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Assessment of Geochemical Characteristics and Geomicrobiology of Cave Spring Water from Jaintia and East Khasi Hills of Meghalaya, India

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Abstract. The present study was undertaken to know the concentration of various trace elements and the condition of water quality parameters in the cave water samples besides studying the role the microbes play in the precipitation of minerals in caves. The results revealed that the concentration of various trace elements such as copper, zinc, nickel and cadmium were low and below the water quality standard limits given by WHO (2006). While that of manganese it was exceptionally high, may be due to erosion of the manganese minerals deposits by the spring cave water. The results also revealed that phosphates are present in very low concentration while sulfates are present in high concentration which again may be due to erosion of secondary sulfate minerals. The co-relation matrices and one tailed analysis of variance of physic-chemical factors have been computed and analyzed. The positive correlation coefficient was observed between pH and alkalinity, hardness and conductivity, sulfates and turbidity. The one tailed ANOVA confirms that site spatial variations have less significant effect on concentration of trace elements. Microbial analysis showed that various types of microbes are present in cave sample which may play an important role in mineral precipitations.

Key words: geomicrobiology, water quality, speleothem genesis, mineral precipitation

Introduction

The Jaintia hills, one of the seven districts of Meghalaya, lies between latitude 25°5'N to 25°4'N and longitude 91°51'E to 92°45'E. The district is bound by the state of Assam on the north and east, the East Khasi Hills on the west and Bangladesh in the south. The district covers an area of 3819 km^2 constituting 17.03% of the total area of the state. The topography of the district is composed of undulating hilly landscapes dissected by numerous rivers and streams. On the northern and western borders, these hills take the form of tumbled ranges, running for the most part of north and south and ranging two to three thousand feet in height. Caves are formed in limestone areas and other rocks of similar composition by the process of weathering and erosion by water. The study of cave microbiology deals with the microscopic life that resides in cave. Without photosynthesis, caves are cut off from most energy that supports life on the surface. As a result, cave microorganisms must look for alternative sources of energy for their survival, such as those found in the

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atmosphere, or present in the very rock itself (BARTON *et al.*, 2004; CHELIUS & MOORE, 2004; SPILDE *et al.*, 2005). In adapting to these extremely starved environments, microorganisms produce elaborate scavenging mechanisms to pull scarce nutrients into the cell (KOCH, 1997). When these organisms are then exposed to the rich nutrients of a Petri plate, they cannot turn down these scavenging mechanisms and quickly gorge themselves to death (KOCH, 1997; 2001). As a result, microorganisms from starved cave environments may have a hard time adapting to rapidly changing nutrient status *in vitro*, and simply die from osmotic stresses (KOCH, 1997).The present study was undertaken to analyze the geochemistry of cave water samples and to analyze the geomicrobiology of speleothems.

Material and Methods

Physic-chemical parameters of water

The samples of caves spring water and rock samples were collected from Meghalaya by the Department of Environmental science and Engineering, Guru Jambheshwar University of Science and Technology, Hissar (Haryana).The physic-chemical parameters of water were carried out as per the standard methods.

The data collected were subjected to Pearson's correlation matrix to study the significant level at $p<0.05$ and $p<0.01$ (two tailed) to note the positive and negative correlation among physic-chemical factors. Similarly one way ANOVA was applied to know variation among trace elements. The SPSS version.16.0 statistical program was used for all statistical analysis throughout this research.

Geo-microbiology of caves

Rock samples were crushed to make powder. One gram of powdered rock sample was dissolved into 100 ml distilled water and agitated in a shaker for 15 minutes. Nutrient agar medium was prepared by dissolving 31 g of nutrient agar in one liter distilled water. The media, glassware like micro tips, 40 test tubes having 9 ml distilled water were autoclaved at 121ºC and 15 psi pressure for 15 minutes. Then, the media was poured in the sterilized Petri plates in laminar flow and allowed to keep undisturbed until the media was solidified.

Table 1. Standard methods used for analyses of physic-chemical parameters of water

S .No.	Parameters	Methods
1.	pH	pH meter(APHA 1998)
2.	Dissolved Oxygen	Winkler's titration method (APHA, 1998; Wetzel and Likens, 2000)
3.	Conductivity	Conductivity meter(APHA 1998)
4	Water Temperature	Thermometer (APHA, 1998)
5	Total Alkalinity	Titrimetric method(APHA, 1998)
6	Total dissolved solids	APHA (1998)
7	Calcium Hardness	EDTA titrimetric method (APHA, 1998)
8	Magnesium Hardness	EDTA Titrimetric method (APHA, 1998)
9	Total Hardness	Complexometric method (APHA, 1998)
10	Chloride	Argentimetric method (APHA, 1998)
11	Nitrite	Spectrophotometric method (APHA, 1998; Wetzel & Likens, 2000)
12	Nitrate	Sodium salicylate (APHA, 1998; Wetzel & Likens, 2000)
13	Ammonias Nitrogen	Phenate method (APHA, 1998; Wetzel & Likens, 2000)
14	Ortho-phosphate	Ascorbic Acid method (APHA, 1998; Wetzel & Likens, 2000)
16	Trace Metals (Cu, Zn, Ni, Cd, Mg)	Atomic Absorption Spectrophotometer (AAS) (APHA, 1998)

After that, dilutions of the order of 10^{-2} of the rock sample were prepared and then inoculation was done. Then the Petri Best wishes, plates were wrapped with paraffin wax and were kept in the incubator at 28°C for five days. Then the colonies were identified using Gram Staining technique and Most Probable Number method (MPN).

Results and Discussion

The present study was undertaken to know the concentration of various trace elements in the cave water samples and to know the condition of water quality parameters. Geochemical analysis of cave waters revealed that there is a significant difference in the concentration of various trace metals from different sampling sites as shown in Fig. 1. The concentration of copper ranged from 0.2466 ppm (sample No. SI-3) to 0 ppm in most of the samples (Table 2). In case of zinc maximum concentration is found in sample No. S1-3 i.e. 3.9417 ppm, followed by MC1W–01 (3.1612 ppm) while minimum concentration is detected in sample No. S2-6 i.e. 0.0787 ppm. Nickel showed overall low concentration being maximum in sample No. MC2W-11 i.e. 1.8784 ppm and minimum in sample No. S3-9 i.e. 0.0247 ppm. In case of cadmium concentration ranges from 0.02423 ppm (sample No. MC2W-03) to 0.001287 ppm (sample No. MC2W–02). However, manganese showed highest concentration ranging from 5.7353 ppm in sample No. MC2W-03, while minimum i.e. 0.114 ppm in MC2W-07. It has been found manganese-oxidizing bacteria such as *Leptothrix* in a stream in Matts Black Cave, West Virginia, and attributed the formation of birnessite in this cave to the precipitation of manganese around sheaths of bacteria BROUGHTON (1971) and MOORE (1981).

The pH ranged from 7.6 to 8.2 indicating slightly alkaline nature. Acidity ranged between 10 to 40 ppm which may be due to free carbon dioxide, trace amount of sulfuric acid and nitric acid (Table 3). However, alkalinity ranged from 40 ppm (sample No. MC2W-02, S1 and S3) to 100 ppm (samples MC2W-01 and MC2W-11) which may be due to presence of free ions of hydroxide, carbonate and bicarbonates.

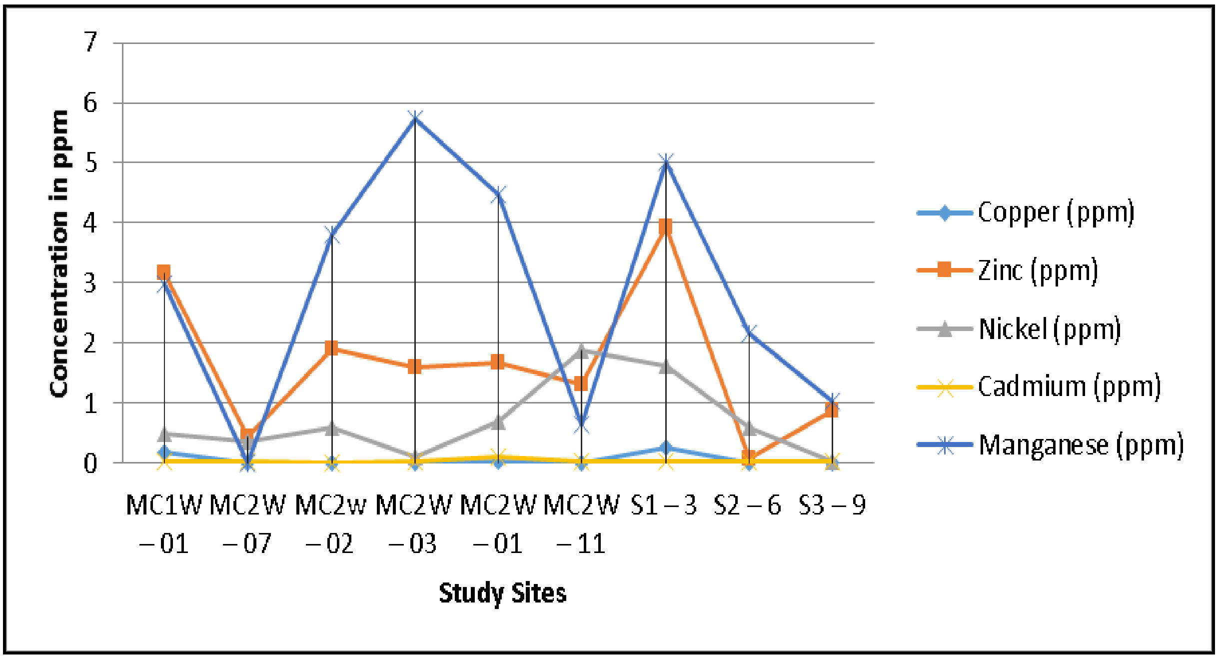

Fig.1. Showing variation in concentration of trace elements at different sites.

Table 2. Microbial colony characteristics.

Sample No.	Viable Count	CFU/g	Colour	Form	Elevation	Margin	Texture	Gram stain	Type
MCIR-01	15	15×10^2	Pale yellow, Creamy white.	Circular, Irregular,	Flat, Convex.	Entire, undulate, erose.	Smooth, Slimy	+ve +ve	*Cocci, Cocci,*
MCIR-02	34	34×10^2	Creamywhite, Slightly pink, Pale yellow	Circular, Irregular, Puntiform	Convex, Pulvinate, Raised.	Entire, Undulate, curled.	Powdry, Glutinous , Slight Slimy	+ve -ve -ve	*Cocci, ccoci cocci*
S-10	32	32×10^2	Creamy white, Pale yellow.	Circular, Punctiform	Convex, Raised.	Entire, Undulate	Smooth, Slimy.	-ve -ve	*Cocci. Cocci*
S-15	19	32×10^2	White, Yellow, Creamy white.	Circular, Irregular Filamentous	Convex, Pulvinate Flat	Lobate, Erose, Curved.	Glutinous, Powdry, smooth.	+ve +ve -ve	*Cocci Cocoi Cocci,*
MC2R-14	45	45×10^2	Orange, Slightly Brown, Creamy white.	Irregular Filamentous. Punctiform.	Convex, Flat, Pulvinate.	Undulate, Lobate, Curved.	Glutinous, Smooth, Powdery.	-ve -ve +ve	*Cocci Cocci cocci*
S-17	14	14×10^2	Orange, Translucent.	Circular, Irregular	Convex, Pulvinate.	Entire, Undulate	Smooth, Powdery,	-ve +ve	*Cocci cocci*
S-7	17	17×10^2	Creamywhite, Greyish black, Pale yellow.	Punctiform, Circular, Irregular.	Convex, Raised. Flat	Entire, Undulate, lobate.	Slimy , Glutinous, Powdry	+ve +ve +ve	*Cocci Cocci Cocci*
S-9	14	14×10^2	Red, Pale yellow	Circular, Irregular.	Raised, Convex.	Undulate, Filamento us	Rough, Slimy.	-ve -ve	*Cocci Cocci*
MCIR-13	17	17×10^2	Pale yellow, grayish white	Circular, Irregular.	Raised, Convex	Entire, Undulate	Powdery, Smooth.	+ve +ve	*Cocci Cocci*
MCIR-24	Uncoun table	Uncounta ble	White, Creamy white.	Punctiform, Circular.	Convex, Pulvinate.	Entire , Erose.	Smooth, Slimy	-ve +ve	*Cocci, Cocci*

Table 3. Geochemistry of Caves wáter.

S. No.	Sample	pH	Temp. (^{0}C)	Acidity (ppm)	Alkalinity (ppm)	Conductivity (µS)	Hardness (ppm)	Turbidity (ppm)	Phosphate (ppm)	Sulfate (ppm)
01.	MC1W-01	7.7	11.5	40	80	209.3	220	0.7	0.02	9.4
02.	MC2W 01	8.2	11.6	20	100	187.9	100	1.5	0.08	12.3
03.	MC2W 02	7.6	13	40	40	227.2	200	2.2	0.04	16.2
04.	MC2W 03	7.7	12.5	20	60	158.5	220	0.1	0.26	5.1
05.	MC2W 07	7.6	13	40	60	162.3	200	0.1	0.12	6.4
06.	MC2W 11	7.9	11	10	100	152	200	0.2	0.06	4.3
07.	S_1	7.6	8	20	40	253	220	0.1	0.07	1.2
08.	S_2	7.8	11.5	40	80	243.1	240	0.4	0.00	0.3
09.	S_3	7.7	12	40	40	74.16	100	0.2	0.05	9.8
10.	Mean	7.7	11.5	30	66.66	185.27	188.88	0.61	0.07	7.22
11.	S.E(±)	2.5	1.5	12.24	24.49	55.86	52.06	0.74	0.076	5.19
12.	CD (5%)	4.9	2.94	23.99	48.98	109.48	102.03	1.45	0.14	10.17

In case of conductivity it ranged from74.16 µs (sample No. S3) to 253 µs (sample No. S1) which may be due various free ions present in the sample. Hardness of caves water samples ranged from 240 ppm to 100 ppm which may be due to ions such as, carbonate and bicarbonates of calcium and magnesium. However the turbidity ranged from 0.1 to 2.2 which may be due to suspended matter ranging from pure inorganic substance to those that are organic in nature. In case of phosphate ions the concentration is extremely very low ranging between 0.02 ppm in sample No. MC1W-01 to 0.26 ppm in sample No. MC2W-03. However in case of sample No. S2 phosphate ions are absent. In case of sulfates the concentration varied from 0.3 ppm in the sample No. S2 to 16.2 ppm in the sample No. MC2W-02 thus being maximal in MC2W-02 cave sample. The results revealed that the concentration of various trace elements such as copper, zinc, nickel and cadmium were low and below the standard water parameter limits. While that of manganese it was exceptionally high, may be due to erosion of the manganese minerals deposits by the spring cave water. The results also revealed that phosphates are present in very low concentration while sulfates are present in high concentration which again may be due to erosion of secondary sulfate minerals.

Morphological characteristic of microbial colonies (Fig. 2-1 and 2-2, Table 4) revealed that both gram positive and gram negative microbes were existing in different forms. They play an important role in mineral precipitation (FRANKEL & BAZYLINSKI, 2003). Studies revealed that calcite was the dominant mineral and an abundant microbial community was detected by direct microscopic observation after DAPI staining which were indicative of microbial involvement in the speleothem genesis (BASKAR *et al.* 2005, 2006, 2007). The iron-oxidizing species *Gallionella ferruginea* and *Leptothrix sp*. has been recovered from cave samples (PECK, 1986). Further detailed investigations are required involving *in vitro* culture experiments and molecular techniques to quantify the extent of microbial participation in speleothem genesis. Progress in the field will depend on cross-disciplinary studies involving the abilities of biologists to recognize assuredly biological structures and measure these processes within the cave environment; and geologists, who can apply the complex tools of chemistry and geology to the problem.

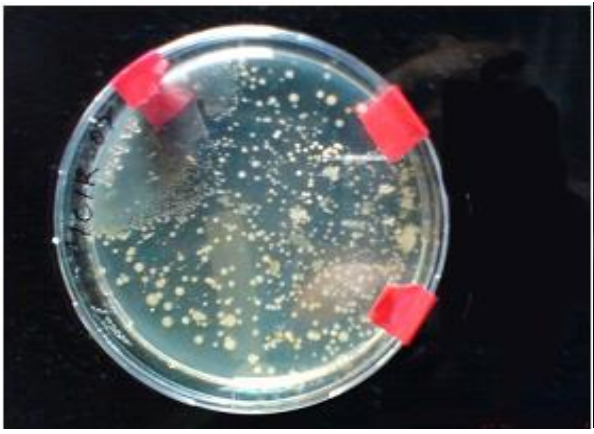

Fig. 2-1. MC2R-14

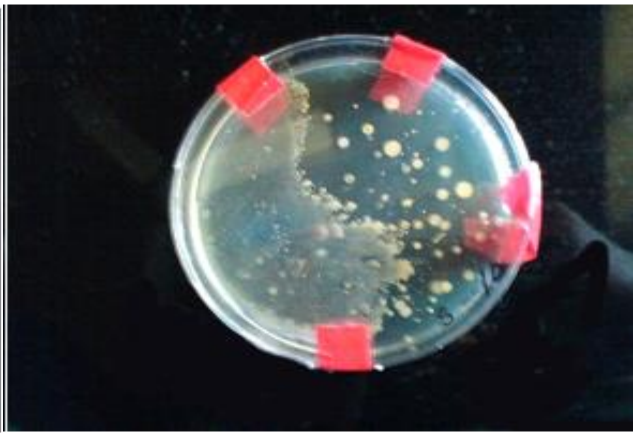

Fig. 2-2. S-10

Table 4. Geochemistry of Caves water

	A	B	C	D	E	F	G	H	I
A	+1								
B	-.014	+1							
C	-.300	+.431	+1						
D	+.866**	-.115	+.000	+1					
E	-.134	+.057	+.247	-.312	+1				
F	+.257	-.106	-.345	+.332	+925**	+1			
G	+.244	+.330	+.044	+.161	-.178	+.149	+1		
H	-.083	+.228	-.576	-.314	-.197	+.126	-.302	+1	
I	+.130	+.550	+.148	-.012	+.175	-.249	+.809**	-.097	+1

** = Correlation is high significant at $p < 0.01$ level, '-' indicate negative correlation, '+' indicate positive correlation,
Where A= pH, B= Temp., C= Acidity, D= Alkalinity, E= Conductivity, F= Hardness, G= Turbidity, H= Phosphate, I= Sulfate

The study of cave microbes has significant implications in the preservation of ancient marble monuments and statues, where microorganisms could be used to deposit a veneer of calcite to protect ancient structures from continued erosion (LAIZ *et al.*, 2003) and can be inoculated into contaminated environments to rapidly degrade pollutants and allow restoration of natural habitats in a process called bioremediation. Cave microorganisms also have the potential to harbour unique antibiotics, with properties that allow efficient ethanol production for fuel, enzymes for environmentally friendly paper processing and even the improved stonewashing of jeans (ONAGA, 2001).

The data collected were subjected to Pearsons's correlation matrix to study the significant level at $p< 0.05$ and $p< 0.01$ (2 tailed) to note the positive and negative correlation among the physic-chemical factors. The statistical analysis of Pearson's correlation coefficient is presented in Table 5. The study of correlation coefficient between various physic-chemical factors indicated that pH values varied with the variation of alkalinity. The rise of carbonate and bicarbonate concentrations increased the level of pH and hence alkalinity enhanced the decomposition of organic matter which in turn increases concentration of nitrite, phosphate and sulfate ions. The abundant of Ca and Mg in addition to nitrite, sulfates and phosphates are responsible for an increase of hardness and a perfect positive correlation with conductivity. The high concentration of sulfates increases turbidity of water.

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Ecological Study of Periphytic Algal Community of Doodh Ganga and Khansha-Mansha Streams of Yusmarg Forests: A Health Resort of Kashmir Valley, India.

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Abstract. The present study on Doodh Ganga and Khansha-Mansha streams of Yusmarg forests deals with the general ecological studies on periphytic algal community in terms of species composition and density. During the present investigation the periphytic algal community of Doodh Ganga and Khansha-Mansha streams were represented by 30 taxa which belonged to 4 major classes namely Bacillariophyceae (14), Chlorophyceae (11), Cyanophyceae (4) and Euglenophyceae (1). The most common periphytic species encountered across all the sites included *Closterium* sp., *Zygnema* sp., *Amphora* sp., *Cymbella* sp., *Epithemia* sp., *Fragilaria* sp., *Navicula* sp., *Synedra* sp., *Tabellaria* sp., *Lyngbya* sp. and *Phormidium* sp. Among the two streams, Doodh Ganga showed large number of taxa (45) and Khansha-Mansha was having 37 taxa of periphyton. Bacillariophyceae was the dominant group both in diversity and density and included 14 taxa contributing 57% of total periphytic algal population. Cyanophyceae forming the second dominant class was represented by 4 genera comprising 22% of the total periphytic algae .Chlorophyceae ranked third in its dominance pattern with 11 genera forming 20% of all the periphytic algae. Euglenophyceae was represented by only one species of *Euglena* sp. forming 1% of all the periphytic algae and found only at site2 (Doodh Ganga downstream).Amongst the study sites the highest (5.69) value of Shannon Weiner Index was found at Doodh Ganga upstream while as lowest (4.38) at Khansha-Mansha downstream. The primary conclusion is that the streams, having crystal clear water, and are free from pollution as Chlorophyceae are better represented in both the streams. Further, as a result of less anthropogenic pressures the quality of water is fairly good.

Key words: Periphyton, Doodh Ganga, Khansha-Mansha, Bacillariophyceae, Chlorophyceae, Cyanophyceae, Yusmarg.

Introduction

Periphyton comprises the organisms living on the substrate. Its assemblages show variations in their nutritional quality. Evidences have suggested that the importance of periphyton in stream food webs is a function of quality than quantity (CROSS *et al.*, 2003). The periphyton community are found to deplete nutrients from waterways, assuming no additional inputs, and communities vary compositionally with nutrient concentrations (MARINELARENA & GIORGI, 2001).And their community structure, species composition, and succession respond to environmental conditions and thus can be used to classify waterways merged substrate (DENECOLA *et al.*, 2004; WARGO & HOLT, 2004). These

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include both the attached forms and the organisms associated therewith. Periphyton play a significant role in the functioning of aquatic ecosystem, producing significant standing crops and hence contribute much to the productivity of fresh water ecosystems (KAUL *et al*., 1980; PANDIT, 1980, 1984; PANDIT *et al*., 1985; SARWAR, 1999). Besides being a major contributor of carbon (energy) fixation, the periphytic algae form a major source of food for fish and waterfowl (PETERS *et al*., 1968; DENNY *et al*., 1978) and are the life of environment of invertebrates and also the commercial fish (PANDIT *et al.,* 1985). The periphyton is useful as biological indicators of pollution as they are mostly sessile and hence cannot avoid contact with the waste effluents. Ecological variables such as nutrient supply, light availability, physical disturbance, and grazing are found to drive or limit algal production in streams and have been studied extensively, both through correlative and experimental approach (WEHR & SHEATH, 2003).In view of the significant role which periphyton play in aquatic ecosystems, the present study was undertaken to assess the status of periphytic algal community in the streams of Yusmarg forests in terms of the species composition and difference in diversity and population density of periphytic flora.

Material and Methods

Study area and site description. Yusmarg is developing Tourist Health resort approximately 47 km from the southwest of Srinagar and lies in the Budgam district of valley of Kashmir, India. It lies between the geographical coordinates of 33°49'42"N Latitudes and 74°39'5"E longitudes and at an elevation of 2712 m a. s. l. Situated amidst Sang-i-Safed valley drained by mighty Doodh Ganga river, it is reputed for having some unique spring flowers and highest mountain peaks in PirPanjal range like Tatakoti 4725 m (a.s.l), Romesh Thong 5000 m (a.s.l) and Sunset Peak 4746 m (a.s.l). It has the potential to be the gateway of some potentially valuable tourist destinations in its South, West and East including Dodpathri, Nilnag, Bargah and Tosaimaidan. In order to assess the present status of periphytic algal community in two different streams of Yusmarg forests namely Doodh Ganga and Khansha-Mansha, four sites viz., site1 (Doodh Ganga upstream), site2 (Doodh Ganga downstream), site3 (Khansha-Mansha upstream) and site4 (Khansha-Manshsha downstream) were selected.

Site 1 (Doodh Ganga upstream). It is located between the geographical co-ordinates of 33° 50' 34.4" N latitude and 74° 39' 12.4" E longitude and at an altitude 2304 m (a.s.l). This site has a large flow of water which form white foam. The water is clear and the bottom consisting boulders and large cobbles with diameter of greater than 0.25 m and 0.256-0.128 m respectively.

Site 2 (Doodh Ganga downstream). This site is located between geographical co-ordinates of 33° 50' 41.2" N latitude and 74° 39' 24.7" E longitude and at an altitude 2264m (a.s.l). It has large discharge of water and bottom is covered wholly and solely with cobbles and pebbles.

Site 3 (Khansha-Mansha upstream). It is located between geographical co-ordinates of 33° 49' 38.9" N latitude and 74° 39' 41.7" E longitude and at an altitude 2414m (a.s.l), dominated by cobbles and pebbles.

Site 4 (Khansha-Manshsha downstream). This site is located downstream of Khansha-Mansha near the entrance of the Sadarmauj reservoir and lies between the geographical coordinates of 33° 49' 39.7" N latitude and 74° 39' 57.2" E longitude and at an altitude 2364m (a.s.l). Bottom is mainly composed of sand, cobbles and small pebbles. The study area and sites are depicted in Fig. 1.

Sample collection, laboratory analysis and methods. The sampling was carried on monthly based from May to December 2010 at four selected sites. The samples were collected by scraping 5cm^2 surface areas of stones and boulders using blade and brushes. The samples were washed into a tray and then transferred into a vial of suitable volume and preserved in 4% formalin and stored in small vials having capacity of 50ml. The process of identification was carried out under the microscope with the standard works of

(PRESCOTT, 1939; 1951; COX, 1996; EDMONDSON, 1992; A.P.H.A., 1998 and BIGGS & KILROY, 2000). Sedgwick- Rafter cell of 1 ml capacity was used for counting of the individuals.

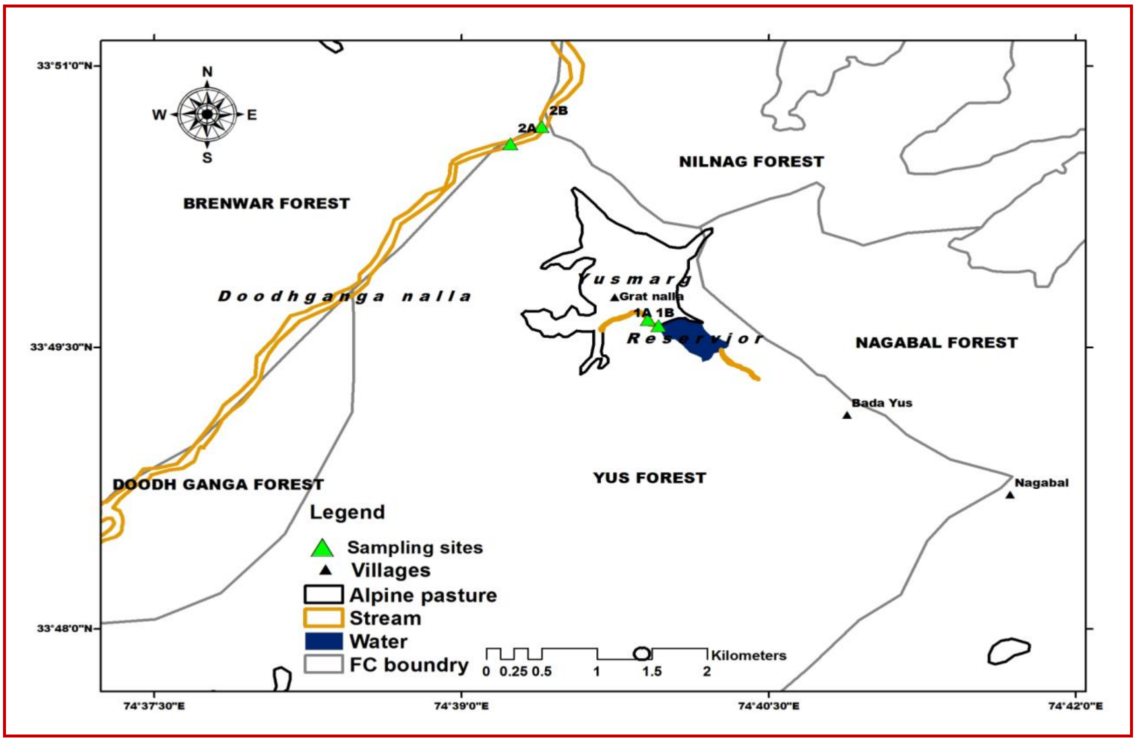

Fig.1. Map of study area and study sites (Note: 2A=Site1, 2B=Site2, 1A=Site3 and 1B=Site4).

Results

Species Composition. The periphytic algal community of Doodh Ganga and Khansha-Mansha streams were represented by 30 taxa, which belonged to 4 major classes namely Bacillariophyceae (14), Chlorophyceae (11), Cyanophyceae (4) and Euglenophyceae (1). The most common periphytic species encountered across all the sites included *Closterium* sp., *Zygnema* sp., *Amphora* sp., *Cymbella* sp., *Epithemia* sp., *Fragilaria* sp., *Navicula* sp., *Synedra* sp., *Tabellaria* sp., *Lyngbya* sp. and *Phormidium* sp. (Table 1, Fig.2). Comparative analysis revealed that at site 1 Bacillariophyceae Chlorophyceae and Cyanophyceae, contributed 13, 8, and 4 respectively. At site 2 10 taxa belonged to Bacillariophyceae 5 to Chlorophyceae, 4 to Cyanophyceae, and 1 to Euglenophyceae. At site 3 10 taxa belonged to Bacillariophyceae, 6 to Chlorophyceae and 2 to Cyanophyceae. Almost Similar contributions were noticed to algal community at site four having Bacillariophyceae (11), Chlorophyceae (5) and Cyanophyceae (3). The present study revealed that among the two streams, Doodh Ganga showed large number of taxa (45) as against Khansha-Mansha having 37 taxa of periphyton. Amongst the 30 species listed, the maximum number of species was noted at site 1, followed by site 2, site 4, and decreasing to the minimum of 18 species at site 3. Among the various periphytic classes, Bacillariophyceae dominated both qualitatively and quantitatively at each site, followed by Chlorophyceae and Cyanophyceae whereas Euglenophyceae was only found at site 2 (Doodh Ganga downstream) being represented by only one species with very low population.

Density

Bacillariophyceae. The population density of Bacillariophyceae varied from a low of 1275 ind./cm^2 at site 2 in December to a high of 4760 ind./cm^2 at site 1 in June. However, the mean density was maintained at its lowest ebb (2209.5 ind./cm^2) at site 2 against the highest (3588.5 ind./cm²) being obtained at site 1. Genera like *Cymbella* sp,*Navicula* sp. and *Tabellaria* sp. were the major contributors to the overall density (Table 2, Fig. 3).

Table 1: Population density (Ind/cm²) of Periphytic flora at different sites of two streams flowing in Yusmarg forests.

S.No.	Genera	Site	Density (Ind/cm²)					
			May	June	November	December	Mean	S.D
Family : Chlorophyceae								
1	*Closterium* sp.	1	370	400	260	240	317.5	79.32
		2	350	410	210	200	292.5	104.04
		3	260	300	210	190	240	49.67
		4	350	410	210	200	292.5	104.04
2	*Cosmarium* sp.	1	180	190	110	90	142.5	49.91
		2	350	410	210	200	292.5	104.04
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
3	*Chlorella* sp.	1	0	0	0	0	0	0
		2	330	390	190	160	267.5	110.26
		3	210	230	105	**0**	181.67	67.14
		4	330	390	190	160	267.5	110.26
4	*Cladophora* sp.	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	150	200	90	80	130	55.98
		4	0	0	0	0	0	0
5	*Geminela* sp	1	200	210	120	110	160	52.28
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
6	*Microspora* sp.	1	190	200	100	90	145	58.02
		2	170	200	100	90	140	53.54
		3	0	0	0	0	0	0
		4	170	200	100	90	140	53.54
7	*Oedogonium* sp.	1	200	220	140	125	171.25	45.89
		2	0	0	0	0	0	0
		3	210	250	110	90	165	77.24
		4	0	0	0	0	0	0
8	*Scenedesmus* sp.	1	180	205	120	106	152.75	47.36
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
9	*Ulothrix* sp.	1	300	370	220	210	275	75.05
		2	250	298	160	120	207	81.46
		3	0	0	0	0	0	0
		4	250	298	160	120	207	81.46
10	*Volvox sp.*	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	150	180	90	81	125.25	47.65
		4	0	0	0	0	0	0
11	*Zygnema* sp.	1	250	300	190	176	229	57.18
		2	200	210	110	105	156.25	56.47
		3	150	200	100	80	132.5	53.77
		4	200	210	110	105	156.25	56.47
Total			**5950**	**6881**	**3715**	**3218**	**4986.42**	**1732.04**

Family: Bacillariophyceae							
12	*Amphora* sp.	1	200	300	120	108	182	88.63
		2	220	256	110	100	171.5	78.28
		3	250	270	140	110	192.5	79.32
		4	260	300	200	180	235	55.07
13	*Bacillaria paradoxa*	1	180	250	112	100	160.5	69.28
		2	200	240	105	94	159.75	71.6
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
14	*Cymbella* sp.	1	210	250	145	130	183.75	56.18
		2	380	410	200	142	283	132.04
		3	500	600	300	250	412.5	165.2
		4	620	750	410	380	540	176.07
15	*Diatoma* sp.	1	240	270	185	160	213.75	50.22
		2	0	0	0	0	0	0
		3	220	260	190	170	210	39.16
		4	210	250	150	120	182.5	58.52
16	*Epithemia* sp.	1	180	220	110	100	152.5	57.37
		2	160	250	90	80	145	78.53
		3	230	255	130	110	181.25	71.92
		4	290	310	160	140	225	87.37
17	*Eunotia* sp.	1	280	330	200	180	247.5	69.94
		2	0	0	0	0	0	0
		3	210	240	120	0	190	62.45
		4	220	250	140	110	180	65.83
18	*Fragilaria* sp.	1	170	200	120	100	147.5	45.73
		2	230	390	167	120	226.75	117.79
		3	350	400	200	170	280	112.25
		4	310	350	200	180	260	82.86
19	*Frustulia* sp.	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	210	220	95	80	151.25	73.97
		4	150	170	90	70	120	47.61
20	*Gomphonema* sp.	1	680	750	500	480	602.5	133.26
		2	230	300	150	110	197.5	84.6
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
21	*Mastagloia* sp.	1	0	0	0	0	0	0
		2	110	140	80	60	97.5	35
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
22	*Navicula* sp.	1	170	190	110	102	143	43.61
		2	440	600	270	231	385.25	169.5
		3	400	500	210	190	325	150.22
		4	320	400	210	190	280	98.32
23	*Nitzchia* sp.	1	170	190	110	102	143	43.61
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	180	210	85	74	137.25	67.95
24	*Synedra* sp.	1	260	300	186	166	228	62.75
		2	230	305	180	150	216.25	67.75
		3	210	250	100	80	160	82.86
		4	200	220	100	90	152.5	67.02
25	*Tabellaria* sp.	1	670	700	550	500	605	95.39
		2	420	490	210	188	327	150.81

		3	350	400	220	200	292.5	97.76
		4	340	390	250	220	300	78.74
Total			**12060**	**14326**	**7710**	**6617**	**10225.8**	**3622.34**
Family: Cyanophyceae								
26	*Calothrix* sp.	1	310	380	200	180	267.5	94.29
		2	260	300	170	120	212.5	82.21
		3	0	0	0	0	0	0
		4	350	400	210	190	287.5	103.4
27	*Lyngbya* sp.	1	600	650	480	420	537.5	105.94
		2	381	410	200	180	292.75	119.51
		3	300	350	190	170	252.5	86.55
		4	320	350	200	185	263.75	83.4
28	*Oscillatoria* sp.	1	320	370	250	230	292.5	64.48
		2	410	490	280	230	352.5	118.99
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
29	*Phormidium* sp.	1	400	450	322	300	390.66	64.5
		2	380	410	260	220	317.5	91.79
		3	400	410	300	280	347.5	67.02
		4	400	430	310	290	357.5	68.01
Total			**4831**	**5400**	**3372**	**2995**	**4172.16**	**1150.09**
Family: Euglenophyceae								
30	*Euglena* sp.	1	0	0	0	0	0	0
		2	211	310	100	96	179.25	102.17
		3	0	0	0	0	0	0
		4	0	0	0	0	0	0
Total			**211**	**310**	**100**	**96**	**179.25**	**102.17**

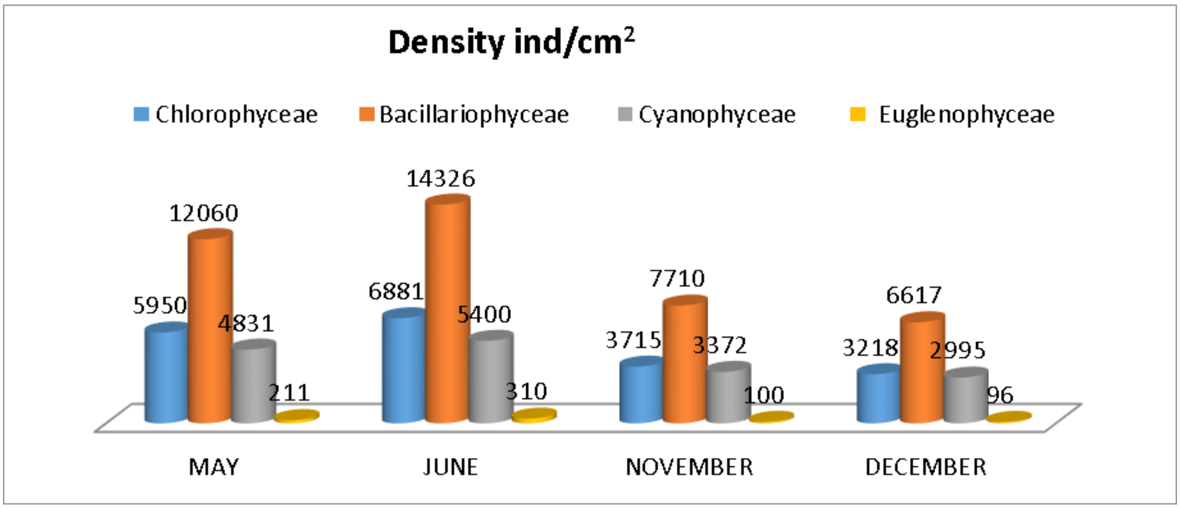

Fig 2. Spatial Variations in mean density (Ind./cm²) of periphytic flora at different sites during study period.

*Chlorophyceae.*Among the sites studied the population density of Chlorophyceae fluctuated from a minimum of 515 ind./cm² at site 2 and 4 in December to a maximum of 2095ind./cm²at site 1 in June. The highest mean population density of Chlorophyceae was noticeable at site 1 (1593 ind./cm²) and lowest (795,75 ind./cm²) was registered at site 2 and 4. The life-forms which contributed their major share in the overall density of Chlorophyceae were *Zygnema* sp. *Closterium* sp. *Chlorella* sp. and *Ulothrix* sp. (Table 2, Fig. 3).

Cyanophyceae. The population density of Cyanophyceae reached its highest peak (1850 ind./cm^2) at Site 1 in June while as the lowest (450 ind./cm^2) was obtained at site 3 in December. However, on spatial basis the group depicted maximum mean population (1488.16 ind./cm^2) at site 1 against its minimum (600 ind./cm^2) at site 3. Taxa like *Phormidium* sp., *Lyngbya* sp. and *Oscillatoria* sp. were the most dominant species contributing the major portion to the overall density of cyanophyceae group (Table 2, Fig 3).

Euglenophyceae. Euglenophyceae was represented by lone species of *Euglena* sp., being recorded at Site 2 and attaining an overall a very low population (highest of 310 ind./cm^2 in June and lowest of 96 ind./cm^2 in December).

Relative density. Bacillariophyceae, dominating both in diversity (Table 3) as well as in density, was comprised of 14 taxa forming 57% of total periphytic algal population in the studied area (Fig. 4). Cyanophyceae formed the second dominant class representing 4 genera and making 22% of total periphytic algal population. Chlorophyceae ranked third in the order of dominance and registered 11 genera forming 20% of the periphytic algal population of the stream. Euglinophyceae was represented by lone taxa being restricted to only Doodh Ganga downstream. The diversity of different algal classes did not vary much among the sites yet, the density showed remarkable variations ranged from a maximum (6669.66 ind./cm^2) at site 1 to a minimum (3969.42 ind./cm^2) at site 3. The Shannon- Weiner index value incorporates both taxa richness and evenness of number of individuals in each taxa. Highest (5.69) value of diversity index was maintained for Doodh Ganga upstream in comparison to Khansha-Mansha downstream (4.38). In general, the index also showed that the diversity of Doodh Ganga stream is greater than the Khansha-Mansha stream.

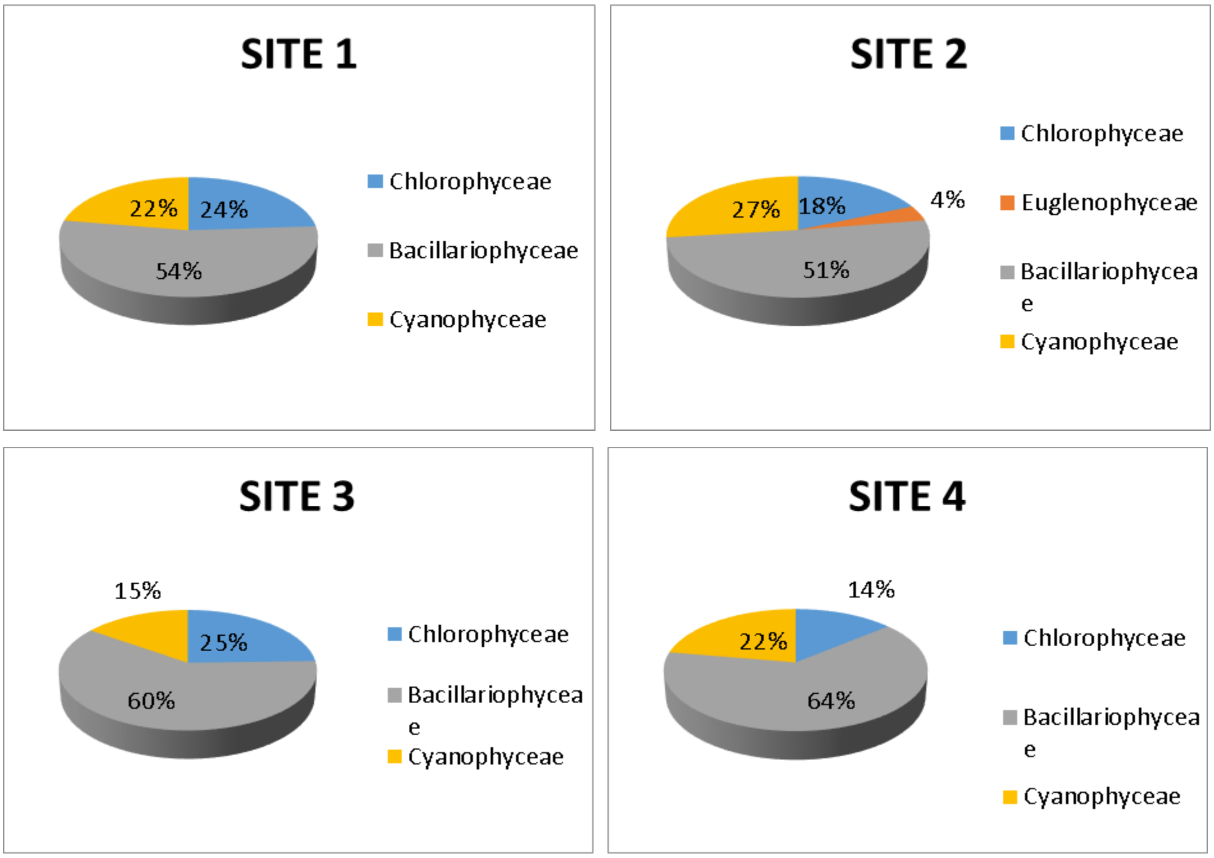

Fig. 3. Relative density of different classes of periphyton at four sites.

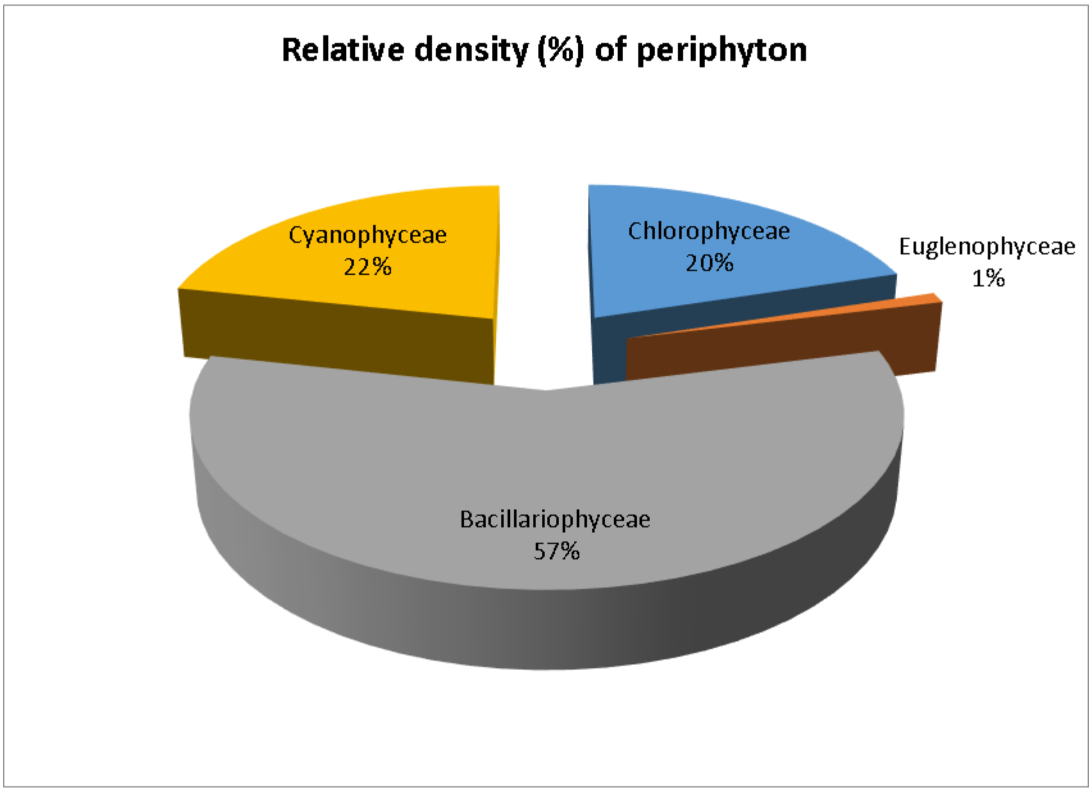

Fig 4. Overall relative density of periphyton at different sites at Yusmarg.

Table 2. Spatio-temporal variation in density (Ind./cm²) of periphytic flora at different sites of two streams flowing in Yusmarg Forests.

S.No.	Class	Site	Density (Ind./cm²)				
			May	June	November	December	Mean
1	**Chlorophyceae**	1	1870	2095	1260	1147	1593
		2	970	1118	580	515	795.75
		3	1130	1360	705	521	974.42
		4	970	1118	580	515	795.75
2	**Bacillariophyceae**	1	3940	4760	2948	2706	3588.5
		2	2620	3381	1562	1275	2209.5
		3	2930	3395	1705	1360	2395
		4	3100	3600	1995	1754	2612.25
3	**Cyanophyceae**	1	1630	1850	1252	830	1488.16
		2	1431	1610	910	750	1175.25
		3	700	760	490	450	600
		4	1070	1180	720	665	908.75
4	**Euglenophyceae**	1	0	0	0	0	0
		2	211	310	100	96	179.25
		3	0	0	0	0	0
		4	0	0	0	0	0

Table 3. Shannon-Weiner Diversity index of periphyton.

