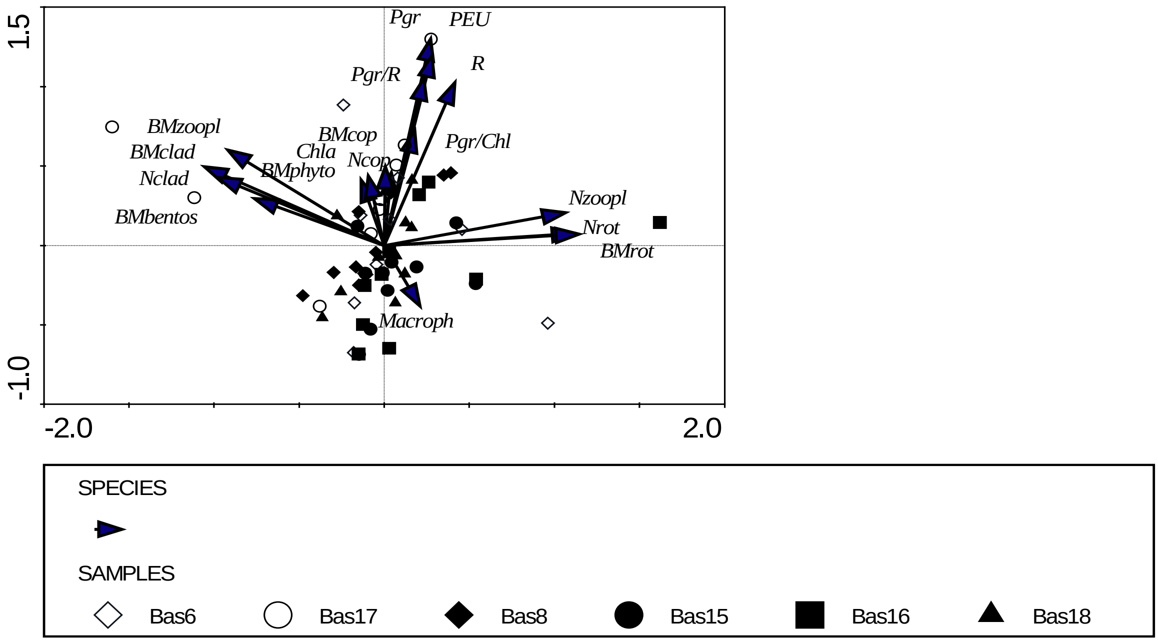

Fig. 1. PCA of the spatial changes between ponds No 6, 17 (manured) and No 8, 15, 16, 18 (control) of the biotic factors (2004).

Table 3. Variation distribution among the main axes for the spatial changes between the ponds of the biotic factors (2004).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.167	0.156	0.111	0.097	1.000
Cumulative variability, %	23.1	44.7	60.0	73.5	
Total Eigenvalues					0.723

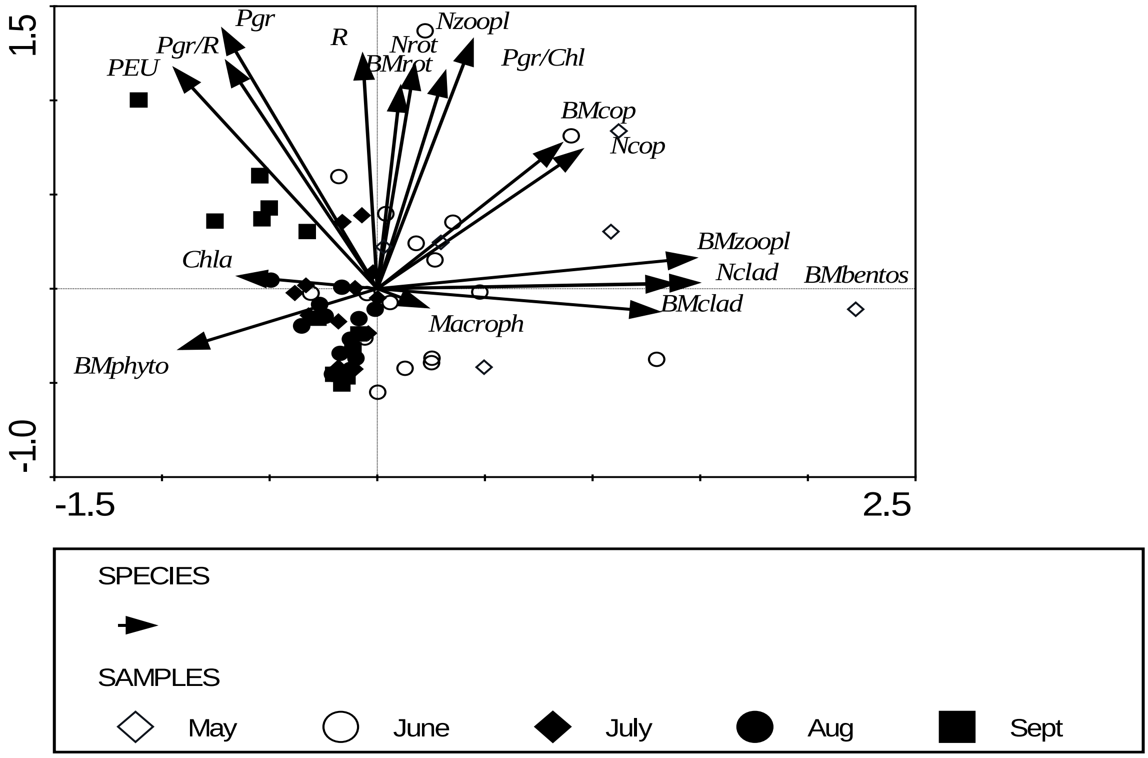

Fig. 2. PCA of the temporal changes (monthly) of the biotic factors (2004).

Temporal changes between the monthly sampling. According to the monthly sampling May and June differed from the rest of the months included in the vegetation season. The Cladocera's number and Cladocera's biomass were higher in this period. Cladocera composed the main part of the total zooplankton biomass, thus the mentioned trend is valid for the biomass of all zooplankton groups (Fig. 2). There was a similar trend for the zoobenthos biomass as well. Usually during the mentioned months the zooplankton and the zoobenthos had a biomass peak. This peak rarely could be influenced by the presence of the ichthyofauna species in the ponds, because the fish in the beginning of the vegetation period are too small in size and have less nutrition needs (TAKAMURA *et al.*, 1995; CAREY & WAHL, 2011). The chlorophyll *a* concentration and the phytoplankton biomass have increased during both August and September. Production factors as PP, respiration, Percent of solar Energy Utilized (PEU), assimilation number (AN), zooplankton number, rotatorian's number and biomass did not contribute for separation between months. The first main axis clearly separate the variation between the months of sampling – 29.2%, (Table 4). The bigger part of the variation is explained by the differences between the months of sampling (0.859) compared to the changes of the investigated factors between the different ponds (0.723), (Tables 3 and 4). The climatic variables explain 58-70% of the variation according to KIPKEMBOI *et al.* (2010).

Spatial changes between the ponds. During 2005 (Fig. 3) the manured ponds showed an increased level of PP, respiration, PEU%, and chlorophyll *a*. Similarly to our results, KIPKEMBOI *et al.* (2010) also reported a high chlorophyll *a* level in manured ponds. There was a trend to higher rotifer numbers and total zooplankton number in this type of ponds. The control ponds had higher PP/respiration and PP/chlorophyll *a* ratios. Macrophyte covering also increased. The biggest part of variation is explained by the first main axis – 34.8%. The next axes explained about 10% each (Table 5).

Principal Component Analysis (PCA) – biotic factors (2005)

Temporal changes between the sampling months. Concerning the first main axis (the abscissa) there was separation between samplings of two vegetation periods: June-July and August-September (Fig. 4). The first two months had a higher zoobenthos and zooplankton (all groups) biomass and a higher chlorophyll *a* values. During August the PP/respiration and PP/chlorophyll *a* ratios increased and there was an increase (a little bit lower) of the bacterioplankton biomass and PEU of the phytoplankton (see also JANA, 1979). The variation between the months of sampling (temporal component, 0.892) is considerably higher compared to the variation between the ponds (spatial component, 0.429) (Table 5 and 6).

Table 4. Main axes distribution of the temporal changes (monthly) of the biotic factors (2004).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.251	0.178	0.135	0.092	1.000
Cumulative variability, %	29.2	50.0	65.6	76.3	
Total Eigenvalues	29.2	50.0	65.6	76.3	0.859

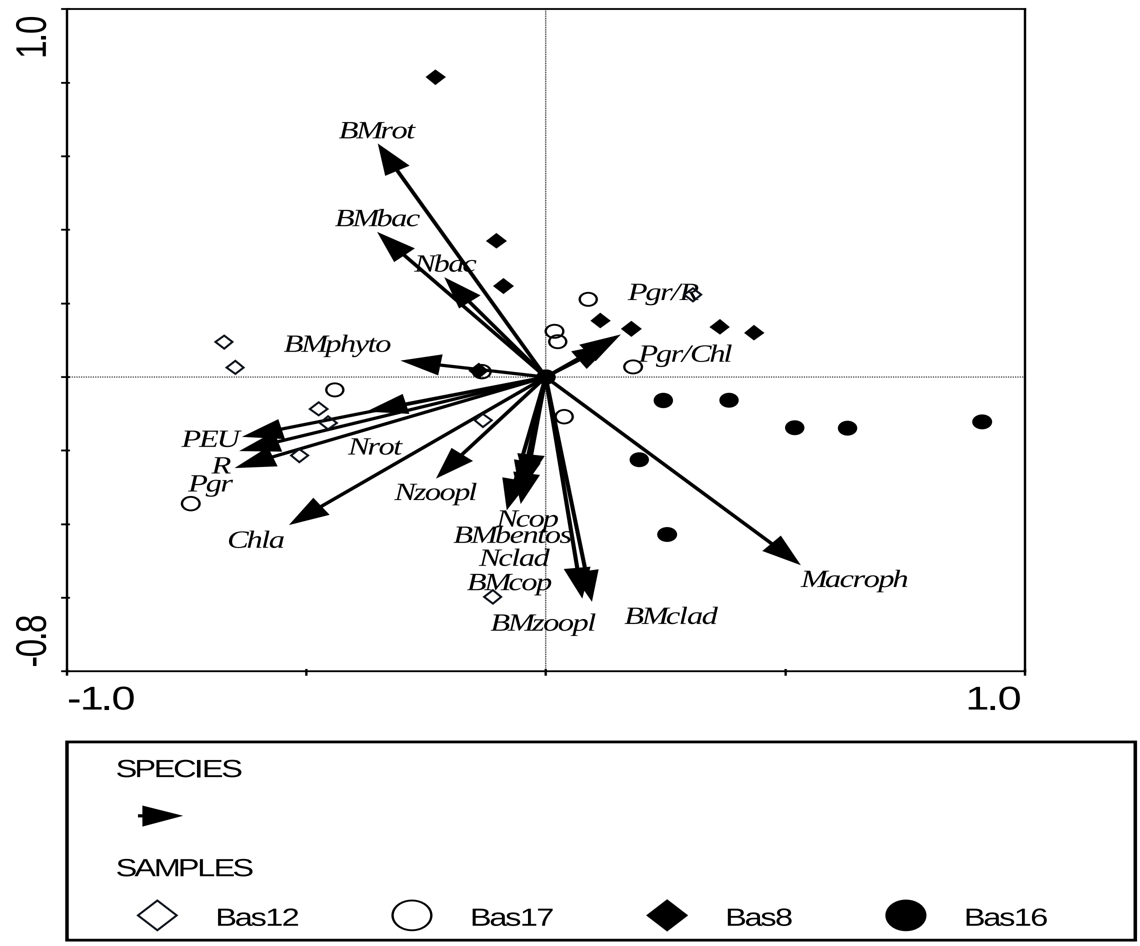

Fig. 3. PCA of the spatial changes between ponds No 12, 17 (manured) and No 8, 16 (control) of biotic factors (2005).

Table 5. Variation distribution among the main axes for the spatial changes of the biotic factors (between the ponds 2005).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.149	0.057	0.049	0.039	1.000
Cumulative variability, %	34.8	48.2	59.6	68.7	
Total Eigenvalues					0.429

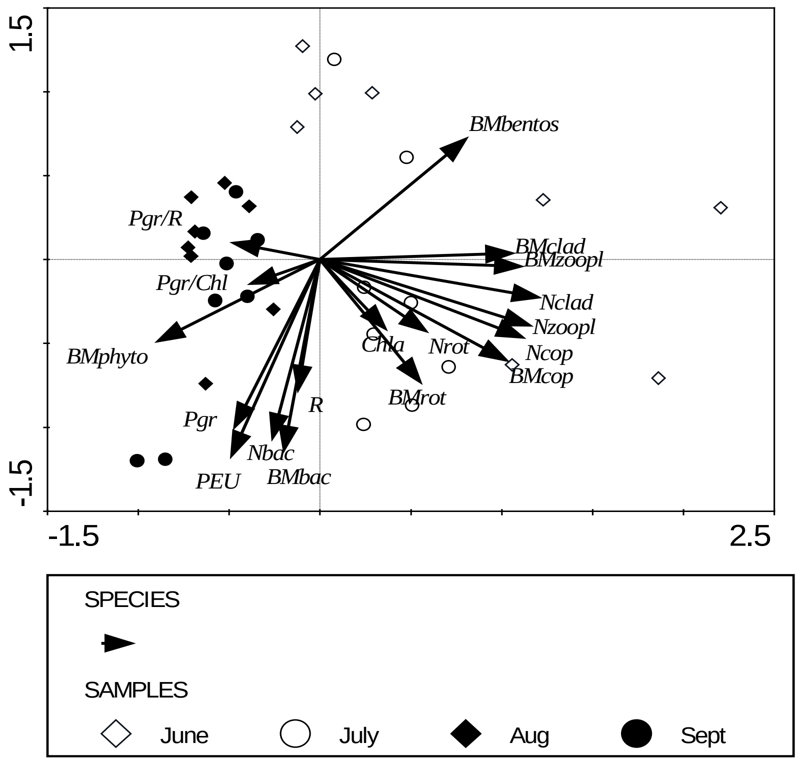

Fig. 4. PCA of the temporal changes (monthly) of the biotic factors (2005).

Table 6. Variation distribution among the main axes for the temporal changes of the biotic factors (2005).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.339	0.186	0.090	0.090	1.000
Cumulative variability, %	38.0	58.9	69.0	77.2	
Total Eigenvalues					0.892

Principal Component Analysis (PCA) – biotic factors (2006). Higher values of the GPP, respiration and chlorophyll *a* were detected during the most of the samplings from both of manured ponds (especially the pond No 12) (Fig.5). The positive relation of the manuring with the GPP and chlorophyll *a* was reported also by other authors (Hepher, 1962; Noriega-Curtis, 1979; Kaggwa *et al.*, 2009). Using different schemes of fertilizing by organic and inorganic fertilizers Diana *et al.* (1991) came to the following conclusions: the high doses of fertilizing lead to similar levels of PP and chlorophyll *a* in all ponds. The fish growth and the yield are significantly higher in the ponds fertilized with organic fertilizers compared to the ponds with inorganic fertilizers used. The control ponds differ with an increased level of macrophyte growth, PP/respiration and PP/chlorophyll *a* ratios. In fact, the separation manured/control ponds by the biotic factors was not highly expressed. The bigger part of the dispersion is explained by the first main axis – 36.1%, the other axes explain about 10 – 16.4%, (Table 7).

Temporal changes between the sampling months. There was an apparent difference between May as a sampling month and the rest of the sampling months within year 2006 (Fig. 6). The benthos biomass, PEU%, zooplankton components and the bacterioplankton biomass increased during May, while in June PP/respiration and PP/chlorophyll *a* ratios and particularly PP were higher (similar to JANA, 1979). There was an increased level of the respiration and the macrophyte covering. The first main axis explains 47.8% of the biotic factor changes month by month. The other axes explain about 8 – 10%, (Table 8).

The bigger variation part (Tables 7 and 8) is explained by the differences between the months of sampling (0.889) compared to the changes of the investigated factors between the ponds (0.594).

Regularly the fish were checked for health problems during the experiments. No diseases were found.

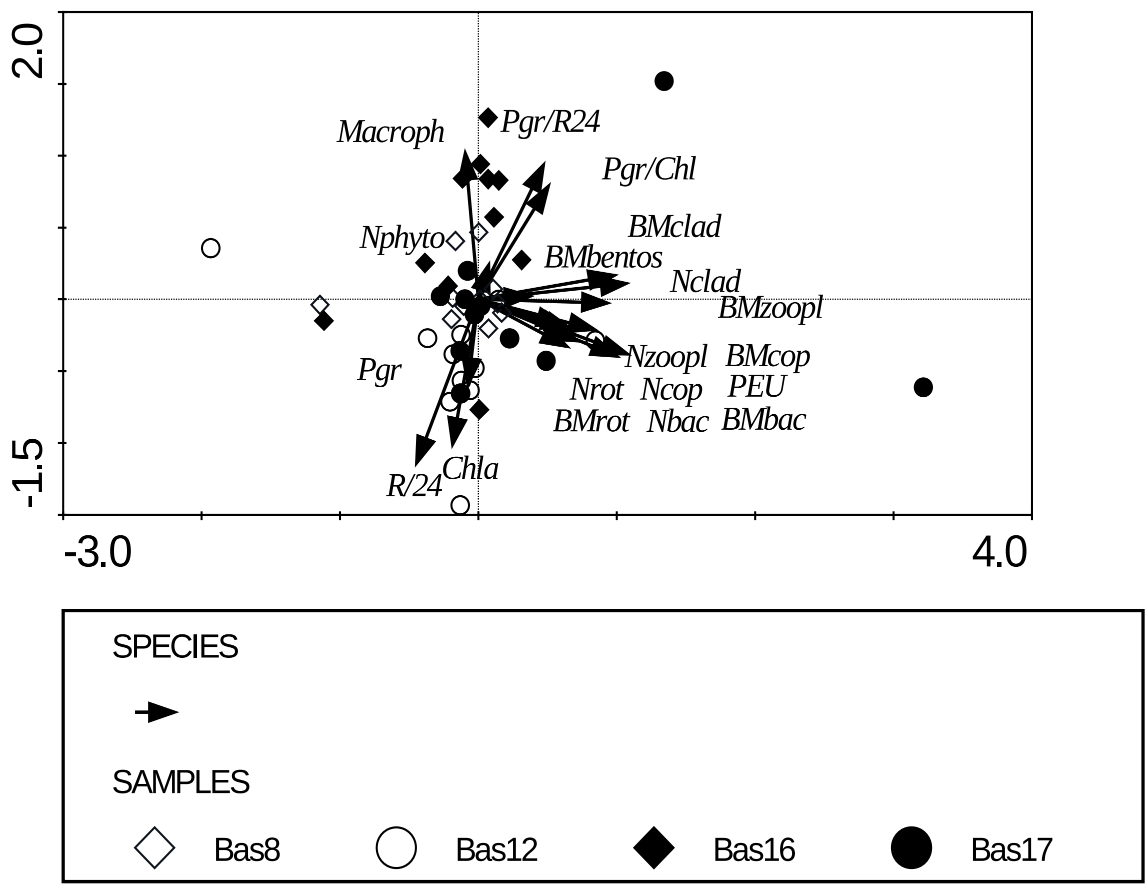

Fig. 5. PCA of the spatial changes between ponds, No 8, 12 (manured) and No 16, 17 (control) of the biotic factors (2006).

Table 7. Variation distribution among the main axes for the spatial changes (between the ponds) of biotic factors (2006).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.214	0.097	0.081	0.058	1.000
Cumulative variability, %	36.1	52.5	66.2	75.9	
Total Eigenvalues					0.594

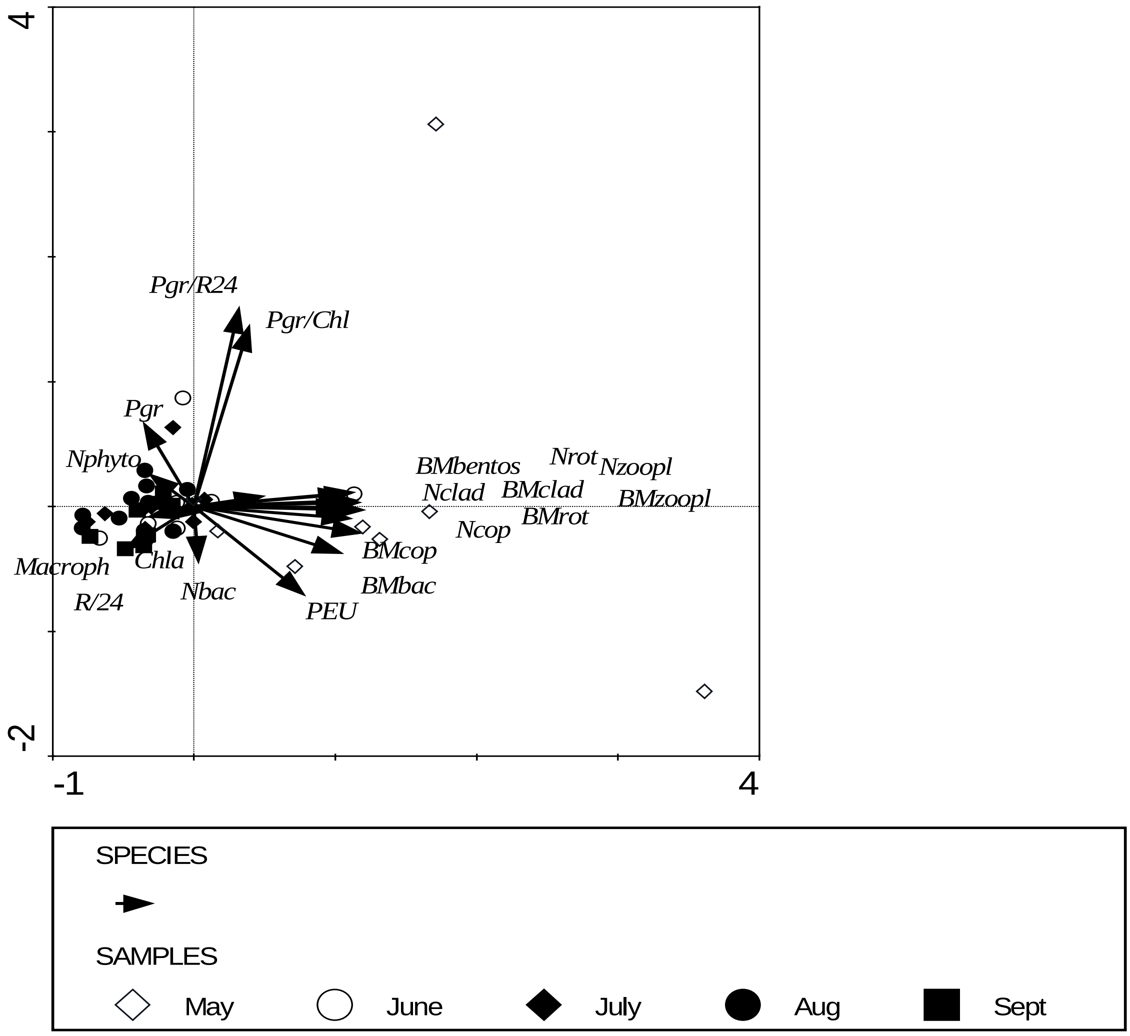

Fig. 6. PCA of the temporal changes (monthly) of the biotic factors (2006).

Table 8. Variation distribution among the main axes for the temporal changes of the biotic factors (2006).

Factor	Main axes, No				Total variation
	1	2	3	4	
Eigenvalues	0.425	0.097	0.071	0.068	1.000
Cumulative variability, %	47.8	58.8	66.7	74.4	
Total Eigenvalues					0.889

Conclusions

1. Differences between the manured and the control ponds concerning the biotic factors observed were not found during 2004. Probably the reason, leading to increased dispersion, is the different number of the manured (2) and the control (4) ponds included in the experiment.

2. The bigger part of the biotic factor variation was defined by the differences between the monthly sampling compared to the changes between the ponds within the period of investigation (2004/05/06). There was a difference between the first and last months concerning the biotic factors. This great seasonal variability decreases the opportunity for revealing of the differences between the manured and the control ponds.

3. The biotic factors as the PP, respiration and PEU in the manured ponds showed higher values compared to the control ponds during 2005. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds.

4. An increased level of the biotic factors as the PP, respiration and chlorophyll *a* was found in the manured ponds during 2006. More macrophytes and higher PP/respiration and PP/chlorophyll *a* ratios were detected in the control ponds.

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Influence of Earthworm (Oligochaeta: Lumbricidae) Populations on Abundance of Soil Fungi

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Abstract. The study explores the influence of earthworms (Oligochaeta: Lumbricidae) on the soil fungi communities. The observation was carried out in poplar forests on three types of soils: Pellic Vertisols, Cromi-Vertic Luvisols and Calcaric Fluvisols. The population of earthworms have a negative effect on the soil fungi. It was revealed that augmentation on the number of lumbricid taxa reduces the soil fungi communities. Earthworm populations and number of soil fungi are in negative correlation (R= -0.9153). In sum, more research is needed to fully understand relationships between earthworms and soil fungi.

Keywords: Earthworms, Lumbricidae, soil fungi, soil microflora, soil fauna.

Introduction

Earthworms take part in various relationships with soil microorganisms (Edwards & Bohlen, 1996). In some cases they are involved in symbiotic or mutualistic, in other cases in antagonistic interactions with soil biota, respectively. Many studies revealed the indirect or direct influence of lumbricid populations on soil microorganisms (Groffman *et al.*, 2004; Suarez *et al.*, 2004).

The gut of earthworms decreased growth of some fungal species (Moody *et al.*, 1996; Parle, 1963). Several species of fungi showed to be ingested preferentially by earthworms (Moody *et al.*, 1995; Cook, 1983; Bonkowski *et al.*, 2000). This implies that the litter-burying or fragmenting anecic and epigeic species may impose some selection pressures on fungal populations in both litter and soils. Earthworms from family Lumbricidae are vectors of distribution of mycorrhizal fungi (Gange, 1993; Reddell & Spain, 1991), symbiotic nitrogen-fixing bacteria (Doube *et al.*, 1994; Madsen & Alexander, 1982) and actinomycetes (Reddell & Spain, 1991) in soil.

The aim of the current study is to explore the influence of earthworm populations on microscopic soil fungi communities in uncultivated soils.

Materials and Methods

The study was carried out over the 2011 - 2013 year period on uncultivated soils in Sofia Plain (Bulgaria). Sampling points: Pellic Vertisols from Bozhurishte town - 42° 44′ 52N 23° 13′ 09E, Chromi-Vertic Luvisols from Chelopechene village - 42° 44′ 24N 23° 28′ 15E and Calcaric Fluvisols from Negovan village - 42° 44′ 33N 23° 24′ 07E.

The soil samples were analyzed for densities of microscopic fungi by Grudeva *et al.* (2007). The numbers of colony forming units (CFUs) were determined after 7 day incubition at 28°C using the dilution plate technique. Chapek growth medium was used for development of fungi species (CFUs). The chemical reaction (pH=4) for fungal growth was achieved with adding a lactic acid. The count of soil fungi was calculated per 1 g absolutely dry soil.

Earthworms were collected by the diluted formaldehyde method (RAW, 1959) complemented with digging 0.5 × 0.5 m quadrates, hand sorting and searching under stones and the bark of fallen logs. The abundance of all collected earthworms was adjusted to one square meter.

The main chemical and physical soil characteristics were estimated. The soil pH was measured potentiometric in water, the soil organic carbon was determined applying TURIN (1937) method and the soil texture was estimated by KACHINSKII (1970) method, respectively. Statistical data were presented with correlation analyses.

Results and Discussion

Explorations of soil fauna and microflora were carried out on three types of uncultivated soils. In Pellic Vertisols earthworm populations had a higher density, ind./m^{-2}. In this soil type the count of soil microscopic soil fungi was 1.0 x 10^3 CFU/g. In Chromi-Vertic Luvisols the lumbricid abundance was lower - 45 ind./m^{-2} and fungi communities were – 5.0 x 10^3 CFU/g. In Calcaric Fluvisols the earthworm density was lowest - 32 ind./m^{-2}m but the soil fungi populations in this soil was highest - 11.0 x 10^3 CFU/g (Fig. 1).

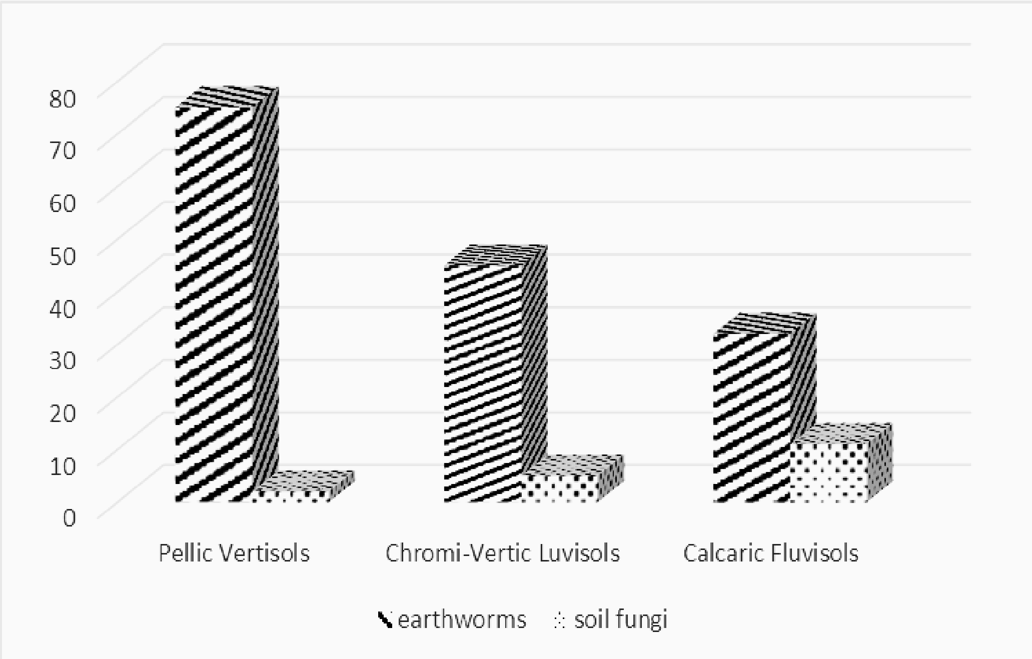

Fig. 1. Abundance of earthworms and soil fungi in explored uncultivated plots.