SITES	S.W Diversity index
Doodh Ganga upstream	5.69
Doodh Ganga downstream	5.17
Khansha- Mansha upstream	5.06
Khansha -Mansha downstream	4.38

Discussion

Bacillariophyceae was found the most dominant taxa in terms of density as well as diversity, being represented by 14 taxa, followed by Chlorophyceae(11), Cyanophyceae (4) Euglenophyceae (1). The quantitative increase of Cyanophyceae is attributable to the relatively higher temperature and lower values of conductivity, alkalinity and hardness (BHAT *et.al.*, 2011).The growth and abundance of Chlorophyceae in the present study also reflects the oligotrophic nature of the Doodh Ganga and Khansha- Mansha streams as also reported earlier in river sindh (BHAT *et.al.*, 2011; BABA *et.al.*, 2011; RASHID & PANDIT, 2008). Bacillariophyceae has been reported to be dominant among periphytic flora in a number of streams studied for periphyton composition (RASHID & PANDIT, 2008; ALLAN, 1997; ALBAY & AYKULU, 2002; MOORE, 1979). Dominance of Bacillariophyceae may be attributed to the presence of good concentration of SiO_2 in water bodies which probably helps in the frustule formation (WETZEL & LINKINS, 1991) and its ability to thrive well in cold waters (RAO, 1955; SARWAR & ZUTSHI, 1988). Diatom communities have been extensively used in the assessment of past and present ecological conditions in the aquatic habitats in which they live (STOEMER & SMOL, 1999). Their indicative utility resides in that sediments and many species form characteristic assemblages under different trophic or diversely contaminated conditions (LANGE & BERTALOT, 1979; PATRICK, 1949; PATRICK, 1951). Diatoms to some extent in streams of Kashmir Himalaya have been poorly studied and a review of the literature reveals that only a fraction of this literature is purely taxonomic in nature, which hinders the potential use of diatoms for bio indication or bio monitoring. Only a few articles have focused on the diatoms from the bio indication point of view which are insufficient to cover vast and extensive array of habitats in Kashmir Himalaya.

Cymbella sp. and *Navicula* sp. dominated at all the study sites. The dominance of diatoms which are good colonizers of bottom stones seems to be favoured by low temperature and high light penetration (VASISHIT & SHARMA, 1975). In terms of abundance, Bacillariophyceae was the most dominant followed by Chlorophyceae and Cyanophyceae which is generally the trend found in the lotic system (HYNES, 1970). On monthly basis the maximum density of periphyton was obtained in the month of June at all the study sites. This may be attributed to the warm conditions and more light intensity as the growth and abundance of Chlorophyceae during warm water periods and at sites having high light intensity may be related to its excessive reproduction. KANT & KACHROO, 1980) reported that rise in temperature provides optimum conditions for the growth and reproduction of Chlorophyceae. In terms of relative density, Chlorophyceae and Cyanophyceae were better represented in both streams; the most probable reason for the greater proportions of Chlorophyceae may be attributed to the clear water in the studied streams, which provide better light conditions for the growth of group (ALLAN, 1995). The seasonality of periphytic flora is found to be governed by many factors especially discharge, light and the release and availability of plant nutrients. In general, there was a seasonal trend in the periphytic algae with lowest periphyton density usually recorded during cold months and the highest in warmer months.

Conclusions

The periphytic flora of Doodh Ganga and Khansha - Mansha streams is diverse and comprises a variety of cosmopolitan species adapted to alkaline habitats. The streams having crystal clear water are free from pollution as indicated by modest contributions of Chlorophyceae in both the streams. The streams are experiencing less anthropogenic pressures and hence quality of water is seemingly fairly good. This study shows that the periphytic flora can vary in response to discharge and substrate sampled. The myriad of factors governing the growth and abundance of periphytic flora at a variety of scales suggests that the variance at temporal scale in species composition is more interesting and this feature deserves further investigation with greater replication and extended sampling to evolve a holistic picture of the stream ecosystem.

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Soil Pollution as a Result of Temporary Steel Scrap Storage at the Melt Shop

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Abstract. In this paper pollution of soil (5300 m^2) used as temporarily steel scrap storage at the CMC Sisak Ltd. was investigated. Concentrations of heavy metals, namely Cd, Cr, Cu, Hg, Ni, Pb and Zn in soil were determined after their extraction in *aqua regia*. Concentrations of heavy metals, except Hg, were measured by inductively coupled optical emission spectrometry. Concentration of Hg was determined by atomic absorption spectrometry. For a number of years, steel scrap (raw material for steel production) was temporarily stored in the unprotected steel scrap yard area. To assess pollution level of soil under the scrap yard, comparison was done between levels of heavy metal concentrations in analysed samples and reference sample taken outside the factory ground with the levels representing tolerance for potentially unacceptable risk for industrially used soil according to the Croatian Soil Monitoring Programme. Levels were also compared with the values permitted by some EU member countries. Concentrations of heavy metals in all samples collected from the scrap yard showed higher values of heavy metals compared to the reference sample concentrations. Also, values are higher than those defined as potentially unacceptable risk for industrially used soil according to legislation of some EU member countries. Obtained results qualify analysed soil from the scrap yard as contaminated soil, caused by its use over a long time as a temporarily storage space of steel scrap on unprotected and roofless soil. In future, steel storage areas should be built in a way to prevent soil pollution.

Key words: Soil; Pollution; Steel scrap; Temporarily landfills

Introduction

Industrial manufacturing imposes significant influence on the environment as different types of industries contribute to the air, soil and water pollution with their emissions. In addition, by-products of manufacturing products often negatively affect the entire living and non-living world, especially people and their health. Croatian legislation (Official Gazette of the Republic Croatia, OG 114/08) defines industrial types, which can, with their emissions, cause soil, air, water and sea pollution as well as pollution of other parts of the environment. Those industrial types, to name a few, include energy industry, metal production and processing, mineral industry and chemical industry.

From the environmental point of view, metal production and processing is one industrial branch of particular importance, considering the applied technology (processes of roasting and sintering of metal orca, processes of raw iron and steel production, production and processing of ferrous and non-ferrous metals). In addition, metal working processes of metal treatment, especially processes with chemical procedures, cannot be ignored.

In real life, one less common is environmental pollution caused by breakdown or major ecological disasters when huge amount of pollutants in the environment is immediately released, but more common is the appearance of long-term emission of lower quantities of pollutants from production processes. Pollutants from the above-mentioned processes, which can harmfully influence air, water and soil, are significant amounts of gas and solid pollutants (EUROPEAN COMMISSION, 2010). In addition, pollutants depend on the technology method used. Most common pollutants occurred as dust, SO_2, NO_x, NH_3, H_2SO_4, HCl, HF, HCN, H_2S, CO, CO_2, CH_4, heavy metals in waste waters and gases (Hg, Pb, Cr, Ni, Zn, Cd, Cu), benzene, phenol, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, cyanide, oil, grease etc.

In addition to the air and water pollution, metallurgical processes can quite often pollute soil indirectly near the process itself or indirectly at the area used either for the storage of raw material or disposal of produced waste. Contact with wastewater can also cause soil pollution.

Type of pollution from metallurgical process depends on the nature of technological operation conducted on a given location and their duration. Intensity of pollution depends on the type of pollutant emitted from the process and its interaction with the environment in real life conditions.

For example, steel production in electric arc furnace can negatively influence the environment with emissions in air and water during the production process. Additionally, pollutant influence can be prolonged if by-product and/or waste (raw slag, used heat-resisting material, metal cuttings, different mud, dust from smoke, burnout etc.) are uncontrollably disposed on the unprotected ground. Pollutants can lead to negative physical-chemical properties of soil (pH, availability of biogenic elements, water capacity, air capacity and presence of organic matter in soil) wherewith directly and/or indirectly influence on biogenic and productivity of soil. The largest consequence of polluted soil is transfer of its negative impact on ground water.

Literature commonly (OG 114/08) discusses types of pollution and emission impact from steel production process either during duration of process itself (BARCAN, 2002; SCHULIN *et al.*, 2007; SIKALIDIS *et al.*, 2010; RIZESCU *et al.*, 2011) or of the influence of created waste and of harmful substances they contain on the environment (CHILINGIROVA *et al.*, 2011; LONCNAR *et al.*, 2009; LUXÁN *et al.*, 2000; REMON *et al.*, 2005; RODELLA & CHIOU, 2009; TOSSAVAINEN & FORSSBERG, 2000). However, a limited number of articles discuss the impact raw material for this process has on the environmental pollution (ADAMO *et al.*, 2002; ENE *et al.*, 2011; SALLAKU *et al.*, 2009).

Steel scrap in steel production process with electric arc furnace is used (often and 100%), which is commonly polluted with different inorganic and organic substances. Furthermore, steel scrap, as a basic raw material for steel production, regarding to its physical and chemical characteristics is often categorized according to the European Scrap Grading System (ESGS) in 11 different quality categories (E1-E3, E6, E8, E40, E5H, E5M, EHRB, EHRM and E46) (BAILLET, 2001; GOJIĆ, 2005). According to BAILLET (2001), content of organic and inorganic pollutants in steel scrap should be less than 1.4 % for E1 (light steel scrap prepared for furnace < 6 mm), EHRB (old or new steel scrap prepared for furnace, without Cu, Sn, Pb and their alloys) and 1 % for E3 (heavy steel prepared for furnace, including pipes, hollow profile, without Cu, Sn, Pb and their alloys).

Due to their numerous applications and appearance, unwanted heavy metals can be commonly found in steel scrap. During the melting of steel scrap in a furnace, heavy metals and other unwanted inorganic and organic ingredients participate in a very complicated reaction of pyrolysis and pyrosinthesis resulting in production of an array of compounds. These potentially very dangerous pollutants are then emitted in the environment in the shape of smoke gases from electric arc furnace. Sometimes those pollutants present in steel scrap enter the environment, especially through soil before they come to the electric arc furnace, i.e. during the time of temporary storage of steel scrap at unprotected soil or during the preparation for cutting to demanded dimensions.

Pollutants and/or unwanted ingredients in steel scrap in the shape of different powders, clusters, sludge etc., either inorganic or organic under the influence of the atmosphere, are washed out from steel scrap or simply scattered, respectively spilled during steel scrap attrition by means of cutting.

In this paper soil under the temporary storage, area for steel scrap in Melt Shop CMC Sisak Ltd. which has been exposed to direct influence of steel scrap (during cutting or temporary storage before melting) for a long period of time was examined. Concentrations of heavy metals in the soil were examined of area so called scrap yard (size of 5300 m^2).

Materials and methods

Sampling. Testing soil was sampled from temporary steel scrap storage at scrap yard, where steel scrap is shipped by railroad and trucks (Fig. 1 and 2). Nearby the steel scrap temporary storage, there are no other facilities that can cause soil pollution. The nearest river is Sava located 1.5 km away in the northeast direction.

For the purpose of soil sampling, temporary storage area, i.e. scrap yard was divided into four almost identical areas (PI, PII, PIII and PIV), size of 1350 m^2 each (Fig. 3). From each area a few soil samples were collected from different depths and composite samples prepared.

Depending of the soil digging possibilities and opening of soil sampling profiles, soil samples were collected as follows; from area PI, 9 soil samples from 3 locations; area PII, 6 soil samples from 2 locations, area PIII 6 soil samples from 3 locations and from area PIV 5 soil samples from 2 locations.

Fig. 1. Shipment of steel scrap by railroad.

Fig. 2. Temporary storage of steel scrap on unprotected scrap yard area.

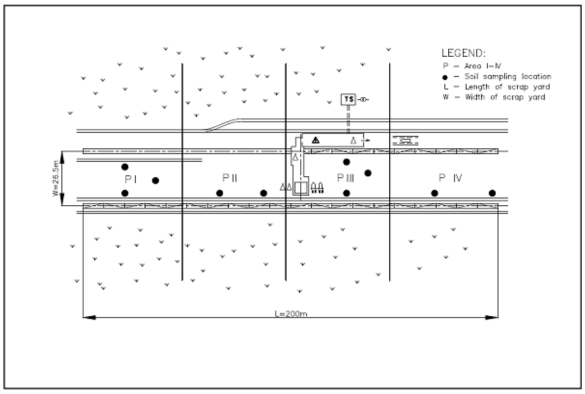

Fig. 3. Soil sampling locations at scrap yard area.

2000g samples were collected using a shovel at 30, 50 and 70 cm depth. For each area, a composite sample was made by mixing identical amounts (1000 g) of individually collected samples, and concentrations of heavy metals were determined.

Presence of water was noticed at a few sampling locations mainly at 0.5–0.7 m depth, and even layering of soil at all locations was noticed as follows:

- surface layer (callow) of sand up to 0.5 m depth (slag and mixture of iron oxides – rust);
- dark colour layer on depths between 0.5 and 1.0 m. The colour probably comes from washing of pollution from used mineral oils on the surface layer, indicated from hydrocarbon essence;
- clay layer on average depth of 1 to 1.2 m.

Water presence at some locations (at 0.5 –0.7 m depths), indicates serious problem in such polluted industrial yards, as pollutants can be washed out by a rainfall to deeper soil levels and can directly pollute underlying groundwater.

One reference sample was collected in a nearby park outside the CMC Sisak Ltd. factory ground approximately 500 m airline in the north-west direction. Activities carried out at the scrap yard have no direct influence on the park were reference sample was collected and as such represent soil image of a wider area (Fig. 4). Reference soil sample was collected in the same manner as samples used for preparation of composite samples.

Sampling locations were marked out with sequence numbers. Additionally, on some locations accordingly to the noticed layering, every soil layer by depth was marked with letters a, b, c (a – surface layer, b – median layer etc.). Preparation of samples for analysis was conducted according to the HRN ISO 11464:2004 (Soil quality –Pre-treatment of samples forphysic-chemical analyses) norm. For preparation microwave oven, MARS Xpress Microwave Digestion System CEM Corporation, was used. After preparing, samples were reserved in desiccators.

Fig. 4. Sampling location of reference soil sample in park outside of the factory.

Cd, Cr, Cu, Hg, Ni, Pb and Zn concentrations

Concentrations of heavy metals (Cd, Zn, Cu, Hg, Ni, Pb and all oxidation states of Cr) after extraction in *aqua regia* according to the HRN ISO 11466:2004 (Soil quality – Extraction of trace elements soluble in *aqua regia*) norm were determined. Heavy metal concentrations, except Hg, were determined by inductively coupled optical emission spectrometry according to the HRN EN ISO 11885:2010 (Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry) norm using a PerkinElmer Optima 7000 DV ICP-OESapparatus. Concentration of Hg was determined by atomic absorption spectrometry, HRN EN 1483:2008 (Water quality - Method using atomic absorption spectrometry) norm on a Perkin-Elmer Atomic absorption spectrophotometer with Varian hydride-vapor generation system.

Results and Discussion

Soil as part of an ecosystem is, because of its complexity, a more dynamic system in comparison to the air and water; therefore, defining pollution is more complex. Soil buffering capacity is determined by its physical, chemical and biological properties, which influence the "vulnerability"to pollution. Because of big differences between soils, there can also be significant differences in chemical composition. To determine the pollution level of any ecosystem component including soil, it is necessary to determine pollution influence on human health. This is done in order to determine the limiting value of pollution level of a particular pollutant for which, based on scientific knowledge, there is not even the slightest possible risk of harmful effect on the human health and/or the environment.

Croatian legislation defines Regulation on protection of agricultural soil from pollution (OG 32/10) as the only regulation that regulates quality of agricultural soil. Limiting values of pollutants, including the limits for concentrations of heavy metals, for soil used for industrial purposes are not prescribed. In Croatia, there is no legal regulation which is directly related to monitoring of soil condition and collection of data regarding potentially polluted or polluted soil. Problems with potentially polluted or polluted soil are only indirectly mentioned in legislation. Some EU member countries regulate limited values of pollutants in the soil (CARLON, 2007) according to the land usage (agricultural, residential, recreational, etc.), principle that was adopted in preparation of Croatian soil monitoring programme (CROATIAN ENVIRONMENT AGENCY, 2008).

The objective of the measurements conducted during the research was to determine if the temporary storage of steel scrap at the unprotected area present potential local source of anthropogenic soil pollution (despite its usage for industrial purposes). Croatian soil monitoring programme (CROATIAN ENVIRONMENT AGENCY, 2008) was used for the identification and interpretation of results. Based on potential sources of soil pollution and type of possible pollutant emissions listed in Programme (CROATIAN ENVIRONMENT AGENCY, 2008), in this paper, concentrations of Cd, Cr, Cu, Hg, Ni, Pb and Zn as pollutants originating from metal industry were determined.

The results of soil analysis from scrap yard area were compared to the recommended limited values (LV) of pollutants in soil allocated for industrial and commercial usage according to Croatian soil monitoring programme (CROATIAN ENVIRONMENT AGENCY, 2008) (Table 1).

Table 1. Limited values (LV) of pollutants in soil (metals extracted in *aqua regia)* according to land usage.

Sort of pollutions in soil	Soil for agricultural production	Playground	Resident areas	Park and recreation areas	Areas for industrial and commercial usage
	(mg/kg dry matter)				
Cd	2	5	10	30	50
Cu	60	60	100	300	500
Ni	50	50	70	200	500
Pb	100	100	100	500	1000
Zn	200	200	300	700	1200
Cr	100	100	200	500	750
Hg	2	5	10	30	50

Results for determination of heavy metals in composite samples of soil from scrap yard area (Table 2) show that concentrations were mostly below limited values (LV) of pollutants for industrially and commercially used soils according to Croatian soil monitoring programme (CROATIAN ENVIRONMENT AGENCY, 2008).

Exceptions were concentrations of Pb in soil samples from areas PII, PIII and PIV, Cu from PI, Cr from PIII and Zn from PII, PIII and PIV.

Table 2. Results from composite sample analysis from scrap yard area compared to the LV of pollutants in soil allocated for industrial and commercial usage according to Programme.

Element	Content of metals in soil, mg/kg dry matter				
	P I	P II	P III	P IV	LV
Cd	1.37	25.30	<0.001	<0.001	50
Hg	0.58	2.43	3.23	1.42	50
Pb	558	**2538**	**2620**	**2724**	1000
Ni	25.27	321	477	263	500
Cu	155	**2239**	722	643	500
Cr	158	438	**1333**	437	750
Zn	508	**14851**	**2457**	**2907**	1200

Noted differences in concentrations of metals between individual areas of scrap yard, for example concentrations of Zn and Pb in PI are significantly lower than those in other areas, can be explained by the shortest contact of PI area with steel scrap. Namely, area PI was rarely exposed to direct influence of pollutants contained in the steel scrap as steel scrap was rarely stored on that area. The newly brought steel scrap has mainly been unloaded on areas PIV and PIII, which are closest to the factory hall and the electric arc furnace.

Before comparison of concentrations of heavy metals in composites soil samples from scrap yard area with the concentrations of heavy metals outside of the factory, first it is necessary to compare results obtained for reference samples with the LV in soil allocated for industrial and commercial usage (Table 3).

Values of heavy metals in reference sample shown in Table 3 are significantly lower than the LV of tested metals for industrially and commercially used soil according to Croatian soil monitoring programme (CROATIAN ENVIRONMENT AGENCY, 2008).

Results obtained from the analysis of reference sample were compared with the maximum allowed values for heavy metals in agricultural soil (Table 4), according to the Regulation on protection of agricultural soil from pollution (OG 32/10). Concentrations of all metals, except Zn, were under the maximum allowed values or within the permitted maximum values.

Table 3. Contents of heavy metals in reference sample compared to the LV for in soil allocated for industrial and commercial usage.

Element	Contents of metal in soil, mg/kg dry matter	
	Reference sample	LV
Cd	<0.001	50
Hg	0.162	50
Pb	49.82	1000
Ni	34.14	500
Cu	21.09	500
Cr	57.84	750
Zn	284	1200

Results from determination of heavy metal content in composite samples (Table 5) point to a significant difference in permitted levels when compared to the content determined in reference samples and some of monitored metals in relation to the LV. This can be assigned to the contamination of soil with the heavy metals in the scrap yard area as a consequence of long-term temporary storage of steel scrap at unprotected and unroofed soil under different weather conditions. For pollution assessment, comparison of results for heavy metals of composite soil samples from all four areas of scrap yard with values for potentially unacceptable risk for soil allocated for industry regulated by some EU member countries is given in Table 5.

Table 4. Content of metals in reference samples compared to regulated boundaries of maximum allowed values in agricultural soil.

Soil sample	Maximum allowed values, mg/kg dry matter						
	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Sandy soil	0.0-0.5	0-40	0-60	0.0-0.5	0-30	0-50	0-60
Dusty – clay soil	0.5-1.0	40-80	60-90	0.5-1.0	30-50	50-100	60-150
Clay soil	1.0-2.0	80-120	90-120	1.0-1.5	50-75	100-150	150-200

Table 5. Comparison of results of heavy metals content in composite soil samples with values for potentially unacceptable risk for soil allocated for industry regulated by some EU member countries.

Element	Scrap yardarea	LV of metals in soil, mg/kg dry matter							
		Sample	Reference sample	Belgium /Bruxelles/	Belgium /Wallonia/	Finland	Italy	Poland	United Kingdom
Cd	P I	1.37	<0.001	30	50	20	15	13	1400
	P II	25.30							
	P III	<0.001							
	P IV	<0.001							
Hg	P I	0.58	0.162	30	84	5	5	27	480
	P II	2.43							
	P III	3.23							
	P IV	1.42							
Pb	P I	558	49.82	**2500**	**1360**	**750**	**1000**	**600**	**750**
	P II	**2538**							
	P III	**2620**							
	P IV	**2724**							
Ni	P I	25.27	34.14	700	500	**150**	500	**285**	-
	P II	**321**							
	P III	**477**							
	P IV	**263**							
Cu	P I	155	21.09	**800**	**500**	**200**	**600**	**600**	-
	P II	**2239**							
	P III	**722**							
	P IV	**643**							
Cr	P I	158	57.84	**800**	**700**	**300**	**800**	**475**	5000
	P II	**438**							
	P III	**1333**							
	P IV	**437**							
Zn	P I	**508**	284	**3000**	**1300**	**400**	**1500**	**1650**	-
	P II	**14851**							
	P III	**2457**							
	P IV	**2907**							

Comparison of heavy metals content in composite soil samples from scrap yard area with the values for potentially unacceptable risk for industrial soil regulated by some EU member countries points out that concentrations of Cd and Hg were under the value for potentially unacceptable risk regulated in Belgium, Finland, Italy, Poland

and the United Kingdom i.e. all considered countries which have regulated LV for those heavy metals.

Lead concentration was lower only in part of PI area, compared to the area (PII – PIV) where it was higher than the values marked as potentially unacceptable risk regulated by some countries. Regarding to its Pb content area scrap yard could be considered polluted.

Concentration of Ni in PI was also lower than the values considered as potentially unacceptable risk regulated by the mentioned EU member countries, while concentrations of Ni from PII – PIV were lower than the values considered as potentially unacceptable risk regulated by Belgium and Italy, and higher than those regulated by Finland and Poland.

Pollution of scrap yard area with the Cu, Cr and Zn was more pronounced, indicating determined concentrations which were, except in PI, higher than values for potentially unacceptable risk regulated by Belgium, Finland, Italy and Poland for soil allocated for industrial and commercial use.

Conclusions

Soil pollution in the Republic of Croatia is defined only for agricultural soil by Regulation on protection of agricultural soil from pollution (OG 32/10), while the quality of soil allocated to industrial and other purpose is not regulated.

Soil pollution assessment was carried out at the scrap yard area that was used for temporary storage of steel scrap during a number of years in CMC Sisak Ltd. Obtained results for heavy metals were compared with the values marked as potentially unacceptable risk for industrial soil according to the Croatian Soil Monitoring Programme and regulated values by some EU member countries. Results were also compared with content of heavy metals in reference soil sample in the vicinity of storage, as well as to all obtained data, thus that following conclusions could be drawn:

- concentrations of heavy metals in all composite soil samples exceed concentrations from the reference sample, which can be assigned to soil contamination at the scrap yard area as a consequence of long term temporary steel scrap storage at unprotected and unroofed soil;
- Cd and Hg concentrations were lower than values for potentially unacceptable risk regulated in all considered countries;
- Pb concentrations were lower only in a part of PI area, opposite to areas (PII – PIV) where they were higher than values for potentially unacceptable risk regulated by considered countries. Regarding to Pb levels determined in composite sample area, the scrap yard could be considered polluted;
- Concentration of Ni from PI were also lower than values considered as potentially unacceptable risk regulated by mentioned EU countries, while concentrations from PII – PIV were lower than values for potentially unacceptable risk regulated by Belgium and Italy, and higher than values regulated by Finland and Poland.
- Pollution of scrap yard area with Cu, Cr and Zn was more pronounced than with other metals, indicating determined concentrations which were, except in PI, higher than values for potentially unacceptable risk regulated by Belgium, Finland, Italy and Poland for soil allocated for industrial and commercial purposes.
- On the base of determined differences in metal content in-between individual areas of the scrap yard, PI area has been least polluted with heavy metals, because steel scrap was rarely stored in that area.

As a result, concentrations of heavy metals in all composite soil samples taken from scrap yard area mainly exceed values of same metals in the reference sample, and values for potentially unacceptable risk for industrial soil as regulated by some EU countries, implying this soil can be defined as contaminated. Therefore it is necessary to take measures as specified in Article 8 Regulations on waste management (OG 23/07, OG 111/07) for the purpose of soil protection and prevention of further soil pollution with following methods:

- area for temporary steel scrap storage should be built from solid material, fully sealed or enclosed roofed space protected from rainfall;
- Floor of the storage/temporary landfill should be leak-tight and resistant to effects of stored steel scrap and polluted ingredients present in it;
- Storage/temporary landfill should be equipped to avoid emission of dust, noise, smell and other emissions in the environment.

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Comparative Analysis of Different Types of Bacterial Colonies from the Soils of Yusmarg Forest, Kashmir valley India

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Abstract. The present work was carried out in the soils of Yusmarg forest to study about the bacterial load (density and diversity), to identify and isolate the bacteria from the soils. During the study a total of thirty six isolates were obtained, among thirty-six different isolates obtained at the four sites B_7 and B_8 were present at all the four sites, B_6 and B_9 were present only at site I in November, B_{16} and B_{17} were present only at site II in November, B_{19}, B_{22}, B_{23} and B_{24} were present only at site III in November, B_{32}, B_{33} and B_{34} were present only at site III in December and B_{35} was present only at site IV in December. Comparative analysis of different types of colonies found at the four sites during the study indicates that the bacterial load was dominant in the month of November.

Key words: Bacteria, Yusmarg, Soil, Isolate, Nutrient Agar.

Introduction

On our planet earth, we feel blessed to be surrounded by natural resources which benevolent God has provided us in abundance. Among all natural resources, one that is rendering its valuable support to sustain human race is soil. Soil, one of the greatest gifts of nature is a vital factor for life. It is the best medium for the growth of micro-organisms. Soil microbial population is the key element in the bio-geochemical cycling of nutrients in nature (PELCZAR *et al.*, 1993). The number and kind of bacteria found in different types of ecosystems vary and are influenced by the ecosystem processes maintaining plant primary productivity (GRIFFITHS *et al.*, 2003). Most of the soil bacteria are decomposers that consume simple organic compounds, such as root exudates and fresh plant litter. It has been estimated that there may be as many as 10^9 bacterial cells per gram of soil (KARATHANSIS & HARRIS, 1994) and it is widely accepted that the majority of soil bacteria possibly as many as 99% cannot be cultured using traditional media based techniques. Bacteria are vital in recycling nutrients with many steps in nutrient cycles depending on these organisms such as the fixation of nitrogen from the atmosphere and Putrefaction.

Material and Methods

Study Area. Yusmarg situated at an altitude of about 2743m a.s.l, lying in the Budgam district of Jammu and Kashmir, India, is a small idyllic meadow set in the heart of mountains to the South West of Srinagar. It is situated at a distance of 47 Km from Srinagar city. The destination is witne-ssed by grassy meadow ringed by forests of pine, towering beyond them awesome and snow clad mountains. It is an emerging destination which is completely raw, pristine and still unspoiled, bandied by rivers and the backdrop of snow capped mountains. It mesmerizes tourists with its scenic meadows, sparkling reservoir and mountains comparable to European Alps. Situated amidst Sang Safed valley, it is reputed for having some unique spring flowers. The mighty river Doodh Ganga rises from these peaks and a distributory of the same flows into the reservoir. The climate of this beautiful area is very bracing and enjoys a subtropical climate. Here the precipitation normally occurs in the form of snowfall during the winters. Summers are mild and winters are very cold. The maximum temperature ranges around 30°C and the minimum temperature around 18°C during the summer months. Temperatures start coming down only from September. During winter months this area experiences a maximum temperature of 15°C to 8°C and a minimum temperature of around -2°C (Fig.1).

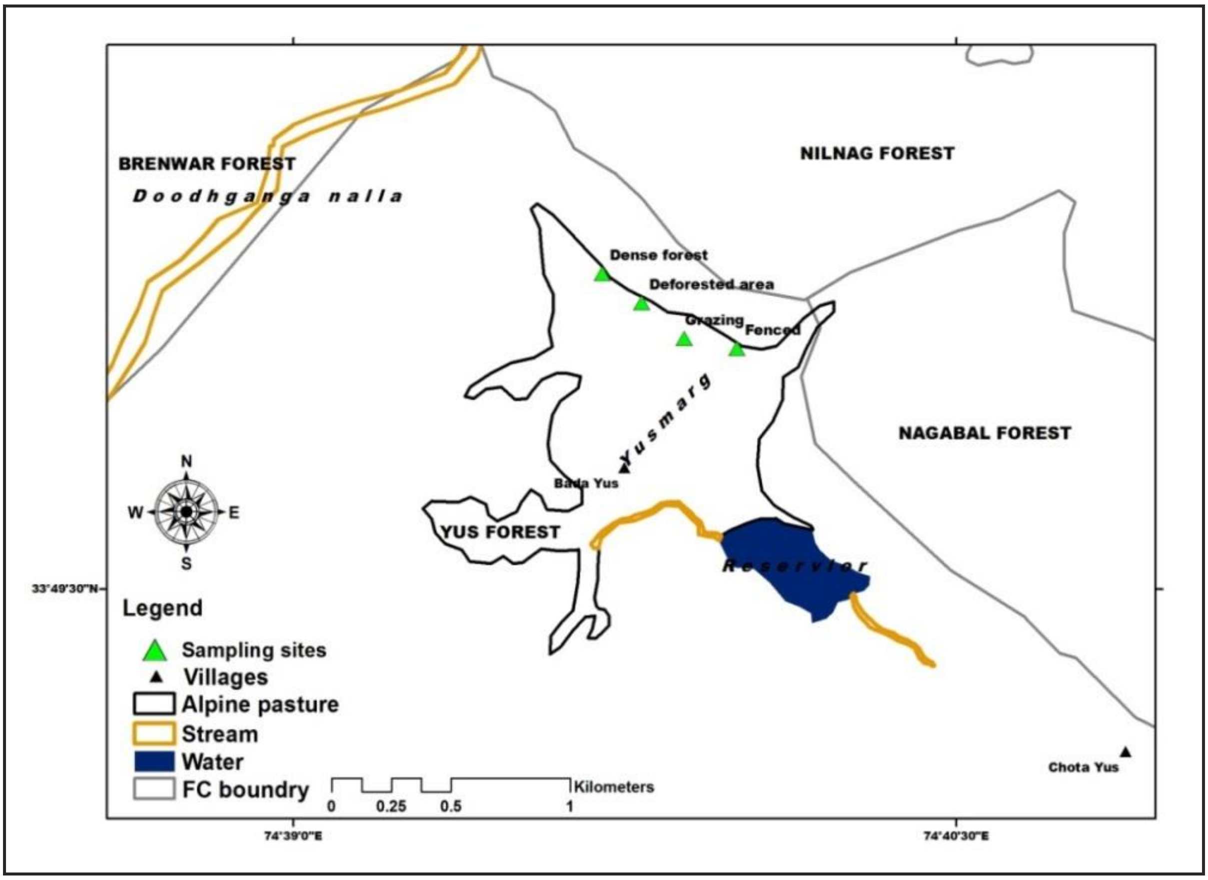

Fig. 1. Map showing the study area and study sites.

Study Sites. Four sites were selected to carry out the work and the brief site descriptions are given below:

Site I (Fenced Area) - The Site renowned for its green pasture least affected by human and animal activities and lies between the geographical co-ordinates of 74° 40′ 1.653″ E and 33° 50′ 0.665″ N, having an elevation of 2418 m. The area was fenced, which prevented it from grazing and anthropogenic impacts.

Site II (Grazing Area) - The site under high grazing pressure and was highly influenced by the human and animal activities, lies between the geographical coordinates of 74° 39′ 57.555″ E and 33°50 ′ 1.768″ N**,** having an elevation of 2411 m and was surrounded by coniferous forest and road on the other sides.

Site III: (Deforested Area) - The site is close to main forest and was marked by deforestation. It lies between the geographical coordinates of 74° 39′ 57.506″ E and 33° 50′

0.034′′ N having an elevation of 2446 m. It was near the grazing area, having vegetation of conifers (*Pinus wallichiana, Abies, pindrow* and *Piscea smithiana*).

Site IV (Forested Area) - This site located in dense forest of conifers. It lies between geographical coordinates of 74° 39′ 56.262′′ E and 33° 49′ 55.747′′ N, having an elevation of 2451 m. The vegetation was dominated by *Pinus wallichiana*, with other conifers and relatively very small number of shrubs and herbs.

Collection Technique. Composite samples of soil from the four sites were collected during the study period, from a depth of 5 inches. Samples were collected in sterile polythene bags and carried to laboratory for bacteriological analysis. The samples were processed using the soil plate method (WARCUP, 1950) and Soil dilution plate Method (WAKSMAN, 1922).

Laboratory Analysis

Soil plate method - About 1g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45°C) agar medium (NA) was added, which was then rotated gently to disperse the soil particles in the medium. The plates were then incubated at 28±2°C for 24 hours.

Soil dilution plate method - The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.1ml inoculum was poured onto Nutrient agar and incubated at 28±2°C for 24 hours. The number of colonies counted was expressed as cfu/g and were calculated by using the formula:

$$\mathrm{cfu/gm} = \mathrm{n} \times \mathrm{d}$$

Where: n= number of colonies; d = dilution factor = 1/dilution.

Results

The recorded temperature and pH at the four sites during November and December 2010 are presented in Table 1.

Different types of colonies were obtained during the study period. Some colonies were circular in shape and some irregular, some rhizoid and some filamentous. A total of 36 colonies were obtained during the study and were assigned the names from B_1 to B_{36} (Table 2).

Table1. Temperature and pH recorded at four sites during November and December 2010.

Site	Temperature (^{0}C)		pH	
	Nov.	Dec.	Nov.	Dec.
I	12.5	2.5	6.26	6.48
II	11.5	2.3	6.6	5.9
III	10.0	1.2	5.25	4.5
IV	9.0	0.3	4.86	4.7
Average	**10.75**	**1.6**	**5.7**	**5.3**

Table 2. Comparative analysis of different types of colonies found at the four sites in the months of November and December 2010.

Isolate number	Site I		Site II		Site III		Site IV	
	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.
B_1	+	-	+	-	-	-	-	-
B_2	+	-	-	-	+	-	-	-
B_3	-	-	-	-	-	-	+	-
B_4	+	-	-	-	-	-	-	-
B_5	-	-	-	-	+	-	+	-
B_6	+	-	-	-	-	-	-	-
B_7	-	+	+	+	-	+	+	+
B_8	+	+	-	+	+	+	+	+
B_9	+	-	-	-	-	-	-	-
B_{10}	+	-	+	-	-	-	-	-
B_{11}	+	-	+	-	-	+	-	-
B_{12}	-	-	+	-	-	-	+	-
B_{13}	-	-	+	-	-	-	-	-
B_{14}	-	-	+	-	+	-	-	+
B_{15}	-	-	+	+	-	+	-	-
B_{16}	-	-	+	-	-	-	-	-
B_{17}	-	-	+	-	-	-	-	-
B_{18}	-	-	+	-	+	-	-	-
B_{19}	-	-	-	-	+	-	-	-
B_{20}	-	-	-	-	+	-	-	-
B_{21}	-	+	-	+	+	+	-	+
B_{22}	-	-	-	-	+	-	-	-
B_{23}	-	-	-	-	+	-	-	-
B_{24}	-	-	-	-	+	-	-	-
B_{25}	-	+	-	+	+	-	+	-
B_{26}	-	-	+	-	-	+	+	-
B_{27}	-	+	-	-	-	-	-	+
B_{28}	-	+	-	-	-	+	-	-
B_{29}	-	+	-	-	-	-	-	+
B_{30}	-	+	-	-	-	-	-	+
B_{31}	-	+	-	-	-	+	-	+
B_{32}	-	-	-	-	-	+	-	-
B_{33}	-	-	-	-	-	+	-	-
B_{34}	-	-	-	-	-	+	-	-
B_{35}	-	-	-	-	-	-	-	+
B_{36}	-	-	-	-	-	-	-	+

The different colonies obtained during the study were tested for gram's reaction and subsequently were examined under microscope to determine the cell shape. Among the different isolates, a total of 8 strains of bacteria were isolated from site I, 12 from Site II, 12 from site III and 7 from site IV during the month of November 2010. During the month of December, 9 strains of bacteria were isolated from site I, 5 from site II, 11 from site III and 10 from Site IV.

Discussion

The increase in the bacterial population at site 1 from November to December may be attributed to the increase in pH from 6.26 to 6.48, the grazing rate also decreased from November to December at this site. A study was carried out by (KOHLER *et al.,* 2005) to study the effect of cattle grazing on bacterial communities in pastures and he showed that bacterial community changes due to simulated effects of cattle grazing. The cattle activities may induce changes in the bacterial community structure. During the study the temperature and pH was recorded at the four sites under consideration, the maximum soil temperature (12.5^{0}C) was at site I in November while as it was minimum (0.3^{0}C) at site IV in December. The average soil temperature decreased from 10.75^{0}C in November to 1.6^{0}C in December. Average pH also decreased from 5.7 to 5.3. This decrease in the count may be attributed to the difference in various biotic and abiotic factors that have been found to influence the density and diversity of soil bacterial communities. The results thus obtained in the present study are confirmed by the findings of PIAO *et al.* (2000) and FIERER & JACKSON (2006). However, the average variation in temperature and pH at the four sites in the months of November and December may also be attributed for the decrease in the bacterial population. The physical properties of soils like temperature and pH recorded at the four sites under consideration during the study period showed a variation of about 10°C in soil temperature from November to December and also a pH change of 0.3 (Table1). From the values of temperature and pH, it is quite clear that the average temperature varied from 10.75°C in November to 1.6°C in December, a variation of about 10°C. Similar results were shown by (MURPHY, 2000) who showed that the bacteria grow faster at higher temperatures; the growth rate slows dramatically at low temperatures. The present findings are also confirmed by (PETTERSSON, 2004) who reported that the soil bacterial community had an optimum temperature for growth and diversity. Another reason for the decrease of bacterial population from November to December may be attributed to the decrease in pH because the average pH varied from 5.7- 5.3.The present study is confirmed by the findings of (LAUBER *et al.,* 2009) who reported that the effect of soil pH on bacterial community composition is evident at even relatively coarse levels of taxonomic resolution. Similar results were also shown by (ROUSK *et al.,* 2010) who reported that the composition of the bacterial communities is closely defined by the soil pH, the apparent direct influence of pH on bacterial community composition is probably due to the narrow pH ranges for optimal growth of bacteria.

Conclusion

From the study it can be concluded that isolate B8 was present at all the four sites during the study period.

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Plant Species Groups in Chestnut (Castanea sativa Mill.) Sites, Hyrcanian Forests of Iran

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Abstract. The aim of this study was to identify floristic composition, introduce main plants species, classify vegetation and determine species groups (types) in the chestnut (*Castanea sativa* Mill.) sites in Guilan province, north of Iran. Sampling was done in selective method and 68 sampling plots were taken to access the information of vegetation cover in Shafarood (10 Plots) and Emamzadeh-Ebrahim (58 plots). Results indicated that there were 45 and 55 plant species in Shafarood and Emamzadeh-Ebrahim, respectively. Two-Way Indicator Species Analysis (TWINSPAN) revealed 6 plant species groups in woody and herbaceous layer separately. Also, the main plant specie were *Ilex spinigera*, *Carpinus betulus*, *Castanea sativa*, *Diospyros lotus*, *Gleditsia caspica* and *Prunus divaricata* in woody species layer and *Poa* sp., *Pteridium aquilinum*, *Oplismenus undulatifolius*, *Mentha aquatica*, *Pteris cretica*, *Oxalis corniculata* and *Hypericum androsaemum* in herbaceous layer according to species important value index (SIV).

Keywords: Chestnut (*Castanea sativa* Mill.), Vegetation classification, TWINSPAN, SIV, Emamzadeh-Ebrahim, Shafarood.

Introduction

The findings of the vegetation study have implications for the design of rehabilitation programs (BARRETT, 2006). Assessment of vegetation changes is an important component of many large-scale environmental monitoring programs (RINGVALL *et al*., 2005). Successful long-term monitoring of habitats is best achieved when using quantitative analysis for precise determination of changes in vegetation over time (BENHOUHOU *et al*., 2003).

Forest managers, forest scientists and forest policy makers all rely on accumulated knowledge of the forest in order to make decisions. This knowledge may be based on their own experience or that of others, but will have originated from information collected from the forest. A quantitative understanding of the forest requires quantitative data to support it. The botanical composition of a forest may affect decisions related to forest management (logging operations or silvicultural planning), forest policy (annual allowable cut and perhaps the minimum logging cycle) or conservation (biodiversity and wildlife habitats). In order to carry out its nature conservation policy in the best possible way, the managers required information on the distribution of plant species and communities (WITTE & MEIJDEN, 2000).

Distinguishing plant associations has been at the heart of vegetation science for centuries, with a traditional focus on the distribution, composition and classification of plant communities. An important subject of vegetation science is 'vegetation

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classification': the derivation of vegetation units from the natural plant covers. Ecological species groups are groups of plants that repeatedly occur together in areas with similar combinations of site factors, and that are perceived to have similar ecological requirements or tolerance ranges. Also, ecological species groups may also provide the ecological basis for distinguishing ecosystems at broader scales. Once species groups are characterized for an area, their distribution can be used for inferring soil properties and other variables relatively difficult to measure (RAD & SHAFIEI, 2010).

Two-Way Indicator Species Analysis (TWINSPAN) is a numerical method for classification of vegetation belonging to similar groups (DAI *et al*., 2006; TICHY *et al*., 2007; LEPS & SMILAUAR, 2009; JELOUDAR *et al*., 2010). This allows the investigator to recognize the homogenous groups (JAFARI *et al*., 2004). TWINSPAN as a vegetation study technique was used to many purposes such as vegetation classification to compare the effects of different management approaches in forest (JANTUNEN & SAARINEN, 2002), to classify the landscape fire succession models and vegetation dynamics (KEANE *et al*., 2002), study on the vegetation associations, structure and composition (OOSTERHOORN & KAPPELLE, 2000; BENHOUHOU *et al*., 2003; BARRETT, 2006; MAINGI & MARSH, 2006), Vegetation restoration patterns and their relationships with disturbance (YONGJIAN *et al*., 2006), relationships of vegetation and environmental factors like landform, soil, physiography, to assess the sites typology and etc. (ROLECEK, 2005; COOPER *et al*., 2006; GARCIA-AGUIRRE *et al*., 2007). In addition, classification of plots using TWINSPAN displayed five vegetation groups of *Alnus glutinosa* ssp. *barbata*, each with specific indicator species in Hyrcanian (Caspian) lowland forests (northern Iran) include alderwood communities, dominated by *Alnus glutinosa* ssp. *barbata* (NAQINEZHAD *et al*., 2008). Identify and compare the forest communities were characterized in deciduous forests in the experimental forests of Tehran University, north of Iran. Four communities, including *Querco-Carpinetum betulii*, *Carpineto-Fagetum oriental*, *Rusco-Fagetum oriental* and *Fagetum Oriental* were recognized and plant species diversity was quantified in the different communities (RAD *et al*., 2009).

The widespread range of chestnut trees in the Europe is mainly related to the fruit production. Chestnut wood is one of the preferred choices in making high quality furniture. Additionally, over the last decade another important income associated with these trees has emerged the collection and commercialization of wild edible mushrooms growing in chestnut stands (BAPTISTA *et al*., 2010). Chestnut (*Castanea sativa* Mill.) is very interesting species for timber production (LUIS & MONTEIRO, 1998; CUTINI, 2001). Chestnut was introduced as the most common species used for building construction in central Italy and it appears to be a suitable species for performing dendrochronological analysis because of missing its annual rings are rarely shown (ROMAGNOLI *et al*., 2004). Chestnut stands have considered because of the spread of disease and decreased the stand areas in the world (MARTINS *et al*., 1997; POURBABAEI, 2002; HEDAYATI *et al*., 2003; NARAGHI, 2003; AREFIPOUR *et al*., 2006; GOMES-LARANJO *et al*., 2006; DINIS *et al*., 2011). Due to the importance of this species, The aim of this study was to identify floristic composition, introduce main plants species, classify vegetation and determine species groups (types) to help the protection of natural vegetation in the chestnut sites in Guilan province, north of Iran.

Material and Methods

Study area. The study areas are located in Shafarood and Emamzadeh-Ebrahim regions in the west of Guilan province, north of Iran. The Shafarood region covers approximately 100 ha of the chestnut stand (37° 02′ 30″ N latitude and 49° 18′ 47″ E longitude) and Emamzadeh-Ebrahim is included about 500 ha (37° 31′ 10″ N latitude and 49° 02′ 48″ E longitude) (Fig.1). General aspect is northern in the both regions, altitude ranges from 261 to 480 m a.s.l. in the Shafarood and from 193 to 745 m a.s.l. in Emamzadeh-Ebrahim region. The Mean annual precipitation and temperature is 1400.6 mm and 15.4°C in the

Shafarood and 1693 mm and 15.4°C in Emamzadeh-Ebrahim region, respectively. Edaphically, soil type is acidic brown forest soil and texture is mostly silt, clay, loam to silt and loam, pH ranges from 5.05 to 6.67 in the studied sites (HEDAYATI, 2001).

Fig. 1. The location of the study areas (Shafarood and Emamzadeh-Ebrahim)

Sampling strategy. The study area was sampled using selective method (ESMAILZADEH *et al.*, 2007). 68 sampling plots were surveyed in the study areas (10 and 58 sampling plots in Shafarood and Emamazadeh-Ebrahim, respectively). The area of plots was 2500 m^2 (50 m × 50 m) (CHIARUCCI *et al.*, 2001; POORBABAEI & RANJAVAR, 2008). In each plot all trees with DBH ≥ 10 cm were measured and the number of shrub species was counted. List and estimate of percent cover of each herbaceous species were recorded using the Braun-Blanquet criterion in 64 m^2 (8 m × 8 m) subplots that obtained minimal area method in the center of each plot (Table 1). The Braun-Blanquet criterion is a method of describing an area of vegetation devised by Braun-Blanquet in 1927. It is used to survey large areas very rapidly. That consists of a plus sign and a series of numbers from 1 to 5 denoting both the degree of presence of a plant species and the proportion of the area covered by that species, ranging from + (sparse and covering a small area) to 5 (covering more than 75% of the area) (http://botanydictionary.org/braun-blanquet-scale.html).

Table 1. Braun-Blanquet cover-abundance criterion (WIKUM & SHANHOLTZER, 1978)

Braun-Blanquet criterion	Ranges of cover (%)
5	75 – 100
4	50 – 75
3	25 – 50
2	5- 25
1	< 5 (numerous individuals)
+	< 5 (few individuals)

Data analysis. Woody and herbaceous layers were separately classified. So, the floristic data matrices consist of 68 plots and 24 woody species (trees and shrubs) and 34 herbaceous species. To classify species groups present in the studied areas, the vegetation data were analyzed using Two-Way Indicator Species Analysis (TWINSPAN) with PC-ORD version 4.17 (MCCUNE & MEFFORD, 1999). The classification was stopped at the third level of division, so that the resulting groups would contain a sufficient number of samples to characterize each vegetation group (KHAZNADAR *et al.*, 2009) and also eigenvalue of each division (JAFARI *et al.*, 2004; MANJILI *et al.*, 2009). Species important value index (SIV) was used to name each group and defined as formula (1) to (5) for woody and herbaceous species (COROI *et al.*, 2004; MESDAGHI, 2006; YONGJIAN *et al.*, 2006; ADAM *et al.*, 2007; ABEDI & POURBABAEI, 2010):

(1) SIV (tree and shrub) = Relative frequency (RF) + Relative density (RD) + Relative dominance (RDo)

(2) SIV (herb) = RF + RDo

$$(3)\ RF = \frac{\text{Number of plots that contain a species}}{\text{Number of all plots}} \times 100$$

$$(4)\ RD = \frac{\text{Individual number of a species in all plots}}{\text{Total individual number of species in all plots}} \times 100$$

$$(5)\ RDo = \frac{\text{Basal area (or cover percentage) of a species in all plots}}{\text{Total basal area (or cover percentage) of all species in all plots}} \times 100$$

Results

Flora. Floristic study revealed that there were 45 plant species including 15 trees, 5 shrubs and 25 herbaceous species in Shafarood, and 55 plant species including 18 trees, 5 shrubs and 32 herbaceous species identified in Emamzadeh-Ebrahim region. The presence and absence of plant species in each family were shown in Table 2 to 4. The families with most abundant plant species were the Lamiaceae and Rosaceae (Fig. 2).

Table 2. List of tree species in the studied areas (+: presence, -: absence)

Scientific name	Family	Shafarood	Emamzadeh-Ebrahim
Acer cappadocicum Gled.	Aceracea	+	+
Acer velutinum Boiss.	Aceracea	+	+
Albizia julibrissin (Willd.) Benth.	Fabaceae	+	-
Alnus subcordata C.A.Mey.	Betulaceae	+	+
Buxus hyrcana Pojark.	Buxaceae	-	+
Carpinus betulus L.	Betulaceae	+	+
Castanea sativa Mill.	Fagaceae	+	+
Diospyros lotus L.	Ebenaceae	+	+
Fagus orientalis Lipsky.	Fagacea	+	+
Ficus carica L. var. *genuine* Boiss.	Moracea	+	+
Fraxinus excelsior Scheele.	Oleaceae	+	+
Gleditsia caspica Desf.	Fabaceae	+	+
Parrotia persica (DC.) C.A.Mey.	Hammamelidaceae	+	+
Quercus castaneifolia C. A. Mey.	Fagaceae	+	+
Robinia pseudoacacia L.	Papilionaceae	-	+
Taxus baccata L.	Taxaceae	-	+
Tilia platyphyllos Scop.	Tiliaceae	+	+
Ulmus glabra Huds.	Ulmaceae	+	+
Zelkova carpinifolia (Pall.) Dipp.	Ulmaceae	-	+

Table 3. List of shrub species in the studied areas (+: presence, -: absence)

Scientific name	Family	Shafarood	Emamzadeh-Ebrahim
Crataegus microphylla C. Koch.	Rosaceae	+	+
Ilex spinigera Loes.	Aquifoliaceae	+	+
Mespilus germanica L.	Rosaceae	+	+
Prunus divaricata Ledeb.	Rosaceae	+	+
Ruscus hyrcanus Juz.	Liliaceae	+	+

TWINSPAN outputs. The results of the TWINSPAN analysis are summarized in Fig. 3 and 4. Based on floristic composition, the 68 sampling plots were classified into six groups in woody and herbaceous layers.

The 68 plots in woody species layer (trees and shrubs) were firstly classified into two groups. In the first level, the negative (left) group (including 39 plots) was determined by species like *Ilex spinigera* and *Diospyros lotus* and the positive (right) group (including 29 plots) by *Tilia platyphyllos*. In the second level, 39 plots divided to 9 plots in negative group and characterized by *Ilex spinigera* and positive group include 30 plots characterized by *Castanea sativa*. Also, 29 plots classified in two groups in this level, negative group including 18 plots characterized by *Carpinus betulus* and positive group (including 11 plots) was characterized by *Prunus divaricata* and *Acer velutinum*. In third level (as the last level of division), 30 plots divided to 18 and 12 plots as negative and positive groups that *Diospyros lotus* in left and *Castanea sativa* in right was determined. In this level, 18 plots classified in two groups including 9 plots that *Gleditsia caspica* was determined in negative group and no species was presented in positive group.

Table 4. List of herbaceous species in the studied areas (+: presence, -: absence)

Scientific name	Family	Shafarood	Emamzadeh-Ebrahim
Acalypha australis L.	Euphorbiacea	+	+
Artemisia annua L.	Astraceae	-	+
Bidens tripartita L.	Astraceae	-	+
Brachypodium pinnatum L.	Poaceae	+	+
Calamintha arvensis Lam.	Lamiaceae	-	+
Campanula rapunculus L.	Campanulaceae	+	+
Centarium minus Moench.	Gentinaceae	-	+
Cerasus avium (L.) Moench.	Rosaceae	+	+
Conyza canadensis (L.) Cronquist.	Astraceae	-	+
Daphne mezereum L.	Thymeleaceae	+	+
Fragaria vesca L.	Rosaceae	+	-
Geum urbanum L.	Rosaceae	+	+
Hypericum androsaemum L.	Hypericaceae	+	+
Hypericum perforatum L.	Hypericaceae	-	+
Lallemantia iberica	Lamiaceae	+	+
Mentha aquatic L.	Lamiaceae	+	+
Nepeta invulucrata (Bunge) Bornm.	Lamiaceae	+	+
Oplismenus undulatifolius (Ard.) P.	Poaceae	+	+
Origanum vulgare L.	Lamiaceae	+	+
Oxalis corniculata L.	Oxalidaceae	+	+
Perilla frutescens (L.) Britt.	Lamiaceae	-	+
Persicaria hydropiper (L.) Delarbre	Polygonaceae	+	+
Pimpinella saxifraga L.	Apiceaceae	+	+
Plantago major L.	Plantaginaceae	+	+
Poa sp.	Poaceae	+	+
Prunella vulgaris L.	Lamiaceae	+	+
Pteridium aquilinum L. Kuhn	Hypolepidaceae	+	+
Pteris cretica L.	Polypodiaceae	+	+
Rumex acetosella L.	Polygonaceae	+	+
Sambucus ebulus L.	Caprifoliaceae	-	+
Solanum nigrum L.	Solanaceae	+	+
Trifolium compester Schreb.	Fabaceae	+	-
Urtica pilulifera L.	Urticaceae	+	+
Xanthum strumarium L.	Astracae	-	+

Thus, woody species were classified into six ecological species group in the studied areas. In order to separate and recognize each group in every site, the number of sampling plots bring out and record in the final level of classification (1 to 58 was located in Emamzadeh-Ebrahim and 59-68 was located in Shafarood) (Fig. 3).

The results of TWINSPAN classification in herbaceous species layer was revealed that 68 plots divided to 22 and 46 plots in negative and positive groups, respectively. At the first level, *Hypericum androsaemum* and *Pteridium aquilinum* were the negative group of species and *Pteris cretica* was the positive. In the second level of division, negative group was classified in two groups that 7 plots were in negative group that *Poa* sp. was characterized the only species in this group and 15 plots were in the positive group that there was no indicator species. In this level, 46 plots were classified to 30 plot groups that were determined by *Brachypodium pinnatum*, *Oxalis corniculata* and *Poa* sp. and 16 plot groups have no species.

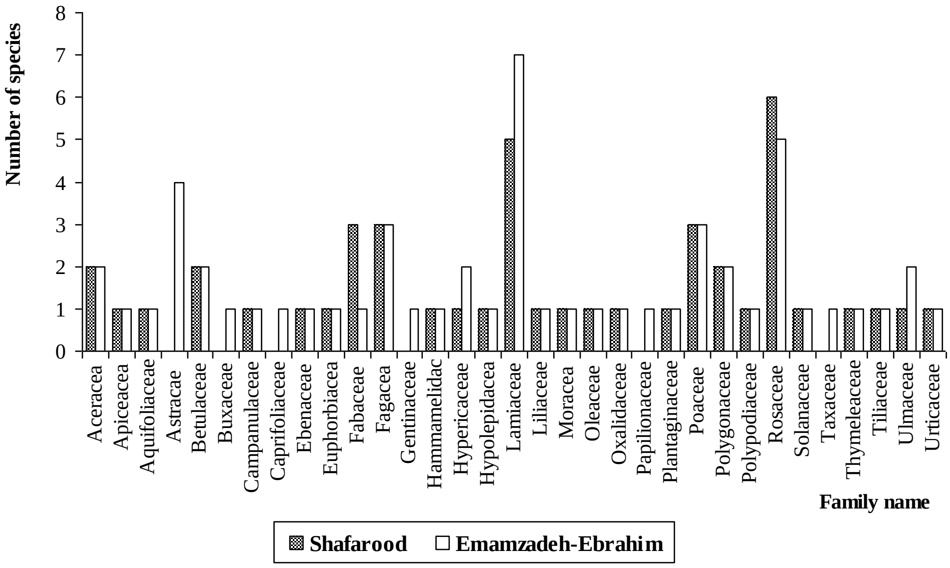

Fig 2. Number of species in each family

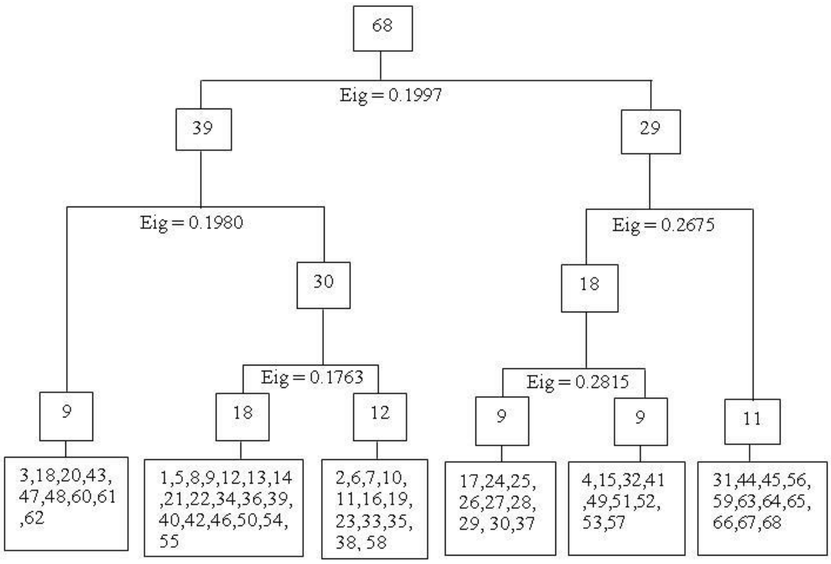

Fig 3. Dendrogram derived from the TWINSPAN analysis in the woody species layer. Numbers in the final level indicated the number of sampling plots in each group. Plant species groups were named according to SIV in woody species layer as follows:

Group 1: *Ilex spinigera - Diospyros lotus*
Group 2: *Diospyros lotus - Ilex spinigera - Castanea sativa*
Group 3: *Castanea sativa - Ilex spinigera - Carpinus betulus*
Group 4: *Carpinus betulus - Gleditsia caspica - Castanea sativa*
Group 5: *Carpinus betulus - Castanea sativa*
Group 6: *Castanea sativa - Carpinus betulus - Prunus divaricate*

In third level of division (as the last level), 15 plots were divided in 7 plot group in negative and characterized by *Daphne mezereum* and *Poa* sp. but there were no indicator species in 8 plots group in positive group. 30 plot groups was classified by 15 plots group as negative and positive group and was determined by *Mentha aquatica* and *Conyza canadensis*, respectively.

Finally, 6 ecological species groups were identified in herbaceous layer. Also, the number of sampling plots recorded in the final level due to recognize the position of plots in each study area (Fig. 4).

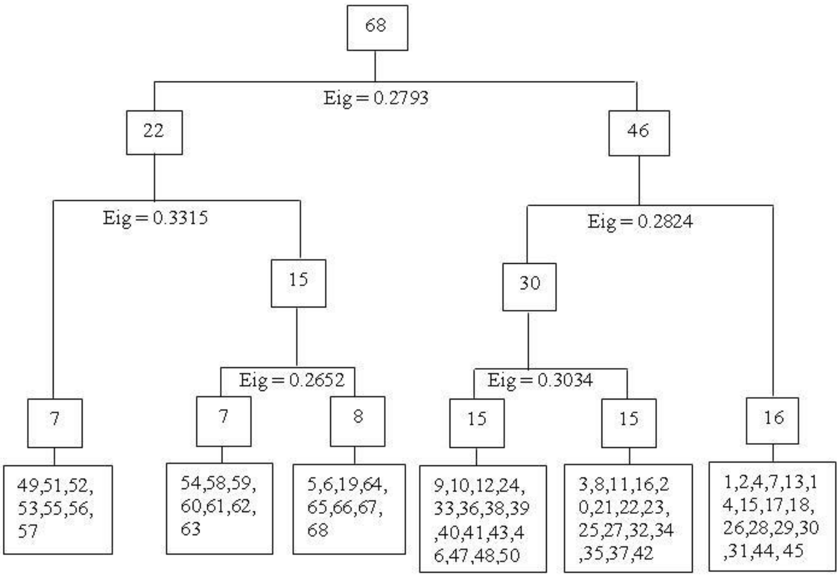

Fig 4. Dendrogram derived from the TWINSPAN analysis in herbaceous layer. Numbers in the final level indicated the number of sampling plots in each groups. Plant species groups were named according to SIV herbaceous layer as follows:

Group 1: *Poa* sp. - *Oplismenus undulatifolius*
Group 2: *Pteridium aquilinum* - *Poa* sp. - *Oplismenus undulatifolius*
Group 3: *Pteridium aquilinum* - *Mentha aquatica* - *Hypericum androsaemum*
Group 4: *Mentha aquatica* - *Oplismenus undulatifolius*
Group 5: *Oplismenus undulatifolius* - *Pteris cretica* - *Oxalis corniculata*
Group 6: *Pteris cretica* - *Oplismenus undulatifolius* - *Mentha aquatica*

In woody species layer, Group 2 was the largest group includes 18 plots. The characterized species in this group was *Diospyros lotus*, *Ilex spinigera* and *Castanea sativa*. This group contains 26.5% of total plots. The smallest groups (group 1, 4 and 5) include just 6 plots (i.e., 13.3% of total plots).

In herbaceous layer, the largest group was contained 16 plots and occupied 23.5% of plots in the study areas (Group 6). The characterized species in the largest group were *Pteris cretica*, *Oplismenus undulatifolius* and *Mentha aquatica*. The smallest group has 7 plots and included 10.3% of plots (Group 1 and Group 2).

Discussion

Some simple stand variables, such as structure and composition can be used as reliable indicators of the conservation status of chestnut woodlands (DIAZ-VARELA *et al.*, 2011). Therefore, this study was carried out to identify floristic composition, introduce main plants species, classify vegetation and determine species groups (types) to help the protection of natural vegetation in the chestnut sites in Guilan province, north of Iran.

According to the results of our study, Group 2 (*Diospyros lotus* - *Ilex spinigera* - *Castanea sativa*) was dominance group in woody species layer in the studied areas because contained the most area of sites. Furthermore, *Castanea sativa* was repeated in five groups that showed the dominance of this species, so SIV index was an efficient index in named of groups in this study. Studies considered that *Castanea sativa* responds well to competitive pressure and show a higher productivity in mixed stands (LUIS & MONTEIRO, 1998).

Buxus hyrcana and *Taxus baccata* had dispersal distribution in Emamzadeh-Ebrahim; these are threatened endangered species in Iran. On the other hand, *Fraxinus excelsior*, *Zelkova carpinifolia* and *Daphne mezereum* will be threatened if they don't be in conservation measures (JALILI & JAMZAD, 1999). Previous study on woody species diversity in these sites showed that *Albizia* *julibrissin*, *Cerasus avium*, *Fraxinus excelsior*, *Tilia begonifolia* and *Ulmus glabra* in Shafarood and *Taxus baccata* and *Zelkova carpinifolia* in Emamzadeh-Ebrahim were introduced as the rare species and sites have remarkable diversity measures thus it is necessary to be considered as protected sites (POURBABAEI, 2002).

Four tree species were characterized as the main species and occurred mostly in groups including *Castanea sativa*, *Diospyros lotus*, *Gleditsia caspica* and *Carpinus betulus*.

Studies have shown that the development of the shrub layer greatly depends on the methods and intensity of forest management (ROLECEK, 2005). So, five shrub species were identified that two species of them were characterized the main species in groups in our studied areas. This seems that the lack of shrub species in our study area was due to the lack of management and sylvicultural treatments. POURBABAEI (2002) has been revealed that woody species richness was higher in Shafarood, because cattle grazing and anthropogenic disturbance were lower in there and the richness and diversity of woody species in the studied sites were found higher than abandoned chestnut (*Castanea sativa*) groves in southern France (GONDARD *et al*., 2001).

Group 6 (*Pteris cretica - Oplismenus undulatifolius - Mentha aquatica*) was dominance group in herbaceous layer in the studied areas and contained the most area of sites.

Conclusion

Based on the results of this study, the natural vegetation cover has a reliable condition in our studied areas but need more conservation. The preserving this species in their native habitat could help their survival and natural regeneration. Changes in the abundance of common species are likely to affect the living conditions of many other species that depend on it and may hence have a high influence on ecosystem processes. Therefore, it has been considered as a major point to protect the natural vegetation of this stands because the first step of design management strategies to maintain stands is investigation and identification the natural vegetation cover. We hope that introduce the main plant species in this study could help the conservation of this stands. In addition, protect the livestock grazing and investigate about pests and diseases of this species are helpful to growth good quality trees, produce healthy seeds and growth the natural regeneration.

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Reproductive Biology of African River Prawn Macrobrachium vollenhovenii (Crustacea, Palaemonidae) In the Lower Taylor Creek, Niger Delta, Nigeria

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Abstract. The sex ratio, Gonadosomatic Index (GSI) and fecundity of *M. vollenhovenii* in the Lower Taylor Creek, Niger Delta, Nigeria were studied between June 2008 and May 2010. The sex ratios showed that *M. vollenhovenii* deviated significantly from the ratio 1:1 and males dominated in *M. vollenhovenii* (X^2 = 68.65, df = 1, P < 0.001). There were no monthly and seasonal variations in the GSI means (P > 0.05), even though there were peaks in September 2008 and January 2009. However, there were significant differences in the GSI values of the size classes (P < 0.05). The 8–9cm size class had the highest GSI value. The overall fecundity of *M. vollenhovenii* ranged from 11, 402 eggs (TL = 6.70cm) to 56,481 eggs (TL = 11.40cm) with a mean of 24,765±3144 eggs (P < 0.001). The correlations of fecundity–total length, fecundity–gonad weight and fecundity–body weight relationships of all the species were positive and gonad weight gave the best predictive values.

Key words: Basket traps, baits, shrimps, Biseni, Gbarain, Nigeria.

Introduction

The African River prawn, *Macrobrachium vollenhovenii* (HERKLOTS, 1857) is endemic to eastern Atlantic, with viable fishery in most of the countries in the West African sub – region (NWOSU & WOLFI, 2006). WILLFUHR-NAST *et al.* (1993) had recommended this species for aquaculture cultivation, as an equivalent of the now widely cultured *Macrobrachium rosenbergii,* de Man, 1879 (FAO, 2000). In the Lower Taylor Creek, the species is an important component of the *Ingo* trap fishery, constituting about 52% by weight of the total catch (KINGDOM, 2012). Little is known about the biology or ecology of the prawn in this creek. This is the situation in most rivers in the Niger Delta, a problem earlier identified by POWELL (1983).

This paper aims to provide information on aspects of the reproductive biology of *M. vollenhovenii* in the Lower Taylor Creek, in order to manage it sustainably.

Materials and Methods

The study was carried out in the Lower Taylor Creek (a freshwater creek) in the Niger Delta, situated between 5º 01ʹN; 6º 17ʹE and 5º 02ʹN; 6º 18ʹE (Fig. 1). Sampling was carried out bi–weekly for twenty-four months (June 2008 – May 2010) using two sets of basket traps at all the stations. The traps, which were made from canes, measured 31 – 62cm in length, a mouth ope-

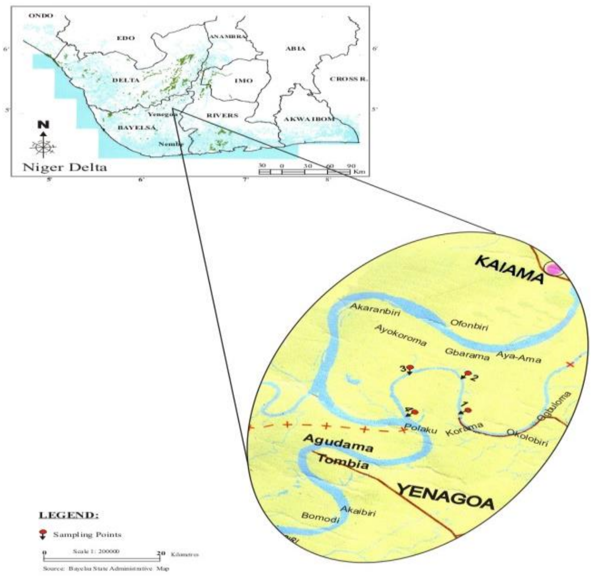

Fig. 1. Map of Niger Delta Showing Bayelsa State and the Taylor Creek Study Area

ning of 3 - 4cm in diameter and rectangular mesh sizes of about 3 by 0.5cm.

Specimens for the study of the reproductive biology were obtained from the samples of *M. vollenhovenii* caught for each month. The total length (from tip of rostrum to tip of telson) of the shrimp was measured in centimeters (cm) with a plastic ruler and individual weights taken in grams (g) using a digital balance (Ohaus; Scout Pro Model SPU 402). The sex of each specimen was determined by visual observation of the base of the fifth pair of periopods (HART *et al.*, 2003). In males, the base of the fifth pair of periopods approximates to the middle while that of females is wide. The weights of the ripe eggs were taken in grams and counted. The numbers of male and female specimens were recorded from the specimens collected. The deviation from the 1:1 ratio was tested using the chi-square test. Monthly ratios were estimated and seasonal and yearly variations of sex ratios were also tested for deviation from the 1:1 ratio with the chi-square test.

The gonadosomatic index (GSI) was determined (KING, 1995) as:

$$GSI = 100 \times \frac{Gm}{Tm},$$

where: Gm = Mass of Gonad,
Tm -= Total mass of fish.

Mean monthly values were computed and plotted to ascertain monthly, seasonal and yearly variation. Means also were computed for size classes and test of significant differences between males and females tested.

Fecundity (absolute fecundity), which is the total number of eggs in the ovaries of a fish prior to spawning (BAGENAL, 1978) was estimated by direct counting of spawnable eggs from the ovigerous females. Whole ovaries were carefully detached for each selected individual with forceps and assessed separately. Total weight of each ovary was determined using digital Ohaus balance, Scout Pro Model SPU 402. A sub – sample of the ovary was weighed and the eggs in each sample were spread on a filter paper and counting was done with the aid of stereomicroscope and hand – held tally counter. The number obtained was then extrapolated to determine the total number of eggs in the ovary.

Relative fecundity (RF) was obtained as the number of eggs per unit length (cm) or the number of eggs per unit weight (g) of shrimps. Scatter diagrams of fecundity against total length, body weight and gonad weight were plotted using linear regression technique and the best predictive equation for fecundity computed as logarithm transformation of the equation (BAGENAL, 1978):

$$F = ax^b$$

$$\text{i.e. } \log F = \log a + b \log x,$$

where: a=constant; b=exponent; F=fecundity; x=total length, total body weight, gonad weight.

Results

The sex ratios of *M. vollenhovenii* are given in Table 1.The overall sex ratio of *M. vollenhovenii* estimated from 341 sexually discernable shrimps was 1:0.38 male/female. The sex ratio deviated significantly from the ratio 1:1 (X^2 = 68.65, df = 1, P < 0.001).

Gonadosomatic index. Monthly GSI values for *M. vollenhovenii* are shown in Fig. 2. There was no significant variation in both

Table 1. Seasonal variation of mean monthly sex ratio of *M. vollenhovenii* in the Lower Taylor Creek

Month/Season Year	Numbers M	Numbers F	Sex ratio M:F
June 2008	22	5	1:0.23***
July 2008	11	10	1:0.91
August 2008	8	6	1:0.75
September 2008	20	32	1:1.60
October 2008	6	2	1:0.33
November 2008	1	2	1:2.00
December 2008	3	7	1:2.33
January 2009	4	6	1:1.5
February 2009	7	2	1.0.29
March 2009	14	4	1:0.29*
April 2009	14	2	1:0.14**
May 2009	10	4	1:0.40
June 2009	17	4	1:0.24**
July 2009	18	1	1:0.06***
August 2009	5	1	1:0.20
September 2009	13	1	1:0.08***
October 2009	4	0	1:0.00
December 2009	8	1	1:0.13*
January 2010	13	0	1:0.00
February 2010	16	2	1:0.13***
March 2010	9	1	1:0.11**
April 2010	15	0	1:0.00
May 2010	9	1	1:0.11**
Dry 2008	4	9	1:2.25
Wet 2008	67	55	1:0.82
Dry 2009	33	13	1:0.39**
Wet 2009	81	13	1:0.16***
Dry 2010	28	3	1:0.08***
Wet 2010	24	1	1:0.04***

*P < 0.05; **P < 0.01; ***P < 0.001

monthly and seasonal GSI means (P > 0.05), even though there were peaks in September 2008 and January 2009. However, there was significant difference in the GSI values of the size classes (P < 0.01). The 8 – 9cm size class had the highest GSI value (Table 2).

Fecundity. The ranges, mean monthly and seasonal fecundity of *M. vollenhovenii* are given in Table 3. The overall fecundity of *M. vollenhovenii* ranged from 11, 402 eggs (TL = 6.70cm) to 56,481 eggs (TL = 11.40cm) with a mean of 24,765±3144 eggs. There were both monthly and seasonal variations in the fecundity (P < 0.001). The eggs per centimetre body length (2495 ± 240 eggs/cm) was higher than the eggs per gram body weight (2115±132 eggs/g).

Fecundity/ total length, fecundity/gonad weight and fecundity/body weight relationships of *M. vollenhovenii* in the Lower Taylor Creek are shown in Figs. 3 to 5 respectively. The relationships of this population expressed in linear regression are as follows:

$F = 1.26TL^{3.16}$ ($r = 0.89$; $r^2 = 0.79$; $P < 0.001$)

$F = 4.21GW^{3.06}$ ($r = 0.94$; $r^2 = 0.89$; $P < 0.001$)

$F = 3.48BW^{0.84}$ ($r = 0.84$; $r^2 = 0.68$; $P < 0.001$)

The correlations were all positive and gonad weight also had the best predictive value of 0.94.

Table 2. Mean GSI values of different size classes of *M. vollenhovenii* in the Lower Taylor Creek

Size classes	Sample size	Range	Mean ±S.E
6.00 - 7.00	1*	-	22.28 ± 0
7.00 - 8.00	1*	-	16.20 ± 0
8.00 - 9.00	5	10.55 - 15.57	12.92 ± 0.79^a
9.00 - 10.00	8	7.49 – 12.14	9.11 ± 0.62^b
10.00 – 11.00	1*	-	6.77 ± 0
11.00 – 12.00	3	6.73 – 8.05	7.42 ± 1.02^b

*Sample with < 2 specimens was not included in analysis

Discussion

Sex ratio. The sexes of *M. vollenhovenii* in this study were not fairly distributed as MARIOGHAE (1982), and MARIOGHAE & AYINLA (1995) reported. The dominance of male *M. vollenhovenii* over females in this study is similar to the observation of MWANGI (1984). The seasonal variation of sex ratio observed in *M. vollenhovenii* may be associated with breeding activities. OLATUNDE (1978) reported that during breeding season, more females are expected to associate with males, particularly in a situation where there are few males in the population. However, this was not the case with *M. vollenhovenii,* which had more males in the population; a situation also reported for some fish species like *Sierrathrissa* *leonensis* (THYS VAN DEN AUDENAERDE, 1969) in the Nun River and Taylor Creek (OTOBO, 1995). REYNOLDS (1974) had already posited that partial segregation of ripe forms, either through habitat preferences or through school formation, could render one sex more vulnerable to capture than the other.

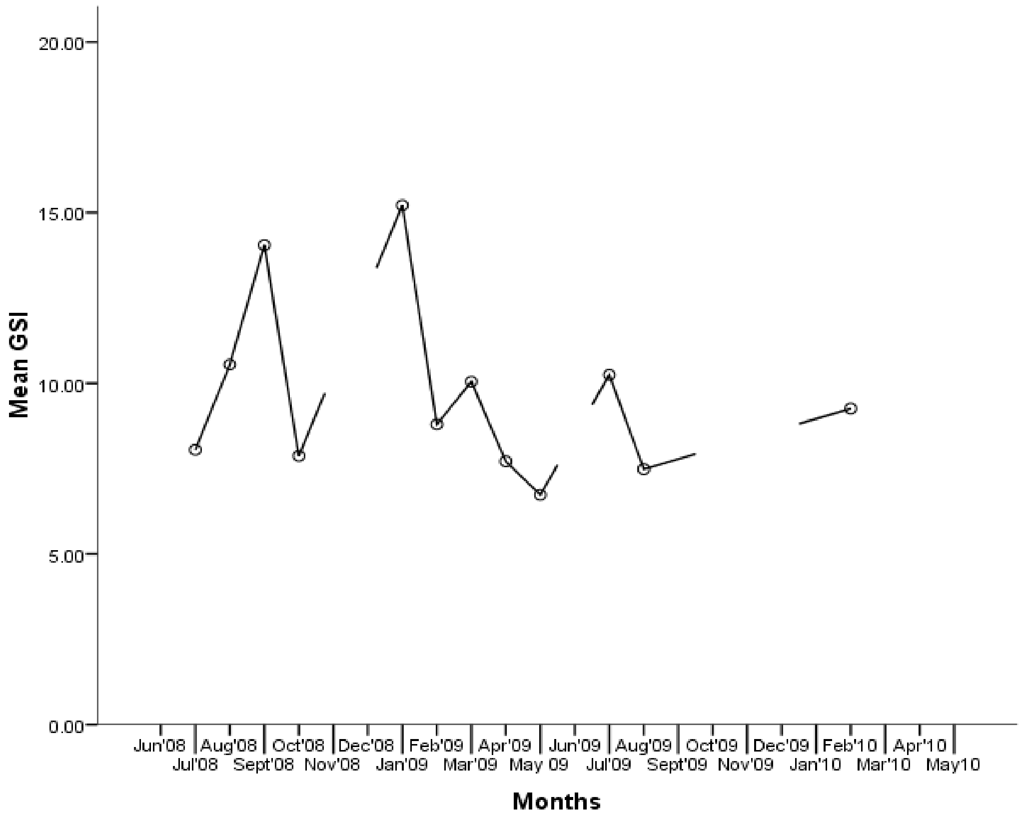

Fig. 2. Mean monthly Gonadosomatic Index (GSI) of *M. vollenhovenii* in the Lower Taylor Creek

Table 3. Monthly and Seasonal variation of fecundity of *M. vollenhovenii* in the Lower Taylor Creek

Month/Year	Sample size	Range	Fecundity Mean ±S.E	Relative Fecundity	
				Mean egg number/cm	Mean egg number/g
Jul-08	1*	-	56481 ± 0	4954 ± 0	3344 ± 0
Aug-08	1*	-	16425 ± 0	1888 ± 0	1805 ± 0
Sep-08	5	13528-20677	17270 ± 8817[b]	1973 ± 311[a]	2363 ± 233[a]
Oct-08	1*	-	18665 ± 0	2007 ± 0	1336 ± 0
Jan-09	2	11482-16041	13760 ± 6234[b]	1675 ± 492[a]	2163 ± 368[a]
Feb-09	3	18006-49145	35200 ± 5090[a]	3266 ± 402[a]	2175 ± 300[a]
Mar-09	1*	-	17210 ± 0	1851 ± 0	1616 ± 0
Apr-09	1*	-	22467 ± 0	2292 ± 0	1561 ± 0
May-09	1*	-	52326 ± 0	4550 ± 0	2689 ± 0
Jul-09	1*	-	25125 ± 0	2513 ± 0	2076 ± 0
Aug-09	1*	-	24828 ± 0	2483 ± 0	1549 ± 0
Feb-10	1*	-	17527 ± 0	1845 ± 0	1546 ± 0
Season					
Dry	7	17527-49145	23310 ± 4489[a]	2321 ± 355[a]	1779 ± 265[a]
Wet	12	11482 -56481	27480 ± 2719[a]	2704 ± 215[a]	2098 ± 160[a]
Overall	**19**	**11482 -56481**	**24765 ± 3144**	**2495 ± 240**	**2115 ± 132**

*Samples with < 2 specimens were not included in analysis

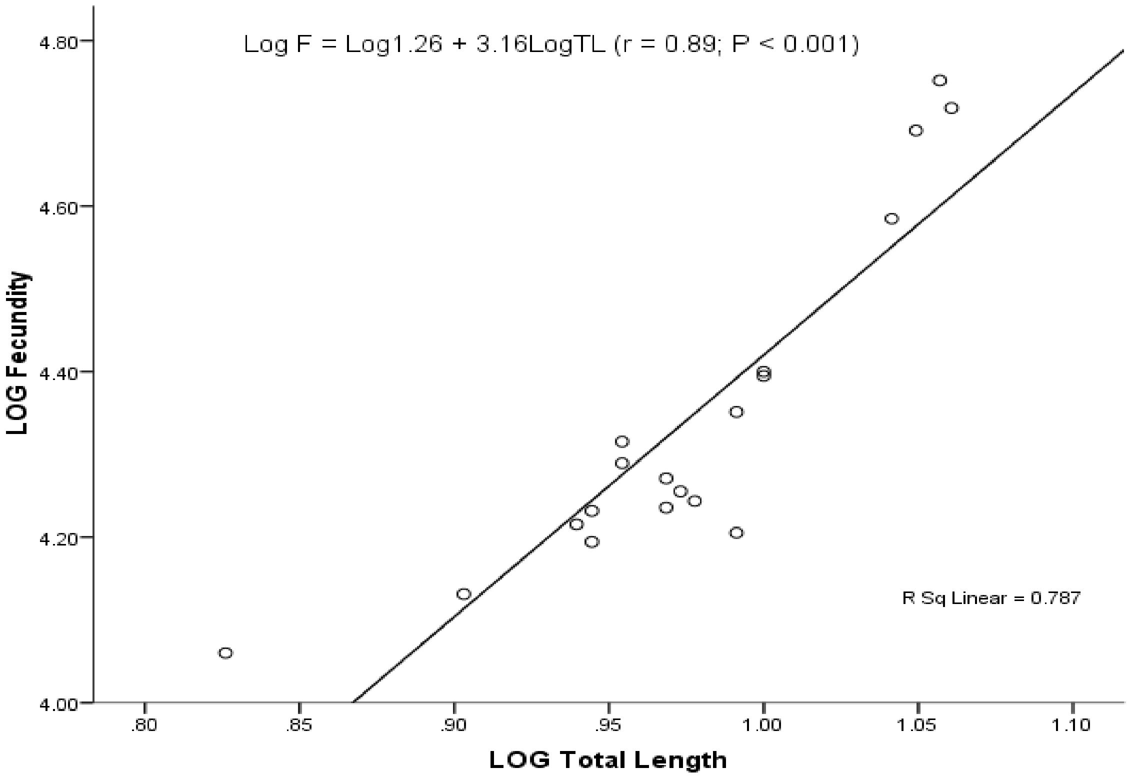

Fig. 3. Relationship of Fecundity and Total length of *M. vollenhovenii* in the Lower Taylor Creek

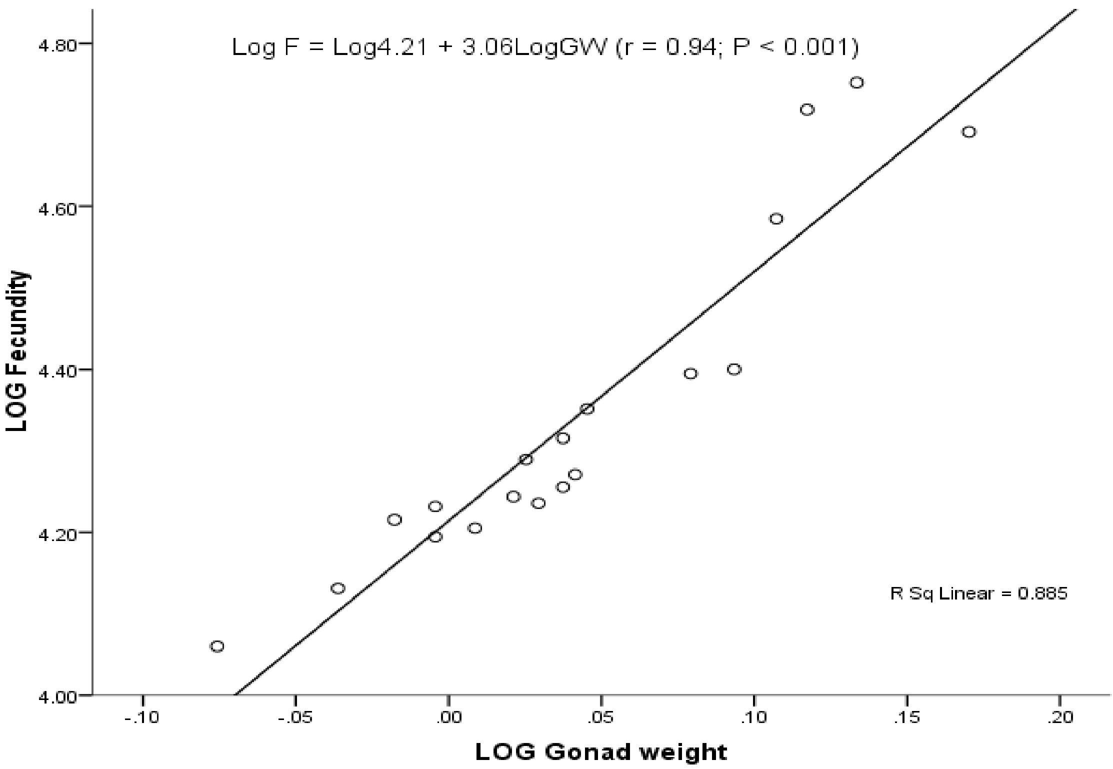

Fig. 4. Relationship of Fecundity and Gonad weight of *M. vollenhovenii* in the Lower Taylor Creek

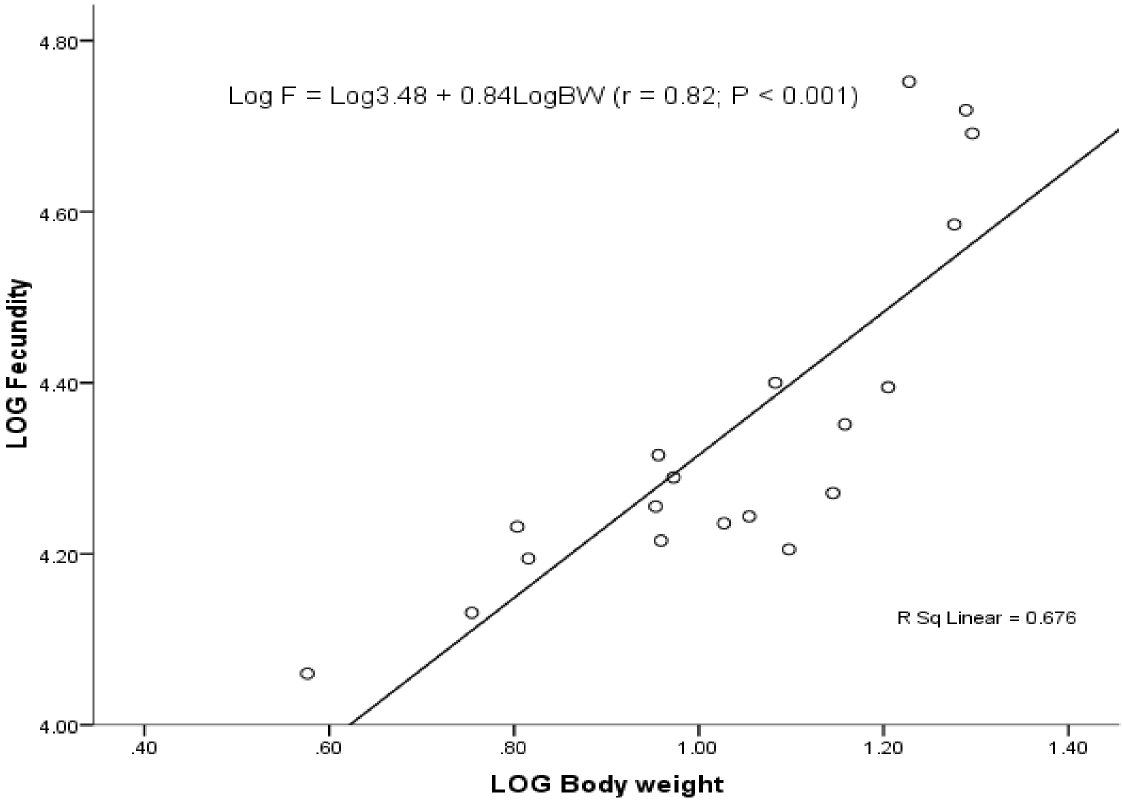

Fig. 5. Relationship of Fecundity and Body weight of *M. vollenhovenii* in the Lower Taylor Creek

Gonadosomatic index. The absence of significant variability in the mean monthly and seasonal GSI for *M. vollenhovenii* may mean all year round reproductive pulses (KINGDOM & ALLISON, 2011), a situation also observed by POWELL (1983) and ENIN (1998).

Fecundity. The absolute fecundity values obtained for *M. vollenhovenii* were highly variable. This situation had been reported by KHMELEVA & GOLOUBEV (1986), who observed that many crustaceans have highly variable absolute fecundity values, even in females of similar size. Various authors have reported the fecundity of *M. vollenhovenii*. VILLE (1970) reported 300 – 1, 000; MILLER (1971) reported 12, 000 – 45, 000, while ANETEKHAI (1986) reported 49, 979 401, 212, with a mean of 173,940 eggs per female. A range of 11,402 to 56,481 eggs per female was estimated in this study. The differences in the absolute fecundity of *M. vollenhovenii* in this study from those of other areas may be due to differences in environment, food supply (BAGENAL, 1978) and egg sizes (BEACHAM & MURRAY, 1993).

The Fecundity/total length relationships showed an increase in number of eggs produced with increasing female size; a situation also observed by ALBERTONI *et al,* (2002) in *M. acanthurus* (EIGMANN, 1836), HART *et al*. (2003) in *M. felicinum* (HOLTHUIS, 1949) and DEEKAE & ABOWEI (2010) in *M. macrobrachion* (HERKLOTS, 1851). The increase of fecundity with body size seems to be a rule that is applicable to many crustaceans (UDO & EKPE, 1991; LLODRA *et al*. 2000). COURTNEY *et al.* (1996), who reported on the decline in number of eggs with an increase in the size of *Penaeus plebejus* and found out that this could possibly be due to ovarian senescence in large (old) females have documented contrary opinion. The increase in the number of eggs with increase in female size of prawns suggests that there are differences in the pattern of allocation of food energy by the prawns at different sizes (TEIKWA & MGAYA, 2003). Usually, in larger individuals, who have low growth rates, much of the energy is devoted to egg production while smaller individuals devote large fraction of their energy to growth rather than egg production. The slopes were all within the range of 2.3–5.3 calculated for a variety of species by BAGENAL (1978). However, gonad weight was the best predictor of fecundity for all species; similar to the observation of ALBERTONI *et al.* (2002) for *M. acanthurus* in a tropical coastal lagoon.

The eggs per centimeter body length was higher than the eggs per gram body weight. Similar situations were also found in the Silver catfish, (*Chrysichthys nigrodigitatus* LACEPEDE, 1803)in Cross River, Nigeria (EKANEM, 2000) and Sharpnose mullet, (*Liza saliens* RISSO, 1810) in Gorgan Bay – Miankaleh Wildlife Refuge, the Southeast Caspian Sea (PATIMAR, 2008). A possible reason for this difference may be as a result of the growth pattern.

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Diet of Threatened Pheasant Species in Himalayas, India – A Faecal Analysis Approach

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Abstract. The aim of this paper is to determine diet composition of threatened pheasant species i.e. Satyr Tragopan *Tragopan satyra*, Himalayan Monal *Lophophorus impejanus*, Kaleej *Lophura leucomelana* and Koklass *Pucrasia macrolopha* in their native forest which was never studied earlier. A study was conducted in the Kumaon region of western Himalaya for two years by collecting dropping material. Faeces were identified through direct sighting of defecating species. The diet items of each pheasant species mainly comprised plant materials followed by invertebrates and grit. A significant difference was observed in consuming food items by all pheasant species. Monal emerged as a specialist feeder on plants which were not eaten by other species. The Satyr and Koklass were more similar in terms of diet composition in both seasons while Kaleej and Monal were least similar, only invertebrates and grit were common in the diet of these species. No significant difference was observed in diet composition in different seasons of all pheasant species. The results expected to provide valuable information for the management of these pheasants in Himalayas.

Key words: Conservation, Faecal, Food Importance Index, Himalaya, Specialist feeder, Pheasants.

Introduction

Pheasant species are amongst the best known and most spectacular species in their ecosystem (GARSON, 2007). They are generally viewed as potentially useful indicators of environmental quality because of used as a source of food and most of them live in forests (FULLER & GARSON, 2000). Our understanding of this importance is facilitated by information on the species' food habits and the extent of their dietary similarities. Very little quantitative information is available on the diet of Himalayan and other Asian pheasants in the wild however they are an important part of ecosystem. But it is far sure that the diet of pheasants includes all types of food matter and it depends to a large extent on their habitats and the availability of food resources (HILL, 1985; RIMLINGER *et al*., 2000).

There is considerable variation in diet composition among pheasants. Kinds of food eaten vary between groups and also by season, and even by habitat in some pheasant species. It includes seeds, roots, leaves, shoots, flowers, stems, buds, invertebrates and even reptiles (MCGOWAN, 1996). Little analogous work (KAUL, 1989 a; b; MOREBY, 1993; KHALING, 1999) has been conducted on avian taxa especially for the phasianidae family in the wild. Many studies have been conducted on direct observation of feeding behaviour of pheasants such as ZHENGJE (1989) studied feeding behaviour of Ring-necked pheasant and found the species to be vegetarian in food habits and 83% food of the species was composed of plant materials, JIANQIANG & YUE (1989) observed the Brown-eared pheasant feeding ecology and recorded 62 plant and animal species. XIANGTAO & XIAOYI (1989) studied the diet and feeding habits of Crimson-bellied Tragopan and found the species to be vegetarian. LELLIOT & YONZON (1980) found the species feeding on the leaf litter and stream debris or on mossy areas during their studies in Central Nepal. They were also found feeding on the fruits of *Berberis* sp., *Symplocos* sp. and *Rhododendron* sp. They found leaf, moss, grass, roots, quartz fragments and an insect wing from faecal analysis, suggesting an omnivorous diet. BHANDARY *et al.* (1986) in Pipar (Central Nepal) found the moss and grass leaves as a main autumn diet of the species. DAVISON (1981) in his studies on the Crested-fireback pheasant in Malaysia observed majority of birds in moist areas with abundant invertebrates.

The present study is on the feeding ecology of Kaleej (*Lophura leucomelana*), Koklass (*Pucrasia macrolopha*), Himalayan Monal (*Lophophorus impejanus*) and Satyr Tragopan (*Tragopan satyra*) in the western (Kumaon) Himalaya, India. The Himalayan Monal and Kaleej pheasant are distributed in the northwestern, western, central and eastern Himalayas. Monal is found mostly between 2300–4875 m and down to 2000 m during winters while Kaleej is found below 2700 m. Koklass pheasant is distributed in the northwestern and western Himalayas between 2700-3300 m and down to 1500 m in winters. The Satyr Tragopan is distributed in the western, central and eastern Himalayas at elevations of 2400–4520 m, descending to 2000 m during late post monsoon season (SATHYAKUMAR & KAUL, 2007). These studied pheasants are protected under Schedule I and III (hunting is prohibited) in Wildlife Protection Act, India, 1972 (RISHI, 1972) so killing of these birds is neither permissible nor practicable for crop and gizzard analysis. Hence, faecal matter analysis was adopted as an alternative method to determine the diet of four pheasant species and to find out the seasonal variation in diet among them.

Materials and methods

Study area. This study site lies in district Bageshwer, Uttarakhand, India (30° 08' N and 79° 57' E) in the Kumaon Himalaya encompassing an area of 58.25 sq. km (Fig. 1). The area represented temperate (1500-3500 m) to alpine (>3500 m) climatic conditions (CHAMPION & SETH, 1968). Being very close to Pindari, Kafni and Sunderdunga glaciers, the area experience extreme weather conditions. Annual rainfall peaks at about 1200 m altitude (4100 mm) and gradually declines to 670 mm at 2700 m. The monsoon starts at the end of June and ceases by the middle of September. The mean temperature varies from 15°C to 25°C in pre monsoon season while below 0°C to 10°C in the post monsoon season (SAXENA *et al.*, 1985). The boundary of the study area touches the Nanda Devi Biosphere Reserve and can be reached by three days trekking from the last motorable road. The vegetation is moist temperate type (CHAMPION & SETH, 1968). The major tree species in the study area included *Quercus semecarpifolia, Quercus floribunda*, *Abies pindrow, Taxus baccata, Betula utilis* in association with *Rhododendron barbatum, Acer caesium, Lyonia ovalifolia, Alnus nepalensis, Aesculus indica*. The dominant shrub species included *Arundinaria falcata*, *Athyrium* sp., *Polystichum* sp., *Pteris cretica*, *Daphne papyracea*., *Urtica dioca* and *Pyrancantha crenulata* etc. (HUSSAIN *et al.*, 2008).

Methods. Faeces collection was made on the selected forest trail and through random search in the study area. These pheasant species occupy different altitudinal range (HUSSAIN, 2002; SATHYAKUMAR & KAUL, 2007) so doubt of mixing of droppings was ruled out. These were identified by direct sightings and directly defecated droppings were collected for reference and further used in data collection. The spots from where faeces were collected were cleared to avoid collection of the same materials during subsequent monitoring. The collection was made for both seasons (pre monsoon; March – June and post monsoon; September –

December, the rest of the year is inaccessible in the study area) for two years for food items comparison. Sixty faeces of each pheasant species for each season per year were collected. They were air dried, labelled and sealed in plastic packets and stored in an airtight container with camphor. Major ground vegetation species were collected in a 10m radius circular plot from the places where feaces were found and in which pheasants were seen feeding directly.