Explored Pellic Vertisols soil plot was with optimal soil moisture, neutral soil pH, high clay content – 76% and organic matter content– 11.87% (Table 1). All those abiotic characteristics favor the earthworm growth and activity. In contrast, Calcaric Fluvisols was with low soil moisture – 6%, low soil organic matter – 1.8% and low clay content – 20.8%. Those parameters are adversely to earthworm populations. Chromi-Vertic Luvisols plot had average soil characteris-tics. In this soil the organic matter content was – 3%, clay content – 44% and soil moisture - 11%, which conduce to balance between the earthworms and soil fungi communities.

The burrowing activity of earthworm species breakdowns the soil fungi mycelium. Many studies showed that the intestinal tract of earthworms suppressed the development of fungal spores. Passage through the gut of earthworms cause decreasing of the soil fungi density (PARLE, 1963; MOODY *et al.*, 1996).

Earthworm casts have a higher bacteria count and lower fungi density in comparison with surrounding soil (VALCHOVSKI, 2011). Bacterial-to-fungal ratios in soils are also often greater in earthworm-worked soils because biotur-bation tends to affect fungal populations negatively more than those of bacteria (HENDRIX *et al.*, 1986).

Influence of earthworm count on microscopic fungi density was estimated using correlation analysis (Fig. 2). The statistical analysis showed that the abundance of earthworms and soil fungi are in a high reverse correlation (R= -0.9153). Therefore, increasing the density of lumbricid populations cause augmentation of soil fungi communities. More explorations are needed in order to reveal the influence of soil fauna on biodiversity of fungi microorganisms in different soil ecosystems.

Table 1. Chemical and physical characteristics of studied types of soils.

Soil	pH (H_2O)	Soil organic matter (%)	Soil moisture (%)	Clay content (%)
Pellic Vertisols	6.8	11.87	30	76
Chromi-Vertic Luvisols	6.5	3.0	11	44
Calcaric Fluvisols	6.9	1.8	6	20

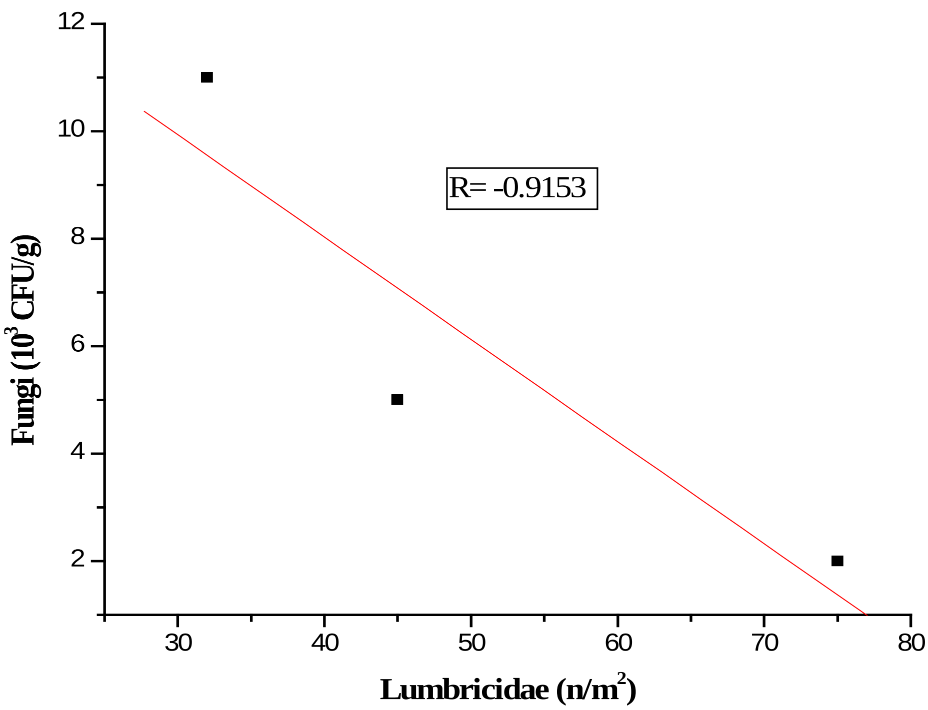

Fig. 2. Correlation analysis between abundance of earthworm populations and density of microscopic soil fungi.

Conclusion

Earthworms are one of the major biotic factors on soil fungi communities. The results from this study revealed a relationship between populations of earthworms and soil fungi. The density of fungi communities decreased with augmentation of lumbricid abundance. Fungivorous and locomotor activity of earthworms cause decreasing of microscopic fungi. Further researches need to estimate properly the functional roles of earthworms in the soil ecosystems.

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Anti-Fungal Activity and Allelopathic Influence of Vitex agnus-castus L. (Verbenaceae) Essential Oils on Actinidia deliciosa in vitro Culture

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Abstract. The results of research of *Vitex agnus-castus* L. plants from the collection of M. M. Gryshko National Botanical Garden, NAS of Ukraine are given. The species belongs to a group of prospective essential oil plants which are characterized by valuable medicinal, food, aromatic, honey, technical and decorative properties, and can be used in many industries, including pharmaceutical, cosmetic and food. The quantitative content and qualitative composition of the vegetating plant's essential oil are determined, its antifungal influence on the cultures of *Aspergillus niger, Alternaria alternata* and *Fusarium culmorum* is assessed. Antifungal properties of *V. agnus-castus* essential oil were used to sterilize *Actinidia deliciosa* (A. Chev) C.F. Liang & A.R. Ferguson explants introduced *in vitro*. *V. agnus-castus* essential oil is shown to have allelopathic effect on *A. deliciosa* plants. The possibility of using the oil *in vitro* to cultivate *A. deliciosa* callus as a source of bioactive substances and to enhance the effectiveness of biotechnological methods of plant propagation is discussed.

Kay words: *Vitex agnus-castus*, essential oils, allelopathic and antifungal activity, *in vitro,* micro clonal reproduction, callus.

Introduction

Micro propagation technologies are an important complement to traditional plant breeding, ensuring the possibility of achieving large numbers of planting material of unique genotypes, valuable species, and new varieties. Biotechnological approaches, based on tissue and organ culture of plants *in vitro,* provide virus-free plants and solve the problem of multiplying of valuable species and varieties that proved difficulties for conventional breeding methods.

Scientific foundation of biotechnological utilization of plant tissue cultures in pharmacognosy is the ability of cells to synthesize various substances *in vitro*: glycosides, phenolic compounds, cardiac steroids, saponins, lignins, flavonoids, terpenoids, alkaloids, etc. Implementation of cell cultures prevents from extinction thousands of rare plant species capable of synthesizing useful substances. It is known that formation of morphological structures (stems, roots, embrioids) in callus is accompanied by increasing generation of bioactive substances in the culture. Currently, *Ephedra monosperma* C. A. Meyer, *Juniperus sibirica* Burgsd., *Cyclamen kuznetzovii* Kotov & Czernova, and *Cyclamen persicum* Mill. *Paeonia anomala* L. are

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successfully cultured *in vitro* in order to produce bioactive compounds in callus (ZARIPOVA *et al*., 2004; PLYNSKAJA *et al*., 2008).

Increased production *in vitro* can be achieved by further studies of selection of specialized cell populations and optimization of culture conditions. Main problem is reception of sterile vegetative planting without fungus infection. Thus, there is a search of natural volatile, environmentally safe substances with antifungal and antibacterial properties in order to use them in plant sterilization *in vitro*.

Among similar antibacterial substances of widely distributed plant species there are phenolic compounds of varying complexity: phenolic acids (gallic, caffeic, benzoic, salicylic, etc.), flavonoids, naphthoquinones, and hydrolyzed condensed tannins (LEVCHYK, 2013), the volatile components of essential oils, etc. If skillfully used, phytoncides can be reliable agents of controlling sanitary conditions of the biosphere and of plant protection against diseases and pests (GRODZINSKIJ, 1965; GRODZINSKIJ *et al*., 1987).

Plant species of the genus *Vitex* L. belong to prospective plants with essential oils, due to their valuable medicinal, nutritive, aromatic, honey, technical and essential oils decorative properties that qualify them for use in various industries such as pharmaceutical, cosmetology and food. The above-ground part of *Vitex* plant appears to contain flavonoid glucoside kasticine, vitexin, and carotene (41.7%), glycoside derivatives of aucubin and p-oxybenzoic acid, iridoid glycosides, essential oils, agnuside, and sytosterol (HOBBS, 1998; HOBERG, 1999; ZASADA & SCHOPMEYER, 2008; STOJKOVIC *et al*., 2011; DOGAN *et al*., 2011). Leaves of *Vitex negundo* L. plants also contain iridoid glucosides, carotene, vitamin C, gluco-nonital, benzoic acid, β-sytosterol, and c-glycoside (HOBBS, 1998; HOBERG, 1999). An important feature, characteristic of leaves of *Vitex* plants is nishindine alkaloid $C_{15}H_{31}ON$, flavones, luteolin 7-glucoside, kasticine (VISHWANATHAN, 2010).

Regarding the content of vitamin C, *Vitex* plants belong to vitamin-rich plants. For example, there are at least 88.9-118.1 mg % of vitamin C in leaves of *V. agnus-castus*, and during secondary growth its content reaches maximum values of 615.8±7.1 mg%. Maximum sugar content is 10.1±0.4 % during secondary growth and budding phases. The amounts of proteins in generative and vegetative organs of *V. agnus-castus* plants are high compared to valuable crops, and reach maximum performance of 23.6 ± 0.6 % during fructification. The proteins mostly consist of aspartic, glutamic, alanine, glycine, and proline amino acids. *V. agnus-castus* essential oils which belong to bioactive substances make up in average 0.36-0.48 % of leaves, in accordance with our research – under 0.65 % and 0.47 % of dried seeds (LEVCHYK & RAKHMETOV, 2015).

In our opinion *Vitex* might be the solution for the mentioned above problem of sterility *in vitro*. The study aimed to determine qualitative and quantitative contents of essential oil of *V. agnus-castus*, introduced in right-bank forest-steppe zone of Ukraine, and its allelopathic and antifungal activity in the conditions *in vitro*.

Materials and Methods

The objects of study were essential oils of plants *V. agnus-castus*, introduced in M. Gryshko National Botanical Garden (NBG) of NAS of Ukraine. Quantitative content of essential oil in vegetative mass was analyzed by Clevenger method according to standards (GOST 24027.2-80, 1999), using SIMAX (Czech Republic) tool for volatile oil analysis. The raw materials for essential oil were sampled according to plant vegetation phases.

Analysis of essential oil was performed according to chromatographic methods (CHERNOGOROD & VINOGRADOV, 2006), using Agilent Technologies 6890 chromatograph with mass-spectrometer detector 5973. INNOWAX capillary CG column with inner diameter 0.25 mm and length of 30 m was used.

Allelopathic activity of the essential oil was studied according to (GRODZINSKIJ, 1973), its antifungal properties analyzed by paper-disc method. Tested fungi culture were represented by phytopathogenic cultures from the collection of Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, *Aspergillus niger* vanTiegh, *Alternaria alternata* (Fr.) Keisser, *Fusarium culmorum* (Sm.) Sacc. Used doses of volatile oil were 2 and 5 μL.

The influence of essential oil on regenerative abilities *in vitro* were studied on the *A. deliciosa* specimens grown in the NBG collection of vine fruit plants. The vegetative reproduction of these plants by traditional breeding methods is difficult and of low efficiency. According to previous studies, shoots of *A. deliciosa* exhibit low coefficient of phytohormone balance and low regeneration abilities, with the maximum percent of rooting explants not more than 10-20% (Skrypchenko *et al.*, 1999).

In order to culture *A. deliciosa,* explants with unopened buds were taken and sterilized using vacuum filter for 1.5-2 min. in 70 % ethanol and 3 min. in 0.05 % sodium thiomersal, then thoroughly washed with distilled water. Then they were planted in the culture medium MS (Murashige & Skoog, 1963). After budding, young leaves were at once placed into Petri dishes to regenerate. For regeneration, MS culture medium with 3 mg/L benzyl aminopurine, 25 mg/L of vitamins B1 and B6, and 12 mg/L of iron sulfate $Fe_2(SO_4)_3$x 9 H_2O was used. The experiment was conducted on the explants of *A. deliciosa*, cultured. The explants were placed in culture containers with MS medium without addition of *in vitro* hormones (Zagoskina *et al.*, 2009).

Additional sterilization of *A. deliciosa* explants for further propagation in culture *in vitro* with essential oil of *V. agnus-castus* was added to the experimental containers in varying volumes: 1 μL (test №1), 5 μL (test №2) and 10 μL (test №3), to prevent the emergence of dormant fungal infection in the tissues of plants. An experimental container without volatile oil was used as control. Experiments were done in triplicate.

Results and Discussion

It is known that quantitative content of essential oil of *Vitex* plants is species-specific and changes according to vegetation phase, reaching maximum at the height of plant metabolic processes (Table 1). In the course of studies, in 2011-2013 the content of essential oil in *V. agnus-castus* plants growing introduced in right-bank forest-steppe of Ukraine was 0.51%-0.65 %. Optimal period for maximum collecting of oil are the flowering, fructification phases, and the end of vegetation.

Qualitative analysis of essential oil of *V. agnus-castus* shows that its main components are 1,8-cineole, sabinene, limonene and the α-pinene (Table 1). Though the percentage ratio of the components does somewhat change in the course of vegetation, the major components per species remain constant regardless of the plantation's location (Levchyk, 2013).

It is noteworthy that 1,8-cineole dominated among oil components in 2012–2013, whereas in 2011 its percentage in the oil was minimum. Qualitative composition of volatile oils is constantly changing, because parallel to the oil secretion and accumulation are the processes of evaporation and losses to the environment.

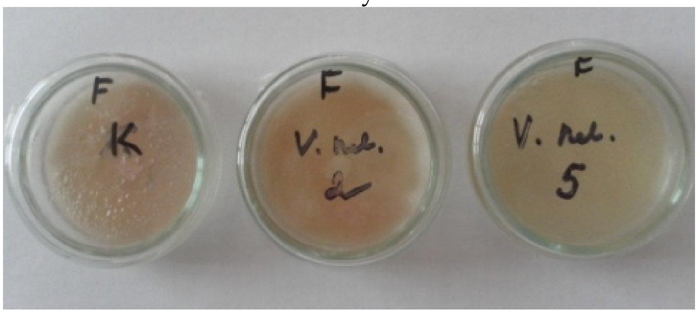
According to our studies, essential oil of *V. agnus-castus* has potent fungicidal and fungistatic influence on test cultures of *A. niger*, *A. alternata*, *F. culmorum* (Fig. 1). The homeostasis of fungal cells was disturbed, resulting in full inhibition or partial suspension of mycelium growth, lack of sporogony, and changes in pigment generation.

It is found that the tested cultures of *A. niger*, *A. alternata*, *F. culmorum* differ in resistance to toxic influence of *V. agnus-castus* essential oil. The most resistant to this influence is the culture of *A. alternata.* The level of antifungal activity of volatile oil changed in accordance with the vegetation phase, highest during flowering, fruiting and at the end of vegetation, relating to the changes in qualitative composition and quantitative contents of essential oil over the course of plant vegetation. The development of fungal culture of *F. culmorum* was most influenced by essential oil of *V. agnus-castus* produced at the end of plants' vegetation. The oil activity persisted for ten days, and the aftereffects continued for two months. It was evidenced by the changed color of mycelium of fungal culture from cherry-pink to beige (Fig. 1).

Table 1. Qualitative and quantitative contents of *Vitex agnus-castus* plants' essential oil according to vegetation phases (%), 2013.

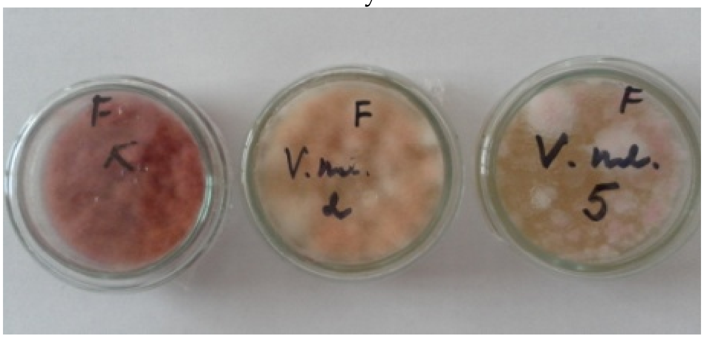
Main components	Vegetation phase			
	Second growth – budding	**Flowering**	**Fructification**	**End of vegetation**
1,8-cineole	23.04	23.08	18.38	24.76
sabinene	12.25	13.45	15.10	15.62
limonene	-	6.71	8.28	9.25
α-pinene	9.14	10.19	11.93	10.14
β-caryophyllene	0.84	1.60	1.95	-
caryophyllene-oxide	23.08	2.17	1.55	0.87

3 days



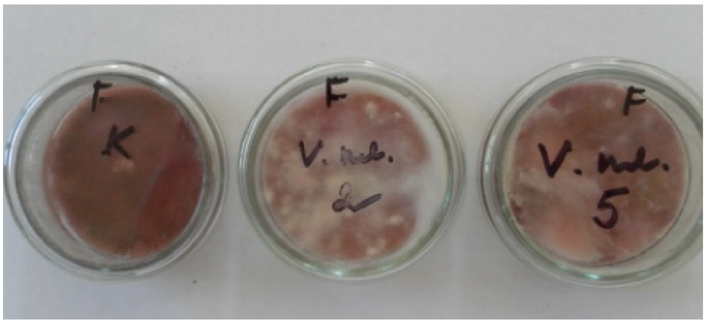
control 2 μL 5 μL

8 days



control 2 μL 5 μL

2 months



control 2 μL 5 μL

Fig. 1. Influence of essential oil of *V. agnus-castus* on growth and development of *Fusarium culmorum* culture depending on the oil volume (2 μL or 5 μL) and duration of culture (3, 8 days or 2 months) versus control (K).

Table 2. Influence of essential oil of *Vitex agnus-castus* L. on culture growth of *Aspergillus niger* and *Alternaria alternata* (diameter of growth suspension zone, mm) depending on vegetation phase and concentration of volatile components per medium volume.

Phenophase	Oil amounts (μL)	*Aspergillus niger*, exposition, days					*Alternaria alternata*, exposition, days	
		3	6	8	10	Aftereffect	3	6
Secondary growth	2	10	-	-	-	-	-	-
	5	*	-	-	-	-	11	10
Budding	2	12	8	8	8	8	*	-
	5	*	*	8	8	8	10	8
Flowering	2	10	8	9	9.5	9.5	9	7
	5	10	8	8	8.5	6	13	8
Fruiting	2	11.5	9.5	8	7	8	9	8
	5	13	10.5	9	10	10	13	11.5
End of vegetation	2	11	-	-	-	-	10	7
	5	13	10	-	-	-	11	10

Note: * poor development of mycelium; - no GSZ (growth suspension zone).

Antifungal activity of *V. agnus-castus* essential oil against *A. niger* and *A. alternata* manifested in suspension of mycelium growth and sporogony and lower density of fungal culture (Table 2). Although, the influence of essential oil of *V. agnus-castus* on *A. alternata* was in less degree than on other fungal cultures.

Application of essential oil suspends the mycelium growth most effectively if the oil was produced by flowering or fruiting plants. Suspension of sporogony of *A. niger* culture due to influence of essential oil of *V. agnus-castus* is observed on all of the plant's vegetation phases and is directly related to the oil dosage. At the end of plant vegetation, fungal sporogony is shown to be suspended, the GSZ becoming unnoticeable. Suspension of sporogony and culture growth of *A. niger* due to influence of the volatile oil of *Vitex* plants continued for two months, that of the *A. alternata* culture – for ten days.

In order to implement the observed antifungal properties of *V. agnus-castus* volatile oil, it was used for maintenance of sterilization of *A. deliciosa* explants that were cultured *in vitro*. The *in vitro* graftage and direct regeneration were ineffective for these plants, due to the dense pubescence of their leaves and stems, whereas the approach of culturing sterile plants through regeneration showed promise.

After rootless young plants formed on the callus of *A. deliciosa*, they were detached and placed in separate containers with MS medium. In one month, the young plants sprouted roots and developed stem system with sufficient foliage. It should be noted that formation of vast amounts of callus is necessary for rooting of *A. deliciosa* plants in case of micro propagation.

Table 3. Growth characters of *A. deliciosa* plants under allelopathic influence of volatile oils of *V. agnus-castus* plants

Version of test	Callus mass, g	Callus, %	Root (1 month)	Root (2 months)
Control	0.613	100	2.67±0.12	4.50±2.50
1 μL	1.226	200	–	–
5 μL	–	35	–	–
10 μL	–	12-15	–	–

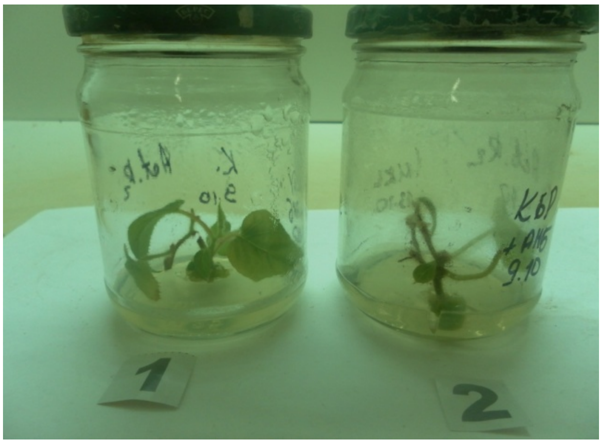


Fig. 2, a. *A. deliciosa* plants
1 month exposition: 1– control;
2 – test №1.

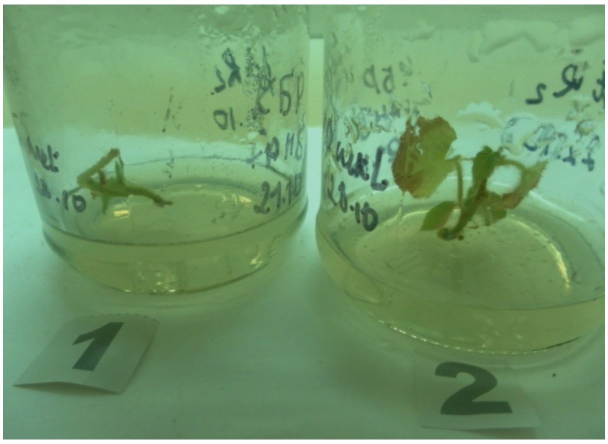


Fig. 2, b. *A. deliciosa* plants
1 month exposition: 1– test №2;
2 – test №3.

Fig. 3. Plants of *A. deliciosa* after two months of experiment (left to right):
control, test №1 (1 μL), test № 2 (5 μL), test №3 (10 μL).

Fig. 4. Plants of *A. deliciosa* at the end of experiment
(left to right: control, test №1, test №2, test №3).

For the first month, all explants developed almost identically regardless of the added volume of essential oil of *V. agnus-castus*. After two months of experimentation, significant differences were observed between samples: the control plants developed callus, roots and sufficient leaf mass of vibrant green color. The plants of test №1 (Fig. 2) developed much more callus tissue compared to control, but formed medium-sized leaves and no roots. Plants of test №2 formed insignificant callus and no roots, their leaves were small-sized and of pale green color. No processes of growth or development of explants were registered. Unlike the aforementioned, plants of test №3 started to dry up without exhibiting any signs of growth or development.

As a result of three months of observations, it was found that the control plants developed into fully functional specimens, test №1 plants formed callus two times heavier of the control mass, but no roots, morphometric parameters of test №2 plants remained unchanged, and test №3 plants lack of vital capacity (Table 3, Fig. 3).

Hence, according to our results, the essential oil of plants of *V. agnus-castus* species has high allelopathic activity (both inhibitory and stimulating), the levels of which depend on the qualitative composition of the essential oil, and concentration of volatile components per volume of medium. The essential oil of *V. agnus-castus* showed the inhibitoriest activity on plants of *A. deliciosa* when added at volumes of 5 and 10 μL.

Conclusions

The qualitative and quantitative contents of essential oil of *V. agnus-castus* plants (the oil making up 0.24-0.65 % of plant composition) are determined. The flowering and fruiting plants have the highest accumulation of the volatile oil. Main components of this oil are 1,8-cineole, sabinene, limonene, and α-pinene.

The essential oil of *V. agnus-castus* exhibits high fungicidal and fungistatic effect on test cultures of *Aspergillus niger*, *Alternaria alternata*, and *Fusarium culmorum*. Its activity level depends on the plant's vegetation phase, and concentration of oil per volume. Test culture of *Alternaria alternata* appears to be the most resistant to the volatile oil's toxic effect. There are implications for practical implementation of this property of the plant species for maintaining sterility of micropropagation and culturing plants *in vitro*. The levels and nature of the *V. agnus-castus* essential oil's effect depend on the oil's concentration per volume.

Antifungal properties of *V. agnus-castus* essential oil were successfully used to sterilize *A. deliciosa* explants introduced *in vitro*.

Allelopathic properties of the *V. agnus-castus* volatile oil is observed *in vitro*, demonstrated by its stimulating or inhibitory effects on plants of *A. deliciosa.* The levels of such activity depend on the concentration of volatile substances per volume of medium. Too high or too low concentrations of the oil in the medium are limiting factors that inhibit or stimulate the new formations.

It was shown that the essential oil of *V. agnus-castus* can be implemented *in vitro* in order to produce callus as a source of biologically active substances, and for bolstering the effectiveness of biotechnological methods of cultivation of *A. deliciosa.*

In order to overcome fungus infection *in vitro* the essential volatile oil of *V. agnus-castus* was suggested. This method is ecological, safe for environment, can substitute harmful antibiotics and chemical active materials.

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Relationships Between Plankton Primary Productivity, Biotic and Abiotic Variables of Carp Fish Ponds

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Abstract. Experiments measuring primary productivity, biotic and abiotic environmental factors in carp ponds were carried out within three consecutive years (2004, 2005 and 2006). The aim of the study was investigation of the relations between the biotic and abiotic variables, their influence on the primary productivity and the effect of manuring on the fish ponds. The influence of environmental factors onto primary productivity was investigated in ponds with and without organic manure. Nitrate nitrogen demonstrated the closest relationship with the gross primary productivity followed by chlorophyll a level and N/P ratio in manured ponds. When no manuring was applied, the importance of the nutrients along the food chain: nutrients– phytoplankton – zooplankton decreased. Trends, which can be used for increasing of productivity in carp ponds, were obtained. They give indications to improve the existing practices for better management of production efficiency and water quality in fish farms.

Key words: primary productivity, carp fish ponds, environmental factors.

Introduction

Fishponds are specific open aquatic systems inhabited by a simple community of living organisms including the main trophic levels (nutrients, phyto-, zooplankton and fish). The primary productivity (PP) is a fundamental biological characteristic being on the lowest trophic level of the food chain and is result of phytoplankton photosynthesis. Therefore, by measuring intensity of photosynthesis we could estimate the PP and get insight into the transformations of the matter and energy in aquatic ecosystems (JANA, 1979; NORIEGA-CURTIS, 1979).

Hence, the investigations of PP in carp fish ponds, its relations to biotic and abiotic environmental factors their strength of influence on PP is of a great scientific interest and have a practical significance.

Measurement of primary production for fish ponds gives the best picture of the outcome of the applied cultivation activities (in particular fertilization) and the rate of photosynthesis, as a first step towards the materialization of the natural fish productivity. That is why one of the prospects for aquaculture is through better utilization of primary productivity to increase the production of filter-feeding fish in extensive or semi-intensive pond systems.

Materials and Methods

The institute of Fishery and Aquaculture, Plovdiv is located in the northern part of the city of Plovdiv, in the

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western Upper Thracian valley. The region is characterized by a transitional-continental climate. The study was carried out in the Plovdiv base during three consecutive years (2004, 2005 and 2006). The ponds are supplied with water from Maritsa River by means of "Eni-Ark" irrigation canal. Seven earthen ponds were involved in the experiment, which individual size varies between 1.8 and 3.9 da (Table 1). According to ZHANG *et al.* (1987) the ponds of this size are among the most productive and easy for management. Their bottom is silty, but the periphery and the shallowest parts of some of them have a strip of 1-2 m width with increased content of sand.

The pond size, their shallowness, vertical and horizontal homogeneity are part of the preconditions for choosing them for model object. About 3000 $kg.ha^{-1}$ mineralized manure once in April each year was applied to ponds No 6, 12 and 17. Additionally to the natural food grain forage was given to the fish according to a scheme related to their seasonal growth rate. The periodical examination did not reveal any fish diseases. The ponds No 6, 12 and 17 were fertilized with mineralized manure (approx. 3000 $kg.ha^{-1}$) once per year (each April). The ponds No 8, 15, 16 and 18 were used as control ponds without manuring. Additionally, the fish were fed with natural grain feeds according to the seasonal growth rate. The periodical examination did not reveal any fish diseases.

The applied polycultural technology (NIKOLOVA *et al.*, 2008a, b) included mixed breeding of 30 individuals per da^{-1} one-year old bighead carp (T_1), (*Aristhichthys nobilis* Richardson, 1845), 50 individuals per da^{-1} one-year old common carp (K_1), (*Cyprinus carpio* Linnaeus, 1758) and 10 individuals per da^{-1} grass carp (one and two-year old) ($A_{1/2}$), (*Ctenopharyngodon idella* Valenciennes, 1844).

Table 1. Design and schedule of the ponds included in the experiments in the period 2004 - 2006.

Year	Variants of breeding	
	Manured ponds, $num.da^{-1}$	Control ponds, $num.da^{-1}$
	Pond No (area in da)	
2004	6 (3.8) 17 (2.6)	8 (3.8) 16 (2.7) 15 (3.1) 18 (1.8)
2005	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)
2006	12 (3.9) 17 (2.6)	8 (3.8) 16 (2.7)

The samples were taken from a station localized 1-2 m away the shore before the outlet device (savak) of each fishpond. The sampling was carried out fortnightly between 8:30 and 11:00 a.m. from May to September (2004, 2006) and from June to September (2005). The final sampling was carried out in the last decade of September. Due to the large number of investigated characteristics some of the samples were taken with one to three days difference.

All the samples were taken from the surface layer (0.3-0.5 m depth) according to the Bulgarian and European standards (e.g. EU Water Framework Directive 2000/60/EC).

The total solar radiation in MJ m^{-2} was recorded by pyranometer type M 80 M. The water column transparency was measured by the Secchi disk method.

The PP ($g.O_2.m^{-2}.24\ h^{-1}$) was determined by a light/dark bottle technique in its oxygen modification. Firstly, the pond water was taken and homogenized in a 10 l plastic bucket and then the bottles were filled with water. Three pairs of light and dark bottles were used for each sample. The determination of the exposure period of the bottles was calculated as the light part of the day was separated in 5 equal time intervals. The bottles were exposed for a period including the second and third part of the above mentioned five time intervals. Within this period about 55-60% of total daily production has been synthesized according to Vollenweider *et al.* (1969). The exposure depths depended on the measured Secchi disk transparency and they usually were in the range 0.25.S - 3.S approximately.

Due to the big productivity of fishponds we frequently had to shorten the exposure time. In fact, the exposure time took one hour in most of the cases. In order to avoid the problem with oversaturation of water with oxygen and bubbles appearance in the bottles an original author's methodology was developed. The water sample was transferred to an empty plastic bottle double bigger volume than the sample. The bottle was pressed by hand until the liquid reached the bottleneck. Then the bottle was tightly closed and vigorously shaken. The elastic bottle walls tried to return to its normal position by creating lower pressure insight the bottle. This drove the excessive oxygen to convert into a gaseous phase. Finally, the oxygen concentration in the water was lower than the saturation value under the instant atmospheric pressure and there were no bubbles in the bottles during the exposure. The obtained productivity values were calculated for 1 m^2.

The diverse characteristics of fishponds presented by a great number of measurements allowed applying statistical methods. The difference between manured and control ponds was tested by Wilcoxon rank paired test and the relations beween the variables were revlealed by multiple linear regression with STATISTICA 7.0 (Sokal & Rohlf, 1997; McGarigal *et al.*, 2000).

Results and Discussion

The relation between pond gross primary productivity (GPP) and the factors influencing it is illustrated on Fig. 1. The effect of these factors was best pronounced in the manured ponds. The correlation coefficient of the multiple regression ($R=0.65$) is highly significant ($p<0.000062$). The relation between pond factors (independent variables) and the gross primary productivity (GPP) in the equation decreased from the right to the left of regression equation. The highest relationship was demonstrated by Nitrate nitrogen ($p=0.002$), followed by the chlorophyll *a* level ($p=0.01$), the N/P ratio ($p=0.014$) and the flow rate of water which regression coefficient was not significantly related with GPP ($p=0.10$).

A similar relation of GPP was revealed by means of multiple linear regression including three independent variables - T, NO_3 and Chl *a* within field experiments at Srebarna Lake (Kalchev *et al.*, 2012). A statistically significant positive correlation with the nitrogen has been found by other researchers as well (Garg & Bhatnagar, 2000). The positive effect of the manuring, leading to nutrient enrichment (especially phosphorus) and increasing of the PP was revealed in different lakes (Kaggwa *et al.*, 2009; Jana *et al.*, 2012). Kipkemboi *et al.* (2010) showed the manure positive effect onto chlorophyll *a*. According to other researchers, GPP is related with the solar radiation and chlorophyll *a*, but not with the total phosphorus or total inorganic nitrogen concentration (Iwakuma *et al.*, 1989). Research works found out that the regression analysis gives predictable relationship between PP and total inorganic nitrogen or total phosphorus in the water (Diana *et al.*, 1991).

The multiple regression for the control ponds delivered the following equation: $lgPgross=0.39 + 0.40lgChl\ a + 0.31lgNH_4 + 0.017TR - 0.17lgPO_4 - 0.09lgBMclad - 0.43lg\ Oxid$. The significance levels of regression coefficients were for lgChl *a* $p=0.0001$, $lgNH_4$ $p=0.01$, TR $p=0.02$, $lgP0_4$ $p=0.046$, lgBMclad $p=0.059$, lgOxid $p=0.052$, respectively.

Legend: BMclad – *Cladocera*'s biomass; Oxid - water oxidability.

The highest correlation with the GPP showed the chlorophyll *a* content in the phytoplankton, ammonium nitrogen, total radiation, phosphate concentration, *Cladocera*'s biomass and water oxidability. The correlation coefficient of the multiple regression ($R=0.69$) was a little bit higher compared to the manured ponds ($R=0.65$). The significance level ($p<0.000043$) was high as well. The GPP in the control ponds was related with more factors compared to manured ponds. Apparently the lack of manure seemed to decrease the role of nutrients along the food chain: nutrients – phytoplankton – zooplankton. As a result, there was an increased water transparency in the control ponds compared to manured ponds (Fig. 2).

Therefore, there was an increased macrophyte growth, which overshadowed the phytoplankton (HOLOPAINEN *et al.*, 1992), utilized the phosphate phosphorus and negatively changed the N/P ratio for the phytoplankton (ABDEL-TAWWAB, 2006). The negative relation with the phosphate phosphorus and the positive relation with the ammonium lead to possible nitrogen limitation (HARGREAVES, 1998). Probably the stronger macrophyte development (more than the phytoplankton) in the control ponds contributed for increasing of oxidation. In fact, this was the negative relation with the PP. It is known that the copepods and rotifers are less efficient phytoplankton filtrators than the cladocerans and the latter are negatively related with the phytoplankton production only (KAGGWA *et al.*, 2009; DODSON *et al.*, 2000; POTUŽÁK *et al.*, 2007).

The multiple regression for chlorophyll *a* in phytoplankton as dependent variable in manured ponds delivered the following equation: lgChl *a*=2.9 – 0.025Secchi – 0.15lgBMbenthos – 0.35lgN/P + 0.36lgNO_3 with regression index significance levels for Secchi p=0.00005, lgBMbenthos p=0.00045, lgN/P p=0.0029, lgNO_3 p=0.0028, respectively, and multiple correlation index R=0.75 with significance level p<0.0000001 (Secchi - Secchi transparency of water column).

Probably the higher turbidity in manured ponds limits both the phytoplankton growth and the immersed macrophyte (HOLOPAINEN *et al.*, 1992). The negative relation with the N/P ratio indicated possible indirect phosphorus influence. The positive regression coefficient of the nitrogen confirmed the results of PP equations for possible nitrogen limitation, while the negative regression coefficient with the zoobenthos most probably was due to the different time occurrence of the growth peaks of both groups of organisms and there was no real casual connection between them.

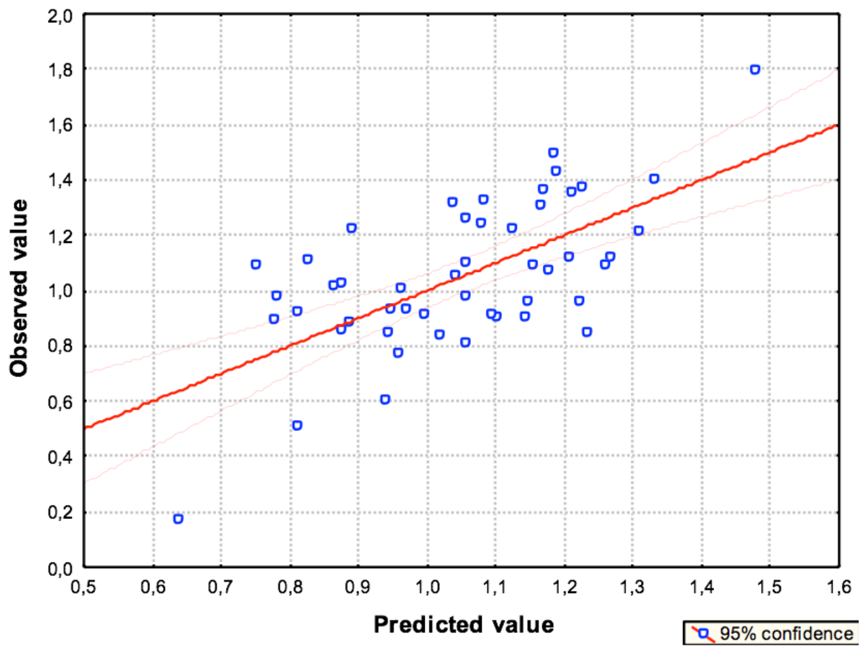

Fig. 1. Observed and predicted values of multiple linear regression of GPP, as a dependent variable, NO_3, Chl *a*, N/P and Flow-rate as independent variables in manured ponds for 2004/05/06 investigation period. The regression equation is lgPgross=0.58 + 0.29lgNO_3 + 0.20lgChl *a* + 0.18 lgN/P +0.056lgFlow-rate with regression coefficient, significance levels for lgN0_3 p=0.002, lgChl *a* p=0.01, lgN/P p=0.14, lgFlowrate p=0.10, respectively, multiple correlation coefficient R=0.65, with level of significance p<0.000062.
Legend: GPP and Pgross – Gross primary productivity; Chl *a* - chlorophyll *a*;

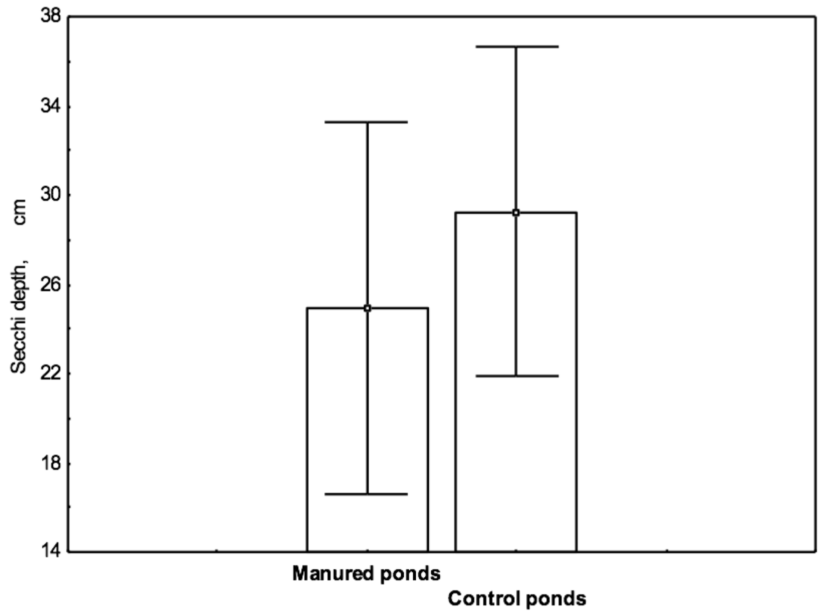

Fig. 2. Average values and standard deviations of Secchi disk transparency in manured and control ponds significantly different for P=0.004 (Wilcoxon test).

The multiple regression of Chl *a* in control ponds delivered the following equation: lgChl *a*=1.92 + 0.28lgBMrot – 0.17lgBMcald – 0.14lgMacroph with regression coefficients, which significance levels were for lgBMrot p=0.0009, lgBMclad p=0.0014, lgMacroph p=0.024, respectively, and multiple correlation coefficient R=0.64, for level of significance p<0.00001 (legend: BMrot - rotatorian biomass; Macroph – macrophyte)

Apparently the lack of enhanced nutrient factors in the control ponds had a significant influence on the regression equation and suggested lower levels of trophy in these ponds. The „top-down" influence was expected to be stronger along the food chain in water of lower trophy (McQueen *et al.*, 1986). Therefore, the biomass of the *Cladocera*, which are more efficient filtrators, had a negative regression coefficient compared to the *Rotatoria*, which have a positive regression coefficient. The rotifers seem to utilize more effectively the phytoplankton and their growth peaks match more closely with the chlorophyll's peaks (Potužák *et al.*, 2007). Macrophytes also demonstrated a negative relationship because of their expressed domination in the control ponds compared to manured ponds causing negative effect on phytoplankton.

Percentage of utilized solar energy (PEU%) in manured ponds repeated more or less the already established relations between the phyoplankton and biotic and abiotic factors from previous two equations. So, the PEU positively correlated with Pgross/Chl *a* (p=0.0000001), chlorophyll *a* (p=0.0000001) and N/P ratio (p=0.009). There was a weak, negative insignificant relation between the PEU and the nitrate ions (p=0.28). The correlation index of multiple regression (R=0.83) was high with significance level p<0.0000001.

The following regression equation for PEU of the control ponds was derived: PEU=3.27 + 1.45lgChl *a* + 1.27lgPgross/Chl *a* – 0.23lgNzoop – 2.1lgT, with significance levels for lgChl *a* p=0.0000001, lgPgross/Chl *a* p=0.0000001, lgNzoopl p=0.0036, lgT p=0.013, respectively (Nzoop - zooplankton numbers).

The correlation coefficient of multiple regression (R=0.81) for the control ponds was high with significance level p<0.0000001. As in the manured ponds, utilizing of solar energy in the control ponds was related with the chlorophyll *a* concentration and Pgross/Chl *a* ratio. The zooplankton numbers and the temperature here were negatively related with PEU. Not the biomass, but the zooplankton numbers was the factor which more actively influenced the phytoplankton. The result was a decreased light utilization. Presumably the reason was the prevalence of small living forms in the zooplankton, surviving under the strong fish pressure.

The multiple regression for the ratio GPP/chlorophyll *a* (lgPgross/Chl *a*), known as assimilation number (AN), repeated some relations observed in the above mentioned equations. On the other hand, it demonstrated markedly weaker relation to the variables selected by the regression analysis.

The correlation coefficient of the multiple regression of respiration in manured ponds was $R=0.71$ with significance level $p<0.000002$, respectively. There was a positive relation with the chlorophyll *a* ($p=0.0047$), nitrate ions level ($p=0.015$) and a negative relation with the rotifer biomass ($p=0.023$) and the total nitrogen ($p=0.076$). The relation between the chlorophyll *a* and the nitrate nitrogen was already discussed above. The green pigment concentration elevation as an indicator for enhancement of the pond trophy led to increasing of the respiration intensity. As they are the largest zooplankton group, the rotifer biomass and total nitrogen are indirectly related by the respiration. It was difficult to establish a causal relationship on the current data basis. The intensity of catabolic processes seemed to decrease with the increase of the total nitrogen in manured ponds, where the anabolic processes prevailed.

The regression equation for respiration in the control ponds is following: $R24= -15.3 + 4.1lgNzoop - 5.6lgNH_4 - 0.15Secchi + 1.9lgFlow\text{-}rate$, with regression coefficient significance levels for lgNzoop. $p=0.002$, $lgNH_4$ $p=0.023$, Secchi $p=0.028$ and lgFlow-rate $p=0.069$. The correlation index of multiple regression ($R=0.52$) was not too high and was with significance level $p=0.004$.

There was a positive correlation of respiration with the zooplankton numbers ($p=0.002$) in the control ponds and a negative correlation with the ammonium nitrogen ($p=0.023$). The respiration increased with the increasing of zooplankton numbers, while the increasing of the ammonium nitrogen was accompanied by the oxygen decrease and reduction of the respiration, respectively. The enhanced macrophytes level in the control ponds apparently suppressed the phyto- and the zooplankton development. An indirect sign for this was the water transparency which demonstrated a negative relation with the respiration. Increasing the flow rate led to the turbulence enhancement of the water followed by the increasing of the oxygen concentration and the respiration, respectively. The mineralization in the flowed ponds was more intensive and there was more expressed turbulent mixing of water compared to the lentic ponds (KONSTANTINOV, 1979).

Conclusions

1. The nitrate nitrogen, followed by the phytoplankton chlorophyll *a* concentration and N/P ratio are the factors which are related with and have a significant influence on the GPP in manured carp ponds with an applied polyculture technology. GPP correlates with more factors in control ponds (without manure) compared to manured ponds. The lack of manure decreases the significance of nutrients along the food chain: nutrients– phytoplankton – zooplankton.

2. The phytoplankton chlorophyll *a* concentration in the manured ponds is significantly influenced by the Secchi disk transparency of the water column, zoobenthos biomass, N/P ratio and nitrate nitrogen. The shortage of nutrient elements in the ponds without manure leads to lower trophy levels. The „top-down" influence on the food chain presented by the zooplankton components is more enhanced.

3. The percentage of utilized solar energy in the manured ponds correlates positively with Pgross/Chl *a* (AN), chlorophyll *a* and N/P ratio. Utilizing of the solar energy in the control ponds is also positively correlated with the Pgross/Chl *a* ratio and the chlorophyll a level, but the zooplankton and water temperature have a negative influence.

4. The plankton respiration in the manured ponds shows a positive relation to

the chlorophyll *a*, the nitrate ions, and the total nitrogen. There is a positive correlation in the control ponds with the zooplankton numbers and the flow rate of the water. A negative correlation is found out with the NH_4-N and the water transparency.

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Stochastic Modeling of Problematic Air Pollution with Particulate Matter in the City of Pernik, Bulgaria

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Abstract. Air quality in urban areas is an important prerequisite for a healthy environment. This paper focuses on the study of the problematic pollutant PM10 in the air over the city of Pernik in order to prevent the worsening of air pollution and to meet the requirements of the applicable regulations and directives, as well as to improve public awareness with regard to health and environmental issues. In this paper, stochastic mathematical models are developed using average 24-hour concentrations of PM10 in atmospheric air over the city for the period from 1 January 2010 until 31 December 2014. The measured values systematically exceed European Union regulations with require that mean daily concentrations should be below 50 μg/m^3. Univariate time-dependent models are derived in the form of time series. The constructed models describe the examined data adequately and also make it possible to forecast future pollution within a timeframe of several days. The selected type of modelling facilitates the decision making needed in the efforts to decrease the pollution levels in future.

Key words: Particulate matter PM10, air pollution, stochastic model.

Introduction

It is well-known that PM10 (particles with an aerodynamic diameter between 2.5 and < 10 μm) can be harmful to human health, which has been shown by epidemiological analysis and toxicological investigations (WHO, 2013). These particles are produced mainly by industrial factories, motor vehicles, and households burning solid fuels, and they can cause asthma, cardiovascular disease, lung cancer, and in some cases, even premature death. The concentrations of PM10 are regulated and monitored in accordance with the provisions of the European Union (EU) as set out in Directive 2008/50/EC (2008) and the air quality standards by the European Commission (2015), pursuant to which the maximum permissible values for PM10 in the air include mean annual values of 40 μg/m^3 and a 24-hour mean value of 50 μg/m^3, which must not be exceeded more than 35 times within one calendar year. Due to the adverse effects on human health caused by PM10 and in order to comply with EU regulations, it is necessary to monitor whether these provisions are met and to undertake measures to eliminate and prevent any violations. In Bulgaria, systematic air pollution with PM10 is problematic in many towns and cities with the worst affected being Pernik (the most polluted one in Europe in 2011), Plovdiv, and Sofia among others, with these often ranking as some of

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the urban areas with the highest concentrations of this pollutant in Europe during the last few years (WHO, 2015).

Observation, control, and forecasting of air pollutant concentrations in Bulgaria are performed by the Executive Environment Agency, and the results of its work are published on the website (ExEA, 2016), along with regular air quality reports for all regions within the country (EEA, 2016).

In addition to the official means to study air pollution, publications also apply various mathematical approaches for modeling accumulated statistical data. A large group of these are stochastic modeling methods for time series, encompassing mostly the ARIMA, SARIMA, and transfer function methods, as well as others. They are based on the Box-Jenkins methodology (Box *et al.*, 1994), which in addition to environmental studies, also finds a wide range of applications in other fields such as economics, econometrics, demographics, etc. ARIMA models in environmental modeling literature have been used, for example in (Sharma *et al.*, 2009; Slini *et al.*, 2006). Hybrid ARIMAs are also applied in combination with the artificial neural network methods, multivariate regression, principle component method, and others, in order to investigate PM10 concentrations (see for example Vlachogianni *et al.* 2011; Vesely *et al.*, 2009; Zwozdziak *et al.*, 2012; Stadlober *et al.*, 2012).

There are very few publications related to the mathematical modeling of PM10 air pollution in urban areas within Bulgaria. We have to note the recent papers (Gocheva-Ilieva *et al.*, 2014; Ivanov *et al.*, 2015; Ivanov & Gocheva-Ilieva, 2013; Voynikova *et al.*, 2015), wherein various methods are applied, such as stochastic modeling, factor and regression analysis, intelligent machine learning methods, etc.

This paper examines the status of air pollution with PM10 in the city of Pernik over a period of 5 years based on average daily measurements. The objective is to construct suitable stochastic models describing actual data, to analyze the quality of the models, and to select an adequate one, as well as to apply the model for estimation and forecasting of future pollution within a timeframe of several days. The availability of data for a relatively long period allows for the application of a method for finding long-term trends, as well as making short-term forecasts for future pollution in order to warn local authorities and the public about any dangers resulting from the exceeded permissible limits for particulate matter concentration.

Data analysis was performed using the statistical software SPSS (IBM Corp., 2013).

Material and Methods

Study area. Pernik is among Bulgaria's cities with the worst air quality, due to the presence of harmful pollutants. The city is located in the south-west of Bulgaria, on the banks of the Struma River. The climate is temperate continental and the average altitude is 750 meters. Rainfall is markedly continental in nature which facilitates pollution and air self-cleaning processes. In 2015, the city's population was around 75,000 people, making it the second most populous city in western Bulgaria after the capital Sofia, and 11th in the country. Pernik is the largest city in Bulgaria where coal is mined. Pan-European transport corridors 4 and 8 pass near the city along the “Lyulin” and “Struma” Motorways, as well as European road E871 and the railway line connecting central Europe with Greece. Among the main sources of air pollution, in addition to vehicle traffic, are large factories such as "Stomana" AD steelworks, fossil-fuel power station "Republika", etc.

Data and initial statistical processing. Our analysis of the situation in Pernik is based on data measured at the "Shahtyor" station, located in close proximity to the city center. The station is equipped with analyzers for continuous measurement of the main and specific atmospheric pollutants such as nitrogen monoxide, nitrogen dioxide, carbon monoxide, carbon dioxide, benzene, PM2.5, and PM10. The data we used cover a period of 5 years – from 1 January 2010 until 31 December 2014 based on average daily observations.

ARIMA Method. ARIMA (Auto-Regressive Integrated Moving Average) models are noted as ARIMA (p,d,q) (BOX *et al.*, 1994). The autoregression element p represents the influence of the data at each moment t of p previous moments within the model. The integrated element d represents trends in the data, and the element q indicates the number of members used to construct the small fluctuations with the help of a moving average.

The main steps of ARIMA methods are:

- Identification – examining the data along with calculation and drawing a graph of auto-correlation functions (ACF) and partial auto-correlation functions (PACF). The smallest values of the parameters are sought. When the value is 0, the element is not needed in the respective model. The element d (trend) is examined first. The goal is to determine whether the process is stationary (d=0), and if it is not, to be transformed into such. The value of p is 0, if there is no connection between every two sequential observations.
- Constructing models and estimating its parameters.
- Diagnostics and selection of model – the residuals and the quality of approximation of the model are examined. The residuals are the differences between the values predicted by the model and the observed data. Theoretically, it is assumed that the residuals are random and normally distributed.
- Application of the predictive model, forecasts, analysis of dependencies, and study problem-solving capabilities.

Results

The results of the initial processing of the data are given in Table 1. As shown, there are no missing values in the measurements. Number of observations N=1826.

The maximum value of 348 μg/m³ for PM10 exceeds 7 times the average daily limit to 50 μg/m³. Such excesses are not isolated occurrences. Table 1 shows that the average value of PM10 of 63,180 μg/m³ for the 5 years exceeds the upper limit for the annual value of 40 μg/m³ as stipulated in European and Bulgarian legislation. Fig. 1 characterizes the behavior of the concentrations of the pollutant over time.

It is known that, the application of parametric models requires normal or close to normal distribution of time variables (WILKS, 2011). The distribution for our original data is shown in Fig. 2 a. In order to improve the distribution and to minimize the variability of the data before constructing the models, various transformations of the initial data are widely used in ecology (WILKS, 2011; BOX *et al.*, 1994).

The initial transformation of the data is performed using the formula (YEO & JOHNSON, 2000):

$$trx = YJlambda(x,\lambda) = \begin{cases} \left\{(x+1)^{\lambda} - 1\right\}/\lambda & x \geq 0,\ \lambda \neq 0 \\ \log(x+1) & x \geq 0,\ \lambda = 0 \\ -\left\{(-x+1)^{2-\lambda} - 1\right\}/(2-\lambda) & x < 0,\ \lambda \neq 2 \\ -\log(-x+1) & x < 0,\ \lambda = 2 \end{cases}, \quad \lambda \in [-2, 2] ,$$

where x is the initial variable, trx is the transformed variable, and λ is an unknown parameter. For our data, the optimal transformation parameter λ is determined using the simple procedure through tests from the values [-2, -1.9, …, 2] and the Kolmogorov-Smirnov test of normality. The effect of the applied transformation is illustrated in Fig. 2 b) – the distribution is close to normal with the chosen optimal parameter λ=*-0.4.*

Table 1. Descriptive statistics of the initial data on PM10 (variable SPM0_1) concentrations in the city of Pernik.

Mean	Median	Std. Dev-iation	Var-iance	Skew-ness	Std. Error of Skewness	Kur-tosis	Std. Error of Kurtosis	Min.	Max.
63.180	47.700	50.273	2527.360	2.272	0.057	5.895	0.114	7	348

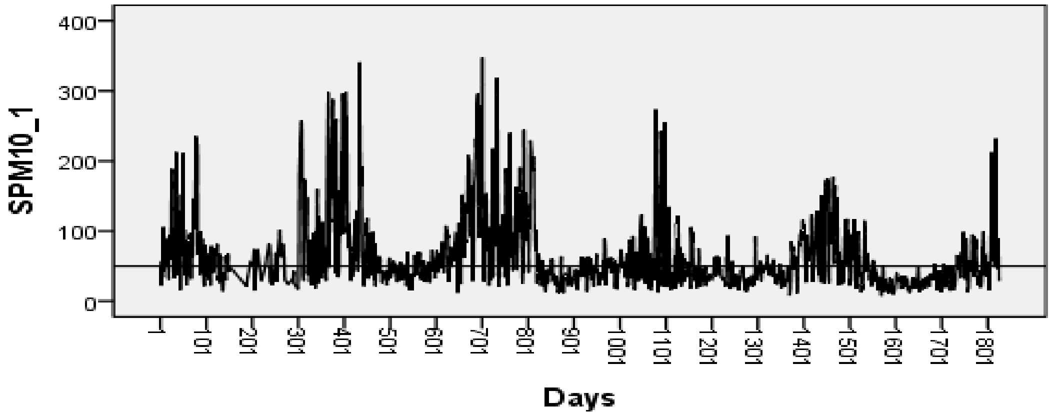


Fig. 1. Graph of the measured average daily data on PM10 concentrations in the city of Pernik. The horizontal line indicates the permissible limit of 50 μg/m³.

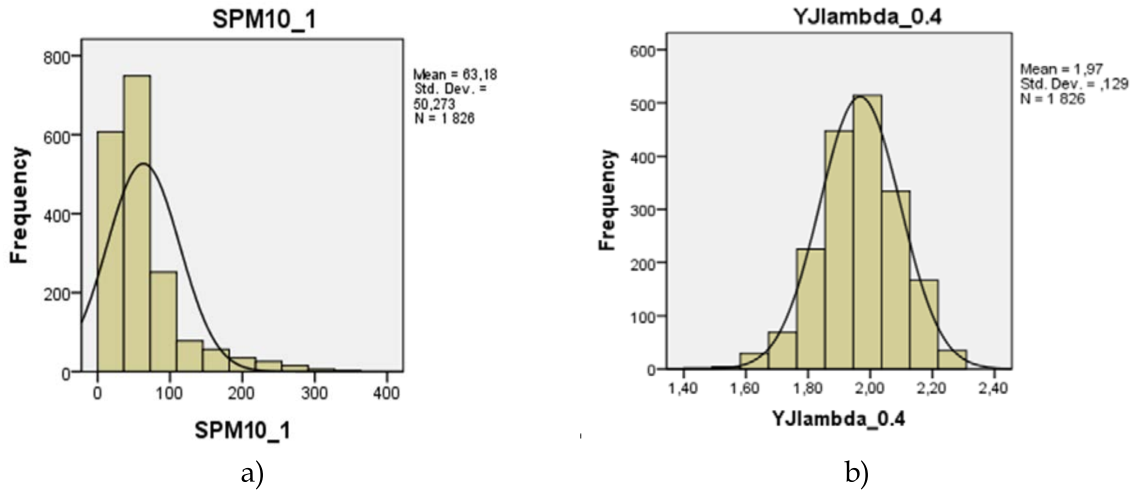


Fig. 2 a, b. Distribution graphs of the initial data of PM10 for the city of Pernik: a) before the transformation; b) after the Yeo-Johnson transformation with λ=*-0.4*.

Construction of ARIMA models and analysis using transformed time series

As part of the analysis of time series, the ACF and PACF functions are used to identify periods and trends in the data. The distribution in the graph of ACF and PACF coincides with the theoretically perfect distribution of a more suitable model describing the main behavior, including the presence of a stationary process, linear or quadratic trends, levels of autoregression, and moving averages, etc.

For the construction of the parametric models, we apply the ARIMA method, looking for models in the form ARIMA(p,d,q) (Box *et al.*, 1994). In Fig. 3 a, ACF goes down slowly, and the PACF function in Fig. 3 b demonstrates three dramatic peaks with delay lags 1, 3, and 6, as well as characteristic distribution with delay in lag 7. This means the PM10 model may be found with three lags back in autoregression (AR) and moving averages (MA) with 5 to 7 terms. Therefore, the expected approximate values of p are: 1<p<5, for ARIMA, it can be considered that there is no trend, i.e. d=0, since it is clear that in PACF under lag 1 there is no value close to +1 or -1, the expected values of q are: 1<q<7. As there is no trend, it can be considered that the series is stationary and there is no trend either towards a reduction, or an increase of PM10 pollution over the examined 5 year period.