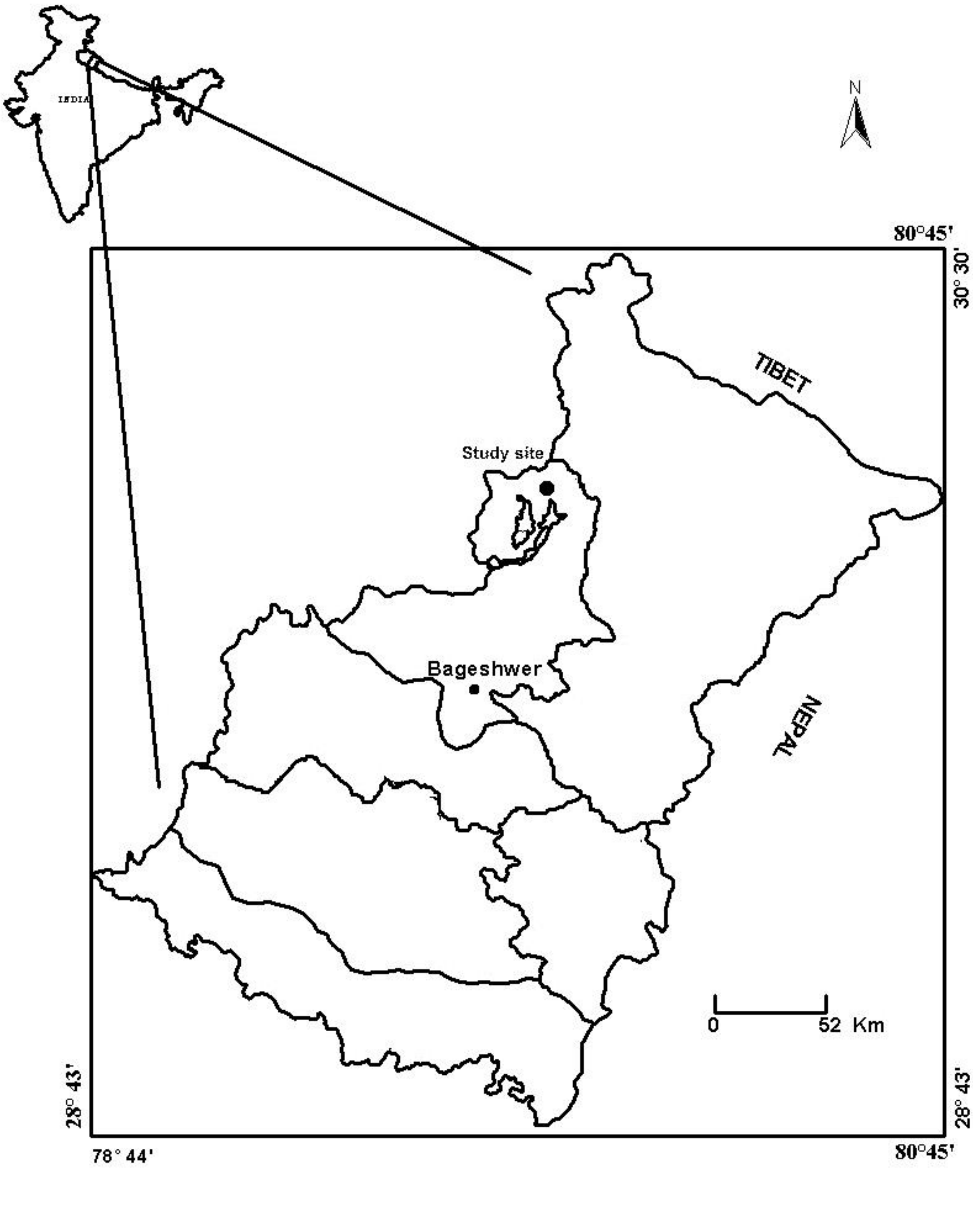

Fig. 1. Location of the study site in District Bageshwer, Kumaon Himalayas, Uttarakhand, India.

Preparation of plant sample slides. Reference slide of each plant species was prepared micro histological and each showed unique epidermal characteristics that allowed easy identification. First the plant species were preserved in 10% formalin. Epidermis of plant species were then stripped off and then passed through the ascending grades of ethanol for dehydration (30%, 50%, 70%, 90% and 100%) (HOLECHEK *et al.*, 1982) and then cleared in xylol. Permanent slides were made by mounting in Canada balsam. A total of 50 reference slides of different plant species was prepared.

Preparation of faecal sample slides. All the collected faeces were pooled season wise and species wise. A total of 120 faecal samples per season per species in two years were ready for preparing major samples accordingly. The samples were gently crushed by hand. Samples were grounded and sieved to homogenize the size of fragments. They were cleared in 10% NaOH solution and boiled for 3-4 minutes with 3-4 changes of the NaOH solution (KHALING, 1998). The excess solution was drained off and the settled material was poured into a petri dish. A dropper extracted 10 sub samples from this sample on 10 glass slides. The sub samples were allowed to air dry later they were mounted in glycerol (BHANDARY *et al.*, 1986). The slides were examined in 50X and 100X magnification levels interchangeably (HOLECHEK & VALDEZ, 1985). The frequency (presence / absence) occurrence of plant fragments was recorded in a microscopic field of view. Every fragment could be identified using reference slides and which fell wholly or partly with the view, was recorded. Although the frequency of occurrence of fragments (plant or animal part) present in the slide tends to overestimate rare and underestimate common species (HANSON, 1970), it is still the most widely used technique. It is expected that the degree of fragility of cuticle differs from species to species (STORR, 1961; STEWART, 1967) so we made ten slides of each pheasant species faecal matter. Then each slide was divided into 20 frequencies to identify and enumerate the proportions of different food items present in the droppings. The identification of invertebrate part in the faecal analysis was also made from the same slides. We have however made no attempt to classify different invertebrate parts but only presence / absence of them was recorded from the microscopic view.

Analyses. The occurrence of each food item was expressed as a Food Importance Index (FII) (BHANDARY *et al.*, 1986). According to BECK (1952), the formula needed specific gravity also, which is possible only by crop analysis. In the present study involving the diet of Himalayan pheasants where crop analysis is not practicable and viable, only the frequency and composition of food items were considered so the following formula was used:

Food Importance Index = % Frequency + % Composition/2

The frequency of each food item was calculated by dividing the occurrence of the particular food item by all frequencies whereas percent composition was calculated by dividing the occurrence of a particular food item in a sample slide by the total occurrence of all food items.

Sorenson similarity index (MAGURRAN, 2004) was calculated for different pheasant species by taking food items into account. This index was calculated by combining both seasons.

$$S = 2A / (B + C)$$

where A is the number of diet components eaten commonly by both species, B is the number of diet components eaten by one species and C is the number of diet components eaten by other species.

Some food item index fluctuated between seasons so paired sample t–test was performed to observe significant differences between seasons for different pheasant species. Kruskal-Wallis one-way ANOVA and t-test were performed (ZAR, 2009) on different food items to observe significant differences among studied pheasant species season wise.

Based on composition, each food item was categorized into three groups, 1) Major food components or food items forming >10% of the total composition; 2) Minor food components or items forming <10% but >3% and 3) Trace items which formed <3% of the total composition (KHALING, 1998).

Results

Diet composition of different pheasant species. Total 38 food items were identified from the faecal matter of Kaleej, Koklass, Himalayan Monal and Satyr Tragopan. Out of these 36 food items were plant materials and the rest were grit and invertebrates (Table 1), other 14 plants were recorded from the area but not found in the faecal matter. Invertebrates were found to be a major food item in the diet of Kaleej (FII = 25.5) and Monal (FII = 8.60) during post monsoon season whereas grit was a major food item of Satyr during pre monsoon season (FII = 14.9). Diet composition of Kaleej, Koklass, Monal and Satyr varied significantly between seasons (Table 1).

The FII of some of the food items fluctuated between seasons such as *Viola* sp., invertebrates, *Geranium wallichianum*, *Rubus biflorus* and *Myrcine africana* in Kaleej and likewise in other pheasant species. So, Paired sample t-test was performed on the composition of these food items (Fig. 2). Some diet components differed significantly between seasons for all species.

Table 1. Food importance index (FII) and different food items found in the droppings of different pheasant species during pre monsoon and post monsoon seasons in Kumaon Himalaya, India. One way ANOVA was performed on different food items for each species in each season. * = significant at 0.00 level. Paired sample t-test showed no significant difference in seasons for each species regarding diet composition. The category has been defined in the methods section.

Species	Food items	Pre monsoon season		Post monsoon season	
		FII	Category	FII	Category
Kaleej	*Geranium wallichianum*	8.28	Minor	26.3	Major
	Rubus biflorus	23.3	Major	6.0	Minor
	Rubus ellipticus	21.1	Major	12.8	Minor
	Viola sp.	13.5	Minor	29.3	Major
	Berginea legulata	15.8	Major	6.75	Minor
	Myrcine africana	16.6	Major	6.0	Minor
	Thalictrium foliolosum	6.77	Minor	9.75	Minor
	Boeninghausienia albiflora	9.03	Minor	6.75	Minor
	Fragaria sp.	15.1	Major	11.3	Minor
	Invertebrates	10.5	Minor	25.5	Major
	Grit	9.03	Minor	9.75	Minor
		Not significant		*$F_{10, 109}$= 5.13	
Koklass	*Daphne papyracea*	4.93	Minor	7.81	Minor
	Skimmia laureola	9.87	Minor	8.59	Minor
	Athyrium sp.	-	-	15.62	Major
	Polystichum sp.	6.58	Minor	12.49	Minor
	Rubus ellipticus	7.40	Minor	5.46	Minor
	Nerium sp.	18.92	Major	25.77	Major
	Mondo intermedius	10.69	Minor	-	-
	Moss	19.74	Major	1.56	Trace
	Myrcine africana	4.93	Minor	7.03	Minor
	Urtica dioica	-	-	6.25	Minor
	Berberis aristata	5.75	Minor	2.34	Trace
	Pteris biaurita	3.29	Trace	7.81	Minor
	Potentilla fulgens	4.11	Minor	5.46	Minor
	Fragaria sp.	13.98	Major	18.74	Major
	Invertebrates	7.40	Minor	1.56	Trace

	Grit	9.87	Minor	12.49	Major
		$*F_{13, 106}= 5.31$		$*F_{14,105}= 7.75$	
Himalayan Monal	*Eulophia compestris*	3.58	Minor	-	-
	Nordostachis jatamansi	9.56	Major	1.72	Trace
	Picrorhiza kurroa	4.78	Minor	3.44	Minor
	Aconitum heterophylum	4.78	Minor	3.44	Minor
	Potentilla fulgens	13.1	Major	6.88	Minor
	Artimisea nilgirica	15.5	Major	6.88	Minor
	Hedychium spicatum	-	-	3.44	Minor
	Skimmia laureola	3.58	Minor	5.16	Minor
	Ainsliaea sp.	7.17	Minor	6.88	Minor
	Satyrium nepalense	2.39	Trace	1.72	Trace
	Gaultheria nummularioides	3.58	Minor	1.72	Trace
	Moss	7.17	Minor	3.44	Minor
	Invertebrates	2.39	Trace	8.60	Major
	Grit	8.36	Minor	17.2	Major
		$*F_{12, 107} = 5.22$		$*F_{12, 107} = 3.56$	
Satyr Tragopan	*Cotoneaster acuminate*	12.9	Major	6.55	Minor
	Rubus biflorus	4.98	Minor	4.09	Minor
	Arisaema flavum	1.99	Trace	9.82	Minor
	Pilia sp.	12.9	Major	4.91	Minor
	Skimmia laureola	1.99	Trace	5.73	Minor
	Polygonum amplexicaule	1.99	Trace	7.37	Minor
	Potentilla fulgens	1.0	Trace	4.91	Minor
	Valeriana wallichii	1.99	Trace	7.37	Minor
	Daphne papyracae	2.99	Trace	5.73	Minor
	Viola sp.	1.0	Trace	9.82	Minor
	Arundinella nepalensis	20.9	Major	17.2	Major
	Poa annua	10.9	Major	4.09	Minor
	Polystichum sp.	1.99	Trace	8.18	Minor
	Pteris biaurita	2.99	Trace	6.55	Minor
	Moss	3.98	Minor	7.37	Minor
	Invertebrates	1.0	Trace	4.91	Minor
	Grit	14.9	Major	13.9	Major
		$*F_{16,103} = 9.01$		$*F_{16,103} = 8.95$	

Few plant species were exclusively consumed in a particular season only; plant species *Athyrium* (major) and *Urtica dioca* (minor) were found only in the post monsoon season diet of Koklass whereas it was *Mondo intermedius* (minor) in pre monsoon season (Table 1). Minor food items were found in higher percentage in most of the species' diet for both the seasons except for Kaleej and Satyr where major items were in more percentage in the pre monsoon season diet (Table 2). *Arundinella nepalensis* and grit were the major food items whereas *Rubus biflorus* and moss were the minor items of Satyr Tragopan for both the seasons. No trace elements were found in Kaleej faeces in both the seasons.

Diet comparison among pheasant species. The Sorenson similarity index showed highest similarity in diet composition between Koklass and Satyr monsoon in both seasons (pre monsoon S = 0.93; post mosoon S = 0.94) while Kaleej and Monal monson were least similar in their diet composition in both seasons (pre monsoon S = 0.2; post monsoon S = 0.2) (Table 3). Some plant species such as *Nordostachis jatamansi, Eulophia* *compestris* and *Gaultheria nummularioides* were found only in Monal's faeces (Table 1). Grit and invertebrates were the common food items for all pheasants for both the seasons. One-way ANOVA showed a significant difference in the percent composition of invertebrates found in all pheasants during pre monsoon season (F = 3.78, d.f. = 3, 36, p = 0.018) and post monsoon season (F = 14.7, d.f. = 3, 36, p = 0.00). The percent grit composition did not differ significantly between seasons among pheasant species (pre monsoon season F = 1.24, d.f. = 3, 36, p = 0.308; post monsoon season F = 0.84, d.f. = 3, 36, p = 0.478)

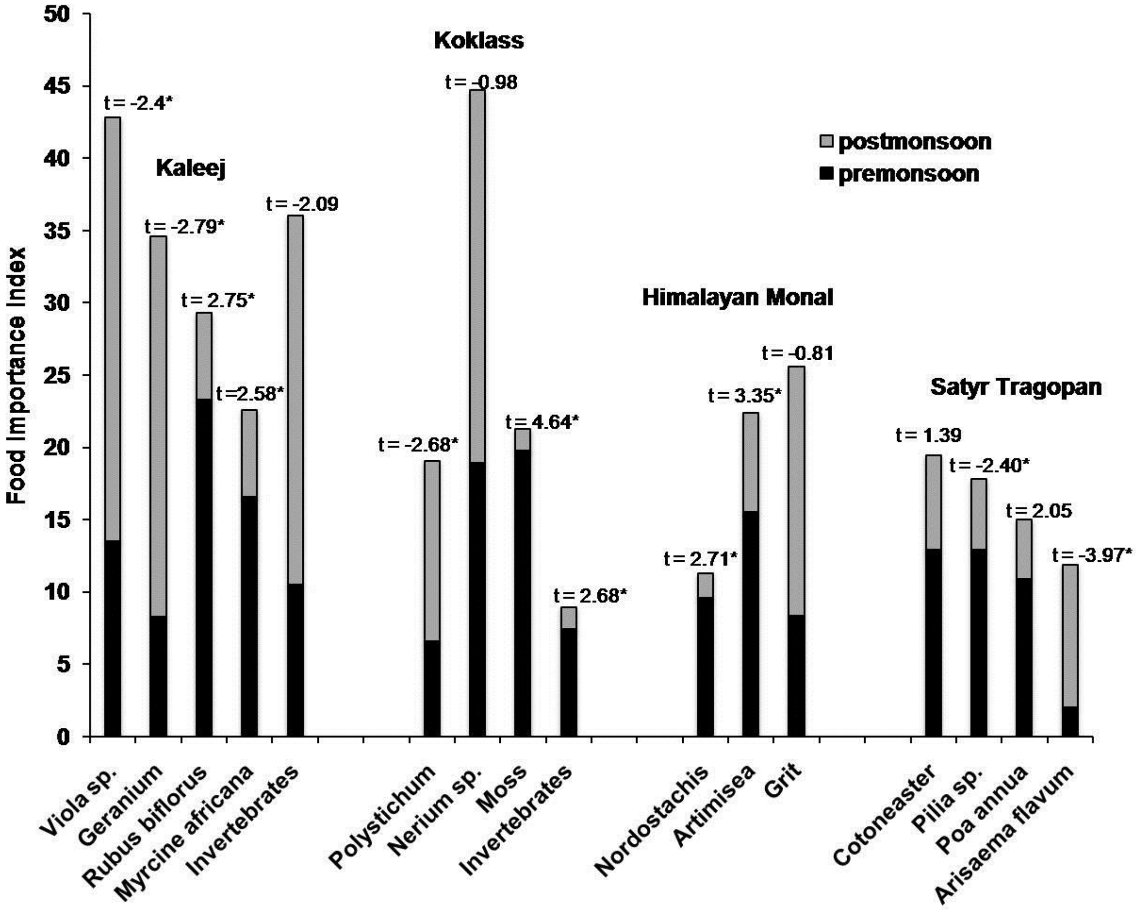

Fig. 2. Food Importance Index of some of diet components which found in pheasant species in Kumaon Himalayas in pre monsoon and post monsoon seasons. t- test was performed for each diet component and value was given. * = significant at p < 0.05

Six food items were found common for Kaleej, Koklass and Satyr so a t - test was performed on these food items between species. The percent composition of *Daphne papyracea* was significantly different in Koklass and Satyr during pre monsoon (t = 3.58, d.f. = 19, p = 0.002) and post monsoon season (t = 5.29, d.f. = 19, p = 0.00) and percent composition of *Pteris biaurita* also differed significantly in these species during pre monsoon and the post monsoon season (Table 4).

Potentilla fulgens and *Skimmia laureola* are highly medicinal value plant and were commonly found in the droppings of Koklass, Monal and Satyr. The percent composition of *Potentilla fulgens* was significantly different among these pheasant species (F = 9.69, d.f. = 2, 27, p = 0.001) during pre monsoon season. Percent composition of *Skimmia laureola* showed significant difference during the premonsoon season (F = 4.66, d.f. = 2, 27, p = 0.018) as well as during the post monsoon season (F = 3.88, d.f. = 2, 27, p = 0.03) in Koklass, Monal and Satyr diet.

Table 2. Mean percent composition (± Standard Error in parentheses) of major, minor and trace food items found in the droppings of different pheasant species during pre monsoon and post monsoon seasons in Kumaon Himalaya, India.

Species	Pre monsoon season			Post monsoon season		
	Major%	Minor%	Trace%	Major%	Minor%	Trace%
Kaleej	61.61 (5.23)	38.38 (3.59)	-	54.0 (3.41)	46.0 (3.09)	-
Koklass	41.29 (3.87)	56.12 (5.18)	2.59 (0.78)	43.26 (4.96)	52.8 (6.12)	3.93 (0.91)
Himalayan Monal	44.44 (4.34)	50.0 (4.05)	5.55 (1.21)	36.58 (3.17)	56.09 (4.94)	7.31 (2.18)
Satyr Tragopan	72.27 (6.19)	8.91 (2.95)	18.81 (2.56)	24.2 (2.17)	75.79 (7.13)	-

Table 3. Sorenson similarity index in different pheasant species for food composition in pre monsoon and post mosoon seasons of Kumaon Himalaya, India.

Pre monsoon season	Kaleej	Koklass	Himalayan Monal	Satyr Tragopan
Kaleej	1.0	0.66	0.2	0.4
Koklass		1.0	0.59	0.94
Himalayan Monal			1.0	0.42
Satyr Tragopan				1.0
Post monsoon season	**Kaleej**	**Koklass**	**Himalayan Monal**	**Satyr Tragopan**
Kaleej	1.0	0.62	0.2	0.4
Koklass		1.0	0.53	0.94
Himalayan Monal			1.0	0.5
Satyr Tragopan				1.0

Table 4. t-test values for for common food items present in different pheasant species in pre monsoon and post monsoon season. *, signi. = significant

Food items	Species	Pre monsoon		Post monsoon	
		t- value	signi.	t- value	signi.
Daphne papyracea	Koklass & Satyr	3.58	0.002*	5.29	0.000*
Pteris biaurita	Koklass & Satyr	2.66	0.15	5.20	0.000*
Rubus biflorus	Kaleej & Satyr	3.53	0.002*	3.11	0.006*
Myrcine africana	Kaleej & Koklass	4.56	0.000*	3.04	0.007*
Fragaria sp.	Kaleej & Koklass	5.24	0.000*	3.93	0.001*
Rubus ellipticus	Kaleej & Koklass	4.39	0.000*	3.77	0.000*

Discussion

Identification is influenced by the distinctiveness of each plant species as well as relative changes in the identifiable characteristics of each plant species as altered by digestion. When plants are succulent, and when the diet is composed of woody and herbaceous plants, the differential rate of digestion lead to highly variable results (VANDYNE, 1968). To obtain a mixture of relative homogenous size, care was taken and samples were screened through appropriate sieves to remove unusually large items. The FII gave a general importance for each food item recorded in the diet of these pheasant species for pre monsoon and post monsoon seasons.

Despite the constraints of techniques which prevented detailed quantitative work on the diet of Kaleej, Koklass, Satyr Tragopan and Himalayan Monal, the results of this study were vital in determining the diet of the species during different seasons. In the present study there might have been a number of plant species present in the study area that were not recorded for faecal analysis. Plant species can be very specific to a particular habitat type and a wide selection of unknown food remains can easily result if a number of birds from different area studied (MOREBY, 1993). The food items identified from the faecal matter of studied pheasants reflected the general if not exclusive diet of these species in the wild because many of the plant species consumed by these species may have undergone complete digestion or may have been reduced to such small fragments that they were not identifiable.

The study revealed that the main diet of all pheasant species was plant matter although invertebrate matter was also present but in low percentage. The dry conditions were still prevalent in early March or the onset of pre monsoon, the evergreen shrubs such as *Rubus biflorus* and *Rubus ellipticus* were the major source of food where as perennials like *Geranium wallichianum*, *Boeninghausienia albiflora* were also present in faecal matter but in minor composition. Such as in Koklass the major food items were *Nerium* sp. Moss and *Fragaria* sp. *Pteris biaurita* and *Polystichum* sp. (fern) were also present but in traces as they were available in the middle or end of the season. In Monal diet, the major sources were rare plants such as *Nordostachis jatamansi*, *Potentilla fulgens*, and *Artimisea nilgirica.* Other minor parts of the Monal diet were also the rare plants. Chinese Monal feeds on the roots and leaves of the plant (HE *et al*., 1988). In Satyr the *Arundinella nepalensis* formed the major portion of the food. It is perennial plant that die after flowering but its leaves were available throughout the year. It had a very high content of indigestible fibre and was loaded with abrasive siliceous compounds, which were difficult to eat and digest (ROBERTS, 1992). The undigested parts were excreted out from the gastrointestinal tract of the Satyr in a highly identifiable form. KHALING (1999) identified 23 food items from the droppings of Satyr tragopan in Eastern Himalayas, India and major portion of food was plant material. She identified *Arundinaria maling* (bamboo species) as a dominant plant species in the diet of Satyr whereas the present study showed another bamboo species *Arundinella nepalensis* in its diet.

During the post monsoon season, the invertebrates formed the major portion of the diet of Kaleej. After monsoon the insect availability increases so as in the diet of Kaleej. Other species were *Geranium wallichianum* and *Viola* sp., which were in full bloom after monsoon. The *Eulophia compestris, Nordostachis jatamansi*, *Picrorhiza kurroa* and *Achonitum heterophylum* were absent or present in minor composition in the diet of Monal during post monsoon season as these herbs were annual. In Satyr Tragopan, the *Arundinella nepalensis* again formed the major component of the diet. Other than these were *Cotoneaster acuminata*, *Rubus biflorus*, *Ariseama flavum*, *Pilia* sp. as these species were found in full-grown stage in the study area.

In the present study invertebrates formed a minor and trace portion in the diet for all pheasant species. It is known from studies in Britain that for high survival rates of Galliform chicks, a protein rich diet is essential (HILL, 1985) and during few weeks of life, this is obtained from insects. Thereafter, the vegetable matter increases until the bird becomes chiefly vegetarian. In this study, we analyzed only adult droppings of all pheasant species and it is apparent that these did not contain a high proportion of invertebrates. However analysis of chick droppings might have revealed a much higher invertebrate content in the diet of the species (GREEN, 1984; HILL, 1985).

Another very significant component found in the diet of all pheasant species was grit fragments. The presence of grit in such high percent composition of the diet of the pheasant species around the year may be attributed to the fact that being mainly vegetarians these fragments helped in the grinding of the vegetative matter in the gizzard for proper digestion.

These pheasant species occupied different altitude with a little niche overlap (HUSSAIN, 2002). The least similarity was found between Kaleej and Monal as Kaleej occupies lower altitude than Monal and plant species also differ on various altitudes. Koklass had a very wide range of habitat use so this species was moderately similar to other species in the context of food items intake. Since more accurate (SANDE *et al*., 2006) but highly invasive methods like crop and stomach draining (PARALIKIDIS *et al*., 2010) are not practicable on threatened and rare Galliformes species. These pheasants used different habitat types in the study area which were not free from biotic pressures (MCGOWAN, 2007). The information on diet items of these pheasants will help to conserve habitat having rare and threatened plant species in general and pheasants of this area and of Central Himalayas in particular.

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Successional Pattern, Stand Structure and Regeneration of Forest Vegetation According to Local Environmental Gradients

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Abstract. Despite the attempted botanical and ecological studies so far, integrated picture of successional and regenerative pattern of the forest vegetation in the studied area have not been achieved. Moreover, stand composition and development of these forests have never been studied in the context of environmental gradients. This study aims to integrate and clarify the accumulated knowledge about the successional pattern, stand structure and regeneration in the studied territory. It has also attempted to test some classical viewpoints about the forest vegetation pattern, placed in the context of environmental gradients. We hypothesized that most forest stands will follow the normal diameter distribution. Gradient-transect sampling procedure was used. Accumulated field samples were classified using TWINSPAN clustering method. Obtained forest community types were tested for consistency. Distribution of stand stem number by diameter classes was tested with Shapiro-Wilk test for normality. Stand successional distribution followed its own trajectory and no convergence has been found. All stands had normal diameter distribution and compromised seed regeneration, i.e. they were in "stagnant" condition. Dominant trees also had normal stem distribution except beech stands from the most xeric habitats, but this was due to their sprouting regeneration. We hypothesized that this regeneration pattern is due to erroneous management and lack of major natural disturbances in the area during the last decades, which could have drew the stands from "stagnancy" and restart the seed regeneration. If this tendency is maintained we suppose that it will lead to continuing degradation of local forest vegetation.

Keywords: Balkans, succession, climax, diameter distribution, inversed *J*-curve, moisture gradient, stand structure, regeneration.

Introduction

Vegetation literature often speaks of plant communities existing in climax condition, but data supporting this hypothesis for the compositional species populations seldom can be found. Vegetation managers traditionally accept the climax idea in theory as well as in practice, therefore identification of climax condition has a great practical significance. Demonstrating vegetation steady state in given community requires repeatable measurements over time greater or comparable with the complete turnover period of all species populations. However, such measurements have been taken only for short-living plants. For the forest communities, dominated by long-living species, documenting of all species population dynamics for the whole turnover period is impossible. This is most frequently done only for a short period. Hence, for the climax condition of given vegetation it can only be supposed based on past and present characteristics of the compositional species populations (VEBLEN, 1992).

When the forest is in relatively steady-state condition it is characterized with

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balanced mortality and regeneration rates. Forest stand, where there are numerous young individuals and lesser mature ones, is recognized as having an inverse *J*-curve diameter distribution (MEYER, 1952; LEAK, 1964, 1965; PEET, 1981). If mortality and birth rate of individuals from the different diameter classes is constant then diameter distribution can be described with negative exponential curve, which has the form of inversed *J*-curve. However, if mortality decreases steadily relative to size, diameter distribution is power function, which is more concave *J*-curve. Because the lower light levels under the canopy and the intense competition between the young individuals in most forests, species early mortality rate is higher (HETT, 1971; HETT & LOUCKS, 1971; PEET, 1981). Mortality slows down with the aging until senescence is reached. However, if senescence phase is included in the diameter class analysis then diameter distribution, more probably, has sigmoid curve form (GOFF & WEST, 1975). No matter what the model is or whether it is applied on diameter or age classes, the result, in principal, is inversed *J*-curve distribution, which indicates the presence of much more young individuals than mature ones (PEET, 1981). Diameter distribution in successional stands, however, has much more variable form. When disturbances destroy great part of the stand, many young individuals establish on the cleared place. With the stand aging, these saplings deplete the limiting resources and impede the new seedling establishment. In these cases diameter distribution curve most often has skewed bell-shaped form (BAILEY & DELL, 1973; BLISS & REINKER, 1964; DAY, 1972; ILVESSALO, 1937; NELSON, 1964; PEET, 1981). The height and breadth of the curve is influenced from the initial density and synchronization of the seedling establishment. The most important here is that the more favorable the habitat conditions are, and the more severe the competition is, the more intensive is the initial resource depletion. This process leads to sapling suppression and seedling elimination until mortality among the mature individuals relaxes the competition (PEET, 1981). Considering these assumptions, for a stand with *J*-curved diameter distribution of principal tree species can be claimed that it is in relatively steady state. Deviations from this condition can be interpreted as evidence for weak reproduction or prior disturbance event (JACKSON & FALLER, 1973; JOHNSON & BELL, 1975; SCHMELZ & LINDSEY, 1965; PEET, 1981).

Three types of forest development under different habitat conditions are known (PEET, 1981), which are defined as points in the continuum of potentials possibilities. These have been applied to: 1) favorable sites, found on moderate elevation in the middle or moesic part of the moisture gradient; 2) unfavorable sites, found on higher elevation or in extremely xeric places on middle and lower elevation; and 3) episodic sites, distributed on lower elevation along the ecotone between forest and open habitats.

Environmental gradient classification results in group of stands with variable age, but with similar habitat conditions and potential for development, in which successive change of stand population structure can be established (PEET, 1981). Bell-shaped curves have been acknowledged (PEET, 1981) as an indicator for even-aged stands, where curve breadth is consequence of the establishment period duration, initial seedling density and habitat quality. The inversed *J*-curve is indication for successful regeneration and approximation of stand steady-state condition. Since the variation of habitat condition, seed arrival and disturbance history may influence successional rate, stand arrangement on the basis of diameter distribution makes them more comparable than their arrangement by age structure alone (PEET, 1981).

The three types of stand development are useful models and referent points in the continuum of stand structural variation. They can be used as frame for the interpretation of multiple aspects of forest ecology. Diversity, basal area, biomass, productivity and stability of forest communities are indicators, which are regulated by the dominant species population dynamics, which in their turn react to the environmental conditions. Studying population dynamics during the forest succession should also be placed in the context of environmental gradients. Due to the extensive data required in the stand development studies, most of them fail in their attempt to bound obtained results with the environmental or successional characteristics (PEET, 1981).

Attempted botanical and ecological studies so far have not brought an integrated picture about the successional and regenerative pattern of the forest vegetation in the studied area. Moreover, stand composition and development of these forests have never been studied in the context of environmental gradients. This study is an attempt to expand and clarify the accumulated knowledge about the forest stand structure and regeneration. It has also attempted to test some classical viewpoints about the forest vegetation pattern, placed in the context of environmental gradients. We hypothesized that most forest stands will follow the normal diameter distribution. We also tried to predict the future successional trajectory of the studied forests as far as possible using this methodology. Accomplishing our tasks will improve vegetation understanding of the investigated territory, which in turn will ease undertaking forest management activities in the future.

Study area

Vitosha Mountain is located in Western Bulgaria. Since it is a relatively young mountain (SHIPKOVA, 2005), it is characterized with compactness and well expressed elevation gradient. The mountain has steep slopes and variable expositions. Its vegetation has varied and rich species composition. Most of the mountain's territory is declared Nature Park by the Bulgarian legislation. The current study embraces the south slope of the mountain, covering all forested habitats. GPS coordinates of that territory are between N42°32′ E23°09′ and N42°26′ E23°21′. This area covers 118 km^2.

Vitosha Mountain is formed during the late Cretaceous and early Tertiary period. The most widely distributed bedrocks are Paleozoic sediments and early Mesozoic sediments. The highest peak is Cherni peak reaching 2290 m (SHIPKOVA, 2005).

Mean annual rainfall is between 650-700 mm in the mountain base and around 1000 mm in the highest parts. Annual rainfall distribution has one peak and the most of the precipitation falls in the April-July period. The most arid period is late summer and early autumn. The highest parts of the mountain (above 1800 m) almost all year round are exposed to strong southwest and west winds, reaching up to 8m/sec (KOLEVA, 2005).

Vitosha Mountain has a great variety of soils. In the lowest mountainous parts dominate *Chromic Cambisols*. In the elevation belt 1400-1750 m the most widely distributed are *Cambisols*. In the highest parts of the mountain (1750-1900 m) prevail *Mollic Cambisols*. In the subalpine and alpine zones are formed *Umbrosols*. *Fluvisols* are present along the lower riverbeds (MALINOV, 2005).

Vitosha's natural vegetation was developed during the last ice age. According to palaeobotanical studies, the floristic composition of the territory has not changed essentially. Substantial vegetation alteration took place later, mainly due to human activity. During the 15-19 centuries, mining, primitive metallurgy and nomadic cattle breeding were developed in the region. The need of wood materials led to forest clearing over most of the mountain. Finding of pasture for the numerous herds necessitated setting of periodical fires in the high mountainous parts. This resulted in almost complete destruction of the natural vegetation. These factors, together with the steep relief and the heavy rain conditions, led to developing of erosion processes and the following degradation of the natural habitats (MESHINEV, 2005).

Material and methods

Sampling. In the summers of 2008 and 2009, systematically, along a preliminary drawn vertical transects on the south slope of Vitosha Mountain, 114 0.1 ha (20×50 m) vegetation samples were taken (Fig. 1).

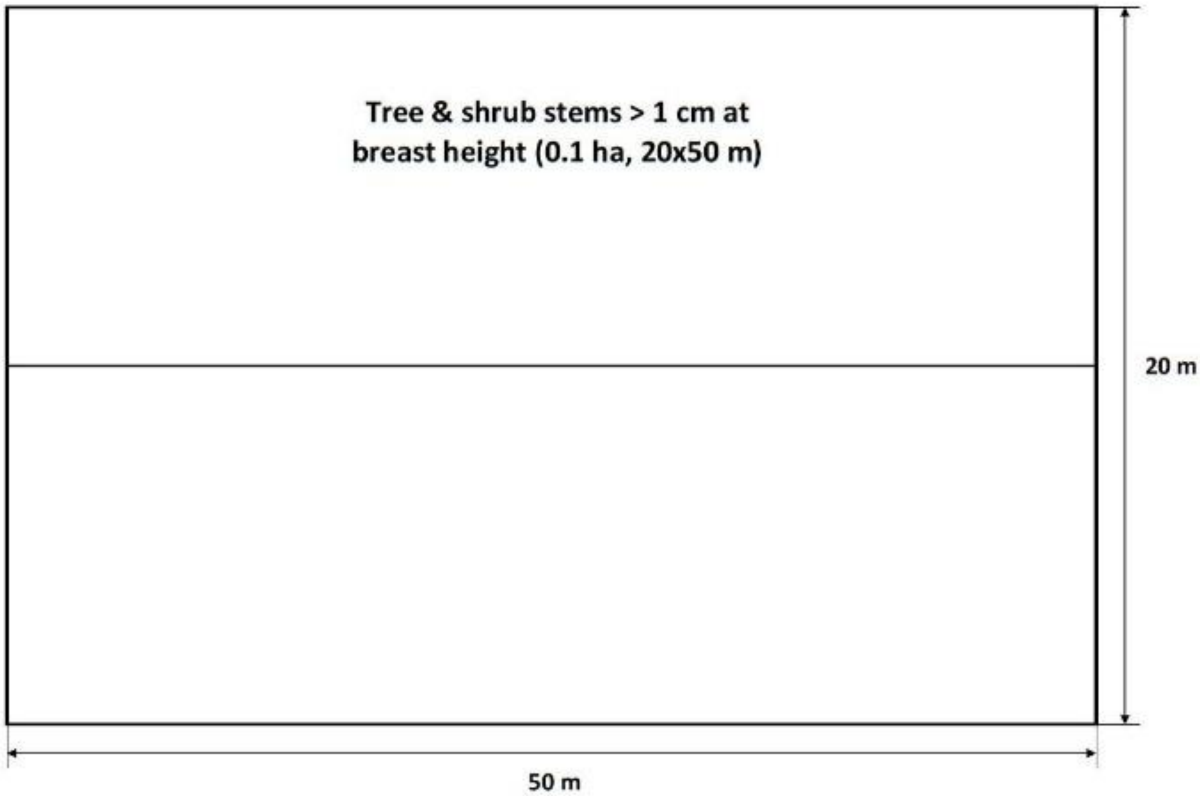

Fig. 1. Sampling plot, modified from WHITTAKER (1956, 1960). For more details on sampling procedure see the text.

Totally nine transects, following the main and intermediate Earth directions (E, EES, ES, SSE, S, SSW, SW, SWW, and W) were laid. Transects start at around 900 m and reach the tree line. Samples were taken at uniform distance of 50 m altitude along the vertical transect. Starting position of transects is randomly chosen after numbering of five possible starting points and pulling one of them. Sampling places are located in such a way so they can cover the maximum variety of expositions, slope inclinations, slope topography and elevations. The exact sampling sites are chosen visually keeping the requirement for vegetation homogeneity, i.e. they should not be located in the forest periphery or in large open forest patches. Forest communities under intensive human influence (intensive livestock grazing or logging) are avoided. In order to avoid spatial autocorrelation the distance between neighboring sampling plots is at least 200 m.

On the place chosen, 50 m plastic tape is laid on the ground, perpendicular to topographic horizontals. From the two ends of the tape toward its two sides, perpendicular to it, 10 m distance is measured and metal stakes are fixed into the ground. Obtained in this way rectangular plot has 20×50 m sides (Fig. 1). First, total tree stratum cover is measured visually in percents. Then, diameter of all tree and shrub stems >1 cm at breast height (≈ 1.30 cm) is recorded by species and grouped by diameter classes.

Undetermined on the field individuals were taken as herbarium specimens and transported to laboratory for species determination. Nomenclature and systematics follows JORDANOV (1989) and KOJUHAROV (1995). DELIPAVLOV (1992), JAVORKA (1975) and Flora Europaea (TUTIN *et al.*, 1968-1993) were also used.

Classification and statistical tests. In the current study TWINSPAN classification (Two Way INdicator SPecies Analysis) (HILL & ŠMILAUER, 2005) was used. The basic idea in TWINSPAN is that each group of samples can be identified based on indicator species, i.e. such species that prevail at the one side of the dichotomy. TWINSPAN gives the opportunity of processing qualitative and quantitative data. The software TWINSPAN not only classify the samples but also produces two-way ordered data table (samples x species). In construction of TWINSPAN table, two-way weighted average algorithm of Correspondence Analysis (CA) (HILL, 1973) was used. Combination of the two has made the method one of the most popular among the vegetation ecologists nowadays (VAN TONGEREN, 2004). Aiming to investigate in details dominant tree stand structure in the context of moisture gradient, all samples where *Fagus sylvatica* and *Pinus sylvestris* dominated were grouped in moisture ecological groups (WHITTAKER, 1956): moesic, submoesic, subxeric and xeric habitats.

Community types were tested for statistically significant difference with nonparametric ANOVA on Ranks, Dunn's method, because they had not normal distribution. Stem distributions for the different community types and moisture groups were checked for normality using Shapiro-Wilk normality test. If not noted otherwise, in all analyses the significance level is $P \leq 0.05$. The following specialized software products are used: STATISTICA, version 8.0 (STATSOFT, 2004), SigmaPlot for Windows, version 11.0 (SYSTAT SOFTWARE INC., 2008).

Results

In the summers of 2008 and 2009 totally 114 0.1 ha samples were taken. These were classified using TWINSPAN software, which results have been published in a previous paper (DYAKOV, 2012). The following analyses were done in the context of that classification scheme. As can be seen from Table 1, six of all forest communities were dominated by *Fagus sylvatica* L. In the other three community types *Pinus sylvestris* L., *Pinus nigra* Arn., *Quercus cerris* L., *Carpinus betulus* L., *Crataegus monogyna* Jacq. and *Cornus mas* L. were dominant. Arranged in this way, community types express the complex environmental gradient, resulting mainly by elevation and habitat moisture gradient interaction. In the very left part of the table, most moesic beech forests are placed, followed by the submoesic beech communities, and then subxeric and xeric ones. In the very right table part are located the subxeric and xeric coniferous forests (plantations), and xeric mixed oak communities.

Forest community types were tested for significant differences in median stem number per 0.1 ha by all diameter classes. Results are shown in Table 1. Significant differences are few. In the thinner diameter classes (1-10 cm) *Fagus* communities are almost indistinguishable one another. Yet, we found significant differences between *Fagus sylvatica-Festuca drymeia* and *Fagus sylvatica-Gallium odoratum* in 5-10 cm diameter class. Another significant difference, between *Fagus sylvatica-Festuca drymeia* and coniferous forests (*Pinus sylvestris-Fragaria vesca* and *Pinus nigra-Crataegus monogyna*) in 5-10 cm diameter classes, was also found. Significantly, higher number of thinner stems in the former community is result of the sprouting mode of regeneration of these forests. Among the middle classes (10-25 cm) significant differences are also few. These are mainly among the coniferous plantations and submoesic beech communities, the former having significantly higher number of stems in 20-25 cm diameter class. The only significant difference among the *Fagus* types is between *Fagus sylvatica-Festuca drymeia* and *Fagus sylvatica-Gallium odoratum* in 5-10 cm diameter class and between *Fagus sylvatica-Gallium odoratum* and *Fagus sylvatica-Hepatica nobilis* in > 35 cm diameter class (Table 1).

Differences in the thickest classes are even lesser. These are between some beech forests and *Pinus nigra-Crataegus monogyna* community type, the later having significantly higher stem number in the 20-25 cm diameter class. Regarding the total stem number per 0.1 ha, forest communities are statistically indistinguishable (Table 1).

According to the obtained results from the statistical comparison of forest community types, we conclude that the forest communities in the studied area are

Table 1. Forest community types in the studied territory. Upper part of the table shows community type names (composed of the Latin names of dominant tree, shrub or herb species) and forest ecological groups. Lower part represents the median stem number by diameter class ± quartile range. In the last row, the total stem number per 0.1 ha in forest communities is given. Stem number per 0.1 ha for the forest community types are tested for differences with ANOVA on Ranks, Dunn's method. Medians with different letters in the rows are significantly different at $P \leq 0.05$ level.

	Forest communities dominated by *Fagus sylvatica*						Mixed forest communities dominated by *Pinus* and *Quercus* genera		
	Moesic beech forests		Submoesic beech forests			Subxeric and xeric beech forests	Subxeric and xeric coniferous forests (plantations)		Mixed xeric oak forests
Forest community types	*Fagus sylvatica-Hepatica nobilis (n = 8)*	*Fagus sylvatica-Physospermum cornubiense (n = 12)*	*Fagus sylvatica-Galium odoratum (n = 13)*	*Fagus sylvatica-Luzula luzuloides (n = 23)*	*Fagus sylvatica-Festuca drymeja (n = 17)*	*Fagus sylvatica-Corylus avellana-Brachypodium pinnatum (n = 15)*	*Pinus sylvestris-Fragaria vesca (n = 15)*	*Pinus nigra-Crataegus monogyna (n = 5)*	*Quercus cerris-Cornus mas (n = 6)*
Diameter classes (cm) (stem number per 0.1 ha)									
1-5	12 ± 23	13 ± 13	17 ± 29	11 ± 27	20 ± 17	28 ± 25	7 ± 19	21 ± 14	32 ± 55
5-10	77 ± 95ab	44 ± 53ab	30 ± 49b	39 ± 60ab	80 ± 143a	68 ± 81ab	13 ± 39b	17 ± 12b	41 ± 97ab
10-15	65 ± 51	34 ± 69	19 ± 26	16 ± 46	69 ± 80	51 ± 74	29 ± 103	12 ± 16	49 ± 81
15-20	31 ± 26	29 ± 31	15 ± 14	15 ± 45	45 ± 39	27 ± 66	32 ± 103	34 ± 45	45 ± 13
20-25	20 ± 10ab	25 ± 14ab	12 ± 11a	15 ± 19a	25 ± 22ab	19 ± 24ab	39 ± 36b	59 ± 40b	25 ± 28ab
25-30	5 ± 8	11 ± 9	9 ± 9	10 ± 16	9 ± 15	9 ± 6	23 ± 32	40 ± 40	9 ± 6
30-35	-	9 ± 5	7 ± 5	5 ± 6	6 ± 10	4 ± 7	10 ± 20	12 ± 12	7 ± 7
>35	1 ± 4a	10 ± 12ab	16 ± 12b	10 ± 12ab	2 ± 6ab	6 ± 10ab	1 ± 7ab	-	1 ± 2ab
Total	**169 ± 344**	**269 ± 250**	**242 ± 165**	**393 ± 469**	**346 ± 1015**	**338 ± 666**	**336 ± 502**	**77 ± 110**	**173 ± 325**

statistically indistinguishable by their stem diameter class distribution.

Forest stand description. Stem number distribution of *Fagus sylvatica-Hepatica nobilis* community type is shown in Fig. 2. Table 2 represents the normality test of stem distribution in the different forest communities.

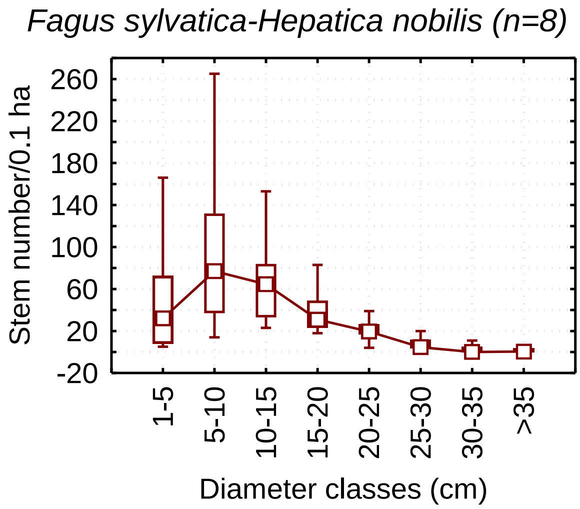

Fig. 2. *Fagus sylvatica-Hepatica nobilis* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Stem distribution by diameter classes of the first community type is normal (Table 2) with greater variance in the small and middle classes (1-20 cm) (Fig. 2). Bell-shaped curve is resultant from the unsuccessful stand regeneration, and particularly of *Fagus sylvatica*.

Most abundant species in the thinner and middle diameter classes are sycamore *Acer platanoides* L., *Acer campestre* L., *Acer pseudoplatanus* L. and *Carpinus betulus*. Forest undergrowth is dominated by species like *Cornus mas, Sorbus aucuparia* L., *Corylus avellana* L., *Viburnum lantana* L., *Prunus avium* L. and *Crataegus monogyna*. With insignificant share in the small and middle diameter, classes are *Ulmus glabra* Huds. and *Fraxinus ornus* L. Because of the weak regeneration of the beech in this community type, it is expected to compete for dominance in the next decades with species like *Carpinus betulus, Acer platanoides, Acer campestre* and *Acer pseudoplatanus*. If the current tendency is retained, the transformation of these forests into coppice beech communities, despite the favorable habitat conditions, is unavoidable.

Fagus sylvatica-Physospermum cornubiense community type also has normal diameter class distribution (Fig. 3, Table 2) with greater variance among the thinner stems (1-15 cm).