In order to determine the most adequate model with the respective values for the parameters (p,d,q), numerous models are constructed with ascending parameter values. When model results are similar, we apply the parsimony principle of choosing the simplest model (Box *et al.*, 1994).

The quality of the models and model fit to data are assessed using the following adequacy tests for time series: the coefficient of determination R^2, root mean square error (RMSE), mean absolute error (MAE), mean absolute percent error (MAPE). Table 2 presents the statistics of two models which provide the best approximation as per these criteria:

$$RMSE = \sqrt{\frac{1}{N}\sum_{t=1}^{N}(y_t - \hat{y}_t)^2},$$

$$MAPE = \frac{100}{N}\sum_{t=1}^{N}\left|\frac{y_t - \hat{y}_t}{y_t}\right|,$$

$$MAE = \frac{1}{N}\sum_{t=1}^{N}|y_t - \hat{y}_t|.$$

where $\hat{y}_t$ is the predicted value at every time t for the transformed variable.

The observed values indicate the percentage of data described by the model which are respectively R^2=0.564 for the model (1,0,5), i.e. the obtained ARIMA describes around 56% of the data and for the model (5,0,7) R^2=0.566. For ARIMA (1,0,5), the autoregression component is (p =1), i.e. the strongest influence on the level of pollution is that of the value for the previous day. The moving average component (q=5) is an indicator that local stochastic changes are correlated with the 5 previous stochastic terms in the time series.

Tables 3 and 4 show that both models have very good statistical significance (Sig.) of the coefficients and the constant considered at level α=0.05.

Table 2. Statistics of ARIMA models for the PM10 pollutant for the city of Pernik.

		ARIMA (1,0,5)	ARIMA (5,0,7)
Model Fit statistics	Stationary R-squared	0,564	0,566
	R-squared	0,564	0,566
	RMSE	0,086	0,085
	MAPE	3,253	3,247
	MAE	0,063	0,062
	Normalized BIC	-4,889	-4,866
Ljung-Box	Statistics	10,543	4,597
	DF	12	6
	Sig.	0,568	0,960

Table 3. Parameters of the ARIMA (1,0,5) model with the transformed data trPM10.

Parameters		**Estimate**	**SE**	**t**	**Sig.**
Constant		1,969	0,022	88,692	0,000
AR	Lag 1	0,987	0,006	176,236	0,000
MA	Lag 1	0,268	0,024	11,099	0,000
	Lag 2	0,352	0,025	14,269	0,000
	Lag 3	0,110	0,026	4,278	0,000
	Lag 4	0,041	0,025	1,656	0,098
	Lag 5	0,077	0,024	3,246	0,001

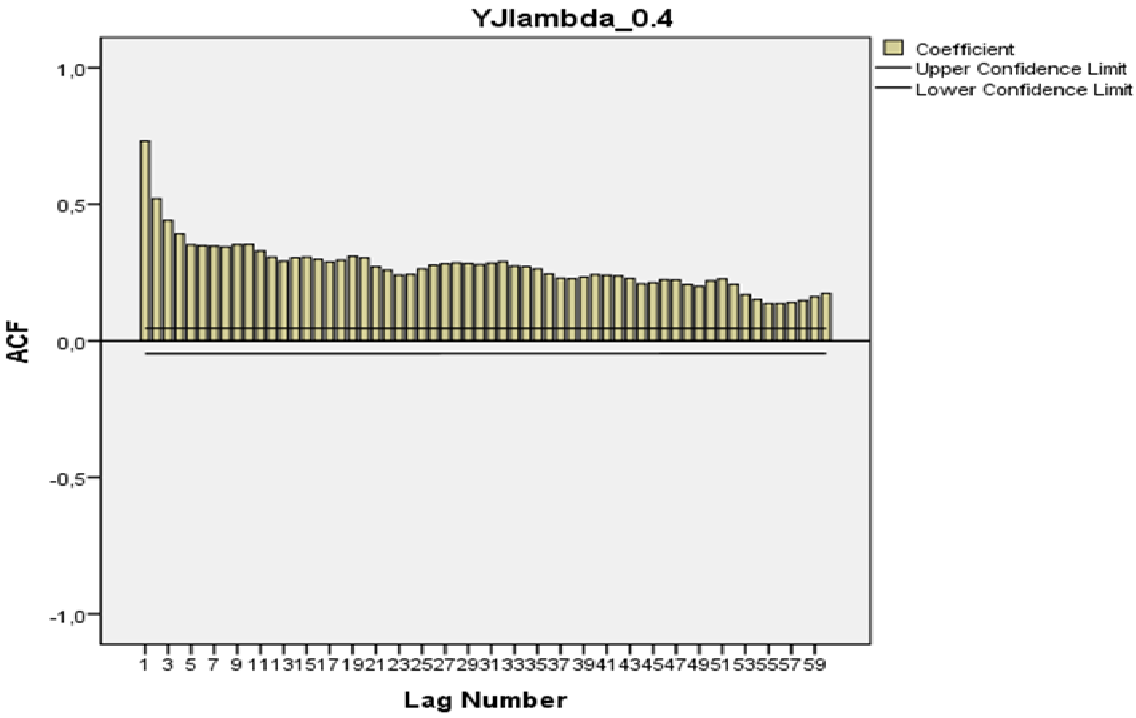
Table 4. Parameters of the ARIMA (5,0,7) model with the transformed data *trPM10*.

Parameters		**Estimate**	**SE**	**t**	**Sig.**
Constant		1,969	0,022	90,176	0,000
AR	Lag 1	1,714	0,237	7,239	0,000
	Lag 2	-2,091	0,504	-4,148	0,000
	Lag 3	1,683	0,595	2,830	0,005
	Lag 4	-0,724	0,453	-1,597	0,110
	Lag 5	0,394	0,179	2,199	0,028
MA	Lag 1	0,998	0,236	4,234	0,000
	Lag 2	-1,220	0,349	-3,501	0,000
	Lag 3	0,553	0,318	1,741	0,082
	Lag 4	-0,042	0,172	-0,242	0,809
	Lag 5	0,196	0,088	2,221	0,027
	Lag 6	0,099	0,056	1,776	0,076
	Lag 7	0,142	0,034	4,226	0,000

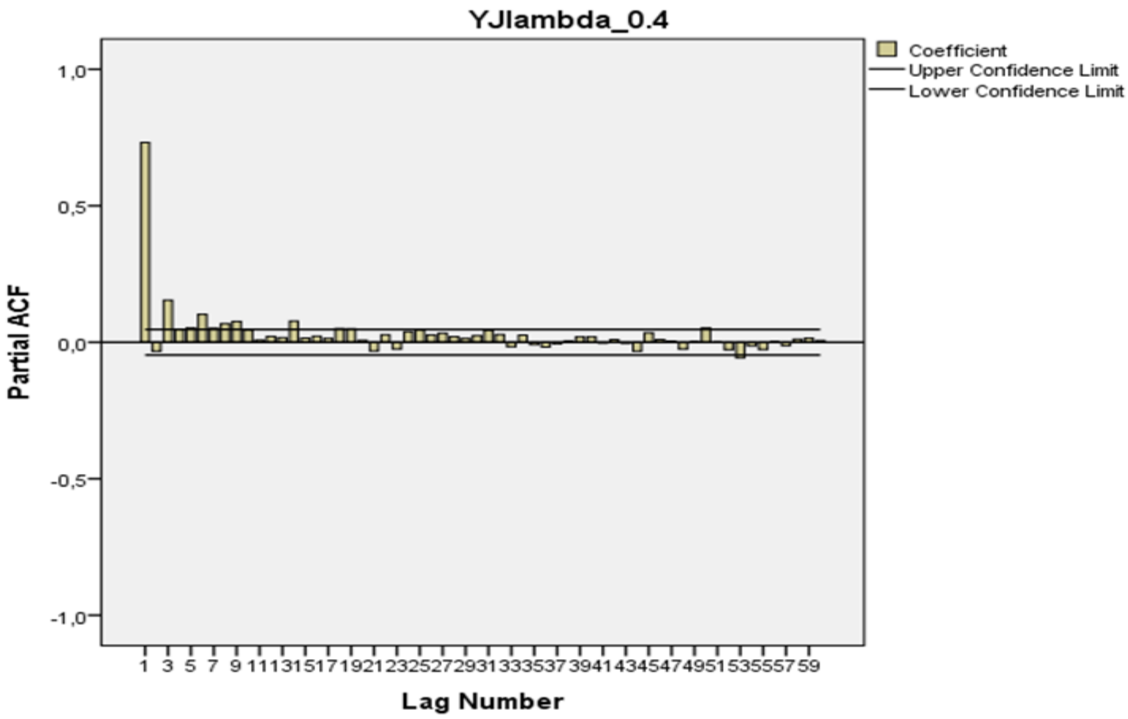
The equation in the case of ARIMA (1,0,5) has the form (see also Table 3): trx_t=1.969 + 0.987trx_{t-1} - 0.268a_{t-1} - 0.352a_{t-2} - 0.11a_{t-3} - 0.077a_{t-5}.

In the first model ARIMA (1,0,5), there is one non-significant parameter in lag MA4, which is excluded from the equation, respectively in the second model ARIMA(5,0,7), there are several non-significant parameters, which are also excluded from the equation, respectively with lags AR4 and MA: 3, 4, 6. The statistical indices in Table 2, demonstrate that the two models provide almost the same approximation, but we chose the simpler model (1, 0, 5) to work with.

Fig. 4 illustrates a comparison of the observed values for the PM10 pollutant with those obtained using the ARIMA (1,0,5) model. The actual measured values for PM10 are given in blue, while the predicted values are in green. Very good fit is observed between the actual data and the values predicted by the chosen model.



a)



b)

Fig. 3 a, b. ACF and PCF for PM10 for the city of Pernik.

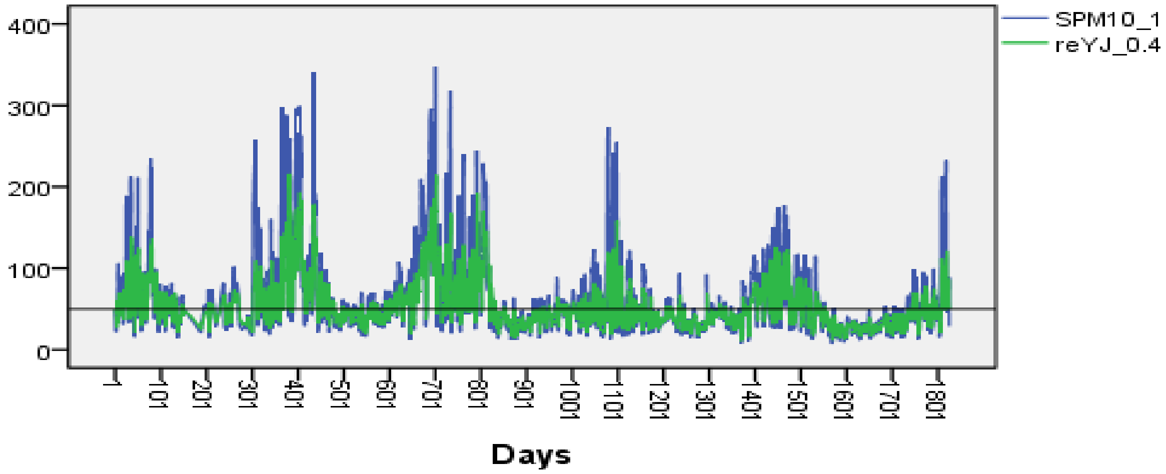


Fig. 4. Comparison of observed values of PM10 against those obtained using the model (1,0,5) following inverse transformation *reYJ*_0.4. The horizontal line indicates the permissible limit of 50 μg/m³.

Diagnostics of the model

As it was described above the model residuals (the differences between the values predicted by the model and the measured data) are assumed to be random and normally distributed. Fig. 5 shows the distribution of the standardized residuals of the model ARIMA(1,0,5), showing zero mean and standard deviation 1. It is very closed to the standard normal distribution N(0,1).

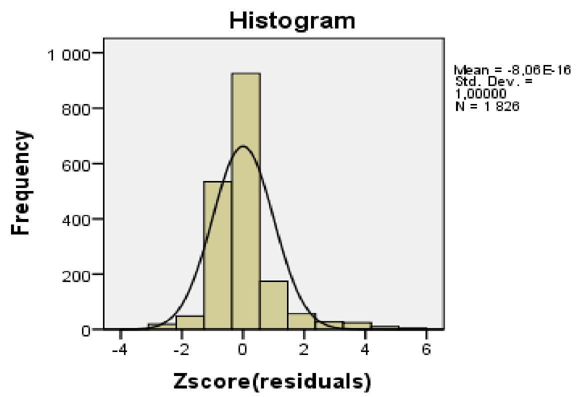


Fig. 5. Distribution of the standardized residuals of the model ARIMA(1,0,5).

Application of the models for forecasting future concentrations

The power of the ARIMA models lies with the good results achieved with predicting future events. In our case, Fig. 6 presents the application of the selected ARIMA(1,0,5) model for short-term forecasting over 7 days – from 25 December to 31 December 2015. To this end, actual additional data is used, which are not included in the construction of the model, and are comparable with the forecasts, obtained using the model. The actual data are colored in green, and the forecast ones in blue. As shown, there is very good fit with the observed variable.

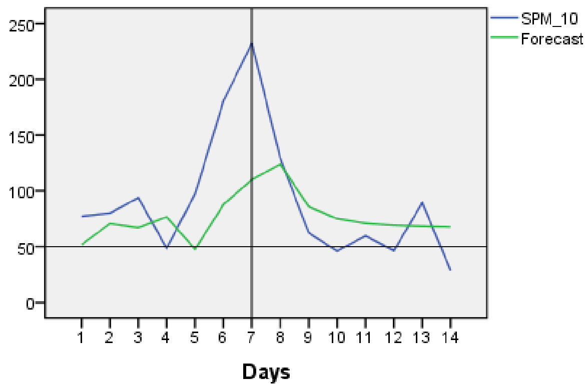


Fig. 6. PM10 values forecast using ARIMA (1,0,5) over a period of 7 days, compared against actual measured values. The horizontal line indicates the permissible limit of 50 μg/m³ and the vertical line is the limit between the last used 7 days (on the left side) and the forecasted 7 days (on the right side of the line).

Discussion

In order to model PM10 concentrations, univariate stochastic ARIMA models of time series are constructed. It is found that the original series does not contain a trend, which indicates that the chosen 5-year period demonstrates no trends for increasing or decreasing pollutant quantities. At the same time, the values of PM10 are high, and their averages exceed the regulatory requirements. It was found that the chosen modeling approach is adequate. The presented graphic forecast of the model compared against the measured concentration fits well with actual measurements. The model is applied for short-term forecasting of air pollution with PM10 for 7 days ahead. The forecast results indicate bad air quality.

In accordance with the regional reports of Pernik Municipality (RIOSV Pernik, 2015) for the air quality, the main reasons for the high pollution levels of fine particulate matter in the city of Pernik are vehicle traffic and households. During winter, heating in the city is provided mainly by solid fuels – coal, briquettes, and wood; with particulate matter remaining at low altitudes and dissipating only slightly. The existing high levels of the pollutant also result from the construction of the „Lyulin" Motorway until 2012, along with the operations of the steelworks "Stomana Industry", etc. The influence of all air pollutants is further worsened by the city's location within a valley, surrounded by mountains. The specific meteorological conditions such as fog, precipitation, also contribute to the worsening of the problem since they cause the accumulation of pollutants close to the ground over a longer period of time.

Conclusions

This study presents the results from the statistical examination of the PM10 air pollutant in the city of Pernik, west Bulgaria. The data are processed using the ARIMA method for time series analysis. The results obtained by applying this method for PM10 show that the pollutant is problematic and exceeds the permissible limit values. The obtained predictive models provide information about future pollution levels which can be used by the respective authorities to take adequate timely measures in order to reduce the concentrations of PM10.

This is a convenient approach for finding long-term future pollution trends and providing warnings in this regard.

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Ecological Plasticity of Apollonia melanostomus (Pisces, Gobiidae) from its Main Habitat Types in Bulgaria

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Abstract. Three native populations of *A. melanostomus* from different Bulgarian habitats were analysed, in view to their morphologic, biochemical-genetic variability and resistance in salinity alterations. Freshwater specimens can survive when fresh water (0‰) is changed rapidly by Black Sea water (16-18‰) and marine vice-versa. In view of the salinity resistance *A. melanostomus* is evaluated as tolerant. Eleven non-enzymatic and 16 enzymatic loci were tested as genetic markers for population identification. Most of them were monomorphic for all populations analysed, with exception of esterases and malate dehydrogenase. These two polymorphic enzyme systems could be used for further analyses of population structure of *A. melanostomus*. The genetic diversity D_{Nei} compared between three populations was low - 0.005-0.016 and the sample from Durankulak occurred to be more genetically divergent. The morphological and biochemical-genetic variability characteristics of the studied populations do not show correlation, but independently vary according to different environmental factors.

Key words: *Neogobius (Apollonia) melanostomus*, genetic markers, morphological variability, invasive species, Black Sea.

Introduction

The round goby *Apollonia melanostomus* Pallas (1814) represents a Ponto-Caspian relict, inhabiting natively Black, Azov and Caspian Seas, as well as rivers, coastal lakes and lagoons connected with these water basins (PINCHUK *et al.*, 2003). In Bulgarian language it is known as "*strongyl*". In its native areal it is a significant target species for the artisanal and commercial fishery (MOSKALKOVA, 1996) and together with other gobiids, it represents an important component of sub-littoral fish communities. The round goby is also a well-known inhabitant of the Bulgarian Danube, although in the past it was comparatively rare (MARINOV, 1966). Although *A. melanostomus* was never attained high population densities in the lower Danube, it is expanded its range to the middle and even upper Danube (ANHELT *et al.*, 1998); STRÁŇAI & ANDREJI, 2004). In the period since 1990, the round goby has extended widely, it has spread through Europe and even North America (PINCHUK *et al.*, 2003; CORKUM *et al.*, 2004; SAPOTA, 2004). The success of the species as an invader has been attributed mainly to its ecological plasticity (HÔRKOVÁ & KOVÁČ, 2014).

A plethora of articles has been published concerning various aspects of the biology and ecology, physiology, behaviour and genetics of the round goby (CORKUM *et al.*, 2004;

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CHARLEBOIS *et al.*, 1997). Morphometric comparison of the species from lower and middle Danube was accomplished by POLAČIC *et al.*, 2012). Genetic analysis by the aims of enzymatic electrophoresis was performed by DOBROVOLOV *et al.* (1995); WALLIS & BEARDMORE (1984a, b); THACHKER & ROJE (2011), etc. Variations in reproductive parameters observed in non-native *A. melanostomus* demonstrate the species' phenotypic plasticity, which appears to be an important attribute that helps potential invaders to establish new populations in unknown environments (HÔRKOVÁ & KOVÁČ, 2014). The available huge amount of information demonstrates the dynamics of the species' populations, as well as their plasticity, and reminds that a population is a constantly changing system. However, the corresponding prediction for the relationship between heterozygosity and morphological variability among populations and species is unclear, and conflicting hypotheses have been suggested (ZINK *et al*., 1985).

The objective of this study was to evaluate the ecological plasticity of *A. melanostomus* from selected areas representing the three main habitat types of the species in Bulgaria on the basis of salinity resistance, morphological and biochemical-genetic data: Danube River, Black sea and a coastal brackish lake. These water bodies differ in their basic hydrology and morphology features including water current, depth and substratum, also in salinity. The followed key assumption was that the possible differences in variation reflect the natural response of the round goby to variable environmental features. Their analysis will also reveal more information on the population dynamics of this highly invasive species.

Materials and Methods

The material was collected from different model ecosystem types: Black Sea, a coastal lake, as well as Danube River during the period 2008–2011 (Fig 1). Part of it was fixed in 4% formaldehyde solution for morphological or frozen at -20°C for genetic analyses.

Adaptation. Salinity adaptation was studied in aquariums with 30 marine and 30 brackish specimens from Black Sea and Durankulak. 1/4 of the initial salinity was lowered daily in a marine aquarium, until it became freshwater. In a second aquarium salinity was decreased to ½ and in a third a rapid exchange of Black Sea water (17‰) with freshwater was performed. The exact opposite operations were performed with the freshwater specimens. The water was tempered before every change; salinity was measured and recorded. Behavior and external morphology was examined two times per day for a ten days period after every manipulation.

Morphometrics. Measurements of 23 continuous external morphological characters from fish pictures were accomplished by the aims of digitizing software as described by SIMONOVIĆ (1999) with abbreviations presented in Table 1.

Genetics. Proteins and enzymes were separated by horizontal starch gel electrophoresis according to SMITHIES (1955) methods, modified by DOBROVOLOV (1973). Besides it, isoelectric focusing (IEF) on thin polyacrylamide Ampholone gel with 3.5-10.0 pH gradients was applied, as well as IEF on ultra-thin polyacrylamide Servalyte gel plates provided by LKB (Stockholm, Sweden) (Fig 2). The proteins were stained with Commassie Brilliant Blue R-250. Staining of different enzymes was undertaken according to SHAW & PRASAD (1970). Buffer systems described by DOBROVOLOV (1976) and CLAYTON & GEE (1969) were used for the electrophoresis. The nomenclature of mentioned loci and alleles followed the recommendation of SHAKLEE *et al.* (1990). Five enzymatic systems were studied: alcohol dehydrogenase (EC 1.1.1.1. – ADH), esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 – LDH (EC 1.1.1.37 – MDH), malic enzyme (EC 1.1.1.40 - MEP), superoxide dismutase (EC 1.15.1.1 – SOD), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8.- G3PDH).

Statistics. Measurements were transformed to ratios, in order to avoid allometric effects, although some basic head lengths do not change according to age SIMONOVIĆ *et al.* (2001). Normality tests for the measured morphometric variables were performed according to SHAPIRO & WILK (1965). Principal Component Analysis (PCA) was used in order to establish which of the measured characters are most flexible. Statistics was computed according to HAMMER *et al.* (2001). The experimental allelic frequencies were tested for deviations with the Hardy–Weinberg equilibrium (HARDY, 1908; WEINBERG, 1908). Intrapopulation genetic diversity Hj was also calculated. Calculation of genetic similarity and genetic distance indices was performed according to NEI (1972). Phylogenetic analysis was done using PAUP, version 4.0 SWOFFORD (1998), visualized with "TreeView" v1.6.6. The morphometric Euclidean distances were calculated according to CULMAN *et al.* (2009).

Fig. 1. Sampling stations: 1 – Danube, Belene 2 – Durankulak Lake, 3, Black Sea, Sinemorets.

Results

Salinity adaptation

The accomplished in aquariums experiments showed that all examined specimens of *A. melanostomus* from a brackish environment are capable to adapt without visual consequences in behaviour or external morphology (white eyes or other abnormal characteristics) when freshwater (0‰) is changed rapidly by Black Sea water (16-18‰). Marine specimens also survived in freshwater without preliminary adaptation in intermediate salinities.

Morphometric analysis

The investigated specimens from the three selected model ecosystems showed comparatively low rates of variability. The Danube group occurred to be the most variable (Table 1). A Principal Component Analysis based on the measured traits (Fig. 4) configures three not well separated groups, in accordance with their site of origin. Most distinctive traits for the three independent groups' delimitation are praeanal length, length to anus and praeopercular width respectively in significance.

The calculated Euclidean distances among the three examined groups (Fig. 5) showed, that the specimens from Durankulak Lake are slightly more distinct from the others (Danubian and marine).

Genetic-biochemical analysis

Eleven non-enzyme protein loci were visualized. In Varna Bay and Shkorpilovtsi region (Black Sea) *A. melanostomus* showed completely different position of *PROT-4**, *PROT-5**, *PROT-6** and *PROT-8** loci in comparison with the other two (freshwater and brackish) samples (Fig 2).

Totally 16 enzyme loci were analyzed and polymorphism was found in three of them (Table 2). *EST-3** and *EST-4**- loci (Fig 3), as well as *MDH-2** were polymorphic, with allelic frequencies given in Table 2. The other studied enzyme systems corresponding to 13 loci (LDH, SOD, MEP, ADH and β-GPDH) were invariant.

All the examined Black Sea specimens were monomorphic in regard to the analyzed enzymatic systems. The sample from Durankulak occurred to be more genetically divergent (Fig 6).

The intrapopulation genetic diversity Hj of the examined groups occurred to be relatively low, varying from 0 to 0.041 (Table 2).

Table 1. Estimated allelic frequencies of polymorphic loci of *A. melanostomus* from different Bulgarian localities: a*- slower (anodal), and b*- faster (cathodal) electromorph. H_o = observed heterozygosity, H_e = expected heterozygosity.

Locus	Allele	Danube River Belene N=10	Durankulak Lake N=12	Black Sea N=42
EST-3*	a*	0.7	1	1
	b*	0.3	0	0
	H_o	0.2	0	0
	H_e	0.42	0	0
EST-4*	a*	1	0.6	1
	b*	0	0.4	0
	H_o	0	0.4	0
	H_e	0	0.48	0
MDH-2*	a*	0.917	0.9	1
	b*	0.083	0.1	0
	H_o	0.2	0.2	0
	H_e	0.152	0.18	0
Percentage of polymorphic loci	P	0.125	0.125	0
Intragroup genetic diversity Hj		**0.036**	**0.041**	**0**

Discussion

In view of the salinity resistance test and taking in mind the established thermal tolerance of the species round goby could be assumed as *superinvasive* CROSS & RAWDING (2009).

The specimens from Black Sea (Bulgarian Black Sea coast) were the most genetically conservative, as being monomorphic in view to the analysed enzymes. The majority of the analyzed enzyme systems (LDH, SOD, MEP, ADH and β-GPDH) are monomorphic for all analyzed populations. Only the high variable system of esterases represents a useful tool for further investigation of the populations' structure of the species. The established comparatively low rates of genetic variability of *A. melanostomus* (0 - 0.041) correspond with the results of WALLIS & BEARDMORE (1984a, b), and these of SMITH & FUJIO (1982).

Durankulak Lake was connected periodically with Black Sea till 60's, so *A. melanostomus* could migrate from the Sea to the lake and vice versa – in case of populations' admixture. This isolation was enough for a genetic distance D_{Nei}= 0.011 between the marine and the lacustrine sample – a higher divergence between this and the marine group, when comparing both with the Danubian one (Table 3). From another point of view, two groups from recently separated ecosystems (Durankulak and Black Sea) are also more morphologically divergent, than the other two analyzed combinations among the analyzed groups (Fig 5). The conclusions of BROWN & STEPIEN (2008) concerning higher established variation in mixed populations could explain this fact, as well as other hypotheses (environmental impact), which should be supported by additional evidence. Morphological traits can be changed according to the environmental factors, mainly the concrete trophic ecology of a species (WOOTTON, 1990; ADAMS *et al.*, 2003; MURAKAEVA *et al.*, 2003). The probability of trophic habits' impact on morphology in this case has to be clarified.

Linear correlation between genetic and morphologic distances between the compared pairs of the round goby from different habitats given in Table 3 was rather strong, but not significant (R=0.73, p=0.4). The obtained results are based on 3 groups only, but still could not confirm Lerner's postulated association between individual heterozygosity and morphological variability if simply extrapolated to higher levels of organization: a negative correlation between allozymic and morphological variance should be observed among populations and higher taxa (LERNER, 1954). Indeed, any particular measures of genetic and phenotypic variability will not be linearly or even monotonically related to one another (STRAUSS, 1989). Due to the lack of a theoretical expectation and many possible confounding factors, the independence of genetic and morphological variances should be by accepted by definition (ZINK *et al*., 1985).

Table 2. Morphologic variability of three *A. melanostomus* populations from Bulgaria.

N		Description	Belene (Danube) N=29 CV	Durankulak lake N=32 CV	Black Sea N=32 CV
1	In % of SL	Head length	2.21	1.14	2.32
2		Height at the beginning of the first dorsal fin	1.24	0.5	0.89
3		Height at the beginning of the second dorsal fin	1.5	0.53	1.55
4		Height at caudal peduncle	0.47	0.19	0.24
5		Caudal peduncle length	2.13	1.48	1.81
6		Praedorsal length to the first dorsal fin	0.6	0.92	0.37
7		Praeedorsal length to the second dorsal fin	1.17	1.19	0.74
8		Praeanal length	2.02	2.23	2.19
9		Praeventral length	3.14	1.09	1.37
10		Jaws length	1.22	0.38	0.82
11		Length from the beginning of the first dorsal fin to anus	1.6	0.66	0.84
12		First dorsal fin length	1.04	0.32	0.75
13		Second dorsal fin length	3.73	2.68	1.63
14		Anal fin length	3.83	2.98	1.95
15		Pectoral fin length	5.92	2.51	3.58
16	In % of Lc	Praeocular length	0.74	0.38	0.77
17		Eye diameter	0.41	0.53	0.41
18		Postocular length	1.18	0.55	0.91
19		Head height	1.31	0.51	1.61
20		Jaws width	1.79	0.73	1.97
21		Praeopercular width measured ventrally	2.73	0.89	1.82
22		Ventral fin length	2.06	1.42	0.97
23		Length to anus measured ventrally	6.53	1.99	5.61
24		Interocular length	0.58	0.22	0.64
25		Praeopercular length measured dorsally	0.7	0.98	0.81
26		Opercular length measured dorsally	0.99	1.06	1.29
		Average morphologic variability	1.96	1.08	1.46

Table 3. Genetic identity/distances according to Nei and morphological Euclidean distances between three *A. melanostomus* groups from Bulgaria. (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene).

	D-B/Dk	D-B/BS	Dk/BS
Genetic identity I_{Nei}	0.984	0.995	0.989
Genetic distance D_{Nei}	0.016	0.005	0.011
Euclidean morphological distance	1.6013	1.3025	1.72

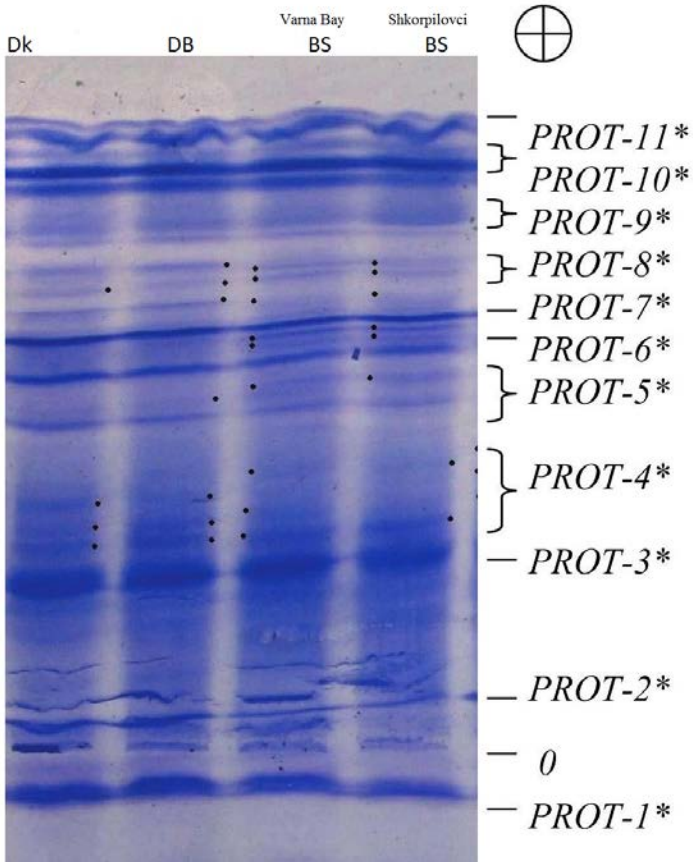

Fig. 2. Isoelectric focusing (IEF) of non-enzyme protein systems on thin polyacrilamide Ampholine gel plate with pH range 3-10 of *A. melanostomus* from different Bulgarian localities: Dk-Durankulak coastal lake, DB-Belene (Danube River), BS-Varna Bay (Black Sea), BS-Shkorpilovci (Black Sea). The differences were marked with black spots, 0 – origin.

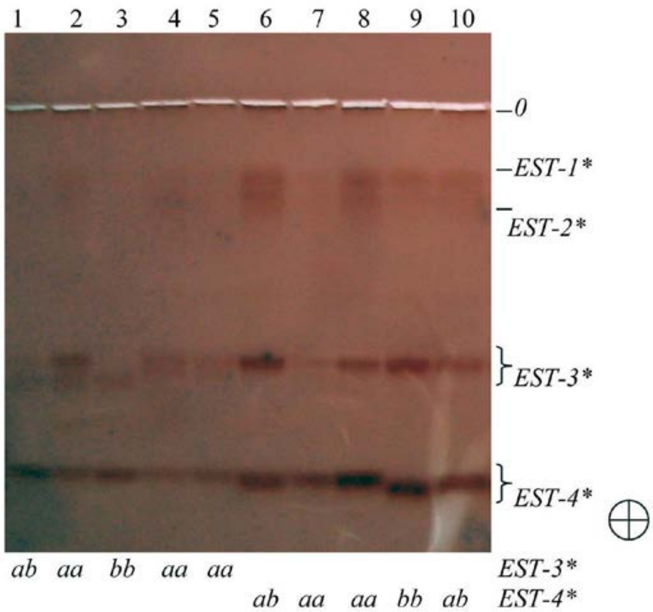

Fig. 3. Electrophoregrams of esterases (EST) on starch gel of *A. melanostomus* from different Bulgarian localities: 1-5-Belene (Danube R.), 6-10-Duranculak lake. *EST-3** and *EST-4** - polymorphic loci and phenotypes are given under the picture, 0- origin.

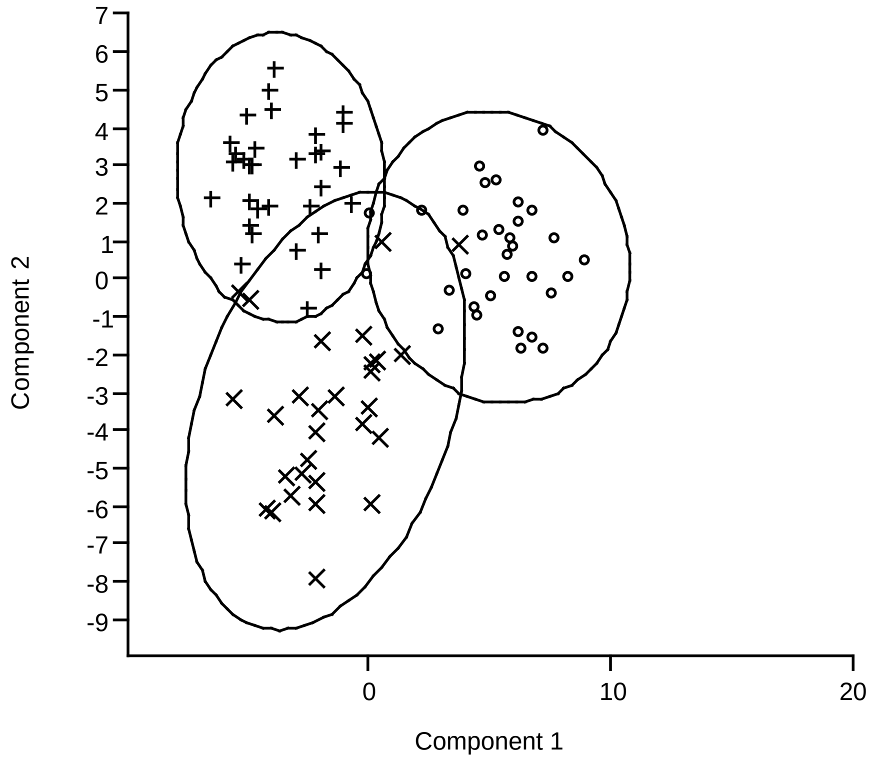

Fig. 4. Comparison of three Bulgarian *A. melanostomus* groups on the basis of 26 morphometric traits. + - Black Sea, o – Durankulak coastal Lake, x - Danube River.

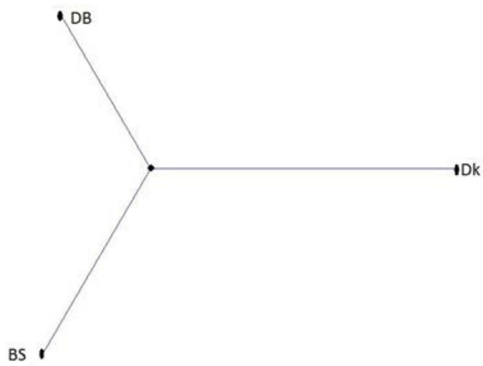

Fig. 5. Euclidean morphologic distances between three *A. melanostomus* groups from Bulgaria on the basis of 26 morphometric traits. (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene).

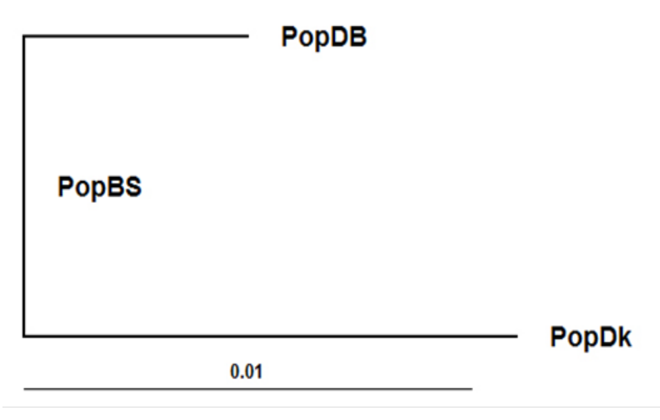

Fig. 6. Genetic distances between three *A. melanostomus* groups (BS-Black Sea, Dk-Durankulak Lake, DB-Danube, Belene), from Bulgaria on the basis of the allelic frequencies of 16 enzymic loci.

Morphologic variability could not be viewed in the "marine-freshwater" axis in this particular case, as well as the correspondence of the environmental salinity with the genetic variability of each group. These data are controversial to SIMONOVIĆ *et al.* (2001), as only praeanal length is most conservative in the freshwater sample, in comparison with the brackish and marine ones.

On the basis of the obtained results it could be concluded that *A. melanostomus* has a remarkable ability for colonization of new habitats of variant salinities from 0 to $17^0/_{00}$. The morphological and biochemical-genetic variability of the species do not show connection, but independently vary according to different environmental factors (salinity, water velocity, depth, and hydrology). Further comparative research of more localities where the species occurs as well as ecology of mixed populations is essential should be done, in order to receive a better picture of its population structure.

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Distribution of Plant Species and Their Relation to Soil Properties in Protected and Degraded Stands of Quercus macranthera in Northern Iran

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Abstract. *Quercus macranthera* is extended in forest areas of the upper elevations in the north of Iran and plays an effective role in conserving soil and water infiltration. The biggest problem in the region is livestock grazing and forest dwellers. The aim of this study was to study the distribution of plant species and their relation to soil properties in protected and degraded stands of *Quercus macranthera*. This study included both protected and degraded areas. Within each of the study areas, we used a random systematic 100 m × 100 m sampling grid to locate 20 circular plots (1000 m^2 plot for tree and shrub species and 8m^2 for herbaceous species) in each area. Also, in each plot, soil samples were collected from 0 to 30 cm depth. The result showed that the distribution pattern of species in both areas was different. Shrub species and invasive herbaceous species were more abundant in the degraded area. Diversity, richness and evenness in the protected area were significantly higher than the degraded area. The soil nutrient factors were significantly more in the protected area. Soil texture was no different between the two areas. It seems that conservation programs such as prevention of livestock need to be carried out in order to protect *Quercus macranthera*.

Keywords: livestock grazing, conservation, soil physical and chemical properties *Quercus macranthera* stand, Roudbar forest.

Introduction

Soil types with different structure and nutrients are important for plant growth and community development. Soil conditions are different in different forest areas and are also related to the restoration process (ZHANG & DONG, 2010). The interactions of environmental factors are important in the restoration process and must be considered in the management of the areas (GATTIE *et al.*, 2003). Well-defined species–environment relationships are important to understand vegetation patterns on forest landscapes (HIX & PEARCY, 1997). The effects of environmental variables on plant species have been the subject of many ecological studies in recent years (RAMIREZ *et al.*, 2007). Research focusing on the relationship between plant communities and environmental variables such as soils has become increasingly important in understanding the ecology of forest communities and especially their ground-layer vegetation (OLANO *et al.*, 1998).

Grazing domestic herbivores are a major ecological factor that shape structure and function of the remaining natural rangelands in the world. Biodiversity of uplands has received particular attention because it relates to productivity and stability. While heavy grazing generally leads to a reduction in diversity, the effects of intermediate grazing pressures differ. For example, sagebrush steppes in Montana reach maximum diversity at intermediate stocking rates; while others show maximum values in exclosures and grazing even at moderate (OLIVA *et al.*, 2016). Grazing is the common land-use throughout the world. It has substantial effects on many ecosystem processes and functions, such as nutrient pool and cycling, soil moisture and structure, vegetation composition and productivity. It has generally been concluded that grazers could affect the floristic composition and diversity in different ways, depending on the type of grazing animals, intensity of grazing and host plant species (BARDGETT & WARDLE, 2010). Livestock overgrazing is considered as the main cause of degradation through lowering both the productivity and resilience of host species, reduction of vegetation cover, increase of unpalatable species, decrease of species diversity, and alteration of soil structure and compactness. Effects of grazing on the plant community and soils are viewed as destructive agents because of the reduction of ground cover, productivity and soil erosion (AL-ROWAILY *et al.*, 2015).

Many abiotic factors, such as soil moisture, light, and nutrition affect composition of vegetation. Human-caused factors include livestock grazing, mining, and timber harvest. These factors affect the plant species composition and the establishment and stability of seedlings. There are significant correlations between human disturbances (such as livestock grazing, anthropogenic wildfire, and logging) and composition, richness and abundance (GILLESPIE *et al.*, 1999). Grazing of macro-herbivores has a great effect on forest plant community structure. Understory perennial plants in protected areas are typically more abundant than in unprotected forest stands (SABO *et al.*, 2009; JAVANMIRIPOUR *et al.*, 2012).

Understanding the relationship between plant diversity and land use history can have important implications in management decisions, especially when these ecosystems were widely used by humans (TALAMO *et al.*, 2012). Nearly 87 % of the Iranian forest and rangeland degradation was caused by human activities such as irregular grazing and wood consumption (HEYDARPOUR *et al.*, 2008). The composition and diversity of plant communities in many of these natural ecosystems were considerably affected, and the extent to which these changes were significant depended on the intensity and frequency of degradation sources and on the ability of plant species to adapt to these new conditions (HERATH *et al.*, 2009). Grazing can change competitive balance between species, composition and abundance of plants, species dominance establishment of plant species, ecosystem processes and biodiversity. These changes can progressively lead to unbalanced ecosystems, which can hardly revert to their initial state (POLASKY *et al.*, 2011; CLARK & COVEY, 2012).

Extant forests of northern Iran consist mostly of broadleaf deciduous species, but some areas are locally covered by a Mediterranean-type vegetation. Moreover, the distribution of forest types in northern Iran is heterogeneous, with forest productivity following a decreasing west-east gradient. Caspian forests appear to be very similar to broadleaf forests typical of central Europe, northern Turkey and the Caucasus (MARVIE MOHADJER, 2006). Forests of the Guilan Province are located in the western part of the Hyrcanian forest region. The dominant species in the northern forests of Iran is beech (*Fagus orientalis* Lipsky), which covers about 565,000 ha and represents the total area of indigenous forests in Guilan Province. Beech forests are the richest, most productive forest communities in Iran because of their economical and environmental value. These forests represent a major carbon pool in the region and are important for their economic value, ability to protect soil, and provide recreation resources. The greatest forest volume occurs in Iran's beech forests (ADEL *et al.*, 2013).

Quercus macranthera covers forest areas of the upper elevations in the north of Iran and plays an effective role in conserving soil and water infiltration. Also, it affects wildlife conservation in the upland. This species grows at altitudes above 1,700 m. In recent years, livestock grazing and human use have reduced and degraded these forests. Studying the effects of livestock grazing and human use on the composition and diversity of vegetation in *Quercus macranthera* forests would yield important information necessary for forest managers.

The aim of this study was to study the distribution of plant species and their relationship with soil properties in protected and degraded areas of *Quercus macranthera* in Roudbar forests in the north of Iran.

Material and Methods

Study area

The study area (Fig. 1) is located in the District 6 (Dasht Daman), at Roudbar City in the south of Guilan Province in northern Iran (36° 45′ 8″ to 36° 55′ 12″ N latitude and 49° 30′ 15″ to 49° 40′ 10″ longitude E). Elevation within the study area ranges from 1800 to 2520 m a.s.l., mean of slope is 50%, and the general aspect is south. Parent materials include lime silt, sandstone, silt stone and shill. Soils texture is loamy clay, with weak acidic pH (6.2-6.8). The maximum temperature is 21.8° C in August and minimum temperature is -3°C in February. The climate, based on the Emberger classification, is humid with mean annual precipitation of 694 mm at the nearest meteorological station (Rasht City). The biggest problem in the region is livestock grazing and forest dwellers.

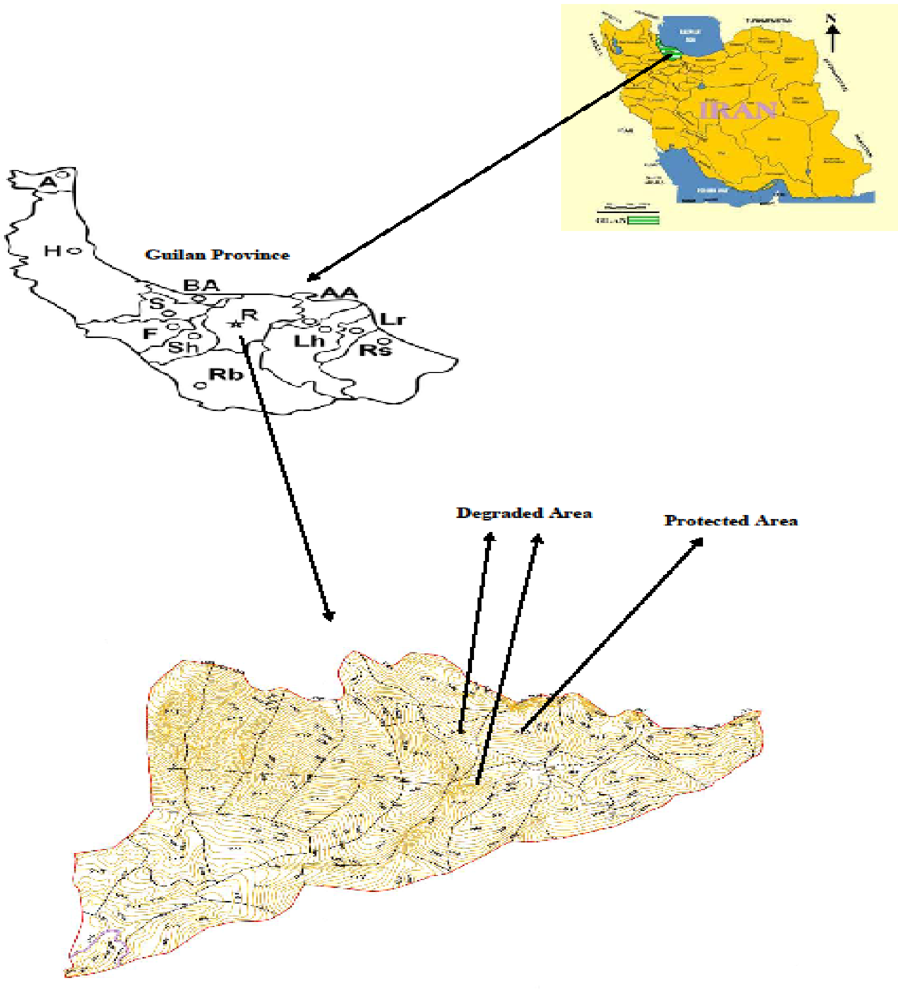

Fig. 1. Map of the study area - District 6 (Dasht Daman), at Roudbar City in the south of Guilan Province in northern Iran.

Data collection

This study included both protected and degraded areas. The two areas had similar elevations, slopes and aspects. In each of the study areas, we used a random systematic 100 m × 100 m sampling grid to locate 20 circular plots (each plot is 1,000 m^2) in each area, resulting in a total of 40 plots. In each plot, type and number of woody (tree and shrub) species were recorded. Also, in a smaller sub-plot of 8m^2, we recorded the percentage of herbaceous species cover. In each plot, soil samples were collected at these 40 plots. Soil samples were collected from 0 to 30 cm depth for physical and chemical properties. In each plot, we collected a composite sample, mixing three sub-samples. Soil samples were prepared for analysis by air-drying and sieving using a 2 mm screen. Sand, silt and clay percentages were determined by the hydrometric method (BOUYOUCOS, 1962). Soil pH and electrical conductivity (EC) were determined using appropriate meters. Total nitrogen (N) was analyzed by the Kjeldahl method (BREMNER, 1996). Available phosphor (P) was determined by colorimetry according to the Bray-II method (BRAY & KURTZ, 1945). Organic carbon (OC) and organic matter (OM) were deter-mined by the WALKLEY & BLACK (1934) method. C/N ratio was calculated. Total potassium (K) was analyzed by flame atomic absorption spectrophotometer (MAPA, 1994).

Data analysis

DCA analysis was used to study plant species composition. RDA and CCA analysis were used to explore the relationship between soil factors and plant species. To determine whether to use linear or unimodal, DCA was used to evaluate the gradient length of the first axis. If gradient length was more than 3, we used CCA and if it was less than 3, we used RDA. A Monte Carlo permutation test based on 499 random permutations was conducted to test the significance of the eigenvalues of the first canonical axis (LIU *et al.*, 2012). DCA, RDA and CCA were performed by CANOCO 4.5. To evaluate herbaceous diversity, we used three indices. Species diversity was assessed with the Shannon–Wiener index, species richness was estimated according to the Margalef index. In addition, the Smith–Wilson index was utilized to calculate species evenness. All three indices were computed using Ecological Methodology and PAST software. The Kolmogorov–Smirnov test was used to evaluate the normality of parameters. Leven test was used to assess the equality of variances. Independent t test were used to compare data that were normally distributed. For parameters that were not normally distributed, the nonparametric Mann-Whitney U-test was used.

Results

DCA ordination of tree species

The distributions of tree and shrub species in the study area are shown in Fig. 1. *Quercus macranthera* is located at the centre of the graph, but was not located during the first and second axes. *Fagus orientalis* and *Sorbus torminalis* species are located at the bottom and right parts of the graph. They exist only in the degraded area. *Carpinus orientalis* and *Quercus castaneifolia* species are located in the bottom and left parts of the graph. These species had a low presence in both areas. They existed near the forest parts with *Fagus orientalis* and *Sorbus torminalis* more than ecotone areas. *Acer campester, Mespilus germanica* and *Pyrus communis* are located in the upper and left parts of the figure, and exist in ecotone and higher elevations. Shrub species of *Juniperus communis, Crataegus microphylla, Cotoneaster nummularia, Prunus spinosa* and *Berberis integgerima* were on the right side of graph. These species are the species that are present in both areas, but mostly distributed in the degraded area.

DCA ordination of Hebaceous species

The distributions of herbaceous species are shown in Fig. 2. Species that are present in the upper part of the graph include *Cruciata laevipes, Stachys byzantiana, Ornithogalum sintenisii* and *Thalspi hastulatum*. These species are found only in the degraded area and have low abundance.

Trifolium repens, located in the upper part of the graph, has a high abundance in both areas. Species that are located on the right side of graph included *Brachypodium silvaticum, Dactylis glomerata, Festuca rechingeri, Poa trivialis, Viola somchetica, Astragalus ureus, Erodium cicutarium* and *Marrubium vulgar*. These species have the highest cover percentage in the degraded area or are only present in the degraded area. Species that are located on the left side of graph included *Asplenium trichomanes, Dryopteris pallida, Viola odorata, Carex divolsa, Geranium molle, Arenaria leptophylla* and *Moehringia trinervia*. These species are only present at lower altitudes and near the forest or were more abundant in this area. Other species that are located in the lower part of the graph, such as *Alium erubescens, Urtica dioica, Polygonum aviculare, Phlomis anisodonta, Veronica persica, Hypericum perforatum, Clinopodium umbrosum, Asyneuma amplexicaule, Medicago lupulina, Crocus gilanicus* and *Milium vernale*, have lower abundance in one of the two protected or degraded areas.

RDA ordination of woody species

The RDA ordination was used because the length of the gradient was calculated (1.19) to be smaller than 3. The first (0.764) and second (0.034) axes had the largest Eigenvalues, which accounted for 97.5% of variation. The results of RDA indicated that *Quercus macranthera* had no correlation with nutrient such as N, P and K. Also, this species had a negative correlation with pH, C.N, clay and sand. *Pyrus communis* and *Quercus castaneifolia* had positive correlation with N, P, K, CEC and Sand. *Carpinus orientalis* and *Acer campester* had positive correlation with pH, clay and C/N. Shrub species of *Prunus spinosa, Berberis integgerima,* Juniperus *communis, Crataeogus microphylla, Mespilus germanica* and *Cotoneaster nummularia* and also *Sorbus torminalis* tree species had a low correlation with clay and silt, but had no correlation with other soil factors. *Fagus orientalis* only correlated with sand.

CCA ordination of herbaceous species

The CCA ordination was used because the length of the gradient was calculated (4.56) to be greater than 3. The first (0.737) and second (0.137) axes had the largest Eigenvalues, which accounted for 79.8% of variation. The results of CCA indicated that the species that are located on negative side of first axis, such as *Brachypodium silvaticum, Dactylis glomerata, Festuca rechingeri, Poa trivialis, Viola somchetica, Astragalus ureus, Erodium cicutarium* and *Marrubium vulgar*, have a low correlation with clay and silt. Species of the positive side of first axis included *Asplenium trichomanes, Dryopteris pallida, Viola odorata, Carex divolsa, Geranium molle, Arenaria leptophylla, Moehringia trinervia, Hypericum perforatum, Asyneuma amplexicaule, Clinopodium umbrosum* and *Trifolium repens*. These species have the most positive correlation with N, P, K, OC, OM, EC and CEC. Also, they have a low correlation with pH and sand. Species on the upper part of graph, *Phlomis anisodonta, Stachys byzantiana, Ornithogalum sintenisii, Thalspi hastulatum, Alium erubescens* and *Urtica dioica*, have the most negative correlation with C.N. Other species on upper part of graph, for example, *Medicago lupulina, Milium vernale, Cruciata laevipes* and *Crocus gilanicus* have no correlation with the soil variables.

Comparison of soil factors between protected and degraded areas

The result of comparisons of soil physical and chemical properties showed that N, P, K, OC, OM, EC and CEC were significantly higher in protected areas than degraded areas. Also, there were no significant differences in pH, clay, silt, sand and C.N between the two areas (Table 1).

Biodiversity indices

Result showed that biodiversity indices (diversity， richness and evenness) in the protected area were significantly higher than degraded area (Table 2).

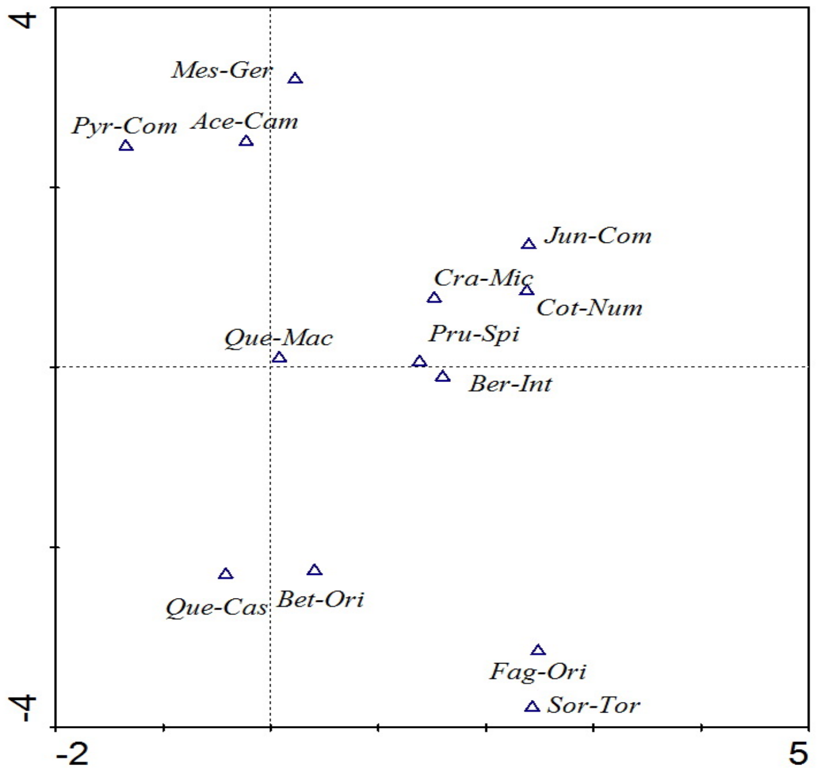

Fig. 2. DCA ordination of woody species in protected and degraded areas.

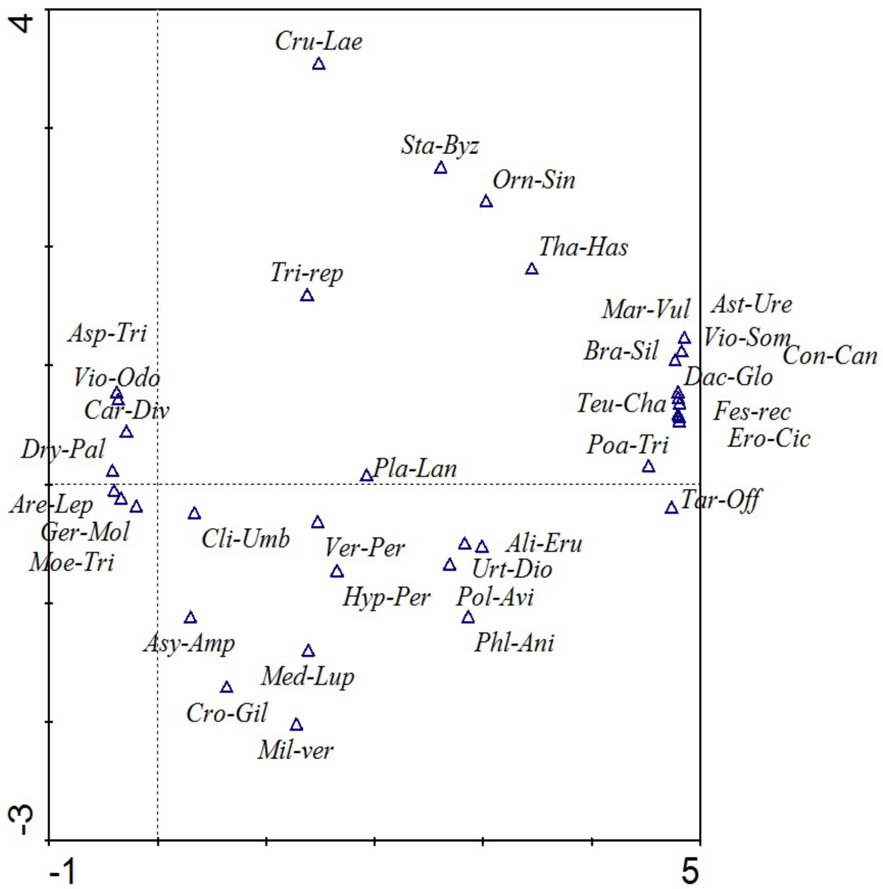

Fig. 3. DCA ordination of herbaceous species in protected and degraded areas.