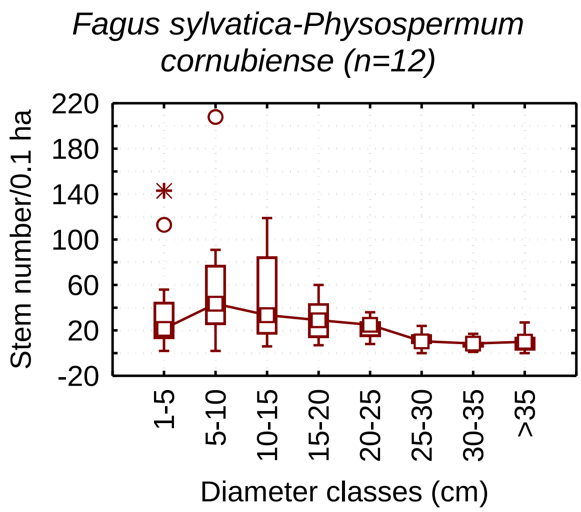

Fig. 3. *Fagus sylvatica-Physospermum cornubiense* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Fagus sylvatica dominates here too, but this is more clearly expressed in the thinner classes. In the diameter classes greater than 15 cm, *Tilia platyphyllos* Scop. prevailed. *Carpinus betulus* and *Acer campestre* are also well represented in the thinner and middle classes. Undergrowth composition is rather diversified, but with prevalence of species like *Corylus avellana, Picea abies* (L.) Karst., *Pinus sylvestris, Ulmus glabra, Prunus avium* and *Crataegus monogyna*. Here can often be found species like *Quercus petraea* (Matt.) Liebl. and *Sorbus aucuparia*, taking part mainly in the middle classes, and rarely in the thickest ones. However, in the thickest diameters only species like *Pinus nigra* и

Populus tremula L. take part. Exceptionally, in the middle and thicker diameter classes, species like *Fraxinus excelsior* L. and *Alnus glutinosa* (L.) Gaertn. can also be found.

Because of its dominance in the thinner diameter classes, mainly with sprouting regeneration, it is expected that *Fagus sylvatica* will dominate in the future successional stages together with species like *Carpinus betulus* and *Acer campestre*. *Tilia platyphyllos* almost completely lacks from the thinner classes, therefore, most probably, it will be excluded from the stand and will be replaced by *Fagus sylvatica* and *Carpinus betulus*.

Community type *Fagus sylvatica-Galium odoratum* looks similar to the previous one, except that the common beech has more weakly expressed dominance in the stand.

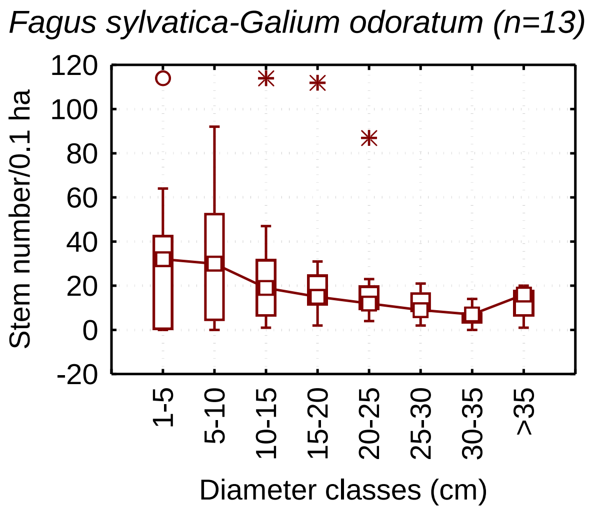

Fig. 4. *Fagus sylvatica-Galium odoratum* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✳ Extremes).

Stem distribution here is normal too (Fig. 4; Table 2). In the thinner classes (1-10 cm) dominate *Fagus sylvatica, Picea abies, Pinus sylvestris* and *Corylus avellana*. With weaker participation are species like *Fraxinus excelsior, Crataegus monogyna, Sorbus aucuparia* and *Prunus cerasifera* Ehrh. In the middle classes (10-25 cm), *Pinus sylvestris* and *Tilia platyphyllos* prevailed, followed in dominance by *Fagus sylvatica.* With small percentage, here is *Picea abies,* but together with it can be found also species like *Betula pendula* Roth, *Salix caprea* L. and *Prunus cerasifera. Fagus sylvatica* and *Tilia platyphyllos*, together with *Pinus sylvestris*, dominate the thickest (25-35 cm) stem diameters. Here are also present *Betula pendula* and *Picea abies*. With negligible proportion are *Acer pseudoplatanus* and *Salix caprea*.

Considering the thinner stem composition, it can be asserted that with the successional progression *Pinus sylvestris* and *Tilia platyphyllos* will be eliminated from the forest stand. They, most probably, will be replaced by *Fagus sylvatica* and *Picea abies*, which have greater participation among the thinner stems.

Stem distribution by diameter classes in *Fagus sylvatica-Luzula luzuloides* type is normal (Fig. 5, Table 2). In this and the next community type, *Fagus sylvatica* reaches its greatest dominance, but the species number reaches its minimum. The beech dominates thinner classes exclusively with the weaker presence of species like *Picea abies, Carpinus betulus, Crataegus monogyna, Salix caprea* and *Prunus cerasifera*. In the middle diameters classes the two principal trees *Fagus sylvatica* and *Picea abies* prevailed too.

With lesser importance, here are species like *Pinus sylvestris* and *Sorbus aucuparia. Fagus sylvatica* and *Picea abies* dominate the thickest classes too, but here larger trees like *Betula pendula* can also be found, forming small groups in some more open sites in the stand. It is certain that for few decades, with the canopy closure, they will be excluded completely. It is doubtless that with the successional progression *Fagus sylvatica* dominance will persist, despite its exclusively sprouting regeneration. *Picea abies* will also have its role in the stand development of these forest communities in the next decades.

Fagus sylvatica-Festuca drymeia community type at greater extent resembles the previous one. Stem distribution of diameter classes of all species is normal (Fig. 6, Table 2).

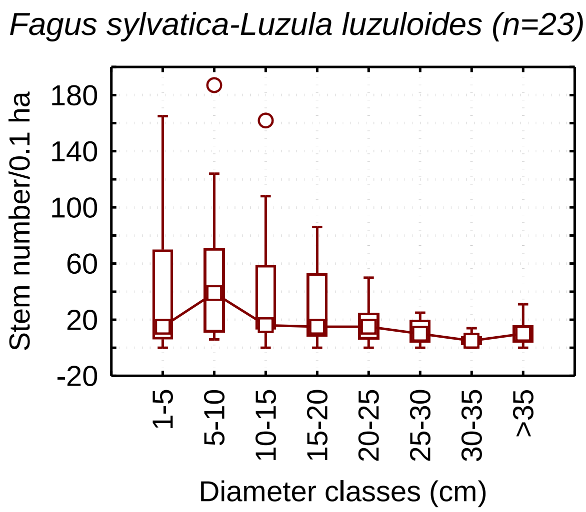

Fig. 5. *Fagus sylvatica-Luzula luzuloides* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Fagus sylvatica dominates the thinner classes exclusively. Here can be found only separate individuals from species like *Carpinus betulus, Sorbus aucuparia* и *Pinus sylvestris.*

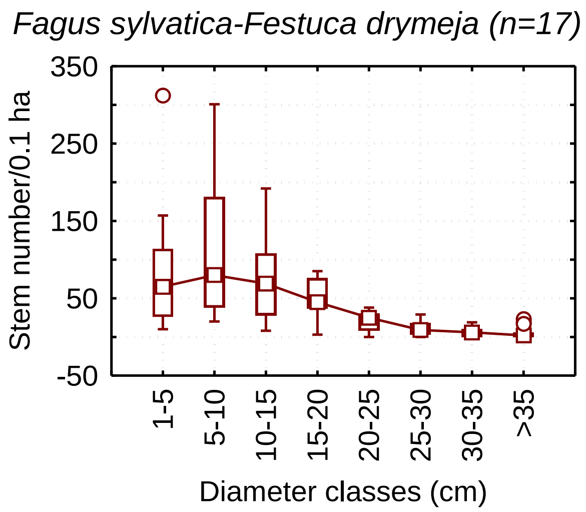

Fig. 6. *Fagus sylvatica-Festuca drymeia* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Species richness in these communities is extremely low, reaching only six tree and shrub species per 0.1 ha. Thicker stem classes are dominated by three tree species, having almost equal importance. These are *Fagus sylvatica, Pinus nigra, Pinus sylvestris* as well as some individuals of *Betula pendula* can also be found.

Successional development of these forests will progress toward beech dominance and pure stand formation. This is so because from the late-successional species only the beech takes part in the thinner stem diameters. *Carpinus betulus* will also be present, but with negligible share. Here, like the former community type, the beech has completely sprouting regeneration.

Fagus sylvatica-Corylus avellana-Brachypodium pinnatum is the most xeric forest type, having greatest diversity of tree and shrub species. *Fagus sylvatica* and *Pinus sylvestris* dominate the stand. Stem diameter distribution is normal (Fig. 7, Table 2).

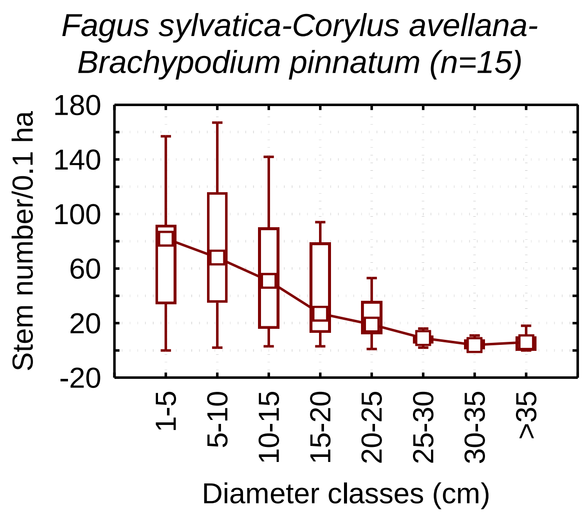

Fig. 7. *Fagus sylvatica-Corylus avellana-Brachypodium pinnatum* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Species composition among the thinner diameter classes is variable, but *Fagus sylvatica, Pinus sylvestris, Betula pendula, Corylus avellana* and *Picea abies* dominate. Less important are *Salix caprea, Prunus cerasifera, Crataegus monogyna, Cornus sanguinea* L., *Sorbus aria* (L.) Crantz, *Malus* *sylvestris* Mill. and *Populus tremula*. In the middle diameter classes dominate *Pinus sylvestris* and *Fagus sylvatica* and less well represented are *Quercus cerris, Quercus petraea, Picea abies* and *Salix caprea*. With negligible share are *Carpinus betulus, Pyrus pyraster* Burgsd. and *Prunus cerasifera*. *Pinus sylvestris* and *Quercus cerris* prevailed in the thickest classes. *Fagus sylvatica* and *Quercus petraea* were more weakly represented. *Picea abies* and *Prunus avium* were also present, but with isolated individuals.

Because the variable composition of the thinner stems, it is impossible to predict the successional direction of these forests and their probable composition in the next decades. The most likely dominant is the beech, accompanied by the oaks and, at some places, for a short period of time, by *Betula pendula*. *Pinus sylvestris* will probably be eliminated completely from the stand.

Pinus sylvestris-Fragaria vesca are co-dominated by *Pinus nigra* with greater stem number variance among the middle diameter classes. Stem distribution of all stems is normal (Fig. 8; Table 2). This forest type has artificial origin (i.e. plantation) and is extremely influenced by human activities – mainly from livestock grazing, insect attacks, forest fires and logging.

Thinner stem classes are dominated by *Fagus sylvatica* together with *Betula pendula*. *Rosa canina* L. has significant share. Because of the more opened canopy of the stand and the excess light reaching the forest floor, great number of shrubs like *Evonymus europaeus* L., *Crataegus monogyna* and *Prunus cerasifera* participate in the undergrowth. *Pinus sylvestris* and *Pinus nigra* sustain less numerous populations in this forest stratum as well as separate individuals of *Quercus petraea, Sorbus aucuparia* and *Acer pseudoplatanus* also can be found.

In the middle classes prevail the pines and, at lesser extent, *Betula pendula*. At some places, along temporal or permanent streams, isolated individuals of *Alnus glutinosa* survive. In the shadiest habitats, some individuals of *Fagus sylvatica* and *Tilia cordata* Mill. can be found. The thickest diameter classes are mainly dominated by the pines with the lesser participation of *Tilia cordata* (in the moister and shadier places). Isolated individuals of *Fagus sylvatica* can also be found.

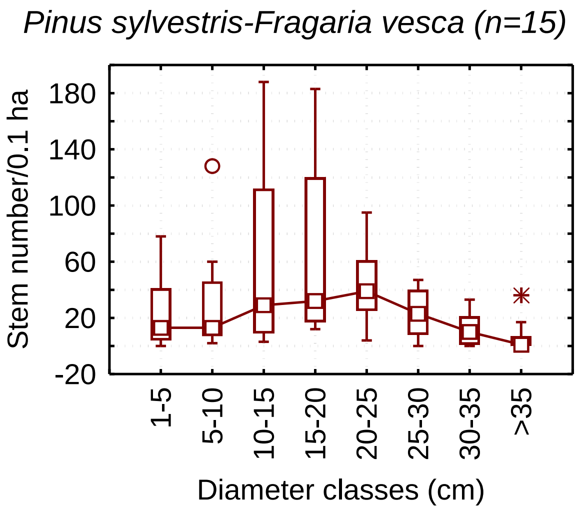

Fig. 8. *Pinus sylvestris-Fragaria vesca* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⊥ Non-Outlier Range ○ Outliers * Extremes).

Because of their poor regeneration, successional development of these forests will be directed toward pine replacement by coppice beech communities. Probable reason for the unsuccessful regeneration of these communities, except unfavorable growth conditions, is the well-developed herb cover of the forest floor, precluding seedling survival. Unless new disturbances take place, this situation will prolong. *Betula pendula* will be probable companion of *Fagus sylvatica* for a few decades until its complete elimination from the stand. Otherwise, succession will be restarted or returned in earlier phase, precluding late-successional species of taking dominance.

Pinus nigra-Crataegus monogyna forests are more moesic, growing more frequently on shadier north-facing slopes. Stem distribution here is normal too (Fig. 9, Table 2).

Generally, *Pinus nigra* and *Pinus sylvestris* are the dominants, followed by *Fagus sylvatica*, which has mostly sprouting regeneration. In the thinner classes, there is no clearly pronounced single dominant. However, with greater importance are species like *Quercus petraea, Carpinus betulus, Acer campestre, Crataegus monogyna* and *Fagus sylvatica*.

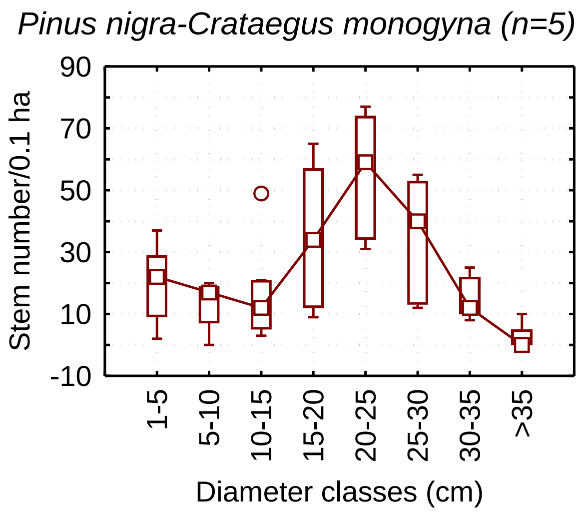

Fig. 9. *Pinus nigra-Crataegus monogyna* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

These forests are characterized with their relative heterogeneity, having multiple open patches in the stand and greater species diversity in the undergrowth. Species with negligible abundance among the thinner stems are *Viburnum lantana, Prunus cerasifera, Cornus sanguinea, Corylus avellana* and *Cornus mas*. In the middle classes oaks and pines dominate. These are *Pinus nigra, Pinus sylvestris, Quercus petraea, Quercus frainetto* Ten. and *Quercus cerris*. The pines prevail almost exclusively in the thickest diameter classes. Some individuals of the oaks also can be found.

Given the thinner and middle class stem composition, successional direction cannot be predicted at this stage. Most probable successional trajectory is toward elimination of the pines, because of their poor regeneration, their replacement by the beech and *Carpinus betulus*, but with the considerable participation of the oaks or even their dominance, especially of *Quercus petraea*. Because the lower elevation of their distribution these forests are strongly influenced by the human activity (for example, livestock grazing and logging). This disturbance regime will be one of the principal factors, determining their future successional development.

Quercus cerris-Cornus mas communities have extremely diversified species composition and stand structure, due mainly to their disturbance regime and the poor habitat conditions.

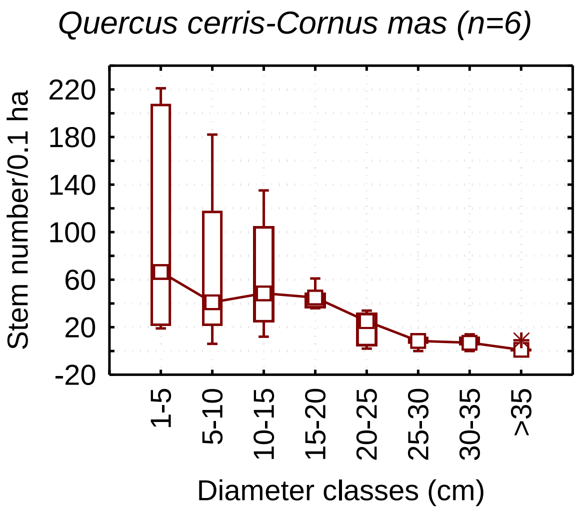

Fig. 10. *Quercus cerris-Cornus mas* community type stem number distribution (> 1 cm at breast height ≈ 1.30 cm) of all trees and shrubs by diameter classes (□ Median □ 25%-75% ⌶ Non-Outlier Range ○ Outliers ✱ Extremes).

Tree stand is heterogeneous with multiple open patches, well developed herb layer, and numerous shrub species. Stem distribution of all species is normal (Fig. 10; Table 2). *Fagus sylvatica* is poorly represented and like the previous two forest types has exclusively sprouting regeneration. Greatest share among the thinner stems have *Carpinus betulus* and *Fraxinus ornus*. Other important species are *Quercus cerris, Quercus petraea, Cornus mas* and *Corylus avellana*. Weakly represented are species like *Cornus sanguinea, Crataegus monogyna, Fagus sylvatica, Ulmus minor* Mill., *Prunus cerasifera, Rosa canina, Malus sylvestris, Sorbus torminalis* (L.) Crantz and *Viburnum lantana*. In the middle diameter classes dominate *Quercus cerris, Quercus* *petraea*, *Carpinus betulus* and *Quercus pubescens* Willd. *Acer campestre* is represented with separate individuals. Species like *Quercus cerris* and *Quercus petraea* completely dominate the thickest classes. Less important are *Carpinus betulus* and *Acer campestre*.

Successional trajectory of this forest type will be predetermined from the heterogeneous stand composition, resulting from natural and anthropogenic disturbances, as well as from the poor and dry conditions of occupied habitats. These complex factors preclude one or few competitively superior species of assuming complete dominance over the rest and their elimination from the stand.

Table 2. Forest community stems distribution. *W* value shows the result of Shapiro-Wilk test of the hypothesis that tested variables have normal distribution. When *P > 0.05* the distribution is normal

Variable (stem number per 0.1 ha)	Distribution	*W* (Shapiro-Wilk test for normality)	*P*
Fagus sylvatica-Hepatica nobilis	Normal	0.911	0.359
Fagus sylvatica-Physospermum cornubiense	Normal	0.924	0.467
Fagus sylvatica-Galium odoratum	Normal	0.943	0.639
Fagus sylvatica-Luzula luzuloides	Normal	0.930	0.516
Fagus sylvatica-Festuca drymeia	Normal	0.885	0.208
Fagus sylvatica-Corylus avellana-Brachypodium pinnatum	Normal	0.887	0.221
Pinus sylvestris-Fragaria vesca	Normal	0.933	0.545
Pinus nigra-Crataegus monogyna	Normal	0.934	0.552
Quercus cerris-Cornus mas	Normal	0.905	0.321

In order to analyze stand development tendency in more details, populations of the two principal tree species in the region (*Fagus sylvatica* and *Pinus sylvestris*) were examined separately. Beech forests from the poorest and driest habitats were separated in fourth group – xeric habitats. *Fagus sylvatica* population from the favorable and subxeric habitats (*sensu* PEET, 1981) is characterized with normal distribution. Only xeric habitats are with lognormal stem distribution. In the latter case, this distribution pattern is caused by the sprouting regeneration of the beech in these places and the numerous thinner stems, producing the inversed *J*-curve (Fig. 11, Table 3).

Table 3. *Fagus sylvatica* stem distribution in the four habitat types. *W* value shows the result of Shapiro-Wilk test of the hypothesis that tested variables have normal distribution. When *P > 0.05* the distribution is normal. Significant *P* values are marked with *italic*

Variable (stem number per 0.1 ha)	Distribution	*W* (Shapiro-Wilk test for normality)	*P*
Moesic habitats	Normal	0.920	0.427
Submoesic habitats	Normal	0.900	0.290
Subxeric habitats	Normal	0.853	0.101
Xeric habitats	Lognormal (inversed *J*-curve)	0.725	*0.004*

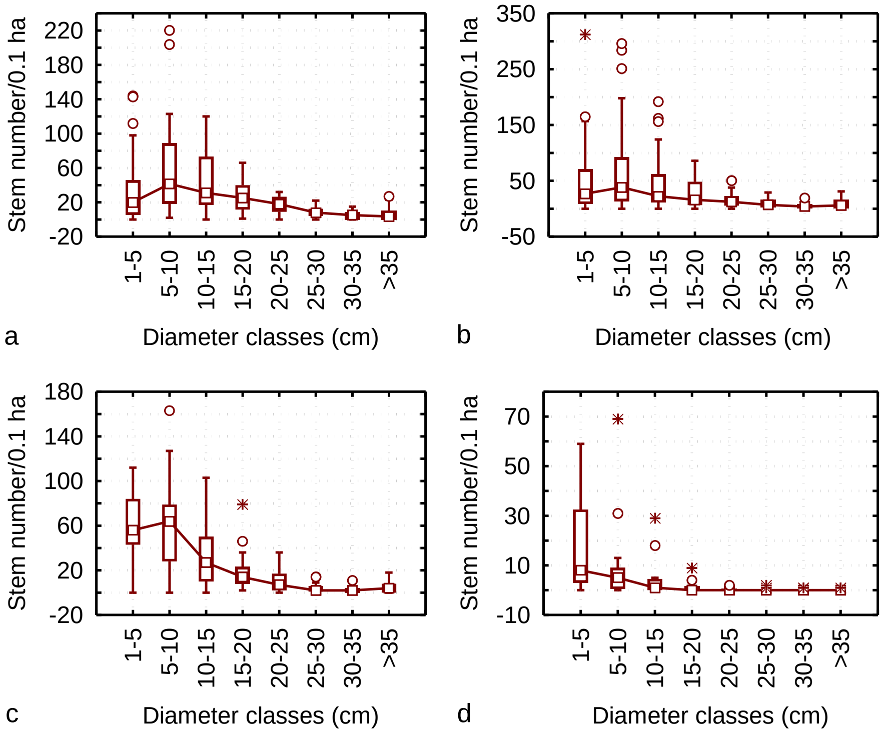

Fig. 11. *Fagus sylvatica* stem number per 0.1 ha (> 1 cm at breast height ≈ 1.30 cm) by diameter classes in four habitat types. a) Moesic habitats (*n = 20*); b) Submoesic habitats (*n = 52*); Subxeric habitats (*n = 13*); d) Xeric habitats (*n = 16*) (Median 25%-75% Non-Outlier Range Outliers Extremes).

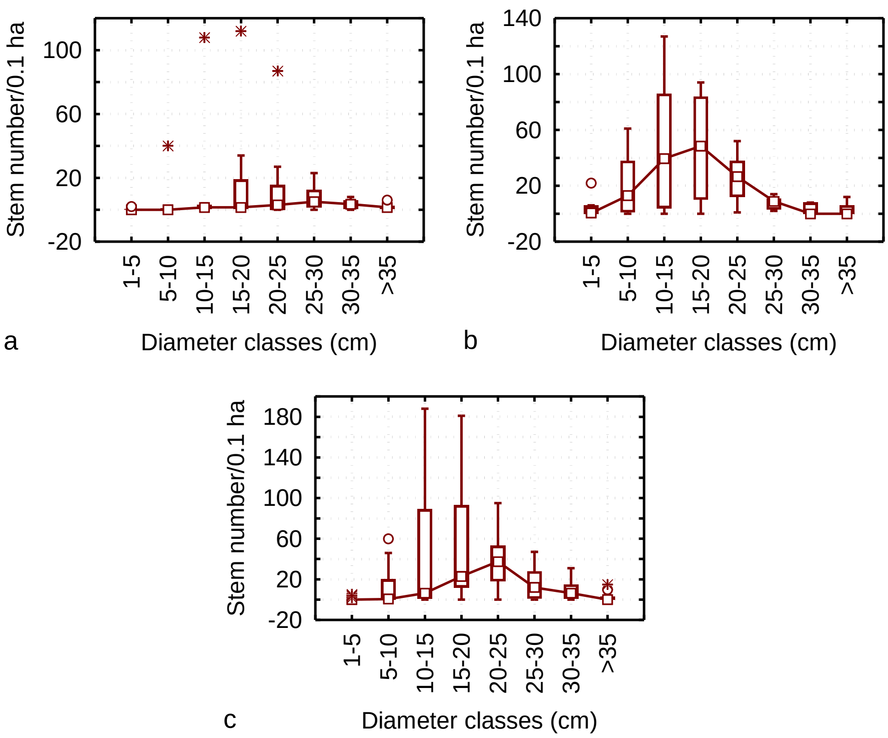

Fig. 12. *Pinus sylvestris* stem number/0.1 ha (> 1 cm at breast height ≈ 1.30 cm) by diameter classes in three habitat types. a) Submoesic habitats; b) Subxeric habitats; c) Xeric habitats. (Median 25%-75% Non-Outlier Range Outliers Extremes).

Table 4. *Pinus sylvestris* stem distribution in the three habitat types. *W* value shows the result of Shapiro-Wilk test of the hypothesis that tested variables have normal distribution. When *P* > *0.05* the distribution is normal.

Variable (mean stem number per 0.1 ha)	Distribution	*W* (Shapiro-Wilk test for normality)	*P*
Submoesic habitats	Normal	0.909	0.348
Subxeric habitats	Normal	0.835	0.068
Xeric habitats	Normal	0.890	0.235

The situation in the favorable places is different. Stems of all diameter classes are present here and the thinner ones prevail (Fig. 11), but this does not contribute for accomplishment of lognormal curve (Table 3).

Stem distribution of *Pinus sylvestris* was also analyzed. We have found normal stem distribution by diameter classes for this species in all habitats (Fig. 12, Table 4). These results also apply to the *Pinus nigra* stands in the region.

Discussion

This study was designed to expand and integrate the accumulated knowledge of the vegetation stand structure and regeneration pattern in the studied area placed in the context of dominant local environmental gradients. It also aimed to investigate the stem diameter distribution of studied stands, which we hypothesized will follow normal distribution. We tried to predict the successional trajectory of forest vegetation in the area based on current stand stem structure.

Consistency of the described forest communities was statistically tested based on their stem number in the different diameter classes. Our results showed that, with few exceptions, forest communities, especially these dominated by the beech, are almost indistinguishable in the environmental space. This result supports the continuum concept of vegetation organization.Most stands that we have analyzed had normal stem diameter distribution as for the total stands as well as for the stem distribution of dominant tree species alone, with the exception of beech stem distribution from the most xeric habitats. This is assumed by some authors to indicate difficulties in regeneration or prior human disturbance events (JACKSON & FALLER, 1973; JOHNSON & BELL, 1975; SCHMELZ & LINDSEY, 1965; PEET, 1981). We suppose that successful regeneration and human disturbances are connected. Possible reason for the unsuccessful seed regeneration is the prevailing even-aged and sprouting stand structure of most beech and pine communities resulting from the management practices (for example, clear-cutting), pushing local vegetation toward homogenization and "stagnancy" (*sensu* WHITTAKER, 1956). Another possible reason is the relative lack of severe natural disturbance (like windfall, for example) events in the area during the last decades, which could have made these forests more heterogeneous.

Beech stands from favorable (moesic) (*sensu* PEET 1981) places have higher stature and most stems are from seed origin. According to Whittaker (1956), the general tendency in the studied by him stands was height and diameter decrease along the moisture gradient from the moistest to the driest habitats. This tendency was in inverse correlation with the stem number per unit area, which increased in the same direction (ILVESSALO 1921, LUTZ 1932). In other words, the more unfavorable (xeric) was given habitat the less was the share of the thickest stems in the stand. Fig. 11 clearly shows the same tendency in our Fagus sylvatica stem distribution. PEET (1981) found inversed *J*-curve distribution for the most stands in the favorite habitats. However, we have found the opposite tendency in all habitats but the most xeric ones where regeneration comes only from sprouting. Canopy closure in the both habitat types is high and the stands have not reached transitional successional phase. This is also evident from the extremely low number of tree and shrub species, which, according to PEET (1981), is minimal in the beginning of second phase. They are arrested in the thinning successional phase, preventing their transition into the transitional stage, characterized by canopy opening and seedling appearance.

Forest practices in the area lean to clear cutting, turning the forest into even-aged stand composed of numerous thin stems with only sprouting regeneration. Low intensity (the so called "regeneration logging") cuttings are also plasticized in the area. We suppose that the stand structural pattern is caused mainly by these activities. Unfavorable growth conditions (extremely dry summers in the last decade) in most places have also played a role for hampering seed regeneration. However, in some distant, inaccessible habitats (mainly moist deep ravines), where forest management is difficult, forest stands are heterogeneous with variable stem numbers from all diameter classes. Because these forest communities are spared from natural disturbances too, canopy opening and overcoming of stagnant forest state is impeded.

According to WHITTAKER (1956) regeneration of stagnant forests, most probably, happens periodically, following partial or complete stand destruction, allowing its replacement in irregular intervals. Unfortunately, forest stands in the studied by us territory, as mentioned above, are relatively speared from severe natural disturbances, which could change the stagnant situation.

In the more xeric habitats, lesser individuals reach the greater diameter classes, which leads to steeper distributional curves (WHITTAKER 1956). The situation in most stands investigated in this study is similar. WHITTAKER (1960) reported that lack of regeneration and normal stem diameter distribution can be result of fire disturbance too. However, we have not found data for severe fires that took place on the studied territory in the last decades.

Coniferous forests in the area have completely artificial origin. They have been planted in these habitats incongruous to the pine species ecology, which led to their even-aged structure and complete lack of regeneration. Stems from the middle diameter classes dominate the stands entirely in these plantations. This is most obvious in the subxeric habitats. The pine stands in the favorable places will be probably completely replaced in the future by the beech. However, in the more xeric habitats, sprouting beech communities together with other broadleaf species (e.g. oaks) will take over. In the most xeric sites, sprouting beech woodlands or/and more open mixed oak forests will dominate.

WHITTAKER (1953) recognized that climax forest can frequently be identified with inversed *J*-curve diameter or age distribution, but the successional one is characterized with discrepancy between the stand and understory composition in particular habitat. However, inversed *J*-curve can also be observed in successional stand or it can be untypical for the unstable climax forests.

Climax communities can be recognized by the regeneration type and by the species dominancy, whether it is stable or variable. Stands can regenerate constantly, regularly or cyclically. Each one of these variants can be seen in forests, where dominant species replaces itself. In other stands, dominant species can alternate cyclically or follow fluctuations and replace themselves irregularly (WHITTAKER 1953).

Similarity between distributional curves from the different moisture gradient parts as for the whole stands as well as for the individual species is regular phenomenon, which according to WHITTAKER (1956) is evidence that none of the stands changes toward another forest type. WHITTAKER (1956) perceived this as lack of convergence toward one climatic climax, with applies to our results too. There is potential for change of one community type to another in the studied territory and it concerns the coniferous plantations, which will be probably replaced by the beech and oaks in the near future. However, this is common and does not constitute exclusion from the rule, given the artificial origin of these plantations.

Conclusion

If the current forest practices continue to operate on this territory, given the xeric habitat condition and continued forest management, we suppose that the current vegetation will change toward more xeric and open one. It could be predicted that all stands, even those from the most moesic places, will be turned into more or less xeric sprouting beech forests. We also presume that in the driest habitats forest vegetation can even turn into woodland composed of sprouting beech or oak accompanied by xeric herb vegetation. Moreover, this structural transition is already obvious in some extremely disturbed and xeric habitats in the studied territory.

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Macrofungal Communities in Hyrcanian Forests, North of Iran: Relationships with Season and Forest Types

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Abstract. The identification of macrofungal genera and species was carried out according to the macroscopic and microscopic characteristics of the specimens, as well as their characteristic responses to some chemical reagents. This study was conducted to identify the forest floor of macrofungal in *Parrotia-Carpinetum* and planted stands of *Cupressus sempervirens* and *Alnus subcordata* in North of Iran (Gorgan, Shast Kalate) during 2010-2011. For this purpose, all macrofungal in the area (0.6 ha in separated stands, three plots) were collected. Measurements were made from slide preparations stained with cotton blue- lacto- phenol contains 100 ml. Lacctophenol, 2ml 2% aqueous solution of cotton blue, by Olympus light microscope. The results show that 35 species were found which were classified into 21 genera from 16 families. Up to 54% of the species were classified into *Parrotia-Carpinetum*, 26% *Alnus subcordata* and 20% *Cupressus sempervirens*. In wet season 30 species of macrofungal and 13 species in dry season were collected. In spring season 12 species, one species in summer season, 23 species in fall season and 7 species in winter season were found.

Key Words: Macrofungi, Agaric, Ecosystem, Macroscopic, Season, Forest stands.

Introduction

Fungi play a vital role in ecosystem functions and have big influence on humans and human-related activities (MUELLER & BILLS, 2004). They are the second largest group of ground creatures (CANNON & HOWKSWORTH, 1995) found in every ecosystem. It is estimated that there are 1.5 million species of fungi, but only about 69 000 species have been studied including, 46,124 species of Basidiomycetes, Ascomycetes. Macroscopic fungi with specific fruiting organs and size, big enough, to be visible by the naked eye, grow on the ground or underground (SAREMI *et al.,* 2005). Although fungi are extremely diverse, they are often ephemeral and cryptic, rendering inventorization difficult (MUELLER *et al.,* 2004a).

Understanding the variation in fungal populations in time and space is important because of its relevance to questions of biodiversity and the roles fungi play in regulating populations of other organisms and ecosystem processes (LODGE & CANTRELL, 1995). HUTTON & RASMUSSEN (1970), compared fungi that were cultured from surfaces of leaves of 20 tree species in moist forest at Fort Clayton, Panama Canal Zone. Although their methods were unclear as to the replication within plant species and their presentation of data make certain analyses difficult, one surprising pattern is suggested by their data. A total of 36 types of fungi were isolated, of which 12 were present only in the rainy season, 12 were present only in the dry season, and 12 were found in both seasons. Three to four fungal species were cultured from leaf surfaces on each tree in the rainy season, although this is probably an underrepresentation of the total epiphyllic mycota. Such a large seasonal turnover in fungal epiphylls was unexpected.

Disturbances induce changes in the environment and the abundance of different substrates, resulting in changes in fungal communities through time, and variation over the landscape. Severe disturbances, as well as the slight daily variations in rainfall, profoundly affect populations of fungal decomposers and their influence on plant nutrient availability (LODGE & CANTRELL, 1995). Some species of agaric fungi are known to live in canopies of rain forests, but fruiting may be rare and confined to the wet season. Disturbances from seasonal changes in rainfall and tree falls to hurricanes can differentially affect fungal species. HUTTON & RASMUSSEN (1970), found in Panama that 8 of the 23 species they sampled had no epiphyllous fungi in common between the rainy and dry seasons and 10 plant species had only one epiphyllous fungus common to both seasons. Epigeous sporocarp productivity greatly increases after the first rains of fall; many species produce sporocarps only during fall (RICHARDSON, 1970).

Hypogenous sporocarp productivity at lower elevations in the Pacific Northwest generally peaks in the spring and fall, although sporocarps of several species either persist through summer and winter, or are produced during these seasons (FOGEL, 1976; HUNT & TRAPPE, 1987; LUOMA, 1991). Numerous studies have successfully used sporophore abundance to assess ectomycorrhizal fungus community composition (MILLER, 1982a; BILLS *et al.*, 1986; VILLENEUVE *et al.*, 1989; NANTEL & NEUMANN, 1992; PALMER *et al.*, 1994). The results produced by these studies appear to correspond well with plausible explanations. NANTEL & NEUMANN (1992) found that the strongest niche dimension of ectomycorrhizal communities was stand composition, but within the range of a stand, fungal assemblages differed in relation to edaphic characteristics.

Predictive equations of forest mushroom yields are quite complex given the dynamics that influence ectomycorrhizal fungal communities. Numerous interactive factors come into play, before and during the autumn fruiting period, including environmental (rainfall, air and soil temperatures, evapotranspiration, relative humidity, and water deficits or excesses), silvicultural (tree species, stand age, density and distribution, canopy cover), ecological (community composition, competitive interactions, reproductive strategies), landscape (altitude, aspect, slope) and anthropogenic (timber removal, controlled burns, wildlife management, grazing, introduced species) (MARTÍNEZ DE ARAGÓN *et al.*, 2007).

The forest tree species, which is host to associated fungal symbionts, influences the fungal community and fungal species richness through host specificity (MOLINA *et al.*, 1992). Each host tree species can only form an ectomycorrhizal symbiosis with a recognized group of fungal species. Therefore the composition of ectomycorrhizal fungi is limited by the range of fungal symbionts recognized by a given host tree under the existing ecological conditions, and the diversity of host trees present in the forest stand. Forest age also has an effect upon succession, diversity and production of certain forest fungi (KALAMEES & SILVER, 1988; SMITH *et al.*, 2002; BONET *et al.*, 2004), and aspect has been shown to influence habitat for some species (BONET *et al.*, 2004).

Human intervention has played a significant role because forest management tools (clearings, pruning, species selection, fire, fertilization) can modify density, canopy cover, primary productivity, basal area, understory plant communities, soil conditions and soil microbial communities. These modifications in turn alter microclimates responsible for the succession and fruiting of numerous fungal species (Ohenoja, 1988; Fernández De Ana *et al.*, 1989; Termorshuizen, 1993; Egli & Ayer, 1997; Hernández & Fernández, 1998).

It is well known that fungal fruiting is a seasonal event that depends on meteorological factors, especially temperature and rainfall. High rainfall and mild temperatures in summer are normally considered to favour the formation of carpophores by the fungal mycelium (Arnolds, 1981). Many authors have tried to find direct relations between fungal fruiting and weather patterns. It has been demonstrated that an important condition is a wet period after a dry one (Becker, 1956; Heim, 1969), and that excess water in the soil inhibits carpophore production (Bujakiwicz, 1969).

Both vegetative communities and animals influence the macrofungal habitat in the forest ecosystem (Crabtree *et al.*, 2010; Hustad *et al.*, 2011), especially the core giant panda habitat, and in turn, the macrofungi are the essential decomposers that maintained regional ecological balance for plants and animals living. The diversity of the macrofungal community reflects the environmental conditions in the region. The data suggests that despite the lagged sporophore peak compared with the highest temperature, macrofungal diversity was positively related to the temperature in the area. More Arbuscular mycorrhizal (AM) fungal biomas and spores were produced during the wet season than during the dry season. The different types of organic matter had similar influence on the amount of AM biomass but the species composition was varied with the types of organic matter. In wet season nine species of AM spores and in dry season ten species of AM spores were found. In dry season *Scutellospora nigra* was found which was different from wet season (Vaidya *et al.*, 2007).This study was conducted to identify the forest floor of macrofungal in natural stands (*Parrotia persica* and *Carpinus betulus*) and planted stands (*Cupressus sempervirens* and *Alnus subcordata*) in North of Iran (Gorgan, Shast Kalate) during 2010-2011.

Material and Methods

Site Description. This investigation was carried out in Shast Kalate (Bahram Nia) forest, experimental forest of Gorgan University of Agricultural Sciences and Natural Resources, a virgin mixed deciduous forest covering an area of about 1 713.3 ha and located in the north of Iran (36°43´ to 36°48′ N and 54°21′ to 54°24′ E), with an average annual precipitation of about 649 mm, and an altitude ranging from 210 to 995 m above sea level (Fig. 1). The study region has an average temperature of 12°C and average humidity of 76.5%. The aforementioned site is a permanent plot for long term studies, established on brown forest soil with mostly sandstone as bedrock (Karim *et al.*, 2012).

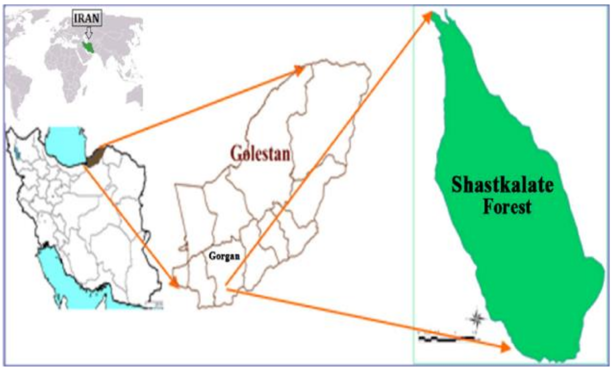

Fig. 1. Indicative map of the study area (Karim *et al.*, 2012).

Identification. Macrofungal identification through the comparison of relevant information is essential (Li *et al.*, 2012). The identification of microfungal genera and species was carried out according to the macroscopic and microscopic characteristics of the specimens, as well as their characteristic responses to some chemical reagents (Smith *et al.*, 1979; Wei, 1979; Moser, 1983; Singer, 1975; Huang, 1998; Mao, 1998, 2000; Diyabalanage *et al.*, 2008; Ouzouni *et al.*, 2009). This study was conducted to identify the forest floor of macrofungal in *Parrotia-Carpinetum* and planted stands of *Cupressus sempervirens* and

Alnus subcordata in North of Iran (Gorgan, Shast Kalate) during 2010-2011. For this purpose, all macrofungal in the area (0.6 ha in separated stands) were collected. Measurements were made from slide preparations stained with cotton blue- lacto-phenol contains 100 ml. Lacctophenol, 2ml 2% aqueous solution of cotton blue, by Olympus light microscope (BH2) (SMITH *et al.*, 1979; MOSER, 1983; SINGER, 1986).

Results

The results show that 35 species were found in the three assessment plots, which were classified into 21 genera (especially genera from Basidiomycetes) from 16 families. Maximum macrofungal were observed at Psathyrellaceae (two genera and six species). Minimum macrofungal (one species and one genus) in Amanitaceae, Cantharellaceae, Coprinaceae, Geastraceae, Hygrphoraceae, Paxillaceae, and Sclerodermataceae were found (Table 1). All macrofungal classified to *Agaricales, Boletales, Cantharellales, Geastrales,* and *Russulales* genera. The maximum of fungal species in *Agaricales* genera were found and distributed in *Parrotia-Carpinetum*, *Cupressus sempervirens* and *Alnus subcordata* stands (Fig. 2). The maximum number of macrofungal in *Parrotia-Carpinetum* (five species and two genera), *Alnus subcordata* (three species, two genera) and *Cupressus sempervirens* (four species and two genera) were found in the Psathyrellaceae family (Fig.3). Up to 54% of the species were classified into *Parrotia persica - Carpinus betulus* (twenty seven species), 26% *Alnus subcordata* stand (eleven species) and 20% *Cupressus sempervirens* (ten species) (Fig.4). In wet season 30 species of macrofungal and 13 species in dry season were found. In spring season 12 species, one species in summer season, 23 species in fall season and 7 species in winter season were observed. In addition to, maximum fungal (43.8%) collected in *Parrotia-Carpinetum* stand in the fall season (Fig. 5).

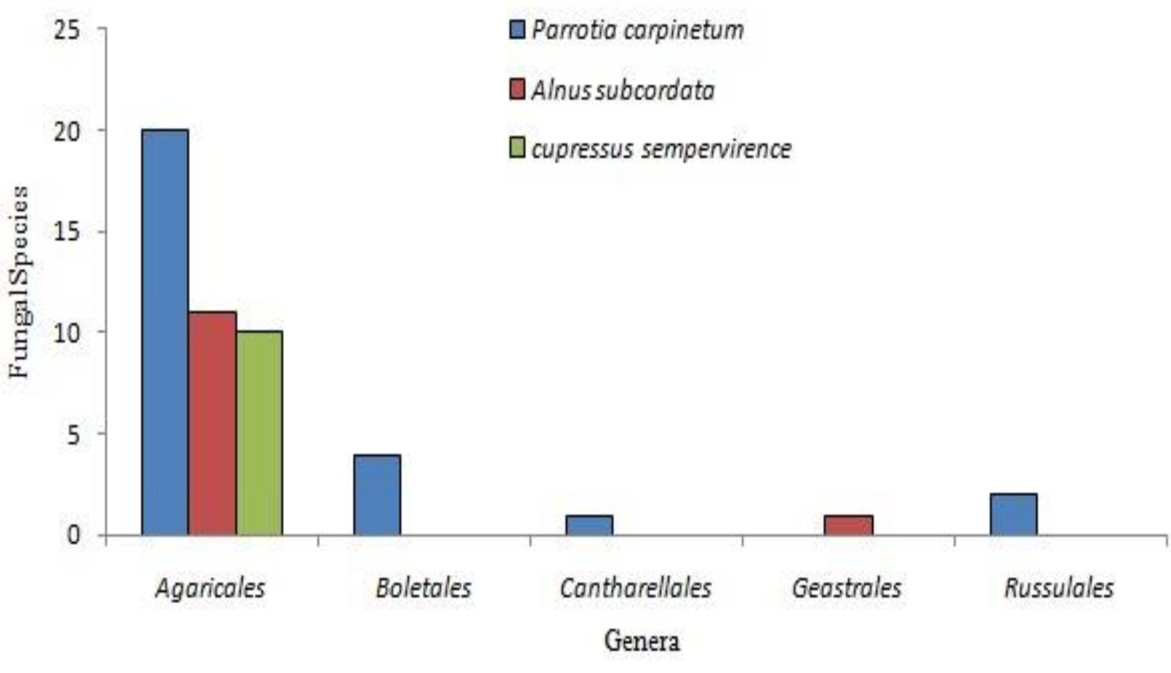

Fig. 2. Frequency of macrofungal species in relation to number of plots in which they were found.

Table 1. Total individual species density of macrofungi in the three plots. Legend:*= Macrofungal species, Pp = *Parrotia persica*; Cb = *Carpinus betulus*; Ag = *Alnus subcordata*.

N	Species name	Family	Genera	Pp	Cb	Ag	Spring	Summer	Fall	Winter
1	*Agaricussilvicola*	Agaricaceae	Agaricales	*					*	
2	*Agaricus xanthodermus*	Agaricaceae	Agaricales	*		*			*	
3	*Agrocybe praecox*	Strophariaceae	Agaricales	*		*	*		*	
4	*Agaricus semiorbicularis*	Strophariaceae	Agaricales	*		*	*			
5	*Amanita rubescens*	Amanitaceae	Agaricales	*					*	
6	*Cantharelluse cibarius*	Cantharellaceae	Cantharellales	*					*	
7	*Clitocybe gibba*	Tricholomataceae	Agaricales	*		*			*	
8	*Clitocybe vibecina*	Tricholomataceae	Agaricales			*			*	
9	*Collybia confluens*	Tricholomataceae	Agaricales		*				*	
10	*Clitocybe dryophila*	Tricholomataceae	Agaricales	*			*			
11	*Coprinus atramentarius*	Psathyrellaceae	Agaricales	*	*	*	*		*	
12	*Clitocybe disseminates*	Psathyrellaceae	Agaricales	*			*			
13	*Clitocybe lagopides*	Psathyrellaceae	Agaricales	*	*	*	*			
14	*Clitocybe micaceus*	Psathyrellaceae	Agaricales		*				*	
15	*Geastrum triplex*	Geastraceae	Geastrales			*	*			
16	*Gymnopilus spectabilis*	Strophariaceae	Agaricales	*						*
17	*Hebeloma sinapizans*	Strophariaceae	Agaricales	*					*	
18	*Hygrocybe unguinosa*	Hygrphoraceae	Agaricales	*						*
19	*Laccaria amethystea*	Hydnangiaceae	Agaricales	*						*
20	*Laccaria laccata*	Hydnangiaceae	Agaricales	*			*		*	
21	*Lepiota cristata*	Agaricaceae	Agaricales		*				*	
22	*Lepiota naucina*	Agaricaceae	Agaricales	*	*	*			*	
23	*Micromphale brassicolens*	Marasmiaceae	Agaricales	*		*	*		*	*
24	*Mycena foetidum*	Marasmiaceae	Agaricales		*		*			
25	*Mycena polygramma*	Mycenaceae	Agaricales		*				*	
26	*Mycena pura*	Mycenaceae	Agaricales		*				*	
27	*Panaeolus sphinctrinus*	Coprinaceae	Agaricales	*						*
28	*Paxillus involutus*	Paxillaceae	Boletales	*			*			
29	*Psathyrella candolleana*	Psathyrellaceae	Agaricales	*	*	*	*	*	*	*
30	*Psathyrella vernalis*	Psathyrellaceae	Agaricales	*						*
31	*Russula delica*	Russulaceae	Russulales	*					*	
32	*Russula heterophylla*	Russulaceae	Russulales	*					*	
33	*Scleroderma verrucosum*	Sclerodermataceae	Boletales	*					*	
34	*Xerocomus badius*	Boletaceae	Boletales	*					*	
35	*Xerocomus chrysenteron*	Boletaceae	Boletales	*					*	

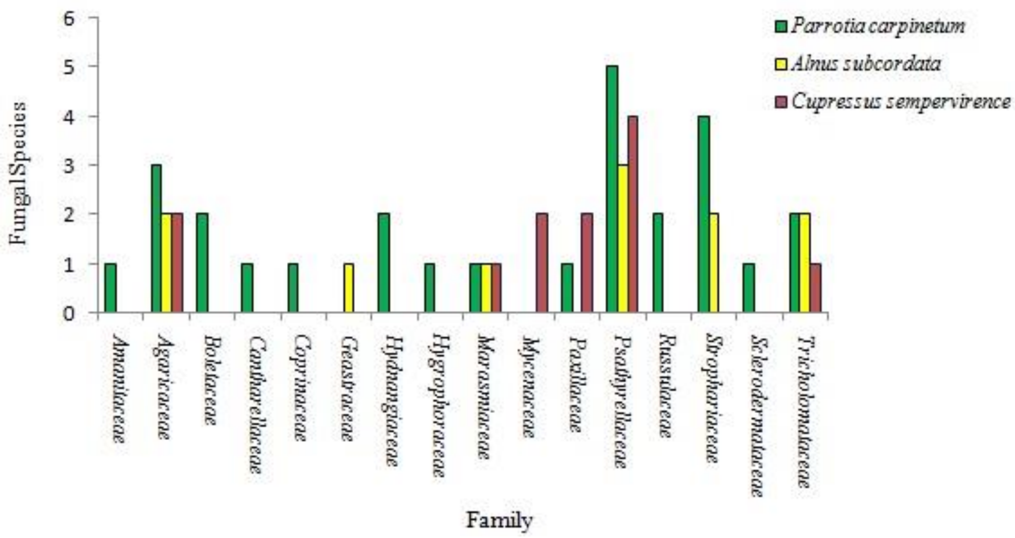

Fig. 3. Number of macrofungal species recorded in different families.