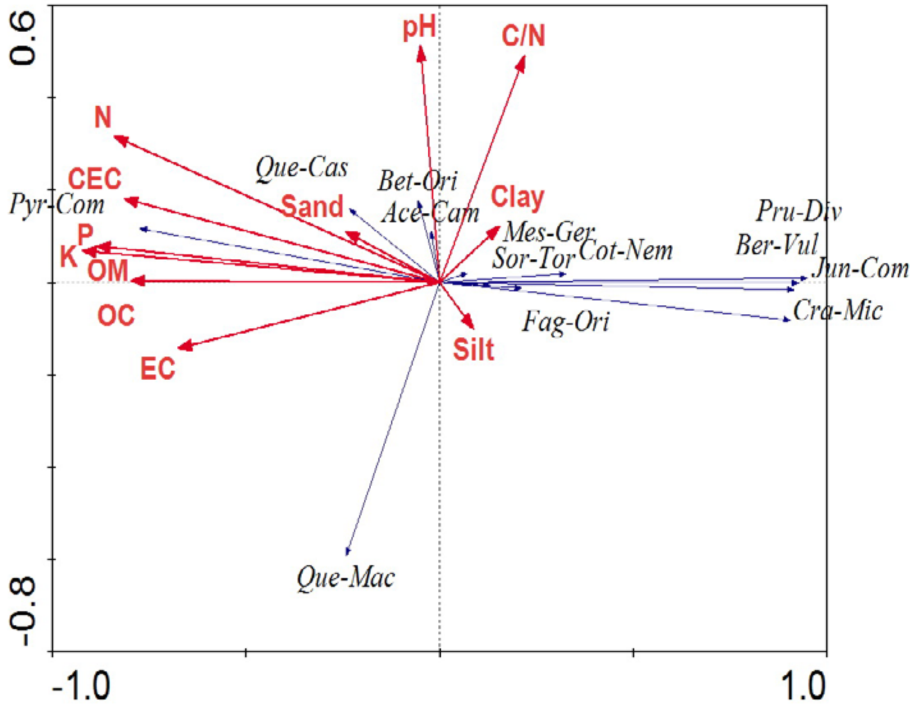

Fig 4. RDA ordination of woody species and soil factors.

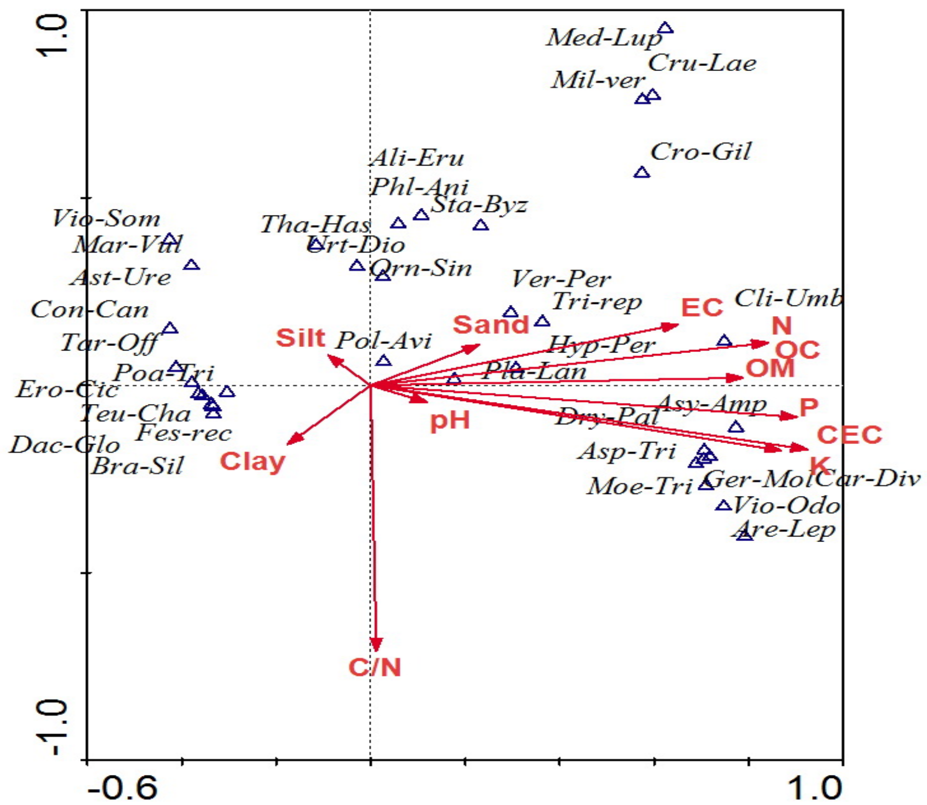

Fig. 5. CCA ordination of herbaceous species and soil factors.

Table 1. Soil properties in protected and degraded areas.

	Protected area	Degraded area
N	0.43[a]	0.3[b]
P	7.14[a]	5.18[b]
K	273.5[a]	178.95[b]
pH	6.6[a]	6.57[a]
EC	0.89[a]	0.58[b]
OC	3.18[a]	2.48[b]
OM	5.41[a]	4.22[b]
Clay	39.3[a]	40.35[a]
Silt	34.05[a]	34.4[a]
Sand	29.15[a]	24.95[a]
CEC	37.95[a]	28.75[b]
C.N	10.05[a]	10.55[a]

Table 2. Biodiversity indices in protected and degraded areas.

	Protected area	Degraded area
Richness	2.92[a]	2.13[b]
Evenness	0.73[a]	0.6[b]
Diversity	2.66[a]	2.08[b]

Discussion

The results of DCA ordination showed that species composition is different between the protected and degraded areas. Indeed, livestock grazing and human interventions have changed species composition. *Fagus orientalis* and *Sorbus torminalis* were two tree species that were only present in the degraded area, thereby marking a different distribution with that of the protected area. In the degraded area, some shrub species—such as *Juniperus communis*, *Crataegus microphylla*, *Cotoneaster nummularia*, *Prunus spinosa*, and *Berberis integgerima*—were present with similar distributions, causing different species composition in the degraded area. In fact, livestock grazing and environmental destruction have paved the way for invasion of the area by such species. *Carpinus orientalis* and *Quercus castaneifolia,* which were present in both areas, recorded the same distribution. In the study area, these two species were mainly found at near-forest areas. *Acer campester, Mespilus germanica* and *Pyrus communis* were among the species with disposition toward higher points; their conditions, however, was almost the same in both areas. *Quercus macranthera*, as the dominant species of both areas, showed the same distribution. Shrub species had, indeed, been the main cause for changed species composition and distribution. Shrub invasion has been considered the main outcome of livestock grazing around the world (PERELMAN *et al.,* 1997). The reaction of shrubs to grazing, however, is not the same for all different species; *Mespilus germanica*, for instance, has shown different behaviour.

Changed species composition was also observed at the herbaceous layer. In the protected area, *Asplenium trichomanes, Dryopteris pallid, Viola odorata, Carex divolsa, Geranium molle, Arenaria leptophylla* and *Moehringia trinervia* were the main components, while in the degraded area, species such as *Brachypodium silvaticum, Dactylis glomerata, Festuca rechingeri, Poa trivialis, Viola somchetica, Astragalus ureus, Erodium cicutarium*, and *Marrubium vulgar* were the common species. Livestock grazing has paved the way for invasion of the area by the aforementioned species, ergo leading to changed herbal composition of the area. These species are mostly annual invading plants, which produce large amounts of seeds and therefore change dominance pattern. MARTINS DA SILVA *et al.* (2009) in Portugal, GODEFROID *et al.* (2005) in Belgium, and MINCHINTON *et al.* (2006) in the USA declared that with their high competitive power and their large amount of seeds, invader species change species composition of grazed-up, destroyed regions.

The results of species relations and soil factors revealed the fact that different species have reacted to soil differently. Herbaceous and woody species, which presence was greater in the degraded area, had no relationship with nutrients such as N, P, K, and C; they, rather, preferred soils with higher clay and silt percentages. Nutrients have, indeed, no effect on such species. Species present in the protected area, however, showed a direct relationship with nutrients. Furthermore, comparative results of soil factors in the two areas showed that nutrients were significantly more abundant in the protected area, which provided chances of better growth for the species. Livestock grazing has imposed a negative effect on the chemical part of the soil. No negative effect was, however, observed for the physical part, based on comparative results of soil pattern for the two areas. In their study on Pol-e-Dokhtar of Iran, SALEHI *et al.* (2011) concluded that amounts of N, P, K, and K were significantly lower in the degraded area. HEIDARIAN AGHAKHANI *et al.* (2010) carried out a study on Bojnord, Iran; they showed how livestock grazing has led to significantly decreased amounts of carbon, N, P, K, and organic materials. In addition, *Quercus macranthera* had no relationship with nutrients; it grew better in areas with lower pH, clay, and C.N ratio. It seems that factors other than soil features are influential in the species distribution. They can include climate factors such as temperature or precipitation, and also topography and latitude.

Livestock grazing had a negative effect on diversity of herbaceous species such that richness, evenness, and diversity were all significantly lower in the degraded area. Apart from trampling, livestock eat from palatable species, ergo leading to the development of non-palatable ones. This alters the dominance pattern of the area; some species cannot well grow and develop in such situations. KEELEY *et al.* (2003) in southern Sierra Nevada, BOUHIM *et al.* (2010) in western Morocco and CESA & PARUELO (2011) in southern Argentina concluded that diversity is higher in the protected areas than degraded areas. Finally, results showed that livestock grazing and human activities can have serious negative effects on the soil. These, furthermore, pave the way for entrance of invader species. That is why programmes for the management and protection of *Quercus macranthera* forests seem especially important. It is also hoped that similar programmes are being strictly planned to prevent entry of animals into similar other areas.

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On the Population Dynamics of the Tomato Leaf Miner Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) in Egypt

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Abstract. The susceptibility of different tomato cultivars during different rotations was determined during the years 2012-2013 under Egyptian field condition. Our evaluation showed that the cultivars coded with GS and 010 appeared to be more resistant to infestation with *Tuta absoluta* in Egypt compared with other strains. Also, the rate of infestation of different host plants and the biology of *Tuta absoluta* on these hosts were investigated and the observations showed that the insect can discriminate between different host plants. It showed more preference to tomato followed by eggplant, potato and pepper and the larval duration was shorter after rearing on tomato leaves with increase in pupal weight and egg production in females previously reared on tomato leaves compared with those reared on other hosts.

Key words: *Tuta absoluta*, ecology, biology, susceptibility of tomato cultivars, host preference.

Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) has been reported in Egypt since 2009, quickly becoming one of the major pests of the tomato crop. It is a multivoltine species that mines leaves, fruits, flowers, buds and stems. The damage is produced when the larvae feed on the leaf mesophyll expanding mines, thus affecting the photosynthetic capacity of the crop with subsequent reduction of yield. Moreover, injury made directly to the fruits causes severe losses (COLOMO & BERTA, 2006).

T. absoluta is a very harmful insect pest with a strong preference for tomato plants, although *T. absoluta* is an oligophagous pest with a strong preference for tomato, it can also attack the aerial parts of Solanaceae plants (NOTZ, 1992). Tomato (*Solanum lycopersicum*) is considered as the primary host of this pest, thus no tomato cultivars are entirely resistant to this insect, but not all cultivars are equally susceptible (BORGORNI *et al.*, 2003; OLIVEIRA *et al.*, 2009; DE OLIVEIRA *et al.*, 2012). However, it can also attack other cultivated Solanaceae plants such as eggplant (*S.melongena*), potato (*S. tuberosum*), pepper (*Capsicum annuum*), sweet pepper (*S. muricatum* L.), tobacco (*Nicotiana tabacum*) and some other non-cultivated Solanaceae (*S. nigrum, S. eleagnifolium*). Also, it infests *Physalis angulata, Phaseolus vulgaris, Datura ferox* and some Chenopodiaceae plants such as *Chenopodium album* (VARGES, 1970; GARÎCA & ESPUL, 1982; FERNANDEZ & MONTAGNE, 1990).

Tomato crop is the first vegetable crop in Egypt; it is grown in four rotations and covers about 3% of Egypt's total planted area. Egypt is classified as the 5th country around the world and second around the Mediterranean countries in production and exportation of tomato (WPTC, 2011). According to the Egyptian Ministry of Agriculture, the tomato cultivations during 2009 -2010 was 385,243 feddans, yielding 7,158,970 tons, with the largest areas located in Beheira, Fayoum, Sharkia and Qena governorates. So, in the present study, host-plant preference and susceptibility of different tomato cultivars to infestation with *T. absoluta* was investigated under Egyptian field conditions with calculation of the thermal requirements for different stages of *T. absoluta*.

This study was designed to evaluate the susceptibility of different tomato cultivars around two seasons to infestation with *T. absoluta* and to determine the host plant preference in the field.

Material and Methods

Field experiments were carried out in Bernucht village, Al-Ayyat centre, located in the southern part of Giza governorate, Egypt; to study the preference of *T. absoluta* to its host plants judged from the rate of oviposition on the plant leaves, number of larvae and mines.

Susceptibility of some tomato cultivars to infestation with T. absoluta.

The susceptibility of different tomato cultivars to insect infestation was also determined in the field according to HARIZANOVA *et al*. (2009); ALLACHE & DEMNATI (2012); TAHA *et al*. (2013). For this purpose, an area cultivated with different strains of tomato 150 m^2 was divided into separate plots, each plot 50 m^2 (5 × 10). The plants were cultivated in 2 rows. A border of one meter was left between plots without planting. Weekly visits were made and in each visit tomato leaves were collected randomly to represent the whole area. The samples were transferred to the laboratory and 60 tomato leaves were selected randomly from each sample for examination and three replicates were used.

The susceptibility of different tomato strains to infestation with *T. absoluta* was studied during 2 years (four seasons or rotations from 2012 to 2014), where the susceptibility to infestation with *T. absoluta* was measured by counting the total number of larvae, its feeding punctures (tunnels or mines) and the number of eggs deposited on the plant leaves of all tested tomato strains.

In the first year (2012), three tomato strains identified as GS, Native and Super hybrid were cultivated on January, 25th, 2012 in three separate plots to represent the early summer rotation. In the nile rotation, the two tomato strains 77 and 010 were cultivated on July, 22nd, 2012 in two separate plots.

In the second year (2013), two tomato strains GS and 2243 were cultivated on March, 14th, 2013 to represent the summer rotation. Two strains 010 and hybrid 5656 were cultivated on September, 19th, 2013 to represent the winter rotation. The same procedure was adopted during different rotations.

Host-Plant preference of T. absoluta.

To determine the host plant preference in the field, an area 200 m^2 was selected in Bernucht village, Al-Ayyat centre, Giza governorate. This area was cultivated on January, 25th, 2012 with four solanaceous host plants namely tomato (*Solanum lycopersicum*, cv GS), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*) and sweet pepper (*Capsicum annuum*) in separate plots (50 m^2/plot, 5 m. length/10 m. width). A border of one meter was left between plots without planting. Weekly visits were made to this area for 4.5 months; in each visit leaves of each host plant were collected randomly using Zigzag methods and the samples were transferred to laboratory. From these samples 60 leaves were selected randomly for examination and three replicates were made. Preference was measured by recording the total number of larvae, its feeding punctures (tunnels or mines) and the number of deposited eggs on the plants (SERAJ, 2000). Differences between different host plants were determined using SPSS program.

In order to determine the effect of host-plants on the biology of *T. absoluta* under laboratory conditions, three solanaceous host plants namely tomato, eggplant and potato were selected for this purpose, since these hosts were more attractive to adult moths of *T. absoluta*.

In this experiment, *T. absoluta* was reared on plant leaves of the tested hosts in separate plastic jars (2 L). The jars were lined at the bottom with filter paper, then provided with leaves of the tested hosts after being washed and dried. One hundred newly deposited eggs were placed on plant leaves of each host. All jars were incubated at room temperature with daily examination for calculating the hatching percentage. The hatched larvae were separated in new jars and divided into 3 groups as replicates to assess the effect of each host plant on different biological aspects according to PEREYRA & SÁNCHEZ (2006) as follows:

- Larval developmental time (from hatching to pupation).
- Pupation percentage and pupal weight (mg) 48 hours after pupation (15 pupae were weighted).
- Adult emergence percentage and fecundity.

Fecundity was estimated for each female. The newly emerged adults were released into rearing containers (50×60 cm) each containing a tomato pot for oviposition. The deposited eggs were counted daily until female death. Adults were fed with sugar solution (15%). The observed values were analyzed statistically to evaluate the effect of the host plants quality on some biological aspects of the insect.

Results

Susceptibility of some tomato cultivars to infestation with T. absoluta.

The susceptibility of tomato cultivars to infestation with *T. absoluta* was estimated in a series of experiments. Five tomato cultivars were cultivated during the first year (2012), in early summer and nile rotations, while four cultivars were cultivated during the second year (2013) in summer and winter rotations.

Early summer rotation (2012)

Three tomato cultivars identified as GS, native and super hybrid were cultivated in 25, January 2012 in three separated plots, 60 tomato leaves were collected randomly to represent each strain, the samples were transferred to the laboratory for examination. Eggs, larvae and tunnels or mines caused by the larvae were counted and the data are reported in Table 1.

It appears that the native strain is more attractive to *T. absoluta* than GS and super hybrid strain, 2 weeks after cultivation, where the average number of eggs was 0.73 eggs/ leaf as compared to 0.25 and 0.22 in samples collected from GS and super hybrid, respectively. The egg number increased gradually in all tested tomato strains with the increase of the plant age, where the average egg number increased to 1.6, 1.63 and 1.45 eggs/leaf of native, GS and super hybrid, respectively, in those samples collected in 20, March 2012 (plant age 8 weeks after cultivation). Then the egg number decreased gradually, while the examination of the collected samples in the first of May 2012 recorded an increase in egg number again to 2.43, 0.98 and 1.37 eggs/leaf for native, GS and super hybrid strains, respectively (when plant age was 14 weeks) and then the egg number decreased again in the following week. The maximum number of eggs was again recorded as 5.7, 2 and 3.8 eggs/leaf for native, GS and super hybrid strains, respectively in the first of June 2012. These data in general showed that the native strain was more susceptible to infestation with *T. absoluta*, where the total number of eggs around the rotation was 15.93±5.5 eggs/leaf being significantly higher as compared to GS and super hybrid, while the total number of eggs around the cycle or the rotation in both GS and super hybrid was 8.03±1.5 and 8.62±3.4 eggs/leaf, respectively. The total number of eggs in the native strain was significantly higher than the corresponding figures in GS and super hybrid strains ($F = 13$ at $df = 8$, $P < 0.05$). No significant differences were obtained between the GS and super hybrid strains with regard to the total number of eggs ($P = 0.87$).

In the meantime, the data showed that the average number of larvae in the native strain was 0.85 larvae/leaf during the first week of February, while it decreased to 0.38 and 0.15 larvae/leaf, respectively in GS and super hybrid strains. The larvae number increased gradually in the tested tomato strains with the increase of deposited eggs, where the average number of larvae increased to 1.8, 1.52 and 1.13 larvae/leaf of native, GS and super hybrid strains, respectively, in the samples collected in 3, April 2012 (plant aged 10 weeks after cultivation) then the larvae numbers were gradually decreased. Samples collected in mid of May 2012 recorded an increase in the number of larvae being 2.9, 0.42 and 1.2 larvae/leaf of native, GS and super hybrid strains, respectively, when the plant age was 16 weeks. The maximum numbers of larvae were recorded in June being 3.45, 2.45 and 1.83 larvae/leaf of native, GS and super hybrid strains, respectively. These data showed that the native strain is more susceptible to infestation with larvae where the total number of larvae around the rotation was 16.78±5.7 larvae/leaf compared to 5.73±1.9 and 8.5±2 larvae/leaf in both GS and super hybrid, respectively. Statistical analysis showed significant differences in the total number of larvae around the rotation in the native strain ($F=17$ at $df = 8$, $P < 0.05$) compared with those recorded in GS and super hybrid leaves ($P < 0.05$), but insignificant differences were recorded between GS and super hybrid ($P = 0.37$).

The number of tunnels in the leaves should be connected with the increase of the deposited eggs, hatched larvae and plant age. The results in Table 1 show that the native strain has a significantly higher number of tunnels around the rotation being 27.6±1.7 tunnels/leaf compared to 17.05±4.2 and 18.57±3.2 tunnels/leaf for GS and super hybrid strains, respectively ($F=29$ at $df =8$, $P < 0.05$). Insignificant differences were detected between the two strains GS and super hybrid ($P = 0.5$).

The nile rotation (2012)

The susceptibility of two tomato strains coded as 77 and 010 were evaluated and the data are given in Table 2 indicating that the infestation symptoms were observed 2 weeks after cultivation in the tested cultivated strains 010 and 77. The strain coded as 77 appeared to be more attractive to *T. absoluta* moths, where the average number of eggs in the collected leaves was 0.45 eggs/leaf compared to an average of 0.16 eggs/leaf in the strain 010. The egg number was increased gradually in the two tested tomato strains with the increase of the plant age, where the average number of eggs increased to 1.13 and 0.8 eggs/leaf in samples collected in 23, August 2012 from the strains 77 and 010, respectively. Following this, the egg number in the collected samples showed fluctuations around the rotation. The total number of eggs around the rotation in the strain 77 was 14.06±3.6 eggs/leaf as compared to 9.69±2.43 eggs/leaf in the strain 010. The difference is statistically significant ($t=2.26$, $df=6$, $P<0.05$).

In the meantime, the average number of the larvae was 0.06 larvae/leaf of the strain coded 77, two weeks after cultivation compared to 0.04 larvae/leaf in 010 strain. The larvae number increased gradually in the tested tomato strains with increase of deposited eggs and plant age, and the average of larvae number increased to 0.18 and 0.13 larvae/leaf of the strains 77 and 010, respectively, in samples collected in 23, August 2012 (plant aged 4 weeks after cultivation). The larvae number increased to 1.1 and 0.28 larvae/leaf of the strains 77 and 010, respectively, six weeks after cultivations. Examination of the collected samples in 23, September 2012 (8 weeks after cultivations) showed reduction in the larvae number to 0.78 and 0.21 larvae/leaf, then the larvae number increased in the next visit to 1.51 and 1.09 larvae/leaf for the strains 77 and 010, respectively. The highest number of larvae was recorded as 3.33 and 2.33 larvae/leaf of tomato strains 77 and 010, respectively, by the end of the cycle. In general, the tomato strain 77 appears to be more attractive and highly infested with *T. absoluta* larvae where the total number of larvae around the rotation was 12.41±2.4 larvae/leaf with significant difference compared to 6.23±1.6 larvae/leaf in the strain 010 ($t = 4.36$ at $df = 6$, $P < 0.05$).

The data revealed that the increase in the number of tunnels was connected with an increase of the deposited eggs, hatched larvae and plant age. It appears that the maximum number of tunnels in the tested tomato strains was 5.03 and 3.32 tunnels/ leaf of the strains 77 and 010, respectively, when the plant age was 18 weeks. The total number of tunnels around the rotation was 21.75±3.4 tunnels/leaf in strain 77 being significantly higher as compared to 14.05±2.2 tunnels/leaf in strain 010 ($t = 4.37$ at $df = 6$, $P < 0.05$).

The summer rotation (2013).

Two tomato cultivars identified as GS and 2243 were cultivated on March, 14th, 2013 in two plots, each plot 50 m² (5 m. length, 10 m. width) and the plants were cultivated as in early summer rotation. Visits were made to the site every 2 weeks and in each visit tomato leaves were collected randomly to represent the whole area, the samples were transferred to the laboratory for examination. Eggs, larvae and tunnels or mines caused by the larvae were counted and the data are reported in Table 3.

The data in Table 3 showed that the infestation symptoms of *T. absoluta* were observed 2 weeks after cultivation of the strains GS and 2243. The strain coded 2243 appeared to be more attractive to *T. absoluta* moths 2 weeks after cultivation where the average number of eggs in the collected leaves was 0.73 eggs/leaf compared to 0.06 eggs/leaf in the strain GS. Fluctuations in the number of eggs around the cycle were observed in both tomato strains. The maximum number of *T. absoluta* eggs was recorded by the end of July 2013 being 4.25 and 3.13 eggs/leaf for the strains 2234 and GS, respectively. These data showed that the strain coded 2234 was more susceptible to infestation with *T. absoluta* where the total number of eggs/leaf around the rotation was 17.79±3.8 eggs/leaf compared to 11.47±3.7 eggs/leaf in the strain coded GS. The statistical analysis showed significant differences between the number of eggs in both GS and 2243 ($t = 0.416$ at $df = 6$, $P < 0.05$).

Table 1. Susceptibility of three different cultivars of tomato to infestation with *T. absoluta* during the early summer rotation (2012).

Time of Examination / week	Average number ± SD / leaf								
	tunnels	larvae	eggs	tunnels	larvae	eggs	tunnels	larvae	eggs
	Super hybrid			GS			Native strain		
7/2/2012	0.43 ± 0.1	0.15 ±0.01	0.22 ±0.05	0.34 ± 0.1	0.38 ± 0.1	0.25 ±0.08	1.15 ±0.26	0.85 ± 0.54	0.73 ± 0.38
21/2/2012	1 ±0.15	0.83 ±0.07	1.17 ± 0.2	0.78 ± 0.39	0.23 ±0.02	0.58 ± 0.1	1.93 ±0.46	1.2 ± 0.53	1.05 ± 0.04
6/3/2012	1.82 ±0.12	0.57 ±0.19	0.06 ±0.01	1.1 ± 0.46	0.1 ±0.06	1.02 ± 0.2	2.25 ±0.15	1.35 ± 0.68	1.22 ± 0.72
20/3/2012	1.22 ±0.28	0.77 ±0.21	1.45 ± 1.1	1.3 ± 0.35	0.13 ±0.07	1.63 ±0.18	2.55 ±0.45	1.2 ± 0.4	1.6 ± 0.87
3/4/2012	1.3 ±0.43	1.13 ±0.21	0.15 ±0.01	1.45 ±0.11	1.52 ±0.89	0.27 ±0.06	2.87 ±0.72	1.8 ± 1.1	0.7 ± 0.04
17/4/2012	2.1 ±0.34	0.83 ±0.31	0.28 ±0.19	1.87 ± 0.18	0 .13 ±0.02	0.75 ±0.16	3.75 ±0.87	1.33 ± 0.7	0.7 ± 0.02
1/5/2012	3.6 ±0.34	1.15 ±0.07	1.37 ±0.13	2.47 ± 0.45	0.37 ±0.26	0.98 ±0.16	3.9 ±0.36	2.7 ± 1.1	2.43 ± 1.2
15/5/2012	2.8 ±0.31	1.2 ±0.34	0.12 ±0.09	2.62 ±1.52	0.42 ±0.06	0.55 ±0.11	4.35 ±0.65	2.9 ± 0.51	1.8 ± 1.1
1/6/2012	4.3 ±1.16	1.83 ±0.47	3.8 ± 1.6	5.12 ±0.62	2.45 ± 0.4	2 ±0.47	4.88 ± 0.7	3.45 ± 0.17	5.7 ± 1.08
Total	**18.57 ±3.2ᵉ**	**8.5 ± 2ᵇ**	**8.62 ± 3.4ᵃ**	**17.05 ± 4.2ᵉ**	**5.73 ± 1.9ᵇ**	**8.03 ± 1.5ᵃ**	**27.6 ±1.7ᴱ**	**16.78 ±5.7ᴮ**	**15.93 ±5.5ᴬ**

Means of total values followed by similar letters within the same row are not significantly different at 0.05 levels.

Table 2. Susceptibility of different strains of tomato to infestation with *T. absoluta* during the nile rotation (2012).

Time of examination / weeks	Average number ± SD / leaf					
	tunnels	larvae	eggs	tunnels	larvae	eggs
	Strain 010			Strain 77		
8/8/2012	0.05 ± 0.01	0.04 ± 0.02	0.16 ± 0.04	0.09 ± 0.04	0.06 ± 0.04	0.45± 0.23
23/8/2012	0.3 ± 0.1	0.13 ± 0.03	0.8 ± 0.19	0.47 ± 0.3	0.18 ± 0.04	1.13± 0.33
8/9/2012	1.08 ± 0.27	0.28 ± 0.08	0.31 ± 0.09	1.15 ± 0.44	1.1 ± 0.2	0.75±0.29
23/9/2012	1.23 ± 0.3	0.21± 0.06	1.43 ± 0.47	1.74 ± 0.25	0.78 ± 0.25	1.3 ± 0.21
7/10/2012	1.5 ± 0.32	1.09 ± 0.35	0.58 ± 0.23	2.5 ± 0.4	1.51 ± 0.09	1.2 ± 0.33
21/10/2012	1.72 ± 0.36	0.27 ± 0.14	1.53 ± 0.48	2.98 ± 0.37	1.4 ± 0.37	1.85± 0.32
5/11/2012	2.16 ± 0.23	1.29 ± 0.41	1.13 ± 0.37	3.48 ± 0.46	2.45 ± 0.49	1.75 ± 0.6
20/11/2012	2.69 ± 0.21	0.59 ± 0.18	1.55 ± 0.27	4.28 ± 0.4	1.6 ± 0.32	2.23 ± 0.5
5/12/2012	3.32 ± 0.37	2.33 ± 0.58	2.2 ± 0.29	5.03 ± 0.7	3.33 ± 0.58	3.4 ± 0.83
Total	**14.05 ± 2.2^{e}**	**6.23 ± 1.6^{b}**	**9.69 ± 2.4^{a}**	**21.75 ±3.4^{E}**	**12.41 ± 2.4^{B}**	**14.06 ±3.6^{A}**

Means of total values followed by similar letters within the same row are not significantly different at 0.05 levels.

Similar results were obtained with the larvae and tunnels where the data given in Table 3 indicated that the strain 2243 was more susceptible to infestation with *T. absoluta* than the strain coded GS. The total number of larvae around the rotation was 12±5.3 larvae /leaf in the strain 2243 compared to 9.48±1.8 larvae/leaf in GS strain, with no significant differences ($t = 0.89$ at $df = 6$, $P = 0.07$). Meanwhile, the total number of tunnels around the rotation was 19.18±4.7 tunnels/leaf in the strain 2243 compared to 17.97±2.8 tunnels/leaf in the strain GS and negatively significant differences were recorded ($t = 0.42$ at $df = 6$, $P = 0.067$).

The winter rotation (2013).

Two tomato strains identified as 010 and 5656-hybrid were cultivated on September, 19th, 2013 in two plots, each plot 50 m^2 (5 m. length, 10 m. width) and the plants cultivated as in early summer rotation. Visits to the site were made every 2 weeks and in each visit tomato leaves were collected randomly to represent the whole area and the samples were transferred to the laboratory for examination. Eggs, larvae and tunnels or mines caused by the larvae were counted and the data are presented in Table 4.

The infestation symptoms of *T. absoluta* were observed 2 weeks after cultivation in both 010 and 5656-hybrid. The strain coded 5656 appeared to be more attractive to *T. absoluta* moths, where the average of egg number in the collected leaves was 0.33 eggs/leaf, compared to 0.07 eggs/leaf in samples collected from strain coded 010. The egg number increased gradually in the tested strains with the increase of the plant age. The average of egg number increased to 1.35 and 2.58 eggs/leaf in samples collected in 12, December 2013 from the strains 010 and 5656, respectively. Then the egg number decreased in the next visit to 1.2 and 1.95 eggs/leaf for the strains 010 and 5656, respectively. The maximum number of *T. absoluta* eggs recorded 2.13 and 2.8 eggs/leaf for strains 010 and 5656, respectively in the samples collected in 23, January 2014. These data showed that the strain coded 5656 appears to be more attractive and highly infested with *T. absoluta* and total number of eggs around the rotation was 14.1±5.2 eggs/leaf, compared to 10.02±3.6 eggs/leaf in the strain 010.

On the other hand, the average number of larvae was 0.11 larvae/leaf for the strain coded 5656 and decreased to 0.04 in the strain 010 at 2 weeks after cultivation. The number of larvae increased gradually in both tested strains with increase of deposited eggs and plant age, where the average number of larvae increased to 1.75 and 1.63 larvae/leaf of 5656 and 010, respectively, in samples collected in 26, December 2012, and then the larvae number decreased to 1.18 and 1.25 larvae/leaf during the next visit. The maximum number of *T. absoluta* larvae was recorded being 2.4 and 1.75 larvae/leaf of the tomato strains 5656 and 010, respectively (the plant age was 18 weeks). These data showed that the tomato strain coded 5656 appears to be more attractive and highly infested with *T. absoluta* larvae and the total number of larvae around the rotation was 10.17±5.8 larvae/leaf compared to 8.5± 3.5 larvae/leaf in strain 010.

The results in Table 4 recorded the maximum number of tunnels in the tested tomato strains in 23, January 2014 when the plant age was 18 weeks, where the average numbers of the tunnels were 3.18 and 2.35 tunnels/leaf of 5656 and 010, respectively. The tomato strain coded 5656 appears to be highly infested with *T. absoluta* where the total number of tunnels around the rotation was 16.5± 1.7 tunnels/leaf, compared with 11.76± 2 tunnels/leaf in the strain 010.

Host-Plant Preference of T. absoluta.

T. absoluta is an important tomato pest that also feeds on other host-plants from the Solanaceae family. Four solanaceous plants i.e. tomato (GS), eggplant, potato and pepper were cultivated on January, 25th, 2012 in Bernucht village, Giza in an area of 200 m^2. Samples were collected weekly at random around the season (4.5 months) to evaluate the host-plant preference. Data presented in table 5 show that tomato plants were highly attractive to the female moths of *T. absoluta* in the field where the average number of deposited eggs and larvae on the tomato leaf were 8.03±1.5 and 5.73±1.9, respectively, while the number of tunnels was 17.05±4.2.

Examination of the samples of both eggplant and potato showed that the eggplant was more attractive to the insect in the field where the average number of eggs on eggplant was more than that deposited on potato being 3.8±1.4 and 2.2±0.7 eggs, respectively. The recorded larvae were 3.7±0.37 and 4.9±1.1 larvae, while the number of tunnels was 5.4±1.8 and 3.7±0.75 tunnels for both eggplant and potato, respectively, with no attractive effects of pepper leaves to the moth of *T. absoluta* since there was no eggs and larvae on pepper plants during the season with low number of tunnels (1.8±1.4 tunnels). The statistical analysis showed that tomato is a more suitable host-plant than the other hosts. It has been demonstrated that there were highly significant effects in the average number of tunnels in tomato as compared with the other hosts ($F=29.4$ at $df=11$, $P < 0.05$), while the variation in the average number of eggs was significant in tomato compared with potato and pepper ($F=32.68$ at $df=11$, $P < 0.05$), while there was negatively significant effects between the number of eggs in both tomato and eggplant ($P = 0.01$). Significant effects were also recorded when comparing the average number of larvae in tomato and pepper ($F=13.77$ at $df=11$, $P < 0.05$), but no significant effects were observed in the number of larvae in either tomato or eggplant ($P = 0.01$), and also in tomato and potato ($P = 0.44$).

The reported data in Table 5 show that tomato plants were more susceptible to infestation with *T. absoluta* than eggplant and potato under Egyptian field conditions. So, the effect of these hosts on the different biological aspects of *T. absoluta* was carried out under laboratory conditions. One hundred newly deposited eggs were exposed to plant leaves of the tested host plants (tomato, eggplant and potato) in plastic jars to evaluate the hatching rate, larval developmental time, pupation, pupal weight and adult emergency percentage and fecundity of females.

The data in Table 6 showed that the larvae which feed on tomato leaves in the laboratory had a significantly shorter developmental time than those fed on eggplant and potato and the average duration of larval development was 11.7±0.47, 13.3±1.3 and 14.7±0.45 days, respectively ($F= 6.8$ at $df = 8$, $P<0.05$). While, there was negatively significant effects between the developmental time of larvae which feed on both eggplant and potato leaves (P = 0.03).

The pupation percentage after feeding the larvae on tomato, eggplant and potato was 70.7, 65.5 and 45.3%, respectively and the pupal weight was significantly higher for individuals reared on tomato and eggplant leaves as compared with those reared on potato, being 3.31±0.54, 3.18±0.41 and 2.55±0.48 mg/pupa, respectively (F= 9.96 at df = 44 P<0.05).

The average number of eggs per female previously fed as larvae on tomato, eggplant and potato during the larval stage was 103.9, 93.2 and 81.4 eggs/female. There were no significant differences between egg production in females fed during larval stage on both tomato and eggplant (P=0.32) as compared with those fed on potato (F=14.08 at df=8, P<0.05).

Table 3. Susceptibility of different strains of tomato cultivars to infestation with *T. absoluta* during the summer rotation (2013).

Time of examination / weeks	Average number ± SD / leaf					
	tunnels	larvae	eggs	tunnels	larvae	eggs
	Strain GS			Strain 2243		
30/3/2013	0.06 ± 0.02	0.05 ±0.03	0.06 ± 0.02	0.28 ± 0.20	0.33 ±0.29	0.73 ± 0.18
14/4/2013	0.70 ± 0.30	0.13 ± 0.04	0.53 ± 0.21	0.68 ± 0.46	0.70± 0.44	1.45 ± 0.05
29/4/2013	1.17± 0.18	0.68 ± 0.33	0.94± 0.42	1.40± 0.68	0.70 ± 0.54	1.70 ± 0.19
14/5/2013	1.50 ± 0.37	1.10 ± 0.19	0.70 ± 0.46	1.53 ± 0.43	1.28 ± 0.55	1.48 ± 0.75
29/5/2013	1.85± 0.33	0.73 ± 0.31	1.35 ± 0.52	1.78± 0.42	1.00 ± 0.40	1.90 ± 0.12
13/6/2013	2.4 ± 0.37	1.35 ± 0.22	1.23 ± 0.51	2.43± 0.49	1.63 ± 0.60	1.58 ± 0.42
27/6/2013	2.96± 0.24	1.28 ± 0.19	1.63 ± 0.43	3.23± 0.55	1.45 ± 0.44	2.05 ± 0.65
11/7/2013	3.40 ± 0.43	1.88 ± 0.16	1.90 ± 0.20	3.60 ± 0.67	2.13 ± 0.87	2.65 ± 0.96
25/7/2013	3.93± 0.55	2.28 ± 0.28	3.13 ± 0.89	4.25± 0.75	2.78 ± 1.12	4.25 ± 0.49
Total	**17.97±2.8^{E}**	**9.48±1.8^{B}**	**11.47±3.7^{a}**	**19.18±4.7^{E}**	**12 ± 5.3^{B}**	**17.79±3.8^{A}**

Means of total values followed by similar letters within the same row are not significantly different at 0.05 levels.

Table 4. Susceptibility of different strains of tomato cultivars to infestation with *T. absoluta* during the winter rotation (2013).

Time of examination / weeks	Average number ± SD / leaf					
	tunnels	larvae	Eggs	tunnels	larvae	Eggs
	Strain 010			Strain 5656- hybrid		
3/10/2013	0.05± 0.01	0.04±0.004	0.07± 0.03	0.1 ± 0.07	0.11 ± 0.6	0.33± 0.19
17/10/2013	0.45 ± 0.1	0.083±0.02	0.87± 0.33	0.6 ± 0.24	0.13± 0.28	0.89± 0.37
31/10/2013	0.83± 0.22	0.83± 0.26	0.68± 0.26	1.13±0.083	1.18± 0.34	1 ± 0.38
14/11/2013	1.3 ± 0.21	0.93± 0.41	1.18± 0.41	1.4 ± 0.3	0.98 ± 0.4	1.05± 0.45
28/11/2013	1.28± 0.19	1 ± 0.38	1.08± 0.35	1.85± 0.27	1.1 ± 0.6	1.38± 0.54
12/12/2013	1.7 ± 0.45	1 ± 0.6	1.35± 0.55	2.23± 0.18	1.43± 0.65	2.58± 0.74
26/12/2013	2.05± 0.08	1.63± 0.53	1.2 ± 0.47	2.93± 0.16	1.75± 0.97	1.95± 0.86
9/1/2014	1.75± 0.38	1.25± 0.63	1.48± 0.55	3.08 ± 0.1	1.18± 1.05	2.075±0.83
23/1/2014	2.35 ± 0.4	1.75± 0.69	2.13± 0.64	3.18± 0.31	2.4 ± 0.87	2.8 ± 0.8
Total	**11.76 ± 2^{e}**	**8.5 ± 3.5^{B}**	**10.02±3.6^{A}**	**16.5 ± 1.7^{E}**	**10.17±5.8^{B}**	**14.1±5.2^{A}**

Means of total values followed by similar letters within the same row are not significantly different at 0.05 levels.

Table 5. Host-plant preference of *T. absoluta* under field conditions.

Host-plant	Average number ± SD / leaf		
	Tunnels	Larvae	Eggs
Tomato	17.05 ± 4.2 [a]	5.73 ± 1.9 [A]	8.03 ± 1.5 [I]
Eggplant	5.4 ± 1.8 [b]	3.7 ± 0.37 [A B]	3.8 ± 1.4 [IJ]
Potato	3.7 ± 0.75 [b]	4.9 ± 1.1 [A B]	2.2 ± 0.7 [J]
Pepper	1.8 ± 1.4 [b]	0 [B]	0 [J]

The values followed by the same letters mean that the differences were not significant at $P< 0.05$.

Table 6. Effects of host-plant on different biological aspects of *T. absoluta* under laboratory conditions.

Host plants	% of egg hatching	Larval developmental (time/day)	% pupation	Weight of pupae (mg)	% of adult emergence	Fecundity (egg/female)
Tomato	85 %	11.7 ± 0.47 [a]	70.7 %	3.31 ± 0.54 [a]	96.3 %	103.9 [a]
Eggplant	77 %	13.3 ± 1.3 [ab]	65.5 %	3.18± 0.42 [ab]	89.8 %	93.2 [a]
Potato	80 %	14.7 ± 0.47 [b]	45.3 %	2.55±0.48 [b]	91.2 %	81.4 [b]

Means followed by similar letters within the same vertical column are not significantly different at 0.05 level.

Discussion

T. absoluta is one of the most important lepidopterous insect pests on tomato in both greenhouses and in the open field. It prefers to lay eggs on tomato leaves and after egg deposition, larvae penetrate the leaves, stems or fruits causing mines and galleries. No tomato cultivars are entirely resistant to this insect, but not all cultivars are equally susceptible. Similar observations were recorded by BORGORNI *et al.* (2003); OLIVEIRA *et al.* (2009); PROFFIT *et al.* (2011); DE OLIVEIRA *et al.* (2012); CHERIF *et al.* (2013).

Investigations were carried out to evaluate the susceptibility of different strains of tomato during the early summer and nile rotations in 2012 and also during summer and winter rotations in 2013. It appears that the native strain was more susceptible to infestation compared to the strain GS and super hybrid in the early summer rotation of 2012. In the nile rotation, the strain 77 was more susceptible to infestation as compared to the strain 010 where the number of eggs, larvae and mines on the leaves of the strain 77 were significantly higher compared to that of the strain 010.

In the summer rotation of 2013, the strain 2243 appears to be more susceptible to infestation with *T. absoluta* as compared to the strain GS. On the other hand, the strain coded 5656 showed to be more susceptible to infestation as compared to the strain 010, judged from the number of eggs, larvae and mines (tunnels) recorded during this season on the leaves of this strain. These findings clearly indicate the variation in the susceptibility of the most dominant tomato strains cultivated in Egypt. Our results agree with observations concluded by BORGORNI *et al.* (2003) and OLIVEIRA *et al.* (2009) who studied the susceptibility of some tomato strains to infestation with *T. absoluta* and showed that no tomato cultivars are entirely resistant to *T. absoluta*, but not all cultivars are equally susceptible. In this concern, FERNANDEZ & MONTAGNE (1990) found that the tomato cultivar "Rome Gigante" was the preferred oviposition host and the best for larval development compared to different tomato variety and other hosts.

The difference in the *T. absoluta* preference between the different tomato cultivars can be attributed to differences in leaf volatile compounds (PROFFIT *et al.*, 2011; CHERIF *et al.*, 2013). Indeed, based on oviposition bioassays, PROFFIT *et al.* (2011) demonstrated that *T. absoluta* females laid more eggs in response to cvs. Santa Clara and Carmen as compared to cv. Aromata. The same authors found that overall leaf volatile composition of cv. Aromata differed significantly from cvs. Santa Clara and Carmen, due to differences in proportions of minor compounds and due to the absence of several compounds, mostly terpenes, in cv. Aromata. DE OLIVEIRA *et al*. (2012) showed that oviposition rate and damage on plants were significantly lower on tomato strains rich in one of the following allelochemicals: 2-tridecanone or zingiberene.

CHERIF *et al*. (2013) tested the susceptibility of different cultivars of tomato to infestation with *T. absoluta* and recommended that selection of lower suitability cultivars for *T. absoluta* female egg-laying in agriculture certainly provide a basis for development and implementation of effective prophylactic and environmentally-sound pest management tools against *T. absoluta*. Also, observations of GHAREKHANI & SALEK-EBRAHIMI (2014) who evaluated the damage of *T. absoluta* on eleven tomato cultivars reported that damaged leaves, active mines and damaged terminal buds were significantly different among the tomato cultivars.

T. absoluta is polyphagous, but it prefers some of its hosts such as tomato. The senses of taste and smell in lepidopterous larvae are important in host plant selection and the acceptance of the larva of *T. absoluta* to a host plant is due probably to chemical volatiles rather than purely physical factors as reported by TORRES *et al*. (2001) and PROFFIT *et al*. (2011). In this concern, Kamel (1969, Cairo University, Egypt, pers. comm.) found that the larvae of *Spodoptera littoralis* bear gustatory receptors that are localized on the labrum epipharynx.

The ecological studies showed that the tomato plants among other host plants were highly attractive to *T. absoluta* under open field conditions; this was followed by eggplant which showed to be more susceptible than potato. On the other hand, pepper was weakly susceptible with very low rates of infestation. VARGES (1970) in South America reported that *T. absoluta* feeds and develops on tomato and other cultivated and non cultivated Solanaceous plants. In Europe, the insect prefers tomato and other Solanaceous crops such as eggplant (MPAAF, 2009; VIGGIANI *et al*., 2009), potato (UNLU, 2012) and pepper (MPAAF, 2009), sweet cucumber (pepino) (FERA, 2009) and common beans (EPPO, 2009; MPAAF, 2009).

Based on these findings, the biology of *T. absoluta* is affected when reared under laboratory conditions on different host plants. Thus the larval duration was shorter when reared on tomato being 11.7±0.47 days as compared to 13.3±1.3 and 14.7±0.45 days when reared on egg plant and potato leaves, respectively. The pupation percentage was 70.7, 65.5 and 45.3% when the larvae fed tomato, eggplant and potato, respectively. Also pupal weight was significantly higher for individuals reared on tomato, eggplant as compared with those reared on potato. Egg production ranged between 103.9 eggs/female previously fed on tomato leaves and 81.4 eggs/female for those fed on potato.

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Comparative Ecophysiology and Photoprotection in Two Stem Succulent Euphorbia Species in Arid Habitat

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Abstract. Stem succulents *Euphorbia fractiflexa* and *Euphorbia triaculeata* (Euphorbiaceae) are common arido-active perennials in arid regions of Arabian Peninsula. Work presented in this paper aimed at investigating Crassulacean Acid Metabolism (CAM) in these two species by studying diurnal and seasonal changes in stomatal conductance and cell sap acidity. Chlorophyll fluorescence was measured to elucidate effect of drought and high irradiance on photosynthetic apparatus. Pigment profile analysis was carried out to provide comparative view of photoprotective mechanisms in these two species. Results indicated that these two *Euphorbia* species are obligate CAM plants that shift to CAM-idling in response to protracted drought. Results also revealed stress induced changes in activity and antenna size of Photosystem II. Changes in Photosystem II associated with increased non-photochemical quenching of chlorophyll fluorescence reflecting operation of photoprotective excess energy dissipation mechanisms. Pigment profile analysis revealed that photoprotection in *E. fractiflexa* relies on operation of xanthophyll cycle while that in *E. triaculeata* relies on anthocyanin accumulation.

Key words: Chlorophyll Fluorescence, Crassulacean Acid Metabolism (CAM), *Euphorbia*, Photoprotection.

Introduction

Arid regions southwest of Arabian Peninsula are among the most inhospitable habitats where climate is a composite of harsh environmental conditions. The dry season is conspicuously long with severe drought and high irradiance, the short wet season features high temperature and high evaporation, and summer erratic rainfall is associated with strong sand storms (FISHER & MEMBERY, 1998; VINCENT, 2008). In such habitats, stem succulents rely for survival on water storage and physiological adaptations of which CAM is prevalent due to improved water economy (SAYED, 2001a; b; LÜTTGE, 2010). In these regions *Euphorbia fractiflexa* and *Euphorbia triaculeata* (Euphorbiaceae) are abundant arido-active stem succulents (MASRAHI, 2012). We studied CAM in these two species by investigating diurnal and seasonal changes in stomatal conductance and cell sap acidity. Seasonal changes in chlorophyll fluorescence and pigment profile were studies to elucidate effects of drought and high irradiance on photosynthetic machinery. Moreover, our filed observations indicated that while *E. fractiflexa* retains its green stem colour, *E. triaculeata* stem acquires an antocyanin-like red colour under protracted drought. Photoprotective properties of carotenoids and anthocyanin

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have repeatedly been reported (GOULD *et al.*, 2010; ADAMS *et al.*, 2004; MURCHEI & NIYOGI, 2011; JAHNS & HOLZWARTH, 2012). We studied seasonal changes in carotenoid and anthocyanin contents and chlorophyll fluorescence quenching to provide a comparative view of photoprotective mechanisms in these two species.

Materials and Methods

Study area. The study site (17°19′N – 42°48′E) in lowland arid region southwest of Saudi Arabia is characterized by nine-months-long dry season and a short wet season (June–August). Mean monthly records of air temperature, precipitation, and evaporation were obtained from Ministry of Electricity and Water (Riyadh, Saudi Arabia). During dry season, daytime changes in air temperature and photosynthetic photon flux density (PPFD) were monitored using digital thermometer ("Kestrel 2000", Philadelphia, USA) and PAR/LAI Ceptometer (AcuPar LP-80, Decagon, Pullman, USA), respectively. Mature plants of *Euphorbia fractiflexa* S.Carter & J.R.I.Wood and *Euphorbia triaculeata* Forssk. (Euphorbiaceae) were used as plant material.

Climatic records (Curtsey of Saudi Meteorological Office, Riyadh, Saudi Arabia) indicated aridity of study site with nine-month-long dry season, short wet season (June-August), and total annual rainfall of less than 100 mm (Table 1).

Measurements. Stomatal conductance was measured using Porometer (AP4, Delta-T, Cambridge, UK). Chlorophyll fluorescence was measured using fluorescence monitoring system (FMS2, Hansatech, Norfolk, UK). Measured chlorophyll fluorescence parameters were F_v/F_m and ΦPSII denoting PSII antenna efficiency and quantum yield, respectively (BAKER, 2008; KALAJI *et al.*, 2012). Fluorescence non-photochemical quenching (NPQ) was calculated using standard nomenclature (SAYED, 2003; BAKER, 2008; KALAJI *et al.*, 2014) and the equation:

$$NPQ = \frac{(Fm - Fm')}{(Fm - Fo)}$$

where: (F_o) minimal fluorescence emitted by antenna chlorophyll molecules, (F_m) maximal fluorescence emitted when PSII traps are closed, and ($F_{m'}$) light-adapted fluorescence maximum.

Acidification-deacidification of CAM were studied in stem chlorenchyma cell sap extracted by grinding known weight of tissue in distilled water, expressing cell sap through muslin, and determining acidity using 0.01N NaOH and phenolphthalein (OSMOND *et al.*, 1991). Chlorophyll and carotenoid contents were determined using spectroscopy (METZNER *et al.*, 1965). Anthocyanin was determined by extraction in ice-cold 10% methanolic HCl (0.1% HCl v/v), determining absorbance at 535, 653 nm, and calculating anthocyanin content as (A535 – 0.24A653) using extinction coefficient 30000 L mol^{-1}cm^{-1} (MURRAY & HACKETT, 1991). Experiments were routinely repeated in samples from five individual plants and standard error was calculated.

Results

Monitoring daytime changes in air temperature and PPFD during dry season indicated that these parameters attained high midday values of 43°C and 2250 µmol m^{-2}s^{-1}, respectively (Table 2). Measurements of stomatal conductance during wet season indicated that *E. fractiflexa* and *E. triaculeata* exhibited high nighttime values and low day-time values (Fig. 1). Measurements during dry season indicated that both species exhibited very low values of stomatal conductance of 2-5 mmol m^{-2} s^{-1} during day and night (Fig. 1). In addition, both species exhibited diurnal acidity changes during wet season and these acidity changes were markedly dampened during dry season (Fig. 2).

Chlorophyll fluorescence measurements during the dry season showed reduction in values of F_v/F_m, and ΦPSII compared to those measured during wet season in both *E. fractiflexa* and *E. triaculeata* (Table 3).

Reduction of chlorophyll fluorescence during dry season associated with markedly increased NPQ in both species (Table 3). Pigment profile analysis indicated that both species showed increased Chl a/b ratio and increased carotenoid and anthocyanin contents during the dry season (Table 4). It was also noticeable that while carotenoid content markedly increased in *E. fractiflexa*, anthocyanin content markedly increased in *E. triaculeata* (Table 4).

Table 1. Mean monthly climatic norms at the study site (1970-2010).

Parameters	Months											
	J	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Air Temperature (°C)	30.8	34.7	35.4	35.5	38.5	39.0	40.0	40.3	38.3	37.3	36.0	34.9
Precipitation (mm)	5.0	4.5	5.0	4.5	5.0	4.4	20.0	8.0	9.0	8.0	7.5	5.5
Evaporation (mm)	4.9	5.6	7.5	8.9	10.2	9.6	10.0	8.6	8.8	9.3	6.3	4.9

Table 2. Daytime changes in air temperature and PPFD during dry season.

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

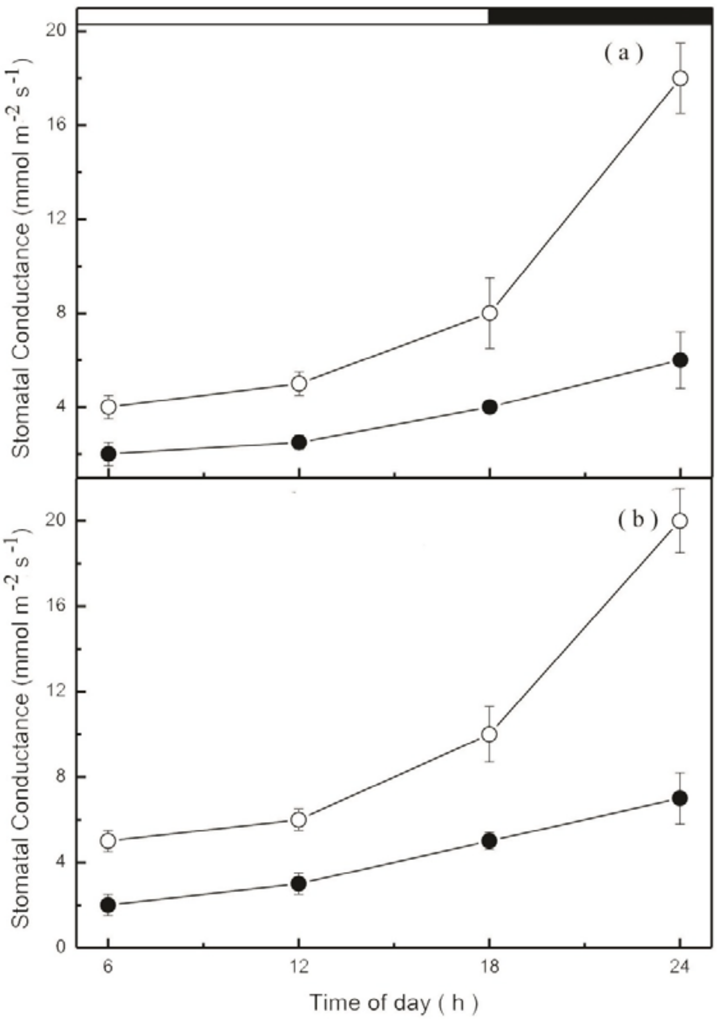

Fig. 1. Diurnal and seasonal changes in stomatal conductance of *E. fractiflexa* (a) and *E. triculeata* (b), during wet season (○) and dry season (●). (± se, n = 5).

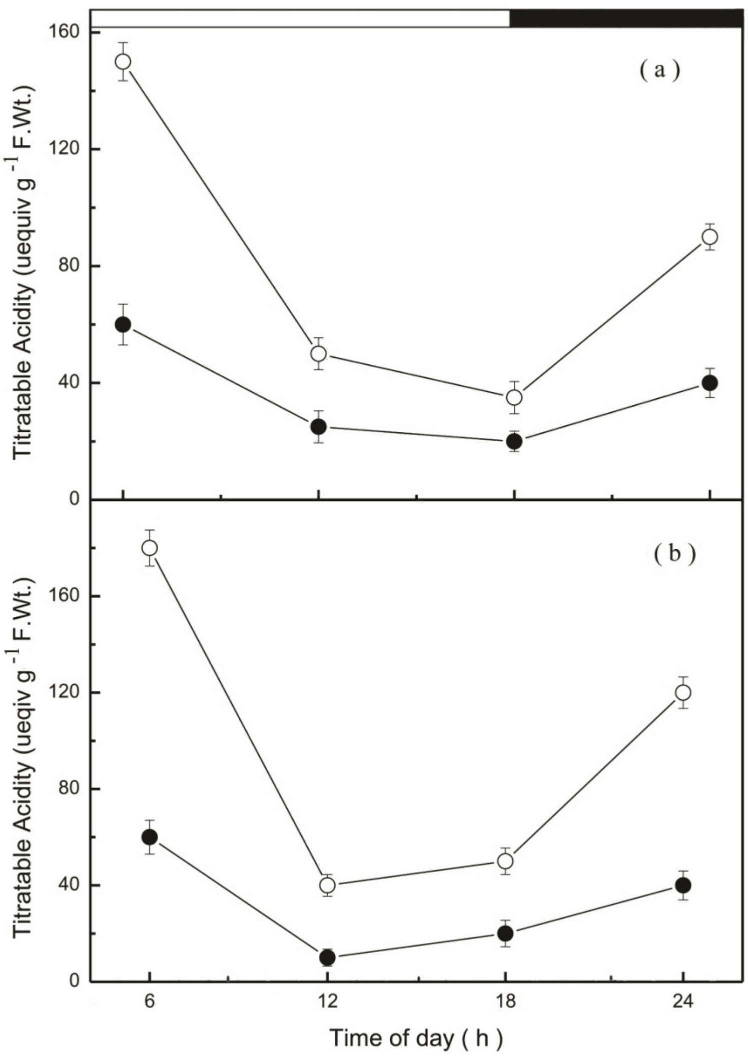

Fig. 2. Diurnal and seasonal changes in chlorenchyma cell sap acidity of *E. fractiflexa* (a) and *E. triculeata* (b), during wet season (○) and dry season (●). (± se, n = 5).

Table 3. Seasonal changes of chlorophyll fluorescence in *E. fractiflexa* and *E. triaculeata* (± se, n = 5).

Parameters	*E. fractiflexa*		*E. triaculeata*	
	Wet Season	**Dry Season**	**Wet Season**	**Dry Season**
Fv/Fm	0.84 ± 0.01	0.68 ± 0.007	0.81 ± 0.006	0.75 ± 0.01
ΦPSII	0.85± 0.003	0.64 ± 0.01	0.83 ± 0.01	0.65 ± 0.006
NPQ	0.02 ± 0.0003	0.07 ± 0.001	0.02 ± 0.004	0.06 ± 0.001

Table 4. Seasonal changes of chlorenchyma pigment profile in *E. fraciflexa* and *E. triaculeata* (± se, n = 5).

Parameter	*E. fractiflexa*		*E. triaculeata*	
	Wet Season	**Dry Season**	**Wet Season**	**Dry Season**
Chl a : Chl b	0.9 ± 0.04	1.5 ± 0.07	2.5 ± 0.05	3.5 ± 0.03
Carotenoides (μg g^{-1} dry weight)	20 ± 1.2	256 ± 1.7	30 ± 2.3	50 ± 3.5
Anthocyanin (μg g^{-1} dry weight)	150 ± 2.5	162 ± 5.5	150 ± 2.1	850 ± 5.4

Discussion

Extreme aridity of the study site was evident from prevailing harsh conditions of nine-month-long dry season and short wet season with scarce water, high temperature, and high evaporation (Table 1). Long dry season and low precipitation are typical features of arid regions of Arabian Peninsula (FISHER & MEMBERY, 1998; VINCENT, 2008). Daytime changes in air temperature and PPFD during dry season showed that these parameters can attain high midday values that add to the harshness of the environment (Table 2).