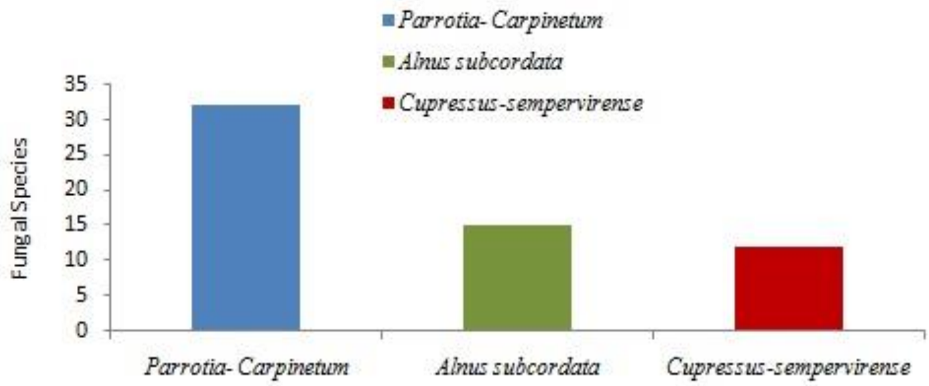

Fig. 4. Distribution of macrofungal species in relation to all forest stands: *Parrotia persica- Carpinus betulus; Cupressus sempervirens* and *Alnus subcordata*.

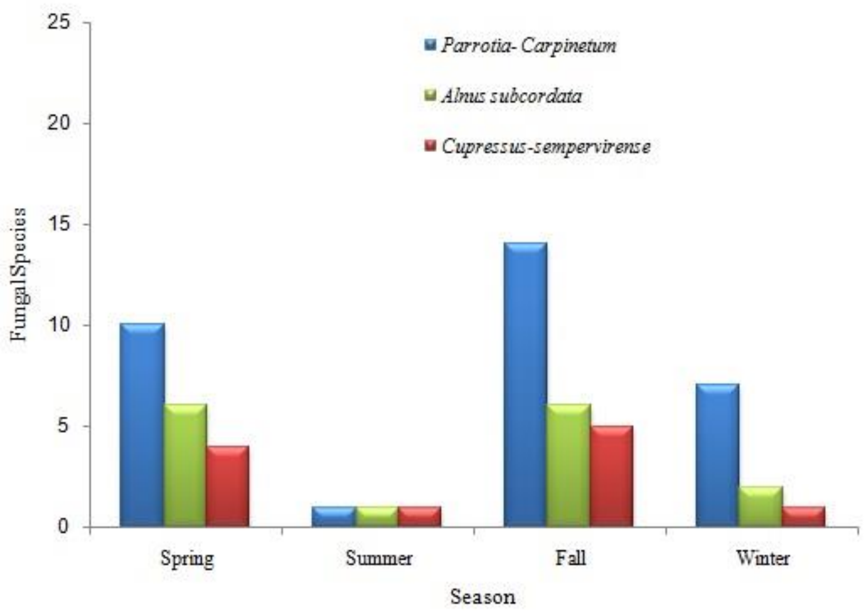

Fig. 5. Collected of macrofungal species in relation to time in season different; spring, summer, fall and winter.

Discussion

Understanding how fungal populations and communities are spatially and temporally distributed is fundamental to estimating their diversity. Such information is also useful in determining how fungal populations affect the abundance and distribution of other organisms and ecosystem processes at the landscape level (LODGE & CANTRELL, 1995). As discussed by BILLS *et al.* (1986), it is difficult to determine the adequate sampling area required to capture the fungal species diversity of a particular vegetation type. Changes in forest composition due to succession, disturbance, or timber harvesting will produce changes in sporocarp abundance and diversity, because ectomycorrhizal fungi that rely on carbohydrates from their tree hosts (HARLEY & SMITH, 1983) produce most sporocarps. However, quantitative data of macrofungal diversity have been used to determine whether mushroom species follow the ecological rule that the local abundance of a species is related to the size of its geographic range (GASTON, 1994; JOHNSON, 1998). In a natural ecosystem different processes could become prominent at different stages of the seasonal cycle, and the changes in the patterns observed might, therefore, have reflected temporal changes in the overall ecosystem functioning (KRIVTSOV *et al.*, 2004). This study has demonstrated that natural stands and *Cupressus sempervirens* – *Alnus subcordata* plantations provide a habitat for diverse macrofungal communities, which vary markedly in composition from site to site. The results of this research showed that number of macrofungal species was found to be correlated with temperature, moisture, and organic matter is consistent with the results (WALKER & MILLER, 2000; KARIM *et al.,* 2011; FISCHER, 2007). Frequency of macrofungal species in relation to forest stands was varied, *Parrotia persica* - *Carpinus betulus* (54%), *Alnus subcordata* plantations (26%) and *Cupressus sempervirens* plantations (20%). This is may be related to tree diversity (FISCHER, 2007), not human effects (FERRIS *et al.*, 2000), exist of litterfall, high decomposition of foliage. This results in consistent with the results of HUMPHREY *et al.,* 2000; LAGANA *et al.,* 2000; MCLAUGHLIN, 1997; BADER *et al.,* 1995; LAGANA *et al.*, 1998.Disturbances from seasonal changes in rainfall and tree falls to hurricanes can differentially affect fungal species. Temperature and moisture are essential factors in distribution of macrofungal. The maximum of macrofungal species were found in wet season. In finally, fungi are extremely diverse, they are often ephemeral and cryptic, rendering inventorization difficult (MULLER *et al.,* 2004) However, knowledge of biodiversity at the community and species level is essential to monitor the effectiveness of, or the need for reservation, and also to follow the effects of natural or artificial disturbance (PACKHAM *et al.,* 2002). The data on fungi diversity and distribution are limited and fragmentary, the consensus is that certain patterns are robust and are worthy of future consideration.

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Demography and conservation of an isolated Spur-thighed tortoise Testudo graeca population in Dobrogea (Romania)

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Abstract. Spur-thighed tortoise is a vulnerable species. The local declines of populations led to an imperative need for conservation. *Testudo graeca* reaches its northern range limit in Dobrogea region, Romania. We studied a population from this region, which occupies an enclosed area of 32 ha within Histria Archaeological Complex. Based on a capture-mark-recapture study we estimated the population size of 221 ± 12.2 individuals. The observed density was 5.1 individuals/ha. The predicted population size suggests a relatively high density in relation to the area thus raising attention for a future conservation strategy. The population structure shows reduced sexual dimorphism and an unbiased sex ratio, implying a young population structure. We suggest correlating the future archaeological studies with conservation requirements of tortoises.

Key words: *Testudo graeca*, estimating population size, density, Romania.

Introduction

Demographic traits of animal populations offer useful information about their conservation status (WILLIAMS *et al.*, 2002) influencing their viability and persistence over time (BOYCE, 1992). The demography and population density are important as they guide the decision making process to establish if a population is suitable for conservation (AKÇAKAYA & SJÖGREN-GULVE, 2000), and help to implement the conservation measures at both local and wider geographical area (BERTOLERO *et al.*, 2007; ROZYLOWICZ & DOBRE, 2010).

Reptiles are a key component of ecosystems as predators, prey, grazers, seed dispersers and/or commensal species. Reptiles are declining worldwide, requiring extensive conservation measures (GIBBONS *et al.*, 2000). European reptiles and especially tortoises are mostly influenced by habitat loss (COX & TEMPLE, 2009). The demographic traits differ among the species of *Testudo* genus and even between populations of the same species (WILLEMSEN & HAILEY, 2003).

The most of demographic studies concerning *Testudo graeca* report the size or the density of the studied population (e.g., KADDOUR *et al.*, 2006; RACHID *et al.*, 2007). However, density can be viewed as a measure of habitat occupancy and is the result of direct observation, dependent of field activity and study site, without offering an estimation of population size or its dynamic in time (INMAN *et al.*, 2009). Therefore, a reliable assessment of population size is required. The size of a reptile population is estimated using different methods (HILL *et al.*, 2007) because of the dissimilarities in activity patterns

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(LAMBERT, 1981) and detectability of adults and juveniles (LAGARDE *et al.*, 2002). One of the widely used techniques is the capture-recapture (KENDALL & POLLOCK, 1992) since is straightforward to implement in small areas (KADDOUR *et al.*, 2006). This method is implemented in population size estimation oriented software (e.g., Mark, U-Care, POPAN; SUTHERLAND, 2006) and allows estimating the size of tortoise population, and detecting patterns of population structure (BESBEAS *et al.*, 2002) and survivability (BERTOLERO *et al.*, 2007).

The Spur-thighed tortoise, *Testudo graeca* Linneaus, 1758, is a flagship species for conservation (WALPOLE & LEADER-WILLIAMS, 2002) due to its ability to attract public attention for conservation measures (BARUA *et al.*, 2011). It is considered a vulnerable species in Europe (COX & TEMPLE, 2009), and a species of community interest that requires the designation of special areas of conservation (HABITATS DIRECTIVE 92/43/EEC).

The populations of this tortoise are spread on a wide range around the Mediterranean Sea living in both dry and Mediterranean wet climate, reaching at altitudes up to 2000 m a.s.l. (ANADÓN *et al.*, 2012). The population densities vary between 2-6 individuals ha^{-1} (HAILEY, 2000; DÍAZ-PANIAGUA *et al.*, 2001; KADDOUR *et al.*, 2006; ROUAG *et al.*, 2007) being reduced when compared to *T. hermanni* populations (i.e., with densities reaching 6.3 ha^{-1} tortoises in France and 12 tortoises/ha in Romania; BERTOLERO *et al.*, 2011).

The fragmented range of *T. graeca* populations is the result of widespread agricultural activities and land use changes that led to the isolation of tortoises in island-like favourable habitats (ANADÓN *et al.*, 2007; SAUMURE *et al.*, 2007; PREDA *et al.*, 2009). Reduced size populations are at risk from being destroyed by vegetation fire (STUBBS *et al.*, 1985; SANZ-AGUILAR *et al.*, 2011) or from illegal collecting for pet trade (LJUBISAVLJEVIĆ *et al.*, 2001; TÜRKOZAN *et al.*, 2008).

In Romania, *T. graeca* is found exclusively in the Dobrogea province (FUHN & VANCEA, 1961) at the northern limit of its distribution range (COGĂLNICEANU *et al.*, 2010), with greatly dispersed populations. The largest population is in the north of the region and a second large population in the forested hills in the south (COGĂLNICEANU *et al.*, 2007, 2008).

This study is assessing the situation of an isolated population of *T. graeca* from Dobrogea. The objectives of this study were (1) to estimate the population structure, size and density, and (2) to provide adequate conservation measures for this isolated tortoise population.

Material and methods

Site description. The studied population is located in an enclosed 32 ha perimeter within the Histria Archaeological Complex (HAC; 44°32'56'' N and 28°45'56'' E), an area of grasslands surrounded by wetlands in the south of the Danube Delta Biosphere Reserve (Fig. 1). The HAC includes an active archaeological and touristic area with exposed ruins, and an archaeological site without touristic activity. The past archaeological activities have resulted in numerous pits, slops and exposed ruins which in conjunction with tumuli and vegetation provide a variety of habitats for tortoises.

Estimation of demographic parameters. Monitoring and inventory were carried out during 2010-2012, throughout the active period of the tortoises (i.e., 29 visits from April to October, between 8 AM and 3 PM) using a capture-mark-recapture design. Animals were captured by active search along transects and marked on a marginal, posterior scute with a small indentation (STUBBS *et al.*, 1984). The animals were sexed based on external morphological characters (CARRETERO *et al.*, 2005), measured for straight carapace length (SCL) and curved carapace length (CCL), weighed and photographed for later identification (TICHÝ & KINTROVÁ, 2010). We used a threshold of 10 cm in SCL for separating juveniles/subadults from adults (STUBBS *et al.*, 1984). Individuals with underdeveloped sexual characters were considered subadults (WILLEMSEN & HAILEY, 2003). We transformed the obtained data in a binary

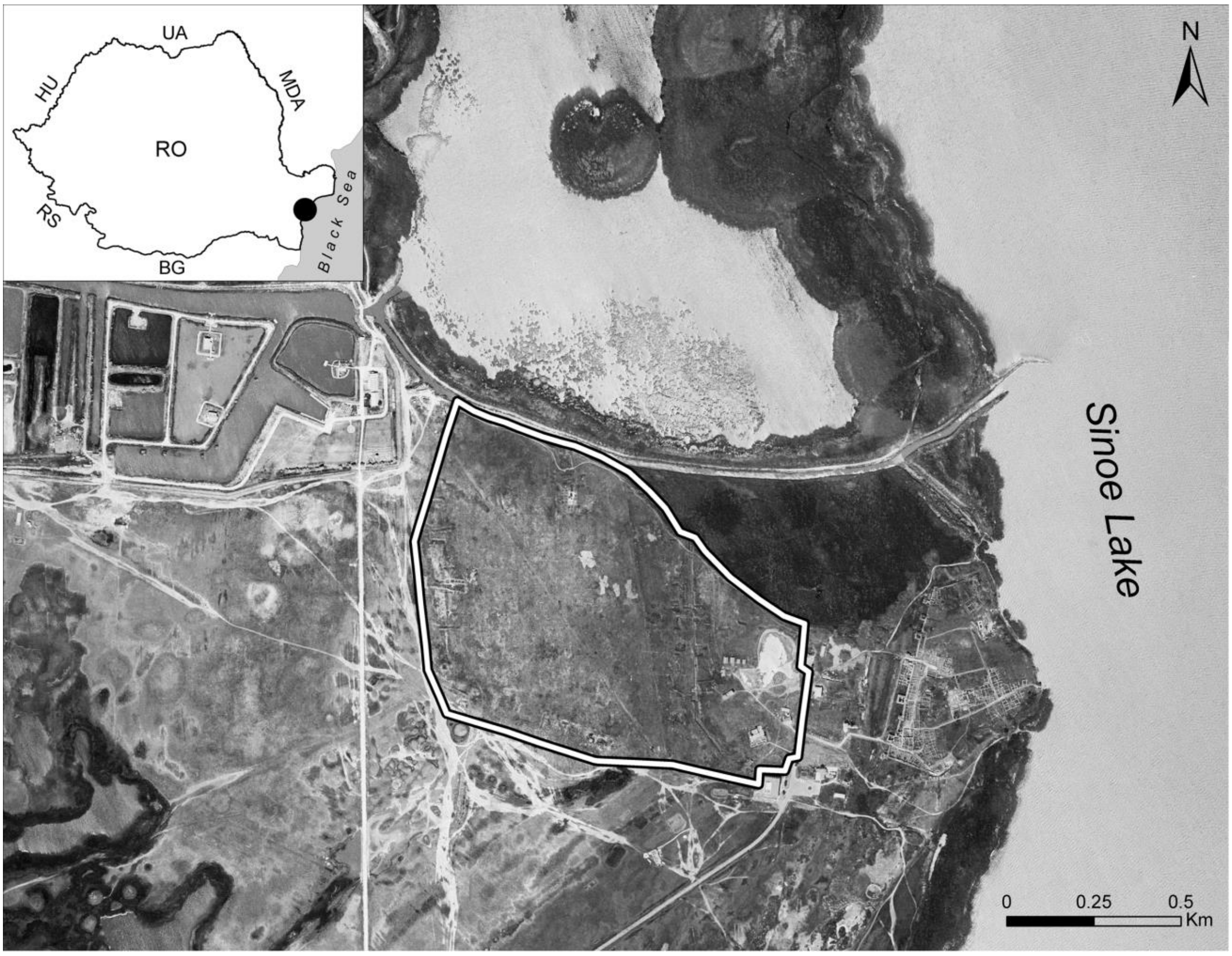

Fig. 1. Location of the study area in Romania (black dot in the upper left map) and the enclosure of Histria Archaeological Complex (white line contour).

string for each tortoise, forming the succession of capture/recapture (1) and the lack of capture (0) events. The number of events is equivalent to the number of occasions of capture-recapture.

The adult population structure was analysed using three biometric parameters (i.e., SCL, CCL and weight). The age (i.e., estimated from scutes growth ring counting; BERTOLERO *et al.*, 2005) was excluded from analyses because good estimation can be obtained only for juveniles and subadults, under 15 years (GERMANO, 1988). We tested for gender specific differences in SCL, CCL and weight using non-parametric Mann-Whitney U test.

We used the capture-mark-recapture design to estimate the population size (WHITE *et al.*, 1982) and analysed the data using Mark 6.2 software (WHITE & BURNHAM, 1999) and Capture module. The population was assumed closed during the study due to: (i) unsuitable habitat surrounding the HAC which limits the emigration or immigration of tortoises, (ii) no dead tortoises observed during the study and (iii) low detectability for juveniles makes the assessment of population growth difficult (GUYOT & CLOBERT, 1997).

We used the models for the estimation of N included in Mark (POLLOCK *et al.*, 1990), the application being able to point to the more appropriate one (SUTHERLAND, 2006). Three estimators were selected to estimate N: a null model Mo, Daroch Mt, and Jacknife Mh. Each of these models is characterised by different features for the estimation of N (OTIS *et al.*, 1978): (1) Null model Mo requires constant probability of capturing for the entire period of the survey, (2) Mt Daroch model assumes variation of captures with time during the survey period, (3) Jacknife Mh implies the variation of captures as function dependent on the individual, other than the capture probability for the entire population. We used these models to estimate the total population size, adult population size and separately for each sex, thus taking into consideration the gender specific differences in behaviour and different capture opportunities of adults and juveniles. Also, for this species there is no positively or negatively subsequent response to capture (PIKE *et al.*, 2005).

We also estimated the population size using temperature at the time of capture as an environmental covariate (HUGGINS, 1989). Only the adult population was estimated, using Mt model and the Huggins Closed Captures data type in the covariation model.

Density estimation was computed using the number of individual captures and the estimated population size obtained from the Mark application in respect to the area being studied.

Results

We inventoried 164 tortoises in the study area and had 72 recaptures. Males had a higher recapture rate than females (males=58.11%, females=34.21%), which instead, attained a higher individual recapture rate. Only 14 juveniles were observed during the survey (Table 1).

Table 1. Number of captured and recaptured *T. graeca* from Histria Archaeological Complex.

	Males	%	Females	%	Juveniles	%	Total	%
Unique captures	74	45.12[a]	76	46.34[a]	14	8.54[a]	164	***
Recaptures (n)	43	58.11[b]	26	34.21[b]	3	21.43[b]	72	43.90[a]
Recaptures n=1	25	58.14[c]	13	50.00[c]	3	1.83[c]	41	56.94[c]
Recaptures n=2	14	32.56[c]	4	15.38[c]	0	0.00[c]	18	25.00[c]
Recaptures n=3	3	6.98[c]	5	19.23[c]	0	0.00[c]	8	11.11[c]
Recaptures n=4	1	2.33[c]	3	11.54[c]	0	0.00[c]	4	5.56[c]
Recaptures n=5	0	0.00[c]	1	3.85[c]	0	0.00[c]	1	1.39[c]

[a] - % of total captures; [b]- % of all captures for each sex; [c] - % of all recaptures for each sex.

Individuals with body weight between 1500-2000 g dominated the population (37.8% of all females and 63.3% of all males; Fig.2). The adult tortoises showed no significant differences between sexes in both SCL (Mann-Whitney U = 2803.50, p = 0.97; $mean_{males}$ = 18.95 cm, $mean_{females}$ = 18.87 cm), CCL (Mann-Whitney U = 2418.00, p = 0.136; $mean_{males}$ = 24.73 cm, $mean_{females}$ = 25.05 cm, Fig. 2) and body weight (Mann-Whitney U = 2492.50, p = 0.23; $mean_{males}$ = 1679.53 g, $mean_{females}$ = 1763.39 g).

Mt model was the most suitable for the used data set (Table 2) and estimated the adult population of tortoises at 197 ± 10.7 individuals, close to the cumulative estimation for sexes (89 ± 5.7 males and 107 ± 9.2 females). The total population, including the juveniles, was estimated at 221 ± 12.2 (Table 2).

There were no differences in estimation, for adults and individually for males and females, when Huggins Closed Capture data type is used, with temperature as covariate (Table 3).

Using direct observations, we estimated a density of 4.8 adult individuals/ha and a total density of 5.1 tortoises/ha including juveniles. Both observed and estimated densities stands within the range observed for other populations of this species (Table 4). Direct observation revealed a balanced sex ratio of 0.97, lower than that observed in other populations of the species (e.g., 1.26 in KADDOUR *et al.*, 2006).

Discussions

Although the population is isolated in a reduced area, our results reveal a population with a high density. The population structure displays a young population with unbiased sex.

Population density is comparable with that found in other populations occupying a reduced area, and similar to the results from other studies from Spain, Greece (HAILEY & WILLEMSEN, 2000) and Morocco.

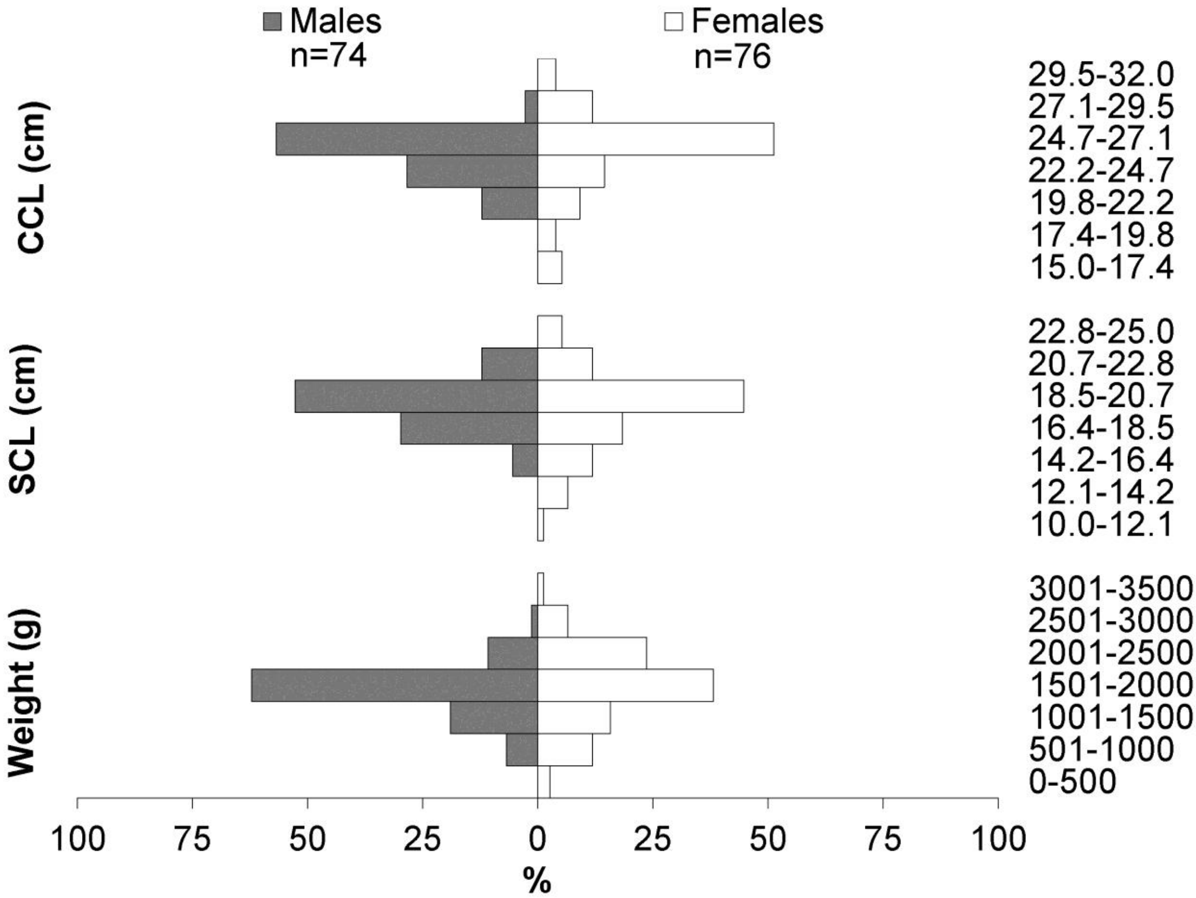

Fig. 2. The distribution of *T. graeca* adults in relation to classes of body weight, SCL and CCL.

Table 2. Estimation of *T. graeca* population size (N) (SD = standard deviation, CI = confidence interval, Mt +1 = number of unique captures and n = total number of captures, including recaptures).

	The used model	M(t+1)	n	N ± SD	CI (95%)	Maximum likelihood range
Adults and juveniles	Mo			255 ± 12.9	205-255	203-253
	Mt*	164	286	**221 ± 12.2**	**202-250**	**200-249**
	Mh[1]			301 ± 31.3	253-377	***
Males	Mo			92 ± 6.5	83-109	82-108
	Mt*	74	139	**89 ± 5.7**	**82-105**	**80-103**
	Mh[1]			100 ± 8.0	88-120	***
Females	Mo			110 ± 9.9	96-135	94-133
	Mt*	76	129	**107 ± 9.2**	**94-131**	**92-130**
	Mh[1]			176 ± 26.4	137-242	***
Adults	Mo			201 ± 11.4	183-228	182-226
	Mt*	150	268	**197 ± 10.7**	**181-223**	**179-221**
	Mh[1]			264 ± 25.6	224-326	***

* Mark-application -Capture module suggested model; [1] interpolated estimation of *N*; in bold the estimation model considers most appropriate.

Table 3. Estimation of *T. graeca* population size (*N*) for adults using as estimation model of N M_t and Huggins Closed Capture data type with temperature as covariate (SD = standard deviation, CI = confidence interval, Mt +1 = number of unique captures and n = total number of captures, including recaptures).

	M (t+1)	n	N ± SD	95% CI
Adults	150	268	197.9 ± 10.9	180.7-224.6
Males	76	139	90.2 ± 6.0	81.7-106.6
Females	74	129	108.0 ± 9.5	94.2-133.1

Table 4. Density of *T. graeca* population in the study area compared with observed and estimated densities of other populations (individuals/ha).

Location	Method for estimation	Observed density	Estimated density (range)	Source
HAC (Romania)	Directly observed	5.1		This study
	Estimated - M_t model (including juveniles)	6.9	6.2-7.7	
	Estimated - M_t model (without juveniles)	6.1	5.5-6.9	
Spain	Directly observed	4.2-12	--	ANDREU *et al.*, 2000; BALLESTAR *et al.*, 2004
Greece	Directly observed	6.2	--	HAILEY, 2000
Morocco	Directly observed	5-7	--	SLIMANI *et al.*, 2002; KADDOUR *et al.*, 2006

The population sex ratio of 0.97 is unbiased than the reported value in other studies (KADDOUR *et al.*, 2006) and lower compared to similar studies on *T. hermanni* reporting values of 1.5 (HAILEY & WILLEMSEN, 2000).

The Histria Archaeological Complex population showed no significant sexual size dimorphism (SSD). A lack of SSD was also observed in Mardin Province, Turkey (TÜRKOZAN *et al.*, 2003), while other studies showed a significant SSD (e.g., DÍAZ-PANIAGUA *et al.*, 2001; KADDOUR *et al.*, 2008).

The data shows a young tortoises population and the differences observed in this study from other population of this species should be correlated with history of the area inhabited by the tortoises. The isolated area offers favourable habitat and protection against human impact. Before this area was part of Danube Delta Biosphere Reserve the human impact was greater with negative impact on the survival of tortoises because of on-going industrial development and agricultural practices limiting their habitat (DOROFTEI *et al.*, 2011; GIOSAN *et al.*, 2012).

The sandy habitats surrounding the study area are overgrazed and covered partly by bare soil are unfavourable for tortoise, providing little or no hiding places. This limits the dispersal of tortoises and contributes to its isolation.

The small number of juveniles captured and the lack of juveniles under age of three is the result of numerous factors and a situation encountered in other studies. The survivability rate for juveniles of tortoises is reduced (GARCIA *et al.*, 2003; DÍAZ-PANIAGUA & ANDREU, 2009) and they exhibit a more reduced activity pattern. In addition, their dimensions and the camouflage colour of carapace makes them hard to be observed (LAGARDE *et al.*, 2002).

The method for population size estimation used in this study is adversely affected by reduced recapture rate, under 50%, and especially by the low number of juveniles. This pattern is not unusual and is determined by age, activity periods (DÍAZ-PANIAGUA *et al.*, 1995) and sex of adults (DÍAZ-PANIAGUA *et al.*, 1996), environmental conditions, vegetation, temperature and precipitations (KADDOUR *et al.*, 2006).

The isolation of the studied population and its relatively high density requires specific conservation measures in the future for its survival. The favourable habitat may be reduced by archaeological activities or touristic development. The collecting of individuals and vegetation fires are risks that may lead to high mortality (STUBBS *et al.*, 1985) and ultimately to the loss of an isolated population.

We consider as conservation measure: (1) the strictly delimitation of the grazing areas closed to HAC to prevent the sheep from accidentally entering inside the tortoises' perimeter, (2) the controlled burning of vegetation (STRICKLAND, 2012) in winter month to reduce the risk of fire, of natural origin or human-made, and (3) a correlation of future archaeological studies with conservation requirements of tortoises. Stray dogs may pose a high risk to juveniles and hatchlings and limiting their access in the area is desirable.

The persistence of a population living in an enclosed area may be altered by the disturbance of a single landscape factor (e.g., changings in land use; RUGIERO & LUISELLI, 2006). Similar, the persistence and viability of our isolated population may depend on the presence of abundant vegetation that offer shelter in midsummer or of the exposed ruins that offer crevices for wintering.

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Combined Effect of Some Bio-Agents on the Grasshopper, Hetiracris littoralis Under Semi-Field Condition

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Abstract. LC50 of the alchoholic-80% extract of *Euphorbia pulchrrima* (0.714%), essential oil of Garlic plant *Allium sativum* (0.067%) and nematodes of *Steinernima sp.* and *Heterorhabditis sp.* (500 IJs/ml), were tested for their solely and/or combined toxic effects and for their effects on some biological aspects against the grasshopper, *Heteracris littoralis* 1st instar nymphs under semi-field condition. The joint action of the mixture of the most effective extract (*E. pulchrrima*) and oil (Garlic oil) exhibit an antagonistic effect with co-toxicity index of (–24), despite the increase the proportion of death in the mixture for all the tested groups the type of interaction were antagonism, all the tested materials had variable mode of action which resulted in significantly antagonistic effects. *Euphorbia* 80% methanol extract and Garlic oil may use separately or in combination as alternatives safe tools against *H. littoralis* grasshopper. Semi-field experiments cleared that nematodes in combination with the oil or extract increased the mortality percentage. The combination mixture of extract, oil and nematode significantly affected development, reproduction and life cycle of *H. littoralis*. Lethal effect varied with regard to the nematode species. Semi-field experiment of the plant extract, plant oil and their mixture revealed some changes on the biological aspects, an increase in the nymphal period, pre-oviposition, oviposition and post-oviposition periods. There is vigorous decrease in the female fecundity and fertility. The control of the insect by nematode and sub-lethal dose of plant extract or plant volatile oil as biological control may enhance their lethal effect on insect pest when applied simultaneously. Combination mixture of the tested bio-agent could be considered as possible means for use in programs of integrated pest management of *H. littoralis* grasshopper.

Keywords: *Heteracris littoralis*, *Steinernema*, *Heterorhabditis sp.*, Biological aspects, *Euphorbia pulchrrima* extract, *Allium sativum* essential oil

Introduction

Botanical insecticides are one of the best alternatives for these hazardous chemicals. They are plant–derive insecticides, either naturally occurring plant materials or the products simply derived from such plants (GUPTA *et al.,* 2005). A number of medicinal plant species like *Euphorbia sp.*, *Dodonea vescosa*, *Eucalyptus sp.* and *Chinus trbinlitolia* etc., are known to possess insecticidal properties (SHARMA & GUPTA, 2009; MAZEN *et al.*, 2009; OPARAEKE, 2004; UWAEZUOKE, 2002; CRUZE *et al.*, 2000). Essential oils are volatile, natural, complex compounds, characterized by a strong odor and are formed by plants as secondary metabolites. In nature, essential oils play an important role in protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivorous by reducing their appetite for such plants. They also may, attract some insects to favor the dispersion of pollens and seeds or repel undesirable others (BAKKALI *et al.,* 2008).

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Therefore, the use of essential oils extracted from aromatic plants to control insect pests has been investigated and is will documented (ISMAN, 2006; KOUL *et al.*, 2008; RAJENDRAN & SRIRANJINI, 2008). The possibility of using pathogenic nematode to control destructive pest has been envisioned for a long time. From time to time efforts to institute the control of certain insects by such measures have been made in various parts of the world. In recent years, there has appeared a need to reappraise the possibilities of this method in the light of new technique and newer knowledge concerning the effect of pathogenic nematodes, plant essential oils and plant extracts on insect population and their control. Recently, most researches tend to use some strains of nematode and extracts of some plants for controlling grasshopper. The grasshopper, *H. littoralis* considered one of the most harmful pests to different cultivated crops in Egypt. Its economic importance comes from attacking many vegetable cultivated areas even trees, feeding on it and causing great losses in quantity and quality of the attacked crops. In some cases, the swarms of grasshopper leaving it as a divested desert may attack thousands of cultivated hectares. *Mistikawy* (1929) and *Nakhla* (1957) had documented the economic injury of *H. littoralis* in Egypt. Insecticide mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance or to combat current resistance in a pest species. Insecticide resistance has become a major obstacle to successful chemical control with conventional insecticides. The evolution of resistance to insecticides is governed by a complex of events and factors; mainly, intense and repeated applications of insecticides which are often from the same chemical group or which employ the same mode of action. To prevent the resistance phenomenon, there is a need for different compounds having different modes of action (FEI YI *et al.*, 2012). Combined mixture of insecticides for 1 - The amount of material used can be reduced without loss of activity, 2 - To delay the selection of resistant strains, 3- Biological evidence alone is not enough, because the observed efficacy of a mixture should be compared with its expected efficacy (GISI, 1991).

The main aim of the present research is to evaluate the combined effect of three bio-agents (Garlic oil, *Euphorbia* extract and nematodes) on the grasshopper, *H. littoralis* under semi-field condition for disrupting growth and development of *H. littoralis* so that they can be used in the integrated handling of pests.

Materials and Methods

Insect culture. Heteracris littoralis grasshopper was reared under laboratory condition for several generations on semi-artificial diet as mentioned by SHARABY *et al.* (2010). Essential oils Garlic, *Allium sativum* (Family: Liliaceae) were obtained from EL-Captain company (CAPPHARM), elcaptain@elcaptain Co., Al-Obour city (Cairo), Egypt. Plant of *Euphorbia sp.* was collected from the garden of National Research Centre, Egypt. The plant parts were dried in shad place then minced into powder in an elecreric mill then extracted with 80% methanol as mentioned by SHARABY *et al.* (2011). The tested nematode were obtained form the Plant Protection Laboratory, National Research Centre, Egypt.

Combined actions of the plant extract, essential oil and entomopathogenic nematode on H. littoralis under laboratory condition. The artificial diet that used for rearing *H. littoralis* and described by SHARABY *et al.* (2010) was mixed with the sub-lethal concentration (LC50) that previously determined by SHARABY *et al.* (2011, 2012) of the different bio-agents (*Euphorbia pulchrrima* plant extract at concentration (0.714%), Garlic, *Allium sativa* plant oil at concentration (0.075%) and *Steinernema carpocabsa* or *Heterorhabditis sp.* nematode (500 IJs/ml)) separately and in combination. The treated diet for each treatment was introduced into the rearing cages each with 100 1st instar nymph of *H. littoralis* and left for feeding on the treated diet for 14 days, then the percentage of mortality was calculated. For the control test untreated diet was used. Corrected mortality was made according Abbott's formula (ABBOTT, 1925). The combined action of the mixtures were expressed as co-toxicity factor which was estimated by equation of MANSOUR *et al.* (1966). To determine the effect of applying pairs of insecticides, the expected LC50 concentration of each insecticide in the paired combination was applied, thus 50% mortality was expected to result when the mixture of 2 insecticides was the sum of the expected mortalities of each of the concentration used in combination. To evaluate the effect of different pairs of the bio-agent used here, the following equation was followed:

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times 100$$

These was used to differentiates the results into 3 categories. A positive factor of 20 or more meant potentiating, a negative factor of 20 or more meant antagonism, and any intermediate value between (-20 and +20) was considered only additive effect.

Semi-field experiment. Effect of combination of different bio-agents on percentage of mortality and some biological aspects for the 1st instar nymph of *H. littoralis* under semi- field condition: A semi-field experiment was conducted outside the laboratory in the garden of National Research Centre under the natural condition during January until April, the average temperature was 29°C (20.5-37.0°C) and the photoperiod 10:14 light to dark. Assay units were pot-planted Clover, *Trifoleum alexandrinum* 40cm high, one month old, twelve treatments were investigated in addition the control (untreated potted Clover), 0.05% Tween-80 was used as emulsifier. Water was used for the control test.

Each treatment was assigned four times. Each potted plant was sprayed with 250ml of the sub-lethal concentration (LC50) of solution of tested material in twelve different treatments (1- Garlic oil, 2- *Euphorbia* extract, 3- *Steinernima sp.* nematode, 4- *Heterorhabditis sp.* nematode, 5- Garlic oil+ *Euphorbia* extract, 6- Garlic oil+*Steinernima sp.* nematode, 7- Garlic oil+ *Heterorhabditis sp.* nematode, 8- *Euphorbia* extract+*Steinernima sp.* nematode, 9- *Euphorbia* extract +*Heterorahabdetis sp.* nematode, 10- Garlic oil + *Euphorbia* extract + *Steinernima sp.* nematode, 11- Garlic oil + *Euphorbia* extract + *Heterorhabditis sp.* nematode, 12- Control (untreated). Spray treatment by using a hand sprayer 1 liter capacity, separately or in combination. The treated potted plants were placed under a wooden screen cages. Hundreds of 50 male with 50 females 1st instar nymphs of *H. littoralis* were obtained from the standard laboratory culture then released inside the cages and left for feeding on the treated potted plant for 14 days, percentages of mortality was recorded for each treatment at intervals each 2 days. The potted plants were changed with another untreated new one. Every treatment and control was replicated four times. Biological aspects were estimated for the remaining insect inside the cages till reached the adult stage and allowed for mating, oviposition and laying eggs, thin calculated the number of the first offspring (F1) (new generation of 1st instar nymphs).

Statistical analysis. Statistical, all data were subjected to analysis of Variance (ANOVA) through "SPSS" Computer Program. To differentiate between means, Duncan's multiple range test (P = 0.05) was used (DUNCAN, 1965).

Results and Discussion

Insecticide mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance or to combat current resistance in a pest species. Insecticide resistance has become a major obstacle to successful chemical control with conventional insecticides. The evolution of resistance to insecticides is governed by a complex of events and factors; mainly, intense and repeated applications of insecticides which are often from the same chemical group or which employ the same mode of action. To prevent the resistance phenomenon, there is a need for different compounds having different modes of action (FEI YI *et al.*, 2012).

Combined mixture of insecticides for 1- The amount of material used can be reduced without loss of activity, 2- To delay the selection of resistant strains, 3- Biological evidence alone is not enough, because the observed efficacy of a mixture should be compared with its expected efficacy (GISI, 1991).

Joint action of different bio-agents

Table 1 revealed the presence of antagonistic effects between Garlic oil and *Euphorbia* extract in the combination mixture that was expressed as co-toxicity factors with (-24), for *Steinernima sp.* nematode and Garlic oil (-44), for *Heterorhabditis sp.* nematode and Garlic oil (-40), for Garlic oil, *Euphorbia* extract and *Steinernima sp.* nematode (-47.9) and finally for Garlic oil, *Euphorbia* extract, *Heterorhabditis sp.* nematode was (-48.88). Despite the increase in the proportion of death in the mixture for all the tested groups the type of interactionwere antagonism. For example, in group1 percentage of mortality for 1st nymph instar of *H. littoralis* that fed on artificial diet treated with Garlic oil as mentioned in the methods cleared 50% and for *Euphorbia* extract was 45% while for their mixture was 55%.

These result indicated that all the tested materials had variable mode of action, which resulted in significantly antagonistic effects. Pesticides are grouped into classes of compounds that have similar chemical structure and modes of toxic action. The term (mode of toxic action) is defined as a series of key processes that begins with the interaction of a pesticide with a receptor site and proceed through on the operational and anatomical changes in an organism that result in sub lethal or lethal effects (EPA, 2000). Gordon and El-DEFRAWI (1960) speculated that antagonism could be produce if one insecticide interfere with the activation of the other by retarding it, so maximal effects were not arrived at simultaneously, and the detoxifying enzymes would have more chance to acting on the less toxic parent compound. HEWLETT (1960) suggested that independence of action, wither partial or complete, might be due to different speeds of action as well as to different sites of action. Mode of toxic action is essential in understanding how mixtures may act jointly. The effect of the tested materials may be due to the presence of some chemical groups in their structure e.g., alkaloids, tannins, mono and triterpenes and sterols (SCHMIDIT & WEMER, 1993). Co-toxicity action of the mixture have been based physical and chemical properties of the compound (ABDE-MAGEED & SHALABY, 2011). MANSOUR *et al.* (1966) recorded that the antagonistic pairs consisted mainly of two relatively strong insecticides, or combinations of one strong and one weak insecticides. None of the pairs was of two weak or two very strong insecticides. One material may interfere with the activation of the other material, antagonism would occur. Table 1 indicated that the all tested materials were relatively strong, or one material was stronger than the other was, or nearly equal in the combined mixture. Reviewing the obtained results, it can be concluded that, the efficiency of the different tested bio agent material mixtures against the first instar nymphs of *H. littoralis* varied tremendously according to the type of component of the tested mixtures. The effect of volatile and plant extracts may be attributed to one or more of the following, fumigants, contact or poisoning effect and may due to the presence of some chemical groups in their structure such alkaloides, tannins, mono and triterpenes, flavonodes, steroles, saponines and glycosides.

Semi-field test

Combined effect of different bio-agent under field condition on the mortality percentage of the 1st instar nymph after feeding on Clove potted plant sprayed with LC50 concentration values.

Table 2 revealed that, the appearance of mortality started form the fourth day after the treatment for all treatments; garlic oil and *Euphorbia* extract gave nearly the same mortality percentage till twelve day then getting a small increase 45% for the oil and 50% for the extract. Mixture of both oil with extract slightly increased mortality to 55%. Mixture of each species of nematode *Steinernema* or *Heterorhabditis sp.* with garlic oil and *Euphorbia* extract increased mortality percentage to 62.5 and 50%, compared with 25 and 17.5% for *Steinernema* and *Heterorhabditis sp.*, respectively if they used separately. This result indicated that the presence of garlic oil and *Euphorbia* extract in one mixture with *Steinernema* or *Heterorhabditis sp.* nematode increased the mortality percentages of the second instar nymphs of *H. littoralis* when they mixed in their diet. These results indicated that the presence of different mode of actions of the tested three bio-agents where the oil or the extract may activated the symbiotic bacteria of nematodes causing septicemia to the insect and increasing of insect mortality. In addition, the symbiotic bacteria of nematode cause damage for the insect tissues and facilitate the oil or the extract penetration into cell tissues and making their effects.**Table 1.** Joint action of different bio-agents after mixed with artificial diet on the percentage mortality of 1st instar nymph of *H. littoralis*.

Table 1. Joint action of different bio-agents after mixed with artificial diet on the percentage mortality of 1st instar nymph of *H. littoralis.* Legend: Nematode (S)* = *Steinernema*, Nematode (H)**= *Heterorhabditis sp.*

Com-bined group	**Material**	**Mor-tality (%)**	**Expected mortality (%)**	**Observed mortality (%)**	**Co-toxicity factor**	**Joint action category**
1	Garlic oil	50	95	55	- 42	Antagonism
	Euphorbia extract	45				
	Garlic oil+ *Euphorbia* extract	55				
2	Nematode (S)*	25	75	42	- 44	Antagonism
	Garlic oil	50				
	Nematode (S)+Garlic oil	42				
3	Nematode (H)**	17.5	67.5	40	- 40	Antagonism
	Garlic oil	50				
	Nematode (H)+ Garlic oil	40				
4	*Euphorbia* extract	45	70	40	-0.43	Antagonism
	Nematode (S)	25				
	Nematode (S)+ *Euphorbia* extract	40				
5	*Euphorbia* extract	45	62.5	32	-0.49	Antagonism
	Nematode (H)	17.5				
	Nematode (H)+ *Euphorbia* extract	32				
6	Garlic oil	50	120	62.5	- 47.9	Antagonism
	Euphorbia extract	45				
	Nematode (S)	25				
	Garlic oil+ *Euphorbia* extract +Nematode (S)	62.5				
7	Garlic oil	50	112.5	57.5	- 48.88	Antagonism
	Euphorbia extract	45				
	Nematode (H)	17.5				
	Garlic oil+*Euphorbia* extract+Nematode (H)	57.5				

These findings agreed with ABDEL-MAGEED *et al.* (2011) they recorded that insecticidal mixture are usually applied in the field to enhance the spectrum of control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance or to combat current resistance in a pest species. To prevent the resistance phenomenon, there are need for different compounds having different mode of action (AYDIN & GURKAN, 2006). The rapid action of oils against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (KOSTYUKOVSKY *et al.*, 2002). Plant extracts include alkaloids, terpenoids, phenols, flavonoids, and other minor chemicals can affect insects in several ways (LIN-ER *et al.*, 1995).

Table 2. Effect of sub lethal concentration of different bio agents on mortality percentage of first instar nymphs of *H. littoralis* at intervals

Tested material (Treatments)	Mean % mortality at intervals (in days)						
	2	4	6	8	10	12	14
Euphorbia extract	0	25	32.5	37.5	42.5	45	45 bc
Garlic oil	0	25	30	37.5	45	50	50 b
Euphorbia extract + Garlic oil	0	15	37.5	50	55	55	55 b
Steinernema nematode	12.5	12.5	20	25	25	25	25 cd
Steinernema + Garlic oil	14.0	22	26	30	38	42	42 c
Sternernema + *Euphorbia* extract	10	20	28	32	36	40	40 c
Stinernema + Garlic oil + *Ephorbia* extract	0	15	47.5	57.5	62.5	62.5	62.5 b
Heterorhabditis sp. nematode	5	12.5	17.5	17.5	17.5	17.5	17.5 d
Heterorhabditis sp. + Garlic oil	12	16	22	32	36	40	40 c
Heterorhabditis sp. + *Euphorbia* extract	6	18	22	26	32	32	32 c
Heterorhabditis sp. + Garlic oil + *Euphorbia* extract	0	17.5	4205	50	50	50	50 b
Control	0	0	0	0	0	0	0 a

Mean mortality percentage after 14 days in the vertical column that have the same letters had no significantly differences.

Combined effect of different bio agent under field condition on the biological aspects of the 1St nymph instar after feeding on Clove potted plant sprayed with LC50 concentration values.