During the wet season, both *E. fractiflexa* and *E. triaculeata* exhibited stomatal conductance that was high during the night and low during the day (Fig. 1). Determination of chlorenchyma cell sap acidity during wet and dry seasons indicated presence of diurnal acidification-deacidification cycles in both species (Fig. 2). This stomatal behaviour and diurnal acidity oscillations are typical of CAM plants (LÜTTGE, 2010; WINTER *et al.*, 2015). Occurrence of these features during both wet and dry seasons reflects operation of obligate CAM in both species. Obligate CAM has previously been reported in stem succulent *Euphorbia* species (PEARCY & TROUGHTON, 1975; WILLERT *et al.*, 1985; MARTIN *et al.*, 1990; VAN DAMME, 1991; MIES *et al.*, 1996; ELHAAK *et al.*, 1997; WINTER *et al.*, 2005; AMEH, 2006). Daytime stomatal closure and nocturnal CO_2 uptake of CAM enhance plant water economy by preventing gas exchange when environmental conditions favor water loss (SAYED, 2001b; LÜTTGE, 2004; 2010; OWEN & GRIFFITHS, 2013). During the dry season, *E. fractiflexa* and *E. triaculeata* showed low stomatal conductance the entire day and night (Fig. 1) and markedly dampened diurnal oscillation of chlorenchyma cell sap acidity (Fig. 2). These results indicated that both species shifted from obligate CAM to CAM-idling under protracted drought. The CAM-idling mode is a CAM modification that further enhances plant water economy by day and night stomatal closure and sustaining diurnal acidity changes by nocturnal re-fixation of respiratory CO_2 (DODD *et al.*, 2002; LÜTTGE, 2004; 2010; WINTER *et al.*, 2015). Drought-induced CAM to CAM-idling shift has previously been reported in several stem succulent species in arid regions of Arabian Peninsula and appears to constitute an important survival strategy in such arid habitats (MASRAHI *et al.*, 2011; 2012a; b; 2015).

Measurement of chlorophyll fluorescence during dry season revealed 10-20% reduction in F_v/F_m and ΦPSII in both species (Table 3). Decreased F_v/F_m and ΦPSII denote reduction of PSII antenna efficiency and quantum yield, respectively (BAKER, 2008; KALAJI *et al.*, 2014). Similar reduction of PSII activity has previously been reported in CAM-idling plants under protracted drought (LÜTTGE, 2004; MASRAHI *et al.*, 2012b; 2015).

Pigment profile analysis indicated increased Chl a/b ratio in both species during dry season reflecting intrinsic changes in PSII (Table 4). The Chl a/b ratio is a measure of light harvesting complex chlorophyll relative to other Photosystem components (BAILEY *et al.*, 2004). Increased Chl a/b ratio reflects changes in PSII antenna size (MURCHIE & HORTON, 1997; BISWAL *et al.*, 2012; DINÇ *et al.*, 2012) and is an integral part of acclimation to high irradiance (KITAJIMA & HOGAN, 2003; KOUŘIL *et al.*, 2013). However, our observed reduction in PSII activity and increased Chl a/b ratio associated with increased NPQ (Table 3) denoting increased non-photochemical quenching and non-radiative excess energy dissipation (BAKER, 2008; PAPAGEORGIOU & GOVINDJEE, 2014; RUBAN & MULLINEAUX, 2014). Increased non-photochemical quenching was reported in CAM-idling plants and was related to increased zeaxanthin content (LÜTTGE, 2010). Our pigment profile analysis also showed increased carotenoid and anthocyanin contents in both *Euphorbia* species during the dry season (Table 4). These results reflect operation of photoprotective mechanisms in both species. Reports in the literature suggest that epoxidation-deepoxidation of xanthophyll cycle carotenoids play a major role in photoprotection under high irradiance (ADAMS *et al.*, 2004; HORTON & RUBAN, 2006; MURCHEI & NIYOGI, 2011; JAHNS & HOLZWARTH, 2012). Anthocyanin is also thought to confer photoprotection by alleviation of oxidative stress via processing reactive oxygen species released when PSII becomes over-energized during CAM-idling (NAGATA *et al.*, 2003; GOULD *et al.*, 2010; ADAMS *et al.*, 2004; NIEWIADOMSKA & BORLAND, 2008; BORLAND *et al.*, 2011). It was also noticeable that while carotenoid content markedly increased in *E. fractiflexa*, anthocyanin content markedly increased in *E. triaculeata* (Table 4). These differences strongly suggest that while photoprotection in *E. fractiflexa* relied on operation of xanthophyll cycle, photoprotection in *E. triaculeata* relied more on anthocyanin accumulation. These views gain support from reports that anthocyanin accumulation under high irradiance compensates for reduced dependence of non-photochemical quenching on xanthophyll cycle pigment conversion (HATIER & GOULD, 2009; FONDOM *et al.*, 2009).

Conclusions

It can be concluded that both *E. fractiflexa* and *E. triaculeata* are obligate CAM plants exhibiting stomatal behaviour and diurnal acidity oscillations typical of CAM plants. Low stomatal conductance both day and night and dampened acidity oscillations during dry season indicate that both species shift from obligate CAM to CAM-idling in response to protracted drought. Under severe drought, PSII activity was reduced in both species due to oxidative stress during CAM-idling with different photoprotective mechanisms operating in these two *Euphorbia* species. Photoprotection in *E. fractiflexa* relies on operation of xanthophyll cycle, while that in *E. triaculeata* relies more on anthocyanin accumulation.

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Removal of Cu^{2+} Ions from Aqueous Medium Using Clinoptilolite/Emeraldine Base Composite

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Abstract. The aim of this study was to investigate the removal efficiency of *in situ* synthesized composites consisted of emeraldine base and clinoptilolite on copper ions removal from aqueous medium. Two composite materials (*Composite I* and *Composite II*) with different quantity of clinoptilolite were synthesised. The influence of the composite dosage, the contact time and the initial copper ions concentration has been studied. The results show that the significant removal of the copper ions becomes at the first minute of the contact between the composite material and the aqueous medium and the longer contact time leads to increasing of the copper ions removal. The removal efficiency at the 1st minute was 57.5% and 77.3% using *Composite I* and *Composite II*, respectively. Maximum removal efficiency of 87.3% and 96.8% was achieved at the same dosage of *Composite I* and *Composite II*, respectively, at contact time of 360 minutes and temperature of 24 °C.

Key words: *in situ* polymerization, polyaniline composite, clinoptilolite, copper ions removal.

Introduction

The wastewater streams, contaminated with heavy metals such as Cu, Pb, Hg, As, etc., are usually released from mining and metallurgical industry as well as from the galvanic production processes. The widely applied processes for their treatment are often either very expensive or cannot meet the health and environmental protection standards. Because of the accumulation of heavy metals in the environment and the newest environmental standards, a development of innovative and cost-effective techniques for their removal is important. Some of the materials such as polyaniline, polypyrrole, polythioamide, chitosan, etc. and their combinations, which have polymeric nature, were used. Combination of different polymeric compounds or their combination with different sorbents leads to increasing of the treatment efficiency (WANG *et al.,* 2009; KAGAYA *et al.,* 2010; OLAD & NASERI, 2010; ZHANG *et al.,* 2010; MANSOUR *et al.,* 2011; BHAUMIK *et al.,* 2012; JIANG *et al.,* 2012; GHORBANIA & EISAZADEHB, 2013; RASHIDZADEH & OLAD, 2013; BEHBAHANI *et al.,* 2014; FAGHIHIAN & RASEKH, 2014; IGBERASE *et al.,* 2014; 2014; SHYAA *et al.,* 2015). This effect is due to the enlarged surface area and greater electron donor property (BHAUMIK *et al.,* 2014). The zeolites are microporous aluminosilicate minerals, commonly used as adsorbents and ion exchangers (SHERY, 1966; ĆURKOVIĆ *et al.,* 1997; ONTHONG & KAREMDABEH, 2010; TOROSYAN *et al.,* 2013; AWUAL *et al.,* 2014; YANEVA *et al.,* 2016). Their lattice structure contains interconnected voids which are filled with cations, usually of elements from

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group I-A or II-A of the periodic system, which can be exchanged with other cations from the surrounding medium.

The aim of this study is to investigate the possibility of clinoptilolite/emeraldine base composites for the removal of copper ions from model aqueous medium. This research includes a synthesis of composites consisted of clinoptilolite and emeraldine base and study on the influence of the initial metal ion concentration, composite dosage and contact time.

Materials and Methods

The experiments were carried out with pure for analysis aniline ($C_6H_5NH_2$), hydrochloric acid (HCl), ammonium persulfate ($(NH_4)_2S_2O_8$), sodium hydroxide (NaOH), $CuSO_4.5H_2O$, sodium acetate ($C_2H_3NaO_2$) and acetic acid (CH_3COOH). Distilled water was also used. The zeolite used in the experiments is clinoptilolite ($(Na,K,Ca)_{2\text{-}3}Al_3(Al,Si)_2Si_{13}O_{36}\cdot 12H_2O$) and was obtained from the eastern part of the Rhodope mountain in Bulgaria. The used material was with a particle size of 0.1-0.8 mm.

Preparation of clinoptilolite/emeraldine base composite

Two types of composites were obtained which were differ in the amount of the clinoptilolite used for their synthesis. In the first case the ratio clinoptilolite/aniline (*Composite I*) was 1:15 and in the second case (*Composite II*) it was 1:4. The preliminary weighted amounts of zeolite were mixed with 1M solution of hydrochloric acid (HCl) at continuous stirring (800 rpm) for 4 hours. After that the aniline was added to the reaction mixture and the stirring continued 24 hours. The solution of the oxidant which was necessary for the preparation of polyaniline from aniline monomer was prepared by dilution of ammonium persulfate ($(NH_4)_2S_2O_8$) with distilled water to a volume of 800 ml. This solution was added to the reaction mixture and the resulting suspension was stirred continuously for another 24 hours. The suspension was filtered and the resulting precipitate was rinsed several times with mixture of water and methanol in ratio 80:20, in order to remove remaining impurities and monomers. The conversion of the synthesized emeraldine salt to emeraldine base is required in order to achieve effective removal of the metal ions. There are more free electron pairs in the emeraldine base, where metal ions can be bound (Fig.1). This was achieved when washing the resulting precipitate with a 0.1 M NaOH solution to pH 10.0 - 11.0. The washed precipitate (composite) was dried at 60 °C to constant weight for 48 hours. Well dried composite is ground to a homogeneous powder.

Preparation of standard solutions of copper ions

Initial model solution with concentration 1 g L^{-1} of Cu^{2+} was prepared, using 3.93 g $CuSO_4.5H_2O$, diluted with freshly distilled water to 1 L. Then standard solutions with concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 30.0, 50.0, 70.0 and 100.0 mg L^{-1} were prepared.

Experimental

In order to determine the influence of the contact time and the composite dosage on the copper ions removal from an aqueous medium by the composite, individual samples with initial copper ions concentration of 50 mg L^{-1} have been prepared. Various amounts of clinoptilolite/emeraldine base composite powder (0.1, 0.5, 1.0, 1.5 g) were introduced into volumetric flasks. In order to achieve pH = 5, proper for the copper ions removal, acetate buffer was added to each sample [10, 19]. The samples were shaken at 24 °C in a plate shaker for 1, 3, 5, 7, 10, 15, 60 and 360 min, respectively. Samples with a volume of 20 ml were taken and filtered through blue ribbon filter paper to remove suspended particles of the composite. The filtrates were analysed by ISP – OES ("Prodigy" High dispersion ICP-OES, Telledyne Leeman Labs) in order to determine the copper ions concentration.

Samples with different concentration of copper ions (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 30.0, 50.0 mg L^{-1}) were prepared in order to determine the influence of the initial Cu^{2+} concentration. The volume of each sample was 50 ml. A certain amount of the composites (0.1 g) was added to each sample. An acetate buffer was added to adjust pH to 5.0. The samples are placed in iodine flasks and were shaken for 30 min. Then samples with a volume of 20 ml were taken and after filtration through a blue ribbon filter paper were analyzed for Cu^{2+} ions concentration.

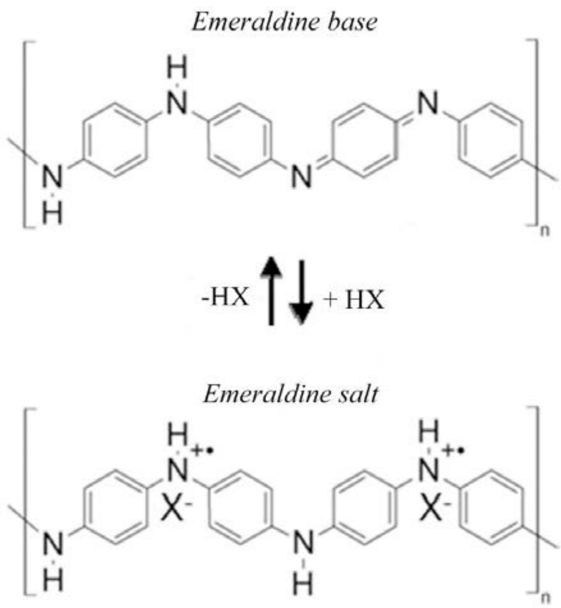

Fig. 1. Chemical structure of emeraldine base and emeraldine salt.

The removal efficiency was determined according to the formula:

$$Removal\ efficiency = 100 - \left(\frac{C_t}{C_o}\right) \times 100$$

where C_o is the initial concentration and C_t is the concentration at time t in mg L^{-1}.

Results and Discussion

Influence of the contact time and the composite dosage on the copper ions removal

The results show that the binding of the metal ions with the composite occurs immediately after mixing the composite with the aqueous medium (Fig. 2). Comparing the removal efficiency, which was achieved using *Composite I* and *Composite II*, correspondingly, it is obvious that the *Composite II* is more effective (Table 1). This is probably due to the fact that for the synthesis of *Composite II* a larger amount of clinoptilolite was used. The larger surface area of the adsorbent, and accordingly the greater number of the active sites are contributing to more effective removal of copper ions from the aqueous medium.

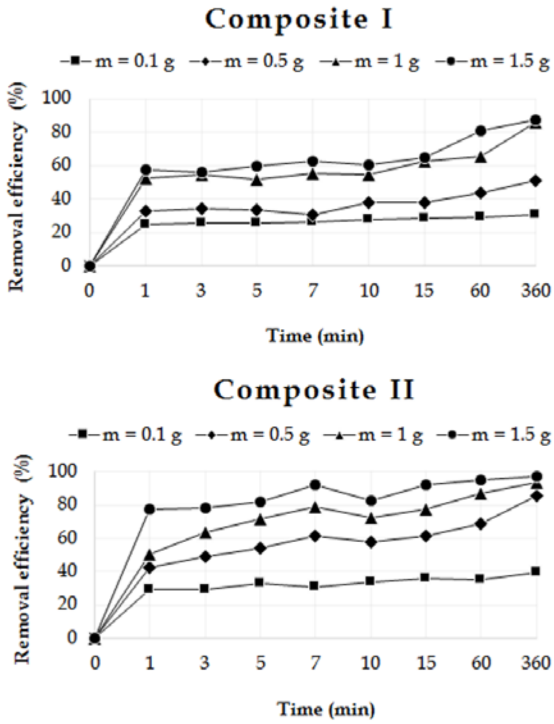

Fig. 2. Influence of the contact time on the copper ions removal at different *Composite I* (a) and *Composite II* dosage (b).

Table 1. Summary of removal efficiency achieved using *Composite I* and *Composite II.*

Dose, g / Time, min	Removal efficiency, %			
	0.1	0.5	1.0	1.5
	Composite I			
1	24.5	33.1	52.1	57.5
60	26.4	43.5	65.4	80.9
360	29.2	51.1	85.9	87.3
	Composite II			
1	29.1	42.1	50.4	77.3
60	34.7	68.5	87.0	95.3
360	39.3	85.8	93.8	96.8

Influence of the coper ions initial concentration on their removal

In the experiments with 0.1 g *Composite I*, the increase of the initial copper ions concentration leads to decreased removal efficiency (Fig.3). For example, after 30 minutes treatment of aqueous solutions with initial copper ions concentration of 1.0, 10.0 and 50.0 mg L^{-1}, the removal efficiency was 78.0%, 54.7% and 27.7%, respectively. The higher removal efficiency of copper ions at lower concentrations can be explained with the presence of sufficient free nitrogen atoms with higher electron density in the polymer chain which are involved in the complexing with copper ions.

With increasing of the metal ions concentration, the possibility of their insertion into the structure of the composite decreases, as the amount of clinoptilolite is low and there is no sufficient free surface area, where to attach the copper ions.

When using 0.1 g *Composite II* by increasing of the Cu^{2+} concentration the effect of their removal was decreased as follows: for 1.0, 10.0 and 50.0 mg L^{-1} copper ions concentration the removal efficiency was 87.0%, 73.1% and 35.6%, respectively. The results show the same tendency as that with *Composite I*. However, it was noted that the usage of *Composite II* leads to greater removal efficiency in comparison with *Composite I*.

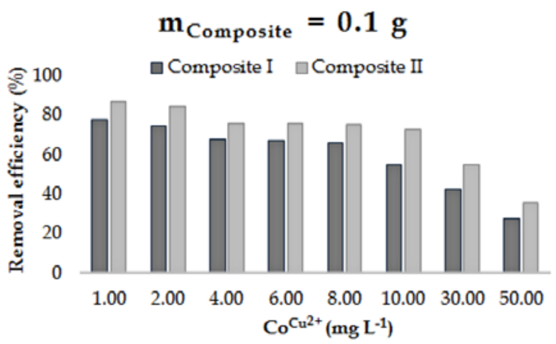

Fig. 3. Influence of the coper ions initial concentration on their removal with *Composite I* and *Composite II.*

Conclusions

The possibility of clinoptilolite/ emeraldine base composites to remove copper ions from model aqueous medium was studied. For this purpose preparation of composites consisted of emeraldine base and different quantities of clinoptilolite was successfully performed by *in situ* polymerization of aniline. It was established that the clinoptilolite/emeraldine base composites have significant potential to remove copper ions from aqueous solutions. The synthesized composites can quickly and effectively to remove the contaminants with a high rate of efficiency. Highest removal efficiency of 96.8% was obtained using 1.5 g *Composite II*.

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Short note

An Exceptional Activity for Darevskia derjugini (Nikolsky, 1898) From Turkey

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Abstract. Observations of reptiles out of their active periods in the field are very surprising for the researchers. The current short note presents data on the exceptional activity of *Darevskia derjugini* for the first time in Turkey. Changing climates and global warming may affect the hibernation period of most lizards in the northern hemisphere.

Key words: Hibernation, *Darevskia derjugini*, lizard, Northern Hemisphere.

Introduction

The climatic changes principally affect summer and winter activity of reptiles (ZUG *et al.,* 2001). Hibernation, as a behavioral response to seasonal change, is most likely a direct response to cold temperatures and changes in food availability (GREGORY, 1982; ADOLPH & PORTER, 1993). After hibernation, ectotherms need to be higher air and body temperature for fecundity, mobility and escape from the predator in their habitats (ADOLPH & PORTER, 1993). In addition, the effect of the photoperiod on seasonal acclimation seems to be significant (RISMILLER & HELDMAIER, 1988).

As known, all members of the *Lacertidae* family in the Northern Hemisphere are hibernating species the winter season. Most of them are active from early April to the middle of October in moderate lowland populations. However, the active periods may be changed from early May to late September in colder highland populations (above 2000 m a.s.l.).

The Derjugin's lizard, *Darevskia derjugini* (Nikoslky, 1898) is distributed from sea level to 1700 m (BISCHOFF, 1982; BARAN & ATATÜR, 1998). This species is active between April and September (ORLOVA & BISCHOFF, 1984). It typically inhabits humid areas in forested habitats, and some individuals are rarely recorded from rocks or walls (TUNIYEV *et al.*, 2009).

Winter activity was observed in some other lizard species as reported for *Podarcis muralis* (BURESH & TSONKOV, 1933; BESHKOV, 1977; BESHKOW & NANEV, 2002; WESTERSTRÖM, 2005; TZANKOV *et al.,* 2014), *Podarcis erhardi* (BURESH & TSONKOV, 1933; BESHKOV, 1977), *Lacerta viridis* (VONGREJ *et al.,* 2008), *Sceloporus jarrovi* (TINKLE & HADLEY, 1973), *Zootoca vivipara* (GRENOT *et al.,* 2000) and *Mediodactylus kotschyi* (MOLLOV *et al.,* 2015).

In the present short note, we present for the first time data about the extraordinary activity of *Darevskia derjugini* in Turkey.

Materials and Methods

The specimen was found during an excursion, during the day, between 10:30 and 11:30 AM. When the observation was made, the air temperature in the locality was 8°C. The specimen was caught by hand. The sex of the individual was diagnosed based on primer sexual character (presence of hemipenis). After the specimen was photographed, it was released back to its natural habitat.

Results and Discussion

A male specimen of *D. derjugini* was observed in the 2 March 2013 from Turkey (Arsin, Trabzon), shown in Fig. 1. The observation site was located at the 210 m a.s.l. (40°55′18′′ N; 39°57′93′′ E). The specimen was found under dry hazelnut leaves and cut nut wood in harvest season.

Fig. 1. A male specimen *Darevskia derjugini* (Nikoslky, 1898), observed from Arsin, Trabzon (Turkey) during the winter period (2 March 2013).

Although active periods during the winter were reported for some lizards (SHTERBACK & GOLUBEV, 1986; OKE, 1982, CAMILLONI & BARROS, 1997; VONGREJ *et al.*, 2008; MOLLOV *et al.*, 2015), this phenomenon was not previously reported for *D. derjugini*. Our data may contribute to the knowledge of the annual activity of *D. derjugini*. In our opinion, the unusual activity may be a result of global warming of the world in the Northern Hemisphere.

Lower air temperatures were very effective to hibernate the lizards because they cannot perform their vital activities such as food availability, mobility, fecundity and escape from predators in lower temperatures (ADOLPH & PORTER, 1993). In parallel with the air temperature in the present study for *D. derjugini* was very low to be carried out its vital activities. The early awakening from hibernation of some lizards may be caused due to constant changing of the air temperatures. The lizards that can cope with lower temperatures may be monitored in different areas of the Northern Hemisphere in the future.

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Short note

Freshwater Pea Clams Pisidium C. Pfeiffer (Mollusca, Bivalvia) From The Alpine Areas of Bulgaria

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Abstract. Distributional data for 3 *Pisidium* species was provided from 19 alpine lakes and 1 dam in Rila and Pirin Mts. (Bulgaria). Most widely distributed was *P. casertanum*. The species *P. globulare* and *P. obtusale* are new records to the area.

Keywords: Rila Mts., Pirin Mts., lakes, Bivalvia, distribution.

Introduction

High mountain massifs of Bulgaria representing an alpine environment are only the Rila and Pirin Mts. situated at the south-west part of the country. The Pea clams (genus *Pisidium* Pfeiffer, 1821) of these areas were poorly studied and only *Pisidium amnicum* (Müller, 1774), and *Pisidium casertanum* (Poli, 1791) were reported (ANGELOV, 2000).

Material and Methods

The material was collected in 75% ethanol between July and September in 1995, 1996, 1998, 2002 and 2005 by the second author. Pea clams were collected by sieving bottom substrate of 20 alpine lakes situated at Rila and Pirin Mts. between 1922 (Belmeken Dam) and 2438 (Tevno Vasilashko Lake) m a. s. l.

Species were determined by anatomical characters or shell morphology considering KORNIUSHIN & HACKENBERG (2000), ZETTLER & GLÖER (2006), and a reference shell collection.

All specimens were deposited in the collection of D. Georgiev.

Results and Discussion

Following 3 species were found (number of localities correspeond with those in Table 1):

- *Pisidium casertanum* (Poli, 1791): 6, 9, 10, 11, 12, 15, 16, 17, 18;
- *Pisidium globulare* Clessin, 1873: 1, 2, 13, 20;
- *Pisidium obtusale* (Lamarck, 1818): 5, 11, 19;
- *Pisidium* sp.: 4, 5, 7, 12, 13, 14.

In all 20 localities studied *Pisidium* species were found. Tree species of Pea clams were registered at the area of the alpine lakes of Rila and Pirin Mts. from

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which most widely distributed was *P. casertanum*. It was found in almost half of all localities studied (N = 9).

The *P. amnicum* was not re-collected from Rila and Pirin alpine zones.

The species *P. globulare* and *P. obtusale* are new records to the areas of both mountains. The species *P. globulare* was known only from one locality in Bulgaria at the W Rhodopes Mts. before this study (HORSÁK, 2006).

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Table 1. Collection localities of the *Pisidium* species from Rila and Pirin Mts.

№	Date	Region	Locality	Coordinates	Altitude (m)
1	25.7.1995	Pirin Mts.	Chairsko Lake	N41 42 29.5 E23 27 35.6	2367
2	25.7.1995	Pirin Mts.	Golyamo Valyavishko Lake	N41 42 31.9 E23 29 00.7	2287
3	26.7.1995	Pirin Mts.	Popovo lake	N41 42 33.8 E23 30 27.1	2248
4	14.8.1995	Pirin Mts.	Gorno Vasilashko Lake	N41 44 19.9 E23 26 35.5	2223
5	14.8.1995	Pirin Mts.	Dolno Vasilashko Lake	N41 44 26.9 E23 27 00.5	2140
6	15.8.1995	Pirin Mts.	Tevno Vasilashko Lake	N41 43 56.3 E23 26 30.6	2438
7	15.8.1995	Pirin Mts.	Gorno Banderishko Lake	N41 44 05.7 E23 25 43.3	2322
8	22.7.1996	Pirin Mts.	Golyamo Gergyisko Lake	N41 44 47.5 E23 22 57.6	2305
9	22.7.1996	Pirin Mts.	Dolno Gergyisko Lake	N41 44 41.7 E23 22 24.9	2210
10	23.7.1996	Pirin Mts.	Sinanishko Lake	N41 43 52.7 E23 21 39.5	2197
11	16.7.1998	Rila Mts.	Bliznaka Lake	N42 12 08.7 E23 18 50.5	2251
12	16.7.1998	Rila Mts.	Trilistnika Lake	N42 12 20.1 E23 19 09.2	2226
13	17.7.1998	Rila Mts.	Dolnoto Lake	N42 12 43.5 E23 19 34.3	2103
14	17.7.1998	Rila Mts.	Skakavishko Lake	N42 12 54.6 E23 18 15.3	2171
15	11.9.2002	Rila Mts.	Gorno Ribno Lake	N42 06 29.8 E23 29 27.7	2237
16	11.9.2002	Rila Mts.	Dolno Ribno Lake	N42 07 05.5 E23 29 40.5	2208
17	12.9.2002	Rila Mts.	Vapsko Lake	N42 05 02.4 E23 30 55.6	2288
18	12.9.2002	Rila Mts.	Belishko (Suho) Lake	N42 04 19.3 E23 33 22.9	2044
19	13.9.2002	Rila Mts.	Marichini Ezera, Dolnoto Lake	N42 09 49.4 E23 35 46.2	2371
20	18.8.2005	Rila Mts.	Belmeken Dam	N42 09 08.7 E23 46 40.0	1922

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Author(s): Full first name(s), middle initials and surname(s) in ***bold italic.*** The corresponding author should be marked with the *-symbol.
Affiliation(s) with postal address: As complete as possible. E-mail address is given only for the corresponding author.
Abstract: Maximum length 300 words. The abstract should state briefly the objective of the research, the primary results and major conclusions, with no description of methods, discussions, references and abbreviations.
Key words: Usually 3–10 words suitable for information-retrieval system.

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

The *Introduction* has to explain the actuality of the researched problem and give the aim of the study.
Materials and Methods have to provide sufficient information to permit repetition of the experiment and/or fieldwork. The technical description of study methods should be given only if such methods are new; otherwise a short presentation is enough.
The *Results* section must be a concise presentation of the finding of the study. **<u>Avoid presentation of the same information as text and/or figure and/or table.</u>**
Discussion section should be separate from the results section at full-length papers and should deal with the significance of the results and

their relationship to the aims of the paper. Also include how the findings of the paper will change or influence the state of our knowledge about the topic at hand. In separate cases a joint section "Results and Discussion" is allowed, but not preferable.
Conclusions should shortly describe the main contributions and recommendations of the study without including citations and statistics.
In the *Acknowledgements* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

Short Notes (generally less than four-five manuscript pages) should be produced as continuous text, preceded by an abstract of no more than 150 words.

Tables: The tables must not repeat information already presented in the figures or in the text. Each table must be self-explanatory and as simple as possible. No fold-outs are accepted. Tables must be numbered consecutively. **They should be placed within the text at the desired position by the author(s).** An explanatory caption, located on the top of the table, should be provided.

Example:

Table 1. Shannon-Wiener indexes in the burned ($\mathbf{H}_{\text{burned}}$) and control ($\mathbf{H}_{\text{control}}$) territory for the total duration of the study (2004–2006).

Figures: They must not repeat information already presented in the tables or in the text. Lines and letters in figures must be able to be enlarged or reduced without reduction in quality. They should conform to the size of the type area (16 × 24 cm) which is the limit for all illustrations. Magnification should be shown by scale bars. Colour illustrations are accepted, but will appear only in the electronic version of the journal (PDF). The illustrations in the hardcopy printed version will be greyscale. All illustrations must be sharp, of high quality with at least 300 dpi. The following formats are acceptable: JPEG, GIF, TIFF, EPS. Figures must be numbered consecutively and should be provided with an explanatory legend below them. *When the figures are presenting maps of the studied area, we recommend using some kind of GIS software for the preparation of the maps, or use of other indicative or topographical maps.*

Satellite or aerial photos (especially from Google Earth) of the studied area will no longer be acceptable! **<u>All figures must be placed within the text at the desired position by the author(s).</u>**

Example:

Fig. 1. Indicative map of the study area.

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

Citations and references

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

Examples:

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

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

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A book:

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

Proceedings or book chapter:

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

Software:

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

Website:

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

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

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

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

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

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Citing references that are still "in press" is also considered frown upon, but not forbidden. If possible, please avoid using such references.

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

significance. The use of the sign is acceptable only at low values of p (e.g. $p<0.0001$).

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

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