This experiment was conducted in order to determine the insecticidal activity of the most effective extract (*Euphorbia*) and the plant oil (Garlic oil) and their combined mixture on the biological aspects of *H. littoralis* under semi-field condition. Data on Table 3 revealed a significant prolongation in the total nymphal period of *H. littoralis* that left for feeding of all the treated potted plants where it was 87.28, 98.93, and 100.13 days on the plants treated with *Euphorbia* extract, Garlic oil and the mixture of both (extract + oil), respectively compared with 63.52 days for the control untreated potted plants. Highly significant increasing was recorded for the pre oviposition period for their adults that emerged from nymphs that fed on the treated plants, it was 51.7 days for the mixture treated plants and 56.9, 59.9 days for the extract and for the oil respectively, when used separately compared to 23 days in the untreated checked plants. Highly reduction occurred in the oviposition periods for all treatment ranged from 16.71 to 23.50 days in comparing with 38.22 days for the control, decreasing of oviposition period making the adult deposited low number of eggs and decreased the population densities of the insect. Egg production by females were greatly decreased by the treatments they were 48.24, 57.90 and 50.00 eggs/female for the extract, oil and their mixture while they were 152.80 eggs/female for control test. Percentages of egg hatchability was vigorously affected by the three different treatments, it decreased form 94% for the untreated plants to 19.3% for the mixture treatment and for 25.6 and 30.5% in case of the extract and the oil treatment, respectively. Adult longevity was significantly decreased by the treatments it decreased from 104 days in the control to 114-127 days for the extract, oil and their combined mixture. Life span was greatly decreased from 183.00 in the control compared to 222.38–236.90 days for the extract, oil and the mixture. From the foregoing data, it will be concluded that *Euphorbia* extract, Garlic oil, separately or in combination causing a great effect on the biological aspects of *H. littoralis* by increasing the total nymphal period, pre-oviposition, oviposition periods, life span, and number of the deposited eggs and percentage of egg hatchability. Combination between the extract and the oil increased the total nymphal period and decreased the percentage of egg hatchability than if the two materials were used separately.

The obtained results showed that the combined mixture gave encouraging results against *H. littoralis*. These findings agrees with CASIDA (1990) who mentioned that the toxicity of phytochemicals depends on several factors among which are the chemical composition of the oil or extract and insect susceptibility. Some other different reported by different scientists. FANG *et al.* (2002) thought the order of toxicity would often vary depending on the particular insecticide. The insecticidal activity of monoterpens, the major components of essential oils, has been reported against stored products insects (GARCIA *et al.,* 2005). Many plant essential oils showed a broad spectrum of activity against pest insect ranging from insecticidal, antiffedant, repellent, oviposition deterrent, growth regulatory and antivector activities (KOUL *et al.,* 2008). In addition, they recorded that the same chemical constituents of the oils interfere with the octopaminergic nervous system in insects. Essential oil constituents are primarily lipophilic compounds that act as toxins, feeding deterrents and oviposition deterrents to a wide variety of insect pests (RICE & COAST, 1994). Secondary compounds in *Euphorbia* extract include alkaloids, terpinoids, phenolics, flavonoids, and their minor chemicals can affect *H. littoralis* in several ways. The mixture of *Euphorbia* extract and the Garlic oil proved possible insecticidal activity against *H. littoralis*. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to plant products based pesticides owing to complex mixture of constituents that characterize many of these products. Ultimately, it is in developing countries, which are rich in endemic plant biodiversity that these pesticides may ultimately have their greatest impact in future integrated pest management programs due to their safety to non-target organisms and environment. These substances are not only of low cost, but also have less environmental impact in term of insecticidal hazard. Our findings indicated that *Euphorbia* 80% methanol extract and Garlic oil may be used separately or in combination as alternatives safe tools against *H. littoralis* grasshopper.

The data in Table 4 show effect of entomopathogenic nematode, *Steinernema carpocapsea* separately or in combination with Garlic essential oil or 80% methanolic *Euphorbia* plant extract on some biological parameters of *H. littoralis*. The data cleared that there was increase in the total nymphal period, it lasted 70.8 day for the mixture of the all tested bio agents (S. carpocapsea +oil + *Euphorbia* extract) and nematode + extract 65.8 comparing with 63.5 day for control. Oviposition period decreased in all treatments if the bio-agents used separately or in combinations, they ranged from 17.8–26.2 day in comparison with 38.2day in control. The mean number of deposited eggs/female significantly decreased in all treatments ranged from 63.5 to 73.0 egg/female comparing with 152.8 for the control. Highly significance obtained in the egg fertility it decreased by about 86% in case of treatment with nematode + extract, where it decreased to 72% in case treatment with mixture *Steinernema* + oil + extract,

Table 3. Effect of *Euphorbia* extract and Garlic oil on biological aspects of *H. littoralis.*

Biological parameters	Treatments (Mean ± SE)				F-value
	Euphorbia	Oil	*Euphorbia*+Oil	Control	
1st instar nymph	10.47±0.12 b	10.50±0.14 b	11.61±0.22 a	8.40±0.10 c	105.710**
2nd instar nymph	15.50±0.41 a	15.43±0.27 a	14.91±0.41 a	10.06±0.12 b	109.221**
3rd instar nymph	18.56±0.22 c	23.00±0.48 b	24.48±0.73 a	12.94±0.12 d	234.326**
4th instar nymph	20.59±0.40 b	24.37±0.48 a	24.17±0.41 a	15.48±0.16 c	174.727**
5th instar nymph	22.25±0.42 b	25.27±0.42 a	25.00±0.37 a	16.54±0.14 c	193.255**
Total nymphal period (in days)	87.28±0.84 b	98.93±0.97 a	100.13±1.36 a	63.52±0.31 c	549.865**
Pre-oviposition period (in days)	56.94±1.43 a	59.90±1.63 a	51.70±1.14 b	23.00±0.15 c	326.665**
Oviposition period (in days)	16.71±0.51 c	19.10±0.59 c	23.50±0.89 b	38.22±1.19 a	113.699**
Post-oviposition period (in days)	40.59±2.10 c	47.30±2.17 b	56.00±0.87 a	42.48±0.68 c	16.865**
No. of deposited eggs/female	48.24±7.14 b	57.90±6.57 b	50.00±5.96 b	152.83±2.68a	111.476**
Percentage of Egg hatchability	25.59±3.78 bc	30.50±2.52 b	19.30±3.34 c	94.04±0.51 a	225.921**
Incubation period	31.65±4.50 b	50.30±5.30 a	24.40±0.93 b	23.00±0.23 b	12.854**
Longevity (in days)	114.03±1.57 c	118.37±1.41 b	127.87±1.01 a	104.20±0.96 d	61.506**
Total mortality percentage	0.56±0.16 c	1.67±0.14 a	1.00±0.22 b	0.00±0.00 d	24.844**
Malformation percentage	0.06±0.04 b	0.00±0.00 b	1.00±0.00 a	0.00±0.00 b	94.542**
Lifespan (in days)	222.38±4.43 b	232.73±5.65 ab	236.91±3.85 a	183.32±3.62 c	27.548**

**= Highly significant
Same letters within the horizontal column were not significantly different P <0.05

Table 4. Combined effect of *Euphorbia* extract + Garlic oil + *Steinernema* nematode on biological aspects of *H. littoralis.*

Biological parameters	Treatments (Mean±SE)					F-value
	Steinern-ema	*Steinern-ema* +Oil	*Steinern-ema* +*Euphorbia*	*Steinern-ema* +Oil +*Eupho-rbia*	Control	
1st instar nymph	9.69±0.15 c	10.35±0.17 b	10.07±0.17 bc	11.27±0.22 a	8.40±0.10 d	52.693**
2nd instar nymph	9.72±0.14 d	11.38±0.14 c	11.83±0.19 b	12.46±0.21 a	10.06±0.12 d	56.265**
3rd instar nymph	12.50±0.20 cd	12.24±0.16 d	13.17±0.17 ab	13.58±0.21 a	12.94±0.12 bc	8.759**
4th instar nymph	16.28±0.23 a	13.10±0.14 d	14.47±0.13 c	15.19±0.24 b	15.48±0.16 b	40.208**
5th instar nymph	16.91±0.17 b	13.97±0.15 d	16.17±0.17 c	18.23±0.40 a	16.54±0.14 bc	48.980**
Total nymphal period (in days)	64.16±0.96 bc	61.07±0.62 d	65.77±0.48 b	70.81±1.10 a	63.52±0.31 c	24.296**
Pre-oviposition period (in days)	23.25±0.31 d	60.60±1.59 a	33.64±0.98 c	50.00±1.05 b	23.00±0.15 d	457.722**
Oviposition period (in days)	26.17±0.58 b	17.80±0.63 c	24.55±0.81 b	25.20±0.95 b	38.22±1.19 a	58.550**
Post-oviposition period (in days)	47.50±1.10 b	45.60±2.52 bc	40.36±1.32 d	51.40±1.61 a	42.48±0.68 cd	9.555**
No. of deposited eggs/female	72.92±4.94 b	64.60±5.95 b	68.64±5.31 b	63.50±5.22 b	152.83±2.68 a	106.699**
Percentage of Egg hatchability	33.75±3.85 b	37.90±3.26 b	20.46±3.84 c	25.50±2.29 c	94.04±0.51 a	191.716**
Incubation period	24.08±2.28 c	44.20±2.47 a	35.64±1.16 b	26.70±0.91 c	23.00±0.23 c	40.828**
Longevity (in days)	92.97±0.86 c	116.90±1.90 a	89.93±1.64 c	120.46±1.31 a	104.20±0.96 b	94.084**
Total mortality percentage	0.56±0.36 a	0.72±0.34 a	0.67±0.39 a	0.92±0.43 a	0.00±0.00 a	1.678 NS
Malformation percentage	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.08±0.05 a	0.00±0.00 b	2.850*
Lifespan (in days)	165.28±3.65 c	193.90±5.93 ab	168.60±4.95 c	201.58±4.69 a	183.32±3.62 b	11.417**

**= Highly significant *= Significant NS= not significant
Same letters within the horizontal column were not significantly different (P <0.05)

The data in Table 5 shows the effect of another species of entomopathogenic nematode (*Heterorhabditis sp.*) on some biological parameters of *H. littoralis* when applied separately or in a combination with the same previous agents, Garlic oil or *Euphorbia* extract. The data clears that application of entomopathogenic nematode, *Heterorhabditis sp.* alone or with other agents led to slight differences in total nymphal period among treatments in relation to control, where there is an increase in nymphal period by about two days as in treatment with nematode alone to about 5 days as in treatment with mixture of nematode and Garlic oil with *Euphorbia* while treatment with nematode and Garlic oil led to decrease in nymphal period as a whole by about 2 days from the control. Treatment with *Heterorhabditis sp.* nematode alone or mixed with the other additives had antagonistic effect on oviposition period where it decreased by about 12, 18, 12, 12 days as treatment with nematode alone, nematode and Garlic, nematode and *Euphorbia* extract and mixture of nematode and Garlic oil with *Euphorbia* extract, respectively. In case of female fecundity, it is observed that all treatments led to decrease the female fecundity by more than 50%, where in the mixture of nematode and Garlic oil with *Euphorbia* treatment the percent decrease was about 68.2%, about 59.2% in both nematode+Garlic oil and nematode+*Euphorbia* extract, and treatment with nematode alone led about 58% off in female fecundity. The incubation period was increased by different treatments in various degrees if compared with the control treatment. Longevity of the resulting adults of *H. littoralis* (F1) increased in some treatments as in case nematode+Garlic oil which increased by about 23 days more than the control, and nematode+Garlic oil+*Euphorbia* extract which increased by about 17 days more than the control, while the other treatments led to decrease in the longevity by about 10.7 and 7.3 day less than the control in treatments of nematode alone and nematode+*Euphorbia*, respectively.

Table 5. Combination of different bio-agent (*Euphorbia* extract + Garlic oil + *Heterorhabditis sp.* nematode) on biological aspects of *H. littoralis*

Biological parameters	Treatments (Mean±SE)					F-value
	Hetero-rhabditis sp.	***Hetero-rhabditis sp.* +Oil**	***Hetero-rhabditis sp.* +*Euphorbia***	***Hetero-rhabditis sp.* +Oil +*Euphorbia***	**Control**	
1st instar nymph	9.65± 0.14c	10.57 ±0.21b	10.32 ±0.13b	11.39±0.19a	8.40±0.10d	65.163**
2nd instar nymph	10.61 ±0.14d	11.33 ±0.19c	11.94 ±0.16b	12.42±0.19a	10.06±0.12e	41.350**
3rd instar nymph	12.97 ±0.21b	12.27 ±0.19b	13.38 ±0.15ab	13.49±0.19a	12.94±0.12c	7.475**
4th instar nymph	15.32 ±0.23a	13.20 ±0.17c	14.27 ±0.14b	14.64±0.21b	15.48±0.16a	25.141**
5th instar nymph	16.68 ±0.22a	14.33 ±0.14c	15.53 ±0.19b	16.27±0.24a	16.54±0.14a	24.954**
Total nymphal period (in days)	65.10 ±0.56b	61.67 ±0.86c	65.44 ±0.67b	68.33±0.95a	63.52±0.31b	13.304**
Pre-oviposition period (in days)	23.83 ±0.35c	50.70 ±2.08a	36.08 ±0.68b	48.33±1.20a	23.00±0.15c	222.972**
Oviposition period (in days)	26.67 ±0.67c	30.60 ±1.61b	26.50 ±0.74c	26.08±1.13c	38.22±1.19a	25.004**
Post-oviposition period (in days)	48.17 ±1.11c	58.50 ±1.64a	39.83 ±1.25d	52.25±1.27b	42.48±0.68d	41.609**
No. of deposited eggs/female	60.42 ±7.29b	62.00 ±5.54b	62.08 ±5.95b	47.92±5.31b	152.83±2.68a	102.460**
Percentage of Egg hatchability	34.58 ±3.11b	27.00 ±3.59bc	30.83 ±4.08b	20.42±3.11c	94.04±0.52a	179.532**
Incubation period	24.92 ±0.94c	43.70 ±2.53a	31.25 ±1.57b	21.42±2.99c	23.00±0.23c	27.239**
Longevity (in days)	93.52 ±1.07d	127.50 ±2.01a	96.97 ±1.19d	121.03±0.90b	104.20±0.96c	132.126**
Total mortality percentage	0.61 ±0.41a	0.67 ±0.35a	0.47 ±0.25a	0.52±0.30a	0.00±0.00a	1.236NS
Malformation percentage	0.00 ±0.00a	0.00 ±0.00a	0.00 ±0.00a	0.00±0.00a	0.00±0.00a	0.000NS
Lifespan (in days)	168.26 ±3.62c	202.90 ±6.09a	173.18 ±3.80bc	195.94±3.40a	183.32±3.62b	12.352**

**= Highly significant NS= not significant at P< 0.05

Same letters within the horizontal column were not significantly different P <0.05

Form the previous findings, we could be concluded that the tested plant extract (*Euphorbia pulcharrima* at 0.714% concentration), essential oil of Garlic plant (*Allium sativum* at 0.067% concentration) may be used separately or in combination for controlling *H. littoralis*, also could be used in combination with the entomopathogenic nematode *S. carpocapsa* or *Heterorhabditis sp.* or in simultaneous application during the integrated pest managements of *H. littoralis* for decreasing the appearance insect resistance and increase the insect mortality.

We believe that these types of studies are critical for realistic estimation of toxicity, because rarely are organisms exposed to only a single chemical in the field. These studies should be the focus of future researches.

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Assessment of the Threats to the Biodiversity and Habitats in "Stara Reka" Reserve (Bulgaria) and Its Adjacent Subalpine and Alpine Areas

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Abstract. The assessment of the threats in the "Stara Reka" reserve and its adjacent subalpine and alpine areas is important since it makes it possible the appropriate conservation measures to be taken in order to prevent or reduce the negative effects on the biodiversity and habitats. The assessment was based on systematic studies and visits in the "Stara Reka" Reserve, located within National Park "Central Balkan" (Bulgaria), during spring, summer and autumn seasons of 2010-2011. A number of threats were recorded, where those by anthropogenic origin were predominating. Tourists have negatively influenced the wild plants such as *Allium ursinum*, *Inula helenium* and *Primula frondosa* by picking them up. Damages were registered on the information system and signs. Waste disposal, fires, poaching and illegal fishing were also some of the recorded threats. Many natural succession changes quite dynamically vary the habitats in the reserve, but the most dangerous for the biodiversity and degradation of habitats remain fires, erosion and introduction of alien species.

Key words: threats, biodiversity, habitats, reserve, subalpine, alpine, "Central Balkan" National park.

Introduction

The "Central Balkan" National Park lies in the heart of Bulgaria, nestled in the central and higher portions of the Balkan Range. The Park contains rare and endangered wildlife species and communities, self-regulating ecosystems of biological diversity, as well as historical sites of global cultural and scientific significance. The park is a favorite place for recreation among tourists from Bulgaria and from around the world. The local people develop traditional livelihoods and crafts here. There is enormous responsibility to preserve this unique environment, following the principles of sustainable tourism and use of resources (YANKOV, 2001).

There are nine Reserves within the "Central Balkan" National Park: Boatin, Tsarichina, Kozya Stena, Steneto, Severen Dzhendem, Peeshti Skali, Sokolna, Dzhendema and Stara Reka. In total, they

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cover 20019.6 hectares, and contain varying examples of natural ecosystems featuring unique wildlife species and their habitats (Central Balkan National Park Directorate, 2012).

Stara Reka (Old River) Reserve was founded on March 19, 1981. With an area of 1974.7 hectares, it preserves the natural scenery of some of the most picturesque Bulgarian rivers, comprising in full the watershed of the Stara Reka River and its tributaries. This Reserve is among the richest in rare plant species in Bulgaria, with over 45 listed in the Bulgarian Red Data Book. Another 20 species are endemic to Bulgaria, including Kerner's thistle (*Cientaurea kernerana*), Troyan campanula (*Campanula thyrsoides*) and Bulgarian betony (*Betonica bulgarica*). The forest contains thermophilic flowering ash (*Fraxinus ornus*) and hornbeam (*Carpinus betulus*) coexisting with beech (*Fagus sylvatica*), fir (*Abies alba*), spruce (*Picea abies*), sycamore (*Acer pseudoplatanus*) and Norway maple (*Acer platanoides*) (Central Balkan National Park Directorate, 2012).

Over 100 species of vertebrate fauna have found sanctuary in this area. The Reserve is home to bears (*Ursus arctos*), wolves (*Canis lupus*), weasels (*Mustela nivalis*) and wild cats (*Felis silvestris*), several predatory birds: the golden eagle (*Aquila heliaca*), large (*Accipiter gentilis*) and small hawks (*Accipiter nisus*), honey buzzards (*Pernis apivorus*), and barn owls (*Tyto alba*), amphibians and reptiles - european tree frog (*Hyla arborea*), fire salamander (*Salamandra salamandra*), blind worm snake (*Typhlops vermicularis*) and many others (YANKOV, 2001).

The purpose of the current study is to identify the real and potential threats to biodiversity and habitats in "Stara reka" Reserve, its adjacent subalpine and alpine areas of "Central Balkan" National Park in Bulgaria, and, to give some recommendations for conservation measures of this protected area.

Materials and Methods

The assessment of the real and potential threats to the biodiversity and habitats of "Stara Reka" Reserve is based on systematic visits and research of different parts of the study area (Fig.1).

Fig. 1. Indicative map of the territory of "Central Balkan" National Park and the study area of "Stara Reka" Reserve and its adjacent subalpine and alpine areas.

The field research is conducted within the territory of "Stara Reka" Reserve and its adjacent subalpine and alpine areas in "Central Balkan" National Park once a month in the active for most animals and plants season – from March to November, during two-year period 2010 – 2011.

Signals for breaches, are received by surveying the local population, including tourists, mountain guides and alpinists. Collaboration with the rangers and administration of "Central Balkan" National Park is established in order to achieve the study's objectives. The Management Plan for the National Park "Central Balkan" for 2001 – 2010 (YANKOV, 2001) and a map of "Stara Reka" Reserve are used, provided by the Directorate to locate the investigated areas at the terrain. Current issues concerning the study area are discussed with experts from the park.

Number of types of habitats identified in the study was 54. Plants species were determined according to Identification guide of plants in Bulgaria (DELIPAVLOV *et al.,* 1992) and Identification guide of bushes and trees in Bulgaria (GRAMATIKOV, 1992). For determination of the fauna Identification guide of vertebrate animals in Bulgaria (PESHEV, 2001) is used. The conservation status of the species in this study are presented according to the contemporary Bulgarian and European legislation (Table 1).

Results and Discussion

The identified threats to the bio-diversity and habitats as a result of the current study within "Stara Reka" Reserve and its adjacent areas are presented in Table 2 and Table 3.

1. Threats arising from natural phenomena and events

1.1. Erosion in treeless zone (to the west of "Ravnets" Hut)

Ditch erosion was found in the treeless zone, adjacent to the reservation in the area "Alay Bozan", to the west of "Ravnets" Hut (over 257 Forest department). The eroded area covers a large acreage. The furrow has several large and smaller branches. The terrain has an average gradient. The furrows have steep banks, which facilitates braking of soil and rocks. When it rains, water runs in the furrows, which carry away the particles torn. At some places, especially at the top, the vegetation in the furrows, which are formed due to the erosion, has begun to recover.

1.2. Large erosion below "Kupena" Peak

Ditch erosion was also found in the treeless zone, adjacent to the reservation in the area, adjacent to the reserve, under Kupena Peak. Reasons for this erosion are large gradient of the slope, which contributes to more rapid sliding of soil and rocks, and a large amount of alluvium from rainwater substrate. The stage of development of the eroded area covers a large area of the hillside. Erosion is in the form of a single groove. The terrain of the erosion is high gradient. Erosion covers an area of great length. There are trends for restoration of the grassy vegetation in the upper area affected by erosion.

Negative influences of both types of the above-mentioned erosion, on the habitat: Erosion reduces the area of alpine meadows. The loss of soil material impact on soil functions at the site of the erosion as well as on its adjacent areas (YANKOV, 2001).

1.3. Wind throw and wind fracture

Phenomena of wind throw and wind fracture are a result of wind action. Wind throw is extracting trees with shallow root system, together with their roots. Wind fracture is breaking stems of the trees. In the forests on the territory of the reserve, wind throw and wind fracture are commonly found on common beech (*Fagus sylvatica*) and white fir (*Abies alba).* Wind throw and wind fracture are intermittent natural threat to forests.

Wind throw of common beech was found at the following places: next to the path between "Vassil Levski" Hut and "Topalitsa" area; next to the path from "Vassil Levski" Hut to Botev Peak (in 242 Forest department); in the habitat of wild garlic between "Balkanski rozi" Hut and "Vassil Levski" Hut.

Wind throw of white fir was found on the right bank Stara Reka River, next to the path between "Balkanski rozi" Hut and "Vassil Levski" Hut.

Wind fracture of common beech was found at the following places: next to the

Table 1. Legal documents in the contemporary Bulgarian and European legislation, determining the conservation status of the registered species in the current study.

<table>
<tr><th>Abbreviation</th><th>Legal Document/Annexes</th><th>Source</th></tr>
<tr><td rowspan="2">BPA</td><td>Biodiversity Protection Act of Bulgaria</td><td>State Gazette Nr 77 of August 9, 2002 (amended - State Gazette Nr 88 of 2005 and State Gazette Nr 94 of 2007)</td></tr>
<tr><td colspan="2">Appendix II - species whose conservation requires the designation of special areas of conservation of their habitats.
Appendix III - species protected in the whole of the country.
Appendix IV - species under the mode of protection and regulated use of nature.</td></tr>
<tr><td rowspan="2">DCE'92/43</td><td>Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora</td><td>Amended by Council Directive 2006/105/EC of 20 November 2006), accessible on-line at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:01992L0043-20070101:EN:NOT</td></tr>
<tr><td colspan="2">Annex II - animal and plant species of community interest whose conservation requires the designation of special areas of conservation.
Annex IV - animal and plant species of community interest in need of strict protection.
Annex V - animal and plant species of community interest who is taking in the wild and exploitation may be subject to management measures.</td></tr>
<tr><td rowspan="2">Bern</td><td>Convention on the Conservation of European Wildlife and Natural Habitats, Bern, 19.IX.1979</td><td>Accessible on-line at: http://conventions.coe.int/Treaty/en/Treaties/Html/104.htm</td></tr>
<tr><td colspan="2">Appendix II - strictly protected fauna species (status in force since 1 March 2002).
Appendix III - protected fauna species (status in force since 1 March 2002).</td></tr>
<tr><td rowspan="2">RDB</td><td>Red Data Book of Republic of Bulgaria, 2011, Vol. 1, Vol. 2.</td><td>Available online at: http://e-ecodb.bas.bg/rdb/bg/</td></tr>
<tr><td colspan="2">Categories are in accordance with IUCN. EX – Extinct, CR - Critically Endangered, EN – Endangered, VU - Vulnerable.</td></tr>
<tr><td rowspan="2">IUCN</td><td>IUCN Red List of Threatened Species. Version 2010.2.</td><td>Accessible on-line at http://www.iucnredlist.org (Downloaded on 30 August 2010)</td></tr>
<tr><td colspan="2">EN - Endangered (a taxon is Endangered when the best available evidence indicates that it meets any of the criteria A to E for Endangered, and it is therefore considered to be facing a very high risk of extinction in the wild).
VU - Vulnerable (a taxon is Vulnerable when the best available evidence indicates that it meets any of the criteria A to E for Vulnerable, and it is therefore considered to be facing a high risk of extinction in the wild).
NT - Near Threatened (a taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future).
LC - Least Concern (a taxon is Least Concern when it has been evaluated against the criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened. Widespread and abundant taxa are included in this category).</td></tr>
<tr><td rowspan="2">CITES</td><td>Convention on International Trade in Endangered Species of Wild Fauna and Flora, July 1 1975</td><td>Accessible on-line at http://www.cites.org/</td></tr>
<tr><td colspan="2">Appendix I - Species threatened with extinction, which are or may be affected by trade. Trade in specimens of these species must be subject to particularly strict regulation in order not to endanger further their survival and must only be authorized in exceptional circumstances.
Appendix II - (a) all species which although not necessarily now threatened with extinction may become so unless trade in specimens of such species is subject to strict regulation in order to avoid utilization incompatible with their survival; and (b) other species which must be subject to regulation in order that trade in specimens of certain species referred to in sub-paragraph (a) of this paragraph may be brought under effective control. Species not necessarily threatened with extinction, but in which trade must be controlled in order to avoid utilization incompatible with their survival.</td></tr>
</table>

Table 2. Threats arising from natural phenomena and events to biodiversity and habitats in “Stara Reka” Reserve and adjacent areas.

Threat	Species/habitats affected	Description
Erosion in treeless zone	Habitats in the alpine zone.	Ditch erosion was found in the zone, adjacent to the reservation in the area "Alay Bozan" to the west of “Ravnets” Hut.
		Ditch erosion was also found below “Kupena” Peak consequently from large gradient of the slope, which contributes to more rapid sliding of soil and rocks.
Wind throw and wind fracture	Common beech (*Fagus sylvatica*)	In the forests on the territory of the reserve, wind throw and wind fracture are commonly found.
	White fir (*Abies alba)*	
Sliding of rocks	Various plant and invertebrate communities.	Sliding of rocks are recorded over the trail, before Barazh site.
Wild boar digging	Grasslands in the alpine meadows.	This digging leads to degradation of grasslands in the alpine meadows.
Invasive plant and animal species	Pine moth *(Thaumetopoea pityocampa*)	A growing population, which may affect seriously pine, trees in the area.
	Siberian juniper *(Juniperus sibirica*)	An extension of the projective covering and conquest of new territories.

path from “Vassil Levski” Hut and “Topalitsa” area; next to the path between “Hubavets” Hut and “Balkanski rozi” Hut; next to the path from “Balkanski rozi” Hut to “Vassil Levski” Hut; on the path from “Vassil Levski” Hut to Botev Peak (in 242 Forest district). The processes of wind throw and wind fracture lead to the formation of open spaces in the forest, located in the surveyed areas. Over time, a secondary succession will occur at the described areas, associated with recovery of the forest of young beech seedlings. These natural phenomena in the ecosystem can have a positive impact in the future due to habitat enrichment with new species.

1.4. Sliding of rocks

Sliding of rocks was recorded over the trail, before “Barazh” site. This phenomenon is a threat to natural habitats in the area of the cluster of slide rocks. This sliding of rocks, in the future will lead to changes in the habitat - accumulated rocks will create new environmental lowers, having suitable conditions for hiding of insects, snails, reptiles, rodents.

1.5. Wild boar digging

There are lands in the treeless area (to the west of “Ravnets” Hut and in “Topalitsa” area, dug by wild boar (*Sus scrofa*). This digging leads to degradation of grasslands in the alpine meadows. At some places (to the west of “Ravnets” Hut) restored grasslands were found, from the damages caused by wild boar.

1.6. Invasion of pine moth (Thaumetopoea pityocampa)

A growing population of the pine moth (*Thaumetopoea pityocampa)* was recorded. Caterpillars of this moth damage many pine trees in the border areas of the reserve. They attack the needles in condition of calamity and the species is able to remove the needles from coniferous trees.

1.7. Extension of the projective cover of Siberian juniper

We recorded an extension of the projective covering of Siberian juniper *(Juniperus*

Table 3. Registered threats of anthropogenic origin to the biodiversity and habitats in "Stara Reka" Reserve and adjacent areas.

Threat	Species/habitats affected	Conservation status	Description
Damages on individual plant and animal species	Fire salamander (*Salamandra salamandra*)	BPA-III; Bern-III, IUCN-LC.	Trampled animals by horses and tourists on the trails.
	Wild garlic (*Allium ursinum*)	-	Picked up by tourists and contamination of its habitats by wastes.
	White elecampane (*Inula helenium*)	The Minister of Environment and Water limits its collection for therapeutic purposes, under the Law on Medicinal Plants (State Gazette, 2000).	Picked up by tourists.
	Balkan primrose (*Primula frondosa*)	BPA-III, RDB-EN, endemic species for Bulgaria.	Picked up by tourists.
	Pine (*Pinus nigra*), Locust tree (*Robinia pseudoacacia)* and Hornbeam (*Carpinus orientalis)*	*Pinus nigra* – IUCN-LR/lc *Robinia pseudoacacia* – IUCN-LC	Human-related forest fire burned the region over "Suchurum" landmark.
	Royal stag *(Cervus elaphus)*	Bern-II,III; DCE'92/43-IV; IUCN-LR/lc	Poaching.
	Roe (*Capreolus capreolus*)	Bern-III; IUCN-LR/lc	Poaching.
	Wild boar *(Sus scrofa*)	Bern-III; IUCN-LR/lc	Poaching.
	Wild goat *(Rupicapra rupicapra)*	BPA-II; RDB-EN; Bern-III; DCE'92/43-II,IV; CITES-II; IUCN-LC	Poaching.
	Brown bear *(Ursus arctos*)	BPA-II,III; RDB-EN; Bern-II; DCE'92/43-II,IV; CITES-II; IUCN-LC.	Poaching.
	Balkan trout *(Salmo trutta fario*)	IUCN-LC	Illegal fishing.
Damages on the information system	-	-	Destroyed signs and information tablets by tourists.
Pollution	?	-	Wastes from tourists around fireplaces and places for rest.
Introduced animal species	Brown Carpathian bear	BPA-II,III; RDB-EN; Bern-II; DCE'92/43-II,IV; CITES-II; IUCN-LC.	Potential genetic contamination of the population of Bulgarian population of bears *(Ursus arctos*).
Non-inherent plant species	Nettle (*Urtica dioica*)	-	These plants were transferred to alpine meadows area by human activities associated with grazing cattle.
	Cherry plum (*Prunus cerasifera*)	-	

sibirica) in the subalpine treeless zone above "Topalitsa" area. Siberian juniper has taken a large area of alpine meadows of the treeless zone in this area. The zone occurs along the tree line, between 1500 and 1850 meters above sea level to the mountain ridge. Strongly reduced, and in many places, fully terminated regime of grazing in recent decades has led to changes in vegetation succession. Siberian juniper conquers new territories.

2. Threats of anthropogenic origin

2.1. Threat for the Fire salamander (*Salamandra salamandra*)

Salamanders, gone out on the trails have been accidentally injured or killed by horses carrying luggage to the huts, due to their weak ability for quick movement and self-preservation. A killed salamander is found on the tourist trail between "Hubavets" Hut and "Balkanski rozi" Hut (May 20, 2011).

2.2. Picking up of Wild garlic, White elecampane and Balkan primrose by tourists

Wild garlic *(Allium ursinum):* In one of the habitats in the Reserve, located between "Balkanski rozi" Hut and "Vassil Levski" Hut is evident that wild garlic is picked up. The leaves are picked up, and along with that, the flowers are eradicated.

White elecampane *(Inula helenium):* There are signs of picking up of white elecampane roots, next to the tourist trail between "Hubavets" Hut and "Balkanski rozi" Hut. The Biological Diversity Act does not protect the species, but the Minister of Environment and Water limits its collection for therapeutic purposes, under the Law on Medicinal Plants (State Gazette, 2000).

A threat for Balkan primrose is determined in one the habitats of this species, located next to the tourist trail between "Hubavets" Hut and "Balkanski rozi" Hut. There, the species is threatened by trampling by animals and destruction by individuals following the passage of tourists through the field.

2.3. Waste disposal by tourists

Wastes from tourists, mainly along the fireplaces, were recorded frequently. The following cases of illegal waste disposal in the area of the reserve and its adjacent areas were registered:

- Wastes of packaging and food scraps in "Krasta" area, which is located next to the reserve. According to the tourists from the local population, sometimes these wastes attract bears. They go outside the reservation area and are threatened by local poachers.
- Wastes on a fireplace next to "Barazh" area.
- Plastic wastes from tourists next to a fireplace on the path between "Hubavetz" Hut and "Balkanski rozi" Hut.
- Wastes in a habitat of wild garlic, located between "Balkanski rozi" Hut and "Hubavetz" Hut.
- Plastic wastes on a fireplace next to "Balkanski rozi" Hut.
- Glass waste, construction and household wastes next to "Balkanski rozi" Hut.
- Metal wastes from an old shepherd hut on the path from "Vasil Levski" Hut to Botev Peak.

There are many wastes at the above places since the main tourist flow is concentrated there.

2.4. Fires

Forest fires are a crucial environmental factor associated mainly with human activity. The combustion of vegetation cover changes almost fully the environmental conditions (POPOV, 2007). On July 29, 2007, a fire burst in the region over "Suchurum" landmark. Much of the mixed forest was burned down, consisting mainly of pine (*Pinus nigra*), locust tree (*Robinia pseudoacacia*) and hornbeam (*Carpinus orientalis)*. The size of the burnt territory is 47 acres; 10 acres of that territory are within the borders of the National Park. The damage to the biota by the fire is not possible to be accurately evaluated, since there is no complete data on biodiversity of the burnt area.

Assessment of the habitat after the fire: secondary succession occurs on the territory of the fire, which consists of restoring the natural deciduous forest, development of which was suppressed by the rapidly growing pine.

2.5. Poaching and illegal fishing

Poaching and illegal fishing are threats that directly affect biodiversity. Perpetrators of poaching and illegal fishing in "Stara reka" Reserve, in most cases remains unpunished. Poachers mostly kill the following species: Royal stag *(Cervus elaphus),* Roe (*Capreolus capreolus*), Wild boar *(Sus scrofa*), Wild goat *(Rupicapra rupicapra*) and Brown bear *(Ursus arctos)*.

According to data, provided by tourists, there are isolated cases of illegal fishing in Stara Reka River. Object of this fishing is Balkan trout *(Salmo trutta fario)*. Balkan trout population in the Central Balkan is an issue of national importance.

2.6. Damages on the information system

Near the trail, old metal signs prohibiting fishing and designating the protected area are found, which were shot with a firearm. The main of this is the lack of possibility for continuous monitoring of signs.

2.7. Introduced animal species

Brown Carpathian bear is an introduced species in "Stara Reka" Reserve. Inhabitation of this species within the reserve leads to the risk of genetic contamination of populations of the local brown bear, which is the bigger one. The park is inhabited by significant part of the Bulgarian population of bears *(Ursus arctos*) - around 60 animals (YANKOV, 2001).

In the 1980's, bears are regarded as an object to hunting in the country. Their breeding in artificial conditions began in Kormissosh (Western Rhodopes), where besides Bulgarian bears; Carpathian bears from Romania were imported. Displacement of bears from Kormisosh was conducted in the Western Rhodope Mountains and Central Stara Planina. This leads to potential contamination of the population. However, it is considered that Carpathian bears existed in our country too short in the wild; practically, they are not likely to have affected the gene pool of local bear (SPASOV *et al.*, 2008).

2.8. Non-inherent plant species

Nettle (*Urtica dioica*) and Cherry plum (*Prunus cerasifera*) were not inherent to the alpine meadows of the area. These plants were transferred to this area by human activities associated with grazing cattle. These species are found mostly along the old abandoned dairies and pens and in meadows amongst woods.

Nettle is found along the ruins of a dairy farm in "Topalitsa" area and in alpine meadows in the treeless zone, to the west of "Ravnets" Hut. Cherry plum is found in the treeless zone, to the west of "Ravnets" Hut.

3. Recommendations for reduction of treats and harmful effects

- Regular monitoring of actual and potential threats must be included in the Management Plan for the park, in order to be achieved timely identification and appropriate response to eliminate the harmful effects.

- To deal with the problems, arising from the threats of anthropogenic origin, it is also recommended some assistance to municipalities in the neighboring settlements, in order that these problems are solved, through organizing awareness events, campaigns for better nature and development of tourism in the region.

Conclusions

The investigated region is characterized by a number of threats, dominated by those with anthropogenic origin. Some naturally occurring successional changes during the years also have changed the habitats as well. The most dangerous for biodiversity and degradation of the habitats remain fires, erosion, poaching and introduction of foreign species.

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Age Structure and Historical Development of Forests in "Bistrishko branishte" Biosphere Reserve in Vitosha Mountain (Bulgaria)

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Abstract. In 2001 the territory of the Bistrishko Branishte reserve of the Vitosha Mountain (Bulgaria) was affected by wind throw. Almost 100% of the *Picea abies* trees on an area of 60 ha were overthrown. After 2003 in the periphery of the wind throw an outbreak of *Ips typographus* has started, which developed at high speed and by 2008 affected 200 ha of the forests in the reserve. These natural disturbances raised questions about the past of the ecosystem and possible relation of these events to the previous history of the forest. To study the age structure and historical development we extracted 165 samples with increment borer from different parts of the forest of the water-catchment of Bistrishka River. Samples were taken from trees representing various diameter classes and perhaps different cohorts. The samples were prepared following standard dendrochronological methodology consisting of gluing to wooden holders, sanding with sandpaper No. 250 and 600, scanning at 1200 dpi and measuring the tree-ring widths with the CooRecorder software. The resulting series were cross-dated using visual characteristics of the tree rings and statistical similarity with the CDendro software.

Our data showed that regardless of the location the age of trees is similar. The majority of dominant trees germinated after the 1870-s. Trees that are visually distinguishable with their larger sizes, had similar ages to neighboring dominants. There is no evidence for large-scale disturbances except for a known wind throw in 1956 in the tree line zone under Skoparnika peak. Over the past 100 years the forest has been affected primarily by small-scale disturbances. The similar age of dominant trees and forest structure are probably a consequence of fast forest recovery after reduced human activity by the end of the 19th century.

Key words: wind throw; bark-beetle outbreak; natural disturbances; tree-ring analysis; Bistrishko branishte; Bulgaria

Introduction

Norway spruce (*Picea abies* Karst.) is one of the most widespread tree species in Eurasia. It is the main tree species in the subalpine zone of most European high mountains and occupies large territories. Norway spruce forests have very high importance as habitats, water catchment areas, protective forests against avalanches, rock falls, landslides, soil erosion and source of valuable timber (SCHUTZ, 1999; AMMAN *et al.*, 2002; RAFAILOV, 2003).

Subalpine forests are among the most frequently affected components by different types of natural disturbances in mountain ecosystems. Until the early 1980-s it was considered that natural disturbance have negative effect on plant biomass (GRIME, 1979). Yet, the summary of available studies made by PICKET & WHITE (1985) demonstrated that natural disturbances such as wind throws, fires and insect outbreaks are an integral part of the ecology of forests. They create conditions for the removal of the

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dominant canopy layer of trees, faster decomposition of dead wood and other cover soil, mineralization of complex organic substances in the soil. This usually creates conditions for establishment of fast-growing pioneer tree species and further on secondary succession and returning of shade-tolerant species such as Norway spruce in the subalpine forests of Europe. In the last decades there were significant efforts for studying in more details the natural disturbance regime of analogous forests such as the mixed-species forests in Central and South Europe (Szwagrzyk & Szewczyk, 2001; Brang, 2005; Firm *et al.*, 2009; Podlaski, 2010), low-elevation spruce-dominated forests in Central and North Europe (Ulanova, 2000; Gromtsev, 2002; Kuuluvainen, 2002; Shorohova *et al.*, 2008) or other coniferous forests in North America (Oliver & Larson, 1996; Franklin *et al.*, 2002; Zenner, 2005). Yet, studies of the disturbance regime of European subalpine forests were scarce mostly due to the perception that the human use had modified these forests and there were no relevant ecosystems. Sharp increase of the number of wind throws and bark-beetles in Central and Eastern Europe in the last 20 decades increased the interest for the disturbance regime of these forests and recently there are a number of studies done mostly in forest reserves in the Carpathians and the Balkan Mountains (Szwagrzyk *et al.*, 1995; Svoboda & Pouska, 2008; Zeilonka *et al.*, 2010; Tsvetanov *et al.*, 2011; Panayotov *et al.*, 2010; Panayotov *et al.*, 2011).

In May 2001 the territory of the Bistrishko branishte Biosphere Reserve in Vitosha Mountain (Western Bulgaria) was affected by very strong winds (Simeonov & Georgiev, 2003) which caused the uprooting of the mature trees on a territory of 60 ha. At the end of the summer of 2002, most of the uprooted trees were infested by *Ips typographus L. (Rossnev et al., 2005). By 2009 the affected territory by the bark-beetle outbreak was more than 200 ha (Panayotov & Georgiev, in press).*

Despite the data for this event and limited data for a wind throw and forest structure in the tree line zone (Panayotov, 2006) there is quite limited information for the past of the Norway spruce forests in Bistrishko branishte. Most of the other information is available from older local people and limited evidence from Zaharieva (1940). She notes that by that period much of the mature trees were aged about 40-50 years and that in many places in the reserve there were visible traces of wood-charcoal production. This is a clue, that the forests of Bistrishko branishte reserve were probably actively managed by local people up to about 100-140 years ago. However, more data on forest structure and age is necessary. This would help to reveal the past history of the forests, the role of human use and probably disturbances. As these forest attributes are directly related to forest stability and resilience, such data would clarify whether there is a possible connection between the wind throw, bark-beetle outbreak and the history of the forest. Therefore, our aim is to study systematically the age structure of different regions in Bistrishko branishte forests.

Materials and Methods

Study site. The study site is the Bistrishka River ínim catchment in the Bistrishko branishte Biosphere Reserve (42°33′N; 23°18′E), located in the "Vitosha" Natural Park in Western Bulgaria (Fig. 1). The total íni of the reserve is 1177.2 ha, of which 600 ha above 1500 m a.s.l. are occupied by natural Norway spruce ecosystems. The local tree line is situated lower ín expected at 1850-1950 m a.s.l. due to frequent strong winds (Panayotov, 2006). The climate is typical for high mountain location with average ínim ínimum ura of 3.3°C, ranging from -5.6°C in January to 12.4°C in July (data for Aleko hut climate station, 1800 m a.s.l.). Annual precipitation amounts to 1228 mm with ínimum in the April –June and ínimum in the August – September periods.

Data collection. Based on preliminary mapping done by satellite images (Panayotov & Georgiev, *in press*) we selected areas with various altitudes, exposure and relief features in the Bistrishka

River water catchment. We excluded from our data collection practically inaccessible locations. We collected samples for dendroecological analysis with increment borer. In each area we located trees with different diameters to try to collect data from possibly different cohorts. In areas with natural disturbances that occurred recently we sampled dead and live trees to establish the year of occurrence of the event. In areas with older windthrow, samples were taken from single surviving trees or trees on the border of adjacent surviving forest areas. Following such scheme it is possible to determine the age of the individual sections, the occurrence of natural disturbances or other events that caused drastic changes in radial growth of trees (TSVETANOV *et al.*, 2010; PANAYOTOV *et al.*, 2011).

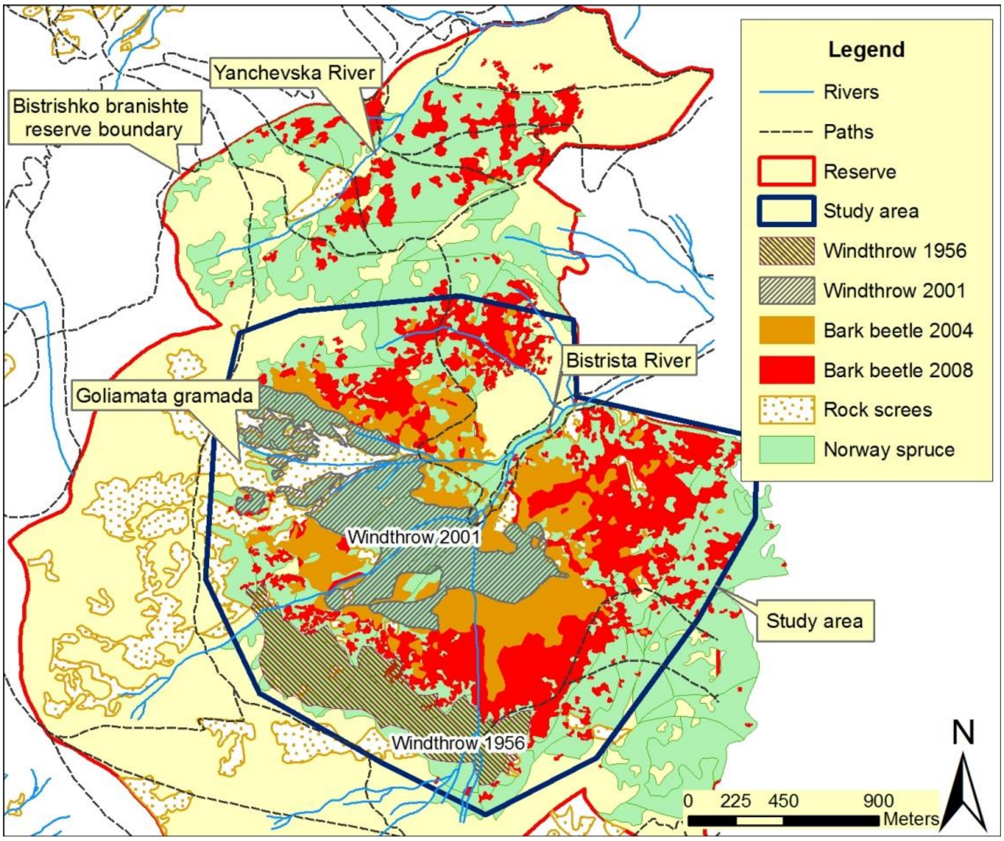


Fig. 1. Study site and location of known large-scale natural disturbances in "Bistrishko branishte" Biosphere Reserve

Samples were mounted on wooden holders, sanded with progressively increasing numbers of sandpaper (250 to 600). Then, they were scanned and cross-dated by visual analysis (STOKES & SMILEY, 1968) and the tree ring width was measured with CooRecorder 7.3 software (CYBIS ELEKTRONIK & DATA AB). The obtained tree-ring width series were cross-dated to establish the exact year of formation of each tree ring. This procedure was performed by visual and statistical comparison of similarities in the variation of the growth curves of individual trees for specific periods of time and tree rings with abnormalities in anatomical texture such as light rings and frost rings (STOKES & SMILEY, 1968; SCHWEINGRUBER, 1996). For the statistical comparisons we used CDendro 7.3 software (CYBIS ELEKTRONIK & DATA AB). Frost rings and light rings were compared with existing local chronology (PANAYOTOV, 2006).

Results and Discussion

Based on the initial mapping we selected eight areas to collect samples (Fig. 2). From three sections - "The Ridge", "Treeline - the Ridge" and "Windthrow 1956" we used initial dataset (PANAYOTOV, 2006) to which we added additional cores.

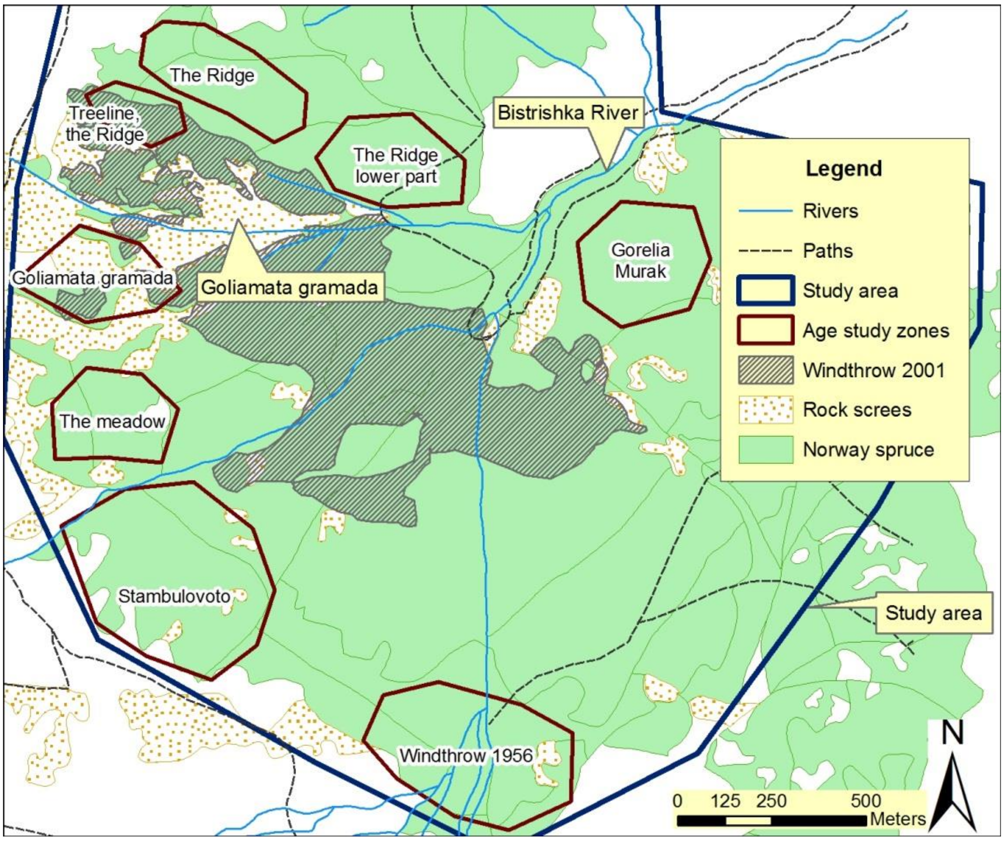
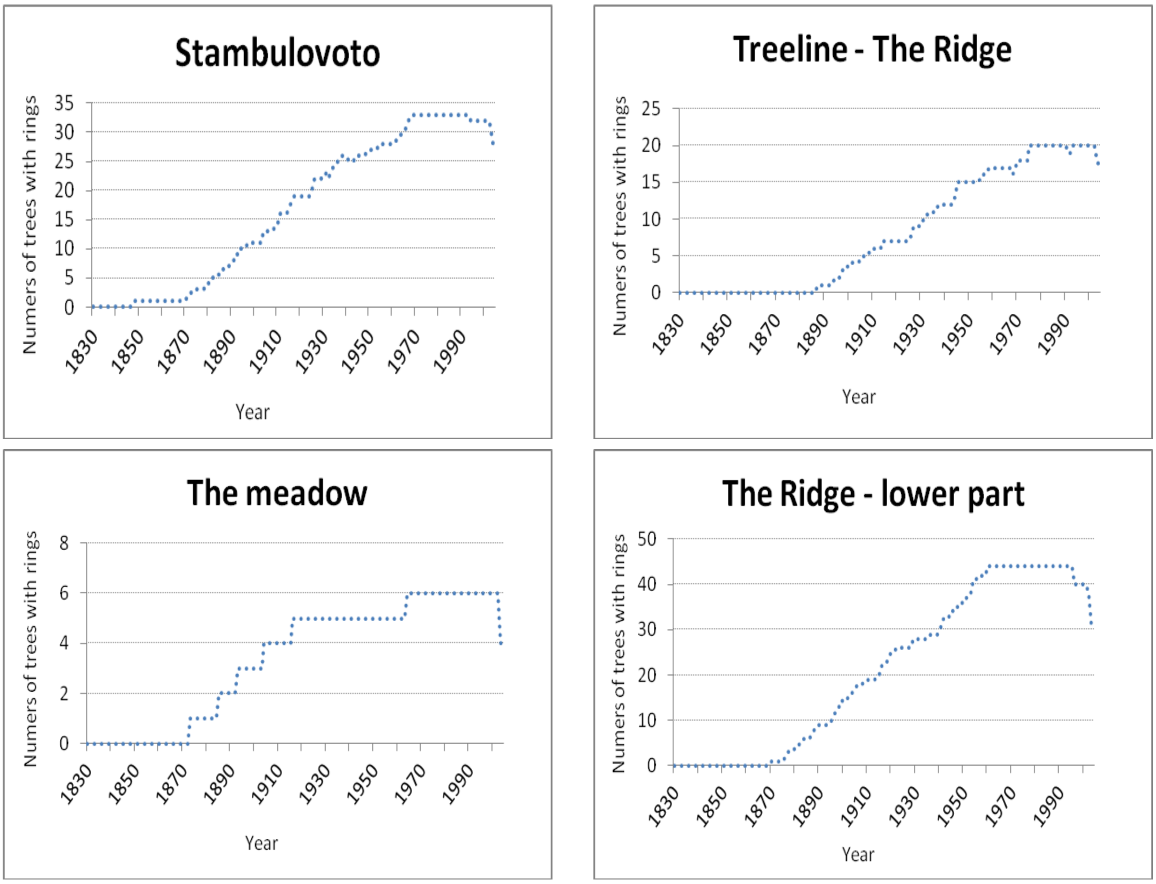


Fig. 2. Age study zones in "Bistrishko branishte" Biosphere Reserve

Data on occurrence of first tree rings (Fig. 3) shows that in all areas the trees were established after the 1870-s. The oldest trees were found in the highest areas of "Stambulovoto" region. Even trees that were visually much thicker than other were actually within the same cohort. Usually such bigger trees had big branches indicating large crowns typical for open growth without side or top shading. This was also demonstrated by tree-ring width patterns. All trees had rather wide tree rings from the very beginning of their growth, which is typical for open growth (SCHWEINGRUBER, 1996; PANAYOTOV, 2011). Our data does not show sharp increase in the number of trees at certain periods, which usually occurs after natural disturbances due to active regeneration in degraded areas (OLIVER & LARSON, 1996; FIRM *et al.*, 2009; TSVETANOV, 2010).

Instead, in the water catchment of Bistrishka River there is a gradually increasing number of trees. Such age structure could be an evidence of two situations – either gradual occupation by trees of areas that were quite spacey populated by few trees or gradual recovery after large-scale stand-replacing disturbance. However, we could not find even a single older tree with sharp release of growth that could be an indication for stand-replacing disturbance. Further, it would be expected that there should be at least limited historical evidence and memories for such large-scale event in the middle of the 19-th century.

Therefore, it seems more likely that the region was used very intensively for grazing and on-site production of charcoal. According to ZAHARIEVA (1940) in 40th of 20th century in the reserve there were numerous traces of charcoal production. Such practice was a traditional way to utilize wood in high-mountain areas where people could not transport large trunks to the valley floors. STEFANOV (1939) stated that the higher parts of Vitosha were actively used for grazing until the 1930-s. Therefore, it is likely that the current territory of the Bistrishko branishte Reserve, which includes extensive grasslands above the tree line zone was used for grazing also at lower altitudes. Perhaps these practices were reduced after the 1880-s when forest legalization limited the use of forests for grazing and the possibilities for salvage logging. This has created conditions for regeneration of spruce and fast occupation of empty spaces between existing small groups of trees and single trees. After initial closure of forests, mostly small-scale natural disturbances (gap formation) have caused loss of a few older trees and their replacement by new ones. This would explain the rather solid number of trees after the 1940s in all areas (Fig. 3).



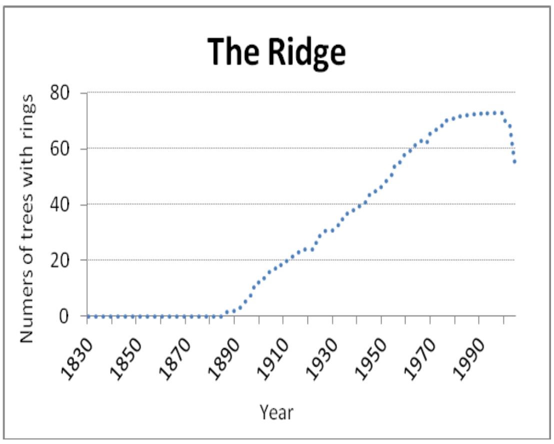


Fig. 3. Number of trees with annual tree rings in the surveyed areas of the "Bistrishko branishte" Biosphere Reserve

The observed reduction of the number of tree rings (and thus of live trees) after 2001 is a reflection both of the wind throw in 2001 and the subsequent *Ips typographus* outbreak. Besides the known disturbances after 2000, the only previous larger-scale natural disturbance was the wind throw in the tree line zone of Stambulovoto region in 1956. Although it affected a territory of approximately 60 ha, the trees were completely overthrown on a smaller area close to the main stream of Bistrishka River. Therefore, its character was quite different from the 2001 wind throw and within the borders of the older disturbances there are groups with older trees (e.g. 120-140 years., Fig. 3, "Stambulovoto") besides patches with younger trees (e.g. 40 years old., data not shown).

Homogeneous forests in which the majority of trees are with similar sizes and age are vulnerable to large-scale disturbances. This is especially true in the case of insect outbreaks, as they are usually specialized for certain species and size class of the trees (TSANKOV, 2005; PANAYOTOV *et al*. 2011). For example, *Ips typographus* prefers Norway spruce trees older than 60 years and seldom attacks younger trees (WERMELINGER, 2004). Indeed, in Bistrishko branishte forest the outbreak affected mostly forest patches with homogeneous structure and age above 100 years (PANAYOTOV & GEORGIEV, *in press*). It is therefore quite probable that the past history of the reserve, which predisposed a forest structure of large territories occupied by trees with similar age and lack of younger patches or patches with higher participation of different species, has facilitated the *Ips typographus* outbreak. Although wind throws are dependent on many factors and mostly on local wind speed, forests with heterogeneous structure are less susceptible to catastrophic damages and more resilient at least because of the presence of many younger groups that are capable of surviving the strong wind and then recover the forest quickly. This is demonstrated by the disturbance model of Parangalitsa Reserve in Bulgaria. There, the much older Norway spruce forest (age of trees > 200 years) has experienced numerous smaller wind throws (e.g. below 10 ha) during the last 150 years which have created a complex mosaic of forest patches with different age and species composition (PANAYOTOV *et al*., 2011). In addition, it has avoided larger insect outbreaks and is much more resilient.

Conclusions

The research conducted within the water catchment of Bistritsa River in Bistrishko branishte Biosphere Reserve indicates that the forest was with relatively similar age before the large-scale wind throw in 2001 and the following bark-beetle outbreak. Most of the bigger trees were 120-140 years old. The only larger natural disturbance was the wind throw in the tree line zone in 1956. The most probable reason for such similar age of the trees is the active use of the current forest territory for grazing and charcoal production on site until the last quarter of the 19th century. The homogeneous forest structure on vast areas occupied by trees of the same species (Norway spruce) with uniform size and age is among the most likely reasons for the scale of the *Ips typographus* outbreak after 2003. Although it was triggered by the 2001 wind throw, the absence of natural barriers as groups of trees of younger age, and other species, assisted the spread of the bark-beetles.

We expect that the disturbances from the last decade, including the recent fire in "Gorelia murak" region, will create conditions for forest heterogeneity at landscape-level and probably assist the occurrence of pioneer species such as *Betula pendula* and *Populus tremula*, besides the increase of the territories occupied by *Fagus sylvatica* for at least the next 50 years.

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Short note

Materials on the diet of the Otter (Lutra lutra L.) in the West Rhodopes Mountain, South Bulgaria

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Abstract. Otter spraints were collected from the West Rhodopes from Batak Dam, Chaya and Parvenetska River areas during 2005-2007. Main food resources in Batak Dam during autumn were the frogs dominated by representatives of the family *Ranidae*, followed by different fish species. In both rivers the main food was the fish with predominance of *Barbus cyclolepis*, the frogs, and the crabs.

Keywords: otter, diet, mountains.

Introduction

The mountain regions of Bulgaria are not so favorable areas for the European Otter (*Lutra lutra* L.) providing mainly temporary usable habitats to the species, and probably low food resources (GEORGIEV, 2005). The diet of the otter in the country was studies only in the lowlands and the hilly areas and there is no any data on its food in the higher mountains (GEORGIEV, 2006).

The aim of this study was to (i) provide information on the number of species exploited by the otter in the mountain regions of Bulgaria and (ii) to point out which are the possible main food resource species.

Material and Methods

Otter spraints (N = 171) were collected from the West Rhodopes from the following localities, dates and seasons: 1. Batak Dam, altitude > 1000 m a. s. l., autumn, 06.10.2006: 40 spraints; 2. Chaya River near Assenovgrad town, 300-400 m a. s. l., winter, 06.01.2007: 35 spraints; 3. Parvenetska River near village of Parvenets, 200-400 m a. s. l., spring, 06.05.2005: 9 spraints, 17.05.2006: 22 spraints; summer, 20.09.2005: 13 spraints, 15.09.2006: 12 spraints; autumn, 08.12.2005: 8 spraints, 15.12.2006: 6 spraints; winter, 30.03.2005: 9 spraints, and 25.02.2006: 17 spraints.

The otter food was studied in a laboratory by considering the minimal specimens found in spraints using the pair bones or body parts of the prey items. The species were determined using a reference collection of hairs, feathers, bones and scales. For the calculations the computer program Biodiversity Professional, Version 2, 1997 was used.

Results

In the study area the otter diet consisted of crustaceans, arthropods, fish, frogs, reptiles, mammals and fruits (Table 1).

Main food resources in Batak Dam during autumn were the frogs dominated by representatives of the family *Ranidae* (34.1% from all specimens registered), followed by different fish species (22.9%). Relatively high percentage had also the mammals and the invertebrates (crustaceans). In both rivers the main food was the fish with predominance of *Barbus cyclolepis* (11.2 to 33.3% from all specimens registered), the frogs (12.3 to 28.5%), and the crabs (16.0 to 18.0%).

Such proportions are typical for the species` diet in areas where the fish resources are scarce (GEORGIEV, 2005). In Bulgaria, mainly the high lands could be considered as rich in water resources but poor on fish populations. Also and the fast changing water levels, long freezing period in winter, the rocky and steep terrains can be considered as characters of temporarily usable habitats by the otters which they visit only during favorable seasons.

Table 1. *Diet of the otter (Lutra lutra) in the studied regions in the West Rhodopes Mountain. Legend: BD – Batak Dam, CHR – Chaya River, PR – Parvenetska River.*

Species	BD		CHR		PR (spring-summer)		PR (autumn-winter)	
	N	%	N	%	N	%	N	%
Potamon ibericum Bieb.	0	0,0	13	16,0	42	16,9	31	18,0
Astacus/*Austropotamobius* sp.	22	9,9	0	0,0	0	0,0	4	2,3
Total crustaceans	**22**	**9,9**	**13**	**16,0**	**42**	**16,9**	**35**	**20,3**
Myriapoda indet.	3	1,3	0	0,0	0	0,0	0	0,0
Coleoptera indet.	29	13,0	0	0,0	12	4,8	0	0,0
Insecta indet.	3	1,3	0	0,0	13	5,2	0	0,0
Total Tracheata	**35**	**15,7**	**0**	**0,0**	**25**	**10,0**	**0**	**0,0**
Salmonidae indet.	14	6,3	0	0,0	0	0,0	0	0,0
Esox lucius L.	0	0,0	0	0,0	0	0,0	0	0,0
Perca fluviatilis L.	8	3,6	0	0,0	0	0,0	8	4,7
Rutilus rutilus L.	2	0,9	0	0,0	4	1,6	3	1,7
Rhodeus amarus Bloch	2	0,9	0	0,0	0	0,0	0	0,0
Barbus cyclolepis Heck.	0	0,0	27	33,3	28	11,2	25	14,5
Gobio gobio L.	0	0,0	0	0,0	0	0,0	4	2,3
Leuciscus cephalus L.	0	0,0	5	6,2	0	0,0	0	0,0
Leuciscus sp.	0	0,0	4	4,9	0	0,0	7	4,1
Carassius gibelio Bloch	0	0,0	0	0,0	5	2,0	0	0,0
Carassius sp.	0	0,0	0	0,0	5	2,0	1	0,6
Hypophtalmychtis sp.	5	2,2	0	0,0	0	0,0	0	0,0
Cyprinidae indet.	7	3,1	10	12,3	22	8,8	21	12,2
Cobitidae indet.	0	0,0	1	1,2	0	0,0	0	0,0
Pisces indet.	13	5,8	11	13,6	18	7,2	17	9,9
Total fish	**51**	**22,9**	**58**	**71,6**	**82**	**32,9**	**86**	**50,0**
Bufo bufo L.	0	0,0	0	0,0	0	0,0	3	1,7
Bufo sp.	0	0,0	0	0,0	4	1,6	0	0,0
Rana temporaria L.	15	6,7	0	0,0	0	0,0	0	0,0
Pelophylax ridibundus (Pall.)	11	4,9	0	0,0	0	0,0	15	8,7
Ranidae indet.	50	22,4	10	12,3	45	18,1	16	9,3
Anura indet.	10	4,5	0	0,0	8	3,2	15	8,7
Total frogs	**86**	**38,6**	**10**	**12,3**	**57**	**22,9**	**49**	**28,5**
Sauria indet.	0	0,0	0	0,0	1	0,4	0	0,0
Natrix sp.	4	1,8	0	0,0	26	10,4	0	0,0
Serpentes indet.	0	0,0	0	0,0	7	2,8	0	0,0
Total reptiles	**4**	**1,8**	**0**	**0,0**	**34**	**13,7**	**0**	**0,0**
Neomys sp.	0	0,0	0	0,0	3	1,2	0	0,0
Insectivora indet.	1	0,4	0	0,0	0	0,0	0	0,0
Arvicola terrestris L.	8	3,6	0	0,0	1	0,4	0	0,0
Microtinae indet.	4	1,8	0	0,0	1	0,4	0	0,0
Rodentia indet.	7	3,1	0	0,0	0	0,0	2	1,2
Mammalia indet.	3	1,3	0	0,0	4	1,6	0	0,0
Total mammals	**23**	**10,3**	**0**	**0,0**	**9**	**3,6**	**2**	**1,2**
Prunus cerasifera L.	2	0,9	0	0,0	0	0,0	0	0,0
Total fruits	**2**	**0,9**	**0**	**0,0**	**0**	**0,0**	**0**	**0,0**
Total specimens	**223**	**100,0**	**81**	**100,0**	**249**	**100,0**	**172**	**100,0**
Simpsons Diversity	**0,2**		**0,5**		**0,2**		**0,4**	

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Short note

On the substrates used for marking by the Stone Marten (Martes foina Erxl.) at the lowland areas of South Bulgaria

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Abstract. At the area investigated the solid materials situated up the ground slightly dominated over the marking just on the ground. The concrete materials were mainly used followed by the stones, and soil. Rare were the cases of marking on the herb vegetation. Occasional marking was observed on a dead grass snake *Natrix natrix* and nylon remains.

Keywords: mustelid, territory, behavior.

Introduction

The Stone Marten (*Martes foina* Erxl.) is the most widespread mustelid in Europe having high ecological plasticity and inhabiting even the big cities. It is having a home range up to 80 ha which is marked by feces deposited on various well visible sites (SIDOROVICH, 1995; POPOV & SEDEFCHEV, 2003). The studies of the proportions of the substrate types used for marking by the species are scarce. Such information from Balkans and especially from Bulgaria is lacking.

The aim of this study is to present some data on the substrate used for marking by the Stone Marten at the lowland areas of South Bulgaria.

Material and Methods

Substrates used for marking by the Stone Marten were signed during 25 field trips made as not repeated occasional transects during all seasons. The lowlands of South Bulgaria near the cities of Stara Zagora and Plovdiv (the Upper Thracian Lowland and the foothills of Sredna Gora and West Rhodopes Mts) were investigated during 2005 and 2006 (Table 1). A total of 93 such sites in anthropogenic and semi-natural (village vicinities) habitats were investigated.

Results

At the area investigated the solid materials situated up the ground slightly dominated by 58% from all registered (Fig. 1). The concrete materials were mainly used with 35% from all (N = 33) followed by the stones with 23% (N = 21). Deposition of the feces on the ground was observed in 32% of the cases, mainly registered on the soil (23%, N = 21) and some on the asphalt roads (9%, N = 8). Single marking was observed on sand. Rare were the cases of marking on the herb vegetation (live grass or dry *Typha* sp. river deposits) with 7%. Occasional marking was observed on a dead grass snake *Natrix natrix* and nylon remains (both 1%).

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Table 1. Dates, sites and numbers of the Stone Marten marking sites studied.

Date	Locality	Region	Number of sites
1.6.2005	Stara Zagora, Park Bedechka	Upper Thracia	5
10.6.2005	Starozagorski Bani	Sredna Gora Mt	11
30.8.2005	Stara Zagora, Park Bedechka	Upper Thracia	1
31.8.2005	West of Kolena vill.	Sredna Gora Mt	11
8.9.2005	Stara Zagora, Zora	Upper Thracia	3
10.9.2005	Sazliika River, Kolarovo vill.	Upper Thracia	2
11.9.2005	Trankovo vill.	Upper Thracia	6
15.9.2005	Plovdiv, vicinities, Trakia	Upper Thracia	10
16.9.2005	Plovdiv, vicinities, Fishfarms	Upper Thracia	4
19.9.2005	Plovdiv, vicinities, west	Upper Thracia	1
20.9.2005	Parvenets vill., near the river	Rhodopes	3
17.10.2005	Canal near Elenino vill.	Upper Thracia	1
22.10.2005	Stryama River, near Manole vill.	Upper Thracia	2
18.11.2005	Sazliika River, Kolarovo vill.	Upper Thracia	6
8.12.2005	Parvenets vill., near the river	Rhodopes	2
12.12.2005	Plovdiv, railway bridge	Upper Thracia	1
13.12.2005	Plovdiv, vicinities, Trakia	Upper Thracia	1
17.12.2005	West of Kolena vill.	Sredna Gora Mt	7
31.1.2006	Plovdiv, vicinities, Fishfarms	Upper Thracia	2
18.3.2006	West of Plovdiv, near round road	Upper Thracia	2
19.3.2006	Konush village	Upper Thracia	1
16.5.2006	Plovdiv, vicinities, Fishfarms	Upper Thracia	2
17.5.2006	Parvenets vill., near the river	Rhodopes	1
11.6.2006	Trankovo vill.	Upper Thracia	3
14.6.2006	West of Kolena vill.	Sredna Gora Mt	1
12.9.2006	Starozagorski Bani	Sredna Gora Mt	3
2.12.2006	Stara Zagora, ZZU area	Upper Thracia	1
Total number of sites studied			**93**

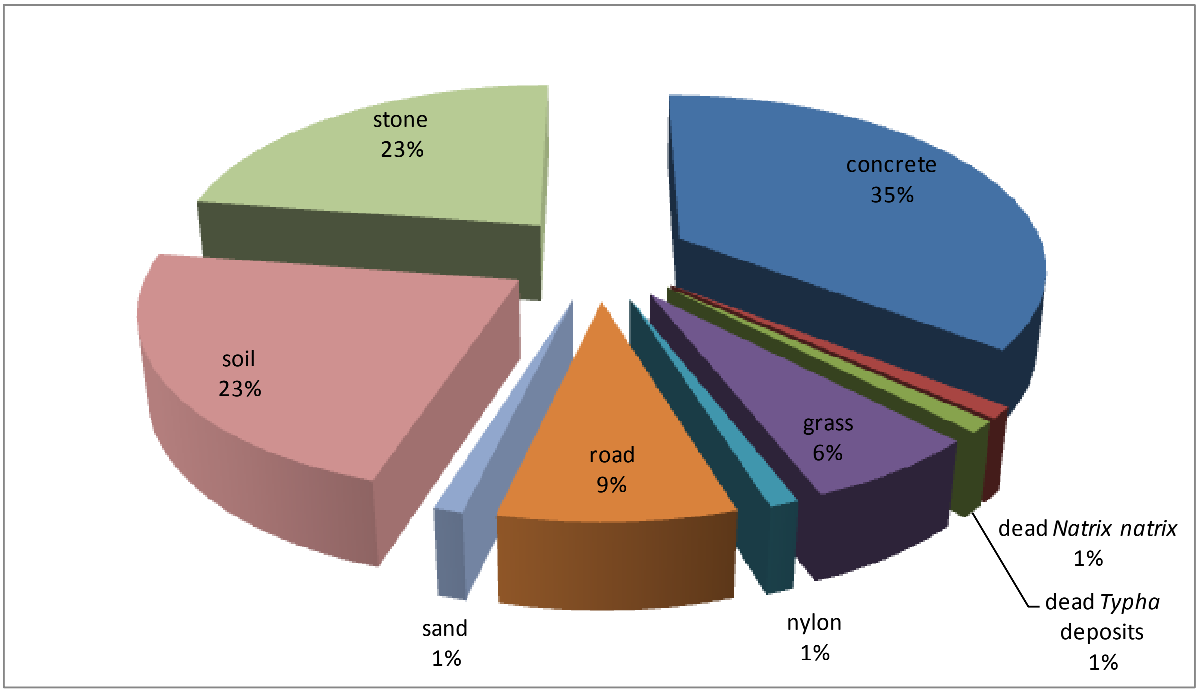

Fig. 1. The proportions of the substrates used for marking by the Stone Marten *(Martes foina Erxl.)* at the study area.

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Short note

Halopeplis amplexicauis (Vahl) Ung.-Sternb (Chenopodiaceae Family). Re-collection in Zarqa of Jordan

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Abstract: *Halopeplis amplexicaulis* is an annual halophytic species that is widespread in salty habitats throughout the countries of the Mediterranean. This species is a Naturalized exotic. In Jordan it was reported that this species was dominant in Wadi Araba, Wadi Rum, Eastern Desert, Shaumari and Al-Azraq Oasis, in the desert (saharo-arabian) around the Dead Sea region, and Abar al Hazim . This species might be considered as invader one in such chorotype area, in that it is distributed in the Mediterranean Woodlands and Shrub lands, Deserts and extreme deserts. as the study area considered a dry habit, and on account of its special edaphic and water requirements, it is rare to see this species in such habitat.

Keywords: *Halopeplis amplexicaulis*, *Chenopodiaceae*, *Biodiversity*.

The genus *Halopeplis* is in the family *Chenopodiaceae* in the major group Angiosperms (Flowering plants), higher Classification of dicotyledons. According to BLANCHE & MOLERO (1987) and TREMBLIN (2000) *Halopeplis amplexicaulis* is an annual halophytic species that is widespread in salty habitats throughout the countries of the Mediterranean.

In Jordan it was reported and according to AL-EISAWI (1996, 1998) that *Halopeplis amplexicaulis* was found in dry land vegetation in Azraq wetland which forms the majority of the total area of the reserve (Al-Azraq Oasis reserve in the Eastern Desert) which was dominated mostly by dome like silt dunes and occupied rarely by this species. The steppe or plateau of Jordan developed at the eastern foot of the highlands. Maximum elevations around the edge of the plateau range from 1,000 m in the south to 700 m in the northeast; the lowest part of the plateau lies at an elevation of 500 m in Azraq Oasis. According to AL-QURA'N (2012) this species was dominant in Wadi Araba, Wadi Rum, Eastern Desert, Shaumari and Al-Azraq Oasis, in the desert (saharo-arabian) around the Dead Sea region, and Abar al Hazim (ABU BAKER et al, 2005). It seems that this species might be considered as invader one in such chorotype area, in that it is distributed in the Mediterranean Woodlands and Shrub lands, Deserts and extreme deserts. They can germinate, grow and reproduce successfully in saline areas which would cause the death of regular plants. This species is located on the salt banks of inland salt lakes in front of other plant communities. It is a pioneer species that colonizes bare salt plains and improves the physicochemical characteristics of the soil, thus preparing the environment for colonization by other perennial halophytes. As the study area considered a dry habit, and on account of its special edaphic and water requirements, it is rare to be found in such habit. This implies the fact of decline pattern in term of its density from the intermediate habit to this pure habit.

The study area is located within the areas adjacent to Al-Hashemyia Municipality (56863208'N, 13143608'E; ca. 603-620 m a.s.l.), Zarqa, in the northern highlands of Jordan (in the Middle Jordan Valley Wetland zones which comprise of Zarqa River and King Talal Dam, Kherbit As-Samra). It is extended from Al-Kherbit Asl-Samra wastewater treatment plant to the north east of Al-Hashemeyia Municipality. Zarqa Governorate lies in the junction of Mediterranean and Irano-Turanian biogeographical regions with semi-arid Mediterranean bio-climate.

Regarding the native vegetation, the study area was dominated by grasses which are herbaceous, rarely woody plants, slightly woody, perennial, or annual. Those plants are generally erect or spread, and some are arising from stolons, tubers, bulbs, rhizomes or seeds. The area was mainly covered by some steppe vegetation especially *Retama raetam; Artemisia herba-alba; and Hammada spp*. For the Mediterranean vegetation, *Capparis spinosa* and *Sacropoterium spinosum* were the expected dominant vegetation type. According to reports of the Ministry of Agriculture, the noteworthy flora indicates the lack of information and scientific researches about that field.

This species was collected from calcareous and sedimentary soil, and according to USDA subgroup and particle size, the study area was dominated with proportions of *Xerochreptic camborthids, Calcixerollic xerochrepts*, and *Lithic xerothent*s. The study area lies at 3210'N, 3610'E; in the northern highlands, with an altitude of 450-500 m a.s.l., about 35 km northeast of Amman, Zarqa Governorate extended from Kherbat As-samra wastewater treatment plant in the East of Al-Hashemyeh Municipality to the West alongside the treated wastewater channel to transport the treated wastewater from Kherbat As-samra, through As-Sukhneh Municipality in the northwest reaching King Talal Dam**.**

The population consisted of about 20 individuals. Individuals of this species were up to 28 cm tall. Agricultural activities in Jordanian highland plateau areas, especially in Al-Hashemyeh Municipality – Zarqa governorate strip, resulting in the general replacement of cropland and pastures with new vegetative cover types, leading to the loss of habitat. Because the species is adapted to extreme halophytic micro-habitats, any changes in those habitats could result in extinction. A picture of the species from its habitat is given in Fig. 1.

Fig. 1. *Halopeplis amplexicauis* in the study area.

According to the results obtained and the literature review, our data fits with the fact that this species was not natively grown in the study area. Interesting fact was the finding of *Halopeplis amplexicaulis* in the study area.

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Plant Essential Oils from Apiaceae Family as Alternatives to Conventional Insecticides

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Abstract. Main method to control insect pest is using synthetic insecticides, but the development of insect resistance to this products, the high operational cost, environmental pollution, toxicity to humans and harmful effect on non-target organisms have created the need for developing alternative approaches to control insect pest. Furthermore, the demand for organic crops, especially vegetables for the fresh market, has greatly increased worldwide. The ideal insecticide should control target pests adequately and should be target-specific, rapidly degradable, and low in toxicity to humans and other mammals. Plant essential oils could be an alternative source for insect pest control because they constitute a rich source of bioactive chemicals and are commonly used as flavoring agents in foods. These materials may be applied to food crops shortly before harvest without leaving excessive residues. Moreover, medically safe of these plant derivatives has emphasized also. For these reasons, much effort has been focused on plant essential oils or their constituents as potential sources of insect control agents. In this context, Apiaceae (Umbelliferae) family would rank among the most important families of plants. In the last few years more and more studies on the insecticidal properties of essential oils from Apiaceae family have been published and it seemed worthwhile to compile them. The focus of this review lies on the lethal (ovicidal, larvicidal, pupicidal and adulticidal) and sublethal (antifeedant, repellent, oviposition deterrent, Growth inhibitory and progeny production) activities of plant essential oils and their main components from Apiaceae family. These features indicate that pesticides based on Apiaceae essential oils could be used in a variety of ways to control a large number of pests. It can be concluded that essential oils and phytochemicals isolated from Apiaceae family may be efficacious and safe replacements for conventional synthetic insecticides.

Keywords: Apiaceae family, essential oils, phytochemicals, natural insecticides, lethal effects, sublethal effects.

Introduction

Currently different kinds of preventive and curative control measures are practiced to get protection from insect pests. Among those, synthetic pesticides such as organochlorines, organophosphates, carbamates, pyrethroids and neonicotinoids have been considered to be the most effective and easy to use tools against insect pests. Most of the farmers are not aware with the ill effect of chemical pesticides and still using most of the systemic and organic insecticides to control insect pests. Although these methods are effective, their repeated use for several decades has its consequences. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually. Repeated applications of synthetic insecticides has disrupted natural enemies in the biological control system and led to

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outbreaks of insect pests, widespread development of resistance and environmental and human health concerns (BENHALIMA *et al.*, 2004; BUGHIO & WILKINS, 2004; SANNA *et al.*, 2004; TAPONDJOU *et al.*, 2005). In this context, efforts are being made worldwide to replace these chemicals with biological alternatives (biopesticides), which are less toxic to the environment.

Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment. Because of the multiple sites of action through which the plant materials can act, the probability of developing a resistant population is very low (ISMAN, 2006). Botanical insecticides degrade rapidly in air and moisture and are readily broken down by detoxification enzymes. This is very important because rapid breakdown means less persistence in the environment and reduced risks to nontarget organisms (ISMAN, 2008). Among natural products certain highly volatile EOs currently used in the food, perfume, cosmetic and pharmaceutical and agricultural industries show promise for controlling insect peat, particularly in confined environments such as greenhouses or granaries. Because of this, much effort has been focused on plant EOs as potential sources of commercial insect control agents. From the standpoint of pest control, one of the most valued properties of *EO*s is their fumigant activity against insects, since it may involve their successful use to control pests in storage without having to apply the compound directly to the insects. In this context, EOs have received much attention as potentially useful bioactive compounds against insects showing a broad spectrum of activity against insects, low mammalian toxicity, degrading rapidly in the environment and local availability (BAKKALI *et al.*, 2008; KOUL *et al.*, 2008; RAJENDRAN & SRIRANJINI, 2008). EOs are secondary metabolites that plants produce for their own needs other than for nutrition. The aromatic characteristics of *EO*s provide various functions for the plants including attracting or repelling insects, protecting themselves from heat or cold; and utilizing chemical constituents in the oil as defense materials. In general, they are complex mixtures of 20-60 organic compounds that give characteristic odour and flavour to leaves, flowers, fruits, seeds, barks and rhizomes. In industrialized countries, EOs could be useful alternatives to synthetic insecticides in organic food production, while in developing countries; they can be a means of low cost protection (Cosimi, *et al.*, 2009). Bioactivity of these EO depends on its chemical composition which varies with plant part used for extraction, harvesting time, plant age, and nature of the soil and growth conditions. EOs are complex mixtures comprised of a large number of constituents in variable ratios. EOs contain natural flavors and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odors. Many EOs isolated from various plant species belonging to different genera, contain relatively high amount of monoterpenes. Jointly or independently they may contribute to the protection of plants against herbivores, although some herbivores have counter adapted to them (DEVI & MAJI, 2011; SAFAEI-KHORRAM *et al.*, 2011). Plant EOs show wide and varied bioactivities against both agricultural pests and medically important insect species, ranging from toxicity with ovicidal, larvicidal, pupicidal and adulticidal activities to sublethal effects including oviposition deterrence, anti-feedant activity and repellent actions as well as they may affect on biological parameters such as growth rate, life span and reproduction (EBADOLLAHI, 2011b; ZOUBIRI & BAALIOUAMER, 2011a,b). Accordingly, the use of plant EOs can lead to the identification of new bioinsecticides.

Some of the plant families known as excellent sources of EOs with insecticidal properties that Apiaceae family is one of them. Apiaceae (Umbelliferae) is one of the best known families of flowering plants, which comprise 300–450 genus and 3000–3700 species. They are aromatic plant and have a distinctive flavor which diverse volatile compounds from the fruits and leaves. The plants of Apiaceae (Umbelliferae) family are occurring throughout the world, but it is most common in temperate regions and rare in the tropics. The most obvious distinctive feature of the family is the inflorescence, which is a simple or compound umbel. Umbelliferae refers to the characteristic umbellate inflorescence. Having bilaterally symmetric flowers towards the outside of the inflorescence tends to make the inflorescence as a whole resemble a single flower (BERENBAUM, 1990; CHRISTENSEN & BRANDT, 2006). The family includes many herbs, spices and medicines, would rank among the most important families of plant.

Although a number of review articles have appeared in the past on the various aspects of *EO*s bioactivities (ISMAN, 2000; BINDRA, *et al*., 2001; PETERSON, & COATS, 2001; BAKKALI *et al*., 2008; ISMAN *et al*., 2008: TRIPATHI, 2009: EBADOLLAHI, 2011b: ZOUBIRI & BAALIOUAMER, 2011b; REGNAULT-ROGER *et al*., 2012) but the present paper emphasizes on the potential of Apiaceae EOs in insect-pest management. In fact, the present study attempted to explain the efficiency of EOs from Apiaceae family and their components as phytochemicals with lethal and sublethal effects against insect pests.

Lethal toxicity

The insecticidal activity of many EOs from Apiaceae has been evaluated against a number of insects. The isolation and identification of the bioactive compounds and EOs from Apiaceae are of utmost importance so that their potential application in controlling insect pests can be fully exploited. Table 1 shows lethal toxicity of *EO*s isolated from Apiaceae family against different insect pests that published since 2000. EOs have several characteristics that improve their efficacy as insecticides. They are both phytochemically diverse (containing many biosynthetically different compounds) and redundant (containing many analogs of one class) (REGNAULT-ROGER *et al*., 2012). *EO* are natural products that contain natural flavors and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odors. Among components of EOs, terpenes especially monoterpenoids and sesquiterpenes have been shown to be toxic to a variety of insects (HUMMELBRUNNER & ISMAN, 2001; LEE *et al*., 2002; ERLER, 2005; STAMOPOULOS *et al*., 2007). Previous studies have also shown that the toxicity of *EO*s obtained from aromatic plants against insect pests is related to the oil's main components such as 1.8-cineole, carvacrol, eugenol, limonene, α-pinene and thymol. For example, EOs from seeds of *Coriandrum sativum*, and *Carum carvi* L. were tested in the laboratory for volatile toxicity against *Sitophilus oryzae*, *Rhyzopertha dominica* (F.) and *Cryptolestes pusillus* (Stephens). Coriander contained linalool (1617 ppm of the oil) as the main product active against the three pests. Camphor-rich fractions (over 400 ppm) were very toxic to *Rhyzopertha dominica* and *Cryptolestes pusillus*. The caraway profile included carvone, limonene and (E)-anethole as major components. Carvone was the most effective (972 ppm) monoterpenoid against *Sitophilus oryzae*. In addition, (E)-anethole at 880 ppm was toxic to *Rhyzopertha dominica* while vapors of limonene (1416 ppm) fractions killed adults of *Cryptolestes pusillus* only (LOPEZ *et al*., 2008). In the study of EVERGETIS *et al*. (2009) insecticidal properties of six different taxa of the Apiaceae family (*Heracleum sphondylium* ssp. *pyrenaicum*, *Seseli montanum* ssp. *tomasinii*, *Conopodium capillifolium* Coss., *Bupleurum fruticosum* L., *Oenanthe pimpinelloides* L. and *Eleoselinum asclepiu*m Bert.) were evaluated against *Culex pipiens* third to fourth instar larvae in order to delineate the relationship between the EOs phytochemical content and larvicidal activity. Results indicated that the oil of *Oenanthe pimpinelloides*, which contains mainly nonoxygenated monoterpenes, possesses the highest activity, displaying a

LC_{50} value of 40.26 mg/l. On the contrary, the EO of *Eleoselinum asclepium*, which is consisted of pinenes and oxygenated monoterpenes, was the less active (LC_{50} value of 96.96 mg/l). These results reveal that the nonoxygenated monoterpenes possess potent insecticidal activities against *Culex pipiens*. Our recent study showed that *Azilia eryngioides* (Pau) Hedge Et Lamond oil had a strong insecticidal activity on adult of *Sitophilus granarius* and *Tribolium castaneum*. Major components in this oil were α-pinene (63.8%) and bornyl acetate (18.9%). The 37.03 µl/l concentration and 48-h exposure time was enough to attain 100 % mortality of all the insects. The EO concentration to cause LC_{50} in *S. granarius* was 20.05 µl/l, whereas it was 46.48 µl/l in *Tribolium castaneum* after a 24-h treatment. Results revealed that the insecticidal activity of *A. eryngioides EO* could be related to these constituents (EBADOLLAHI & MAHBOUBI, 2011). Larvicidal activities of EOs isolated from fourteen different taxa of the Apiaceae family including *Angelica sylvestris* L., *Athamanta densa* Boiss. & Orph., *Chaerophyllum heldreichii* Orph. Ex Boiss., *Ferulago nodosa* (L.), *Laserpitium pseudomeum* Orph., Heldr. & Sart. Ex Boiss., *Peucedanum neumayeri* (Vis.) Reichenb, *Peucedanum officinale* L., *Pimpinella tragium* Vill., *Pimpinella peregrina* L., *Pimpinella rigidula* (Boiss. & Orph.) H. Wolf, *Scaligeria cretica* (Miller), *Seseli parnassicum* Boiss. & Heldr., *Smyrnium rotundifolium* Miller and *Thamnosciadium junceum* (Sibth. & Sm.) Hartvig against 3^{rd}-4^{th} instar larvae of *Culex pipiens* were evaluated by EVERGETIS *et al.* (2012) and their LC_{50} values calculated as >150, 10.15, 53.61, 67.39, 56.73, 47.40, 86.46, 40.13, >150, 40.31, 111.99, 122.54, 80.32 and 44.17 mg/l, respectively. The EO derived from the endemic in Greece plant *Athamanta densa* was determined as the most active since displayed the highest toxicity against mosquito larvae, with LC_{50} value 10.15 mg/l. The EO tested contains a series of compounds which were not found in the other EOs tested, such as bisabolene and the unidentified compounds $C_{14}H_{30}O$, $C_{12}H_{25}O_2N$ and $C_{13}H_{27}O_2N$, which have to study more thoroughly in order to determine their activities. The Apiaceae EOs with toxic effects against insect pests have known to contain the active terpenes such as anethole, camphor, carvone, cymene, linalool, thymol, *α*-pinene and *β*-pinene, which are common constituents of many Apiaceae Eos (Table 2). From above studies, it could be found that the efficacy of EOs varies according to the phytochemical profile of the plant oil and the entomological target. In fact, toxicity of EOs to insects was influenced by the chemical composition of the oil, which in turn depended on the source, season and ecological conditions, method of extraction, time of extraction and plant part used. Bioactivity of EOs is also affected by interactions among their structural components. Even minor compounds can have a critical function due to additive action between chemical classes and synergism or antagonism.

Table 1. Summary of reports indicating lethal toxicity of essential oils isolated from Apiaceae family.

Plant species	Insecticidal activity and tested insect	Reference
Ammi visnaga	Ovicidal activity against *Mayetiola destructor*.	LAMIRI *et al*., 2001
	Adulticidal and ovicidal activity against *Callosobruchus maculatus*.	TRIPATHI *et al*., 2001b
Anethum graveolense	Larvicidal against third instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.	AMER & MEHLHORN, 2006a,b
	Adulticidal activity on *callosobrucus chinensis*	UPADHYAY *et al*., 2007
	Fumigant toxicity against *Callosobruchus chinensis*.	CHAUBEY, 2008
	Fumigant antitermitic activity against *Reticulitermes speratus*.	SEO *et al*., 2009

	Fumigant toxicity against adults of *Callosobruchus chinensis*	CHAUBEY, 2011a
	Fumigant toxicity against *Callosobruchus maculatus* adults.	EBADOLLAHI *et al*., 2012
	Contact and fumigant toxicity against adult male and female of *Blattella germanica*.	YEOM *et al*., 2012
Angelica archangelica	Fumigant toxicity against *Lycoriella mali* adults.	CHOI *et al*., 2006
Angelica dahurica	Contact and fumigant toxicity against adults of *Lasioderma serricorne*.	KIM *et al*., 2003a
	Adulticidal on *Sitophilus oryzae* and *Callosobruchus chinensis*.	KIM *et al*., 2003b
Angelica sylvestris	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Apium graveolens	Adulticidal fumigant toxicity against *Acanthoscelides obtectus*.	PAPACHRISTOS & STAMOPOULOS, 2002
	Larvicidal against *Anopheles dirus* and *Aedes aegypti*.	PITASAWAT *et al*., 2007
	Larvicidal against *Lucilia sericata*.	KHATER & KHATER, 2009
	Adulticidal and larvicidal activity against early fourth instars of *A. aegypti*.	KUMAR *et al*., 2012
Athamanta densa	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Athamanta haynaldii	Larvicidal effect against the second instar gypsy moth larvae.	KOSTIĆA *et al*., 2013
Azilia eryngioides	Fumigant toxicity on adult of *Sitophilus granarius* and *Tribolium castaneum*.	EBADOLLAHI & MAHBOUBI, 2011
Azorella cryptantha	Toxic effects on *Ceratitis capitata*.	LÓPEZ *et al*., 2012
Bifora radians	Adulticidal effect on *Lipaphis pseudobrassicae*.	SAMPSON *et al*., 2005
Bunium persicum	Fumigant toxicity against adults of *Tribolium castaneum*.	MORAVEJ *et al*., 2009
Bupleurum fruticosum	Larvicidal against *Culex pipiens* larvae	EVERGETIS *et al*., 2009
Carum carvi	Fumigant toxicity against eggs, nymphs, and adults of *Trialeurodes vaporariorum*.	CHOI *et al*., 2003
	Larvicidal against *Aedes aegypti* and *Culex quinquefasciatus*.	LEE, 2006
	Larvicidal against *Anopheles dirus* and *Aedes aegypti*.	PITASAWAT *et al*., 2007
	Adulticidal effect against *Sitophilus oryzae*, *Rhyzopertha dominica* and *Cryptolestes pusillus*.	LOPEZ *et al*., 2008
	Fumigant antitermitic activity against *Reticulitermes speratus*.	SEO *et al*., 2009
	Contact toxicity against *Sitophilus zeamais* and *Tribolium castaneum* adults.	FANG *et al*., 2010
	Adulticidal on *Meligethes aeneus*.	PAVELA, 2011
	Contact and fumigant toxicity against adult male and female of *Blattella germanica*.	YEOM *et al*., 2012
Carum copticum	Adulticidal activity against *Sitophilus oryzae* and *Tribolium castaneum*.	SAHAF *et al*., 2007
	Adulticidal activity on *callosobrucus chinensis*.	UPADHYAY *et al*., 2007
	Ovicidal, larvicidal and Adulticidal against *callosobrucus maculatus*.	SAHAF & MOHARRAMI POUR, 2008a
	Toxicity against the workers of the *Odontotermes obesus* termite.	GUPTA *et al*., 2011
	Fumigant toxicity against adults of *Tribolium confusum, Rhyzopertha dominica* and *Oryzaphilus surinamensis*.	HABASHI *et al*., 2011
Centella asiatica	Larvicidal against *Culex quinquefasciatus*.	RAJKUMAR & JEBANESAN, 2005

Chaerophyllum heldreichii	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Cnidium officinale	Contact and fumigant toxicity against adults of *Lasioderma serricorne*.	KIM *et al*., 2003a
	Adulticidal on *Sitophilus oryzae* and *Callosobruchus chinensis*.	KIM *et al*., 2003b
Conopodium capillifolium	Larvicidal against *Culex pipiens* third to fourth instar larvae.	EVERGETIS *et al*., 2009
Coriandrum sativum	Fumigant toxicity against eggs, nymphs, and adults of *Trialeurodes vaporariorum*.	CHOI *et al*., 2003
	Adulticidal effect on *Lipaphis pseudobrassicae.*	SAMPSON *et al*., 2005
	Fumigant toxicity against adults of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Cryptolestes pusillus*.	LOPEZ *et al*., 2008
	Larvicidal activity against *Ochlerotatus caspius*.	KNIO *et al*., 2008
	Toxicity against *Sitophilus granarius* adults.	ZOUBIRI & BAALIOUAMER, 2010
	Larvicidal activity against *Anopheles stephensi*.	SEDAGHAT *et al*., 2011
	Adulticidal against *Tribolium confusum* and *Callosobruchus maculatus*.	KHANI & RAHDARI, 2012
	Contact toxicity on *Diaphorina citri* adults.	MANN *et al*., 2012
Cuminum cyminum	Ovicidal activity against eggs of *Tribolium confusum* and *Ephestia kuehniella*.	TUNC *et al*., 2000
	Larvicidal against *Culex quinquefasciatus*.	PRAJAPATI *et al*., 2005
	Adulticidal activity on *Callosobrucus chinensis*.	UPADHYAY *et al*., 2007
	Fumigant toxicity against *Callosobruchus chinensis*.	CHAUBEY, 2008
	Fumigant activity against *Sitophilus oryzae* adults.	CHAUBEY, 2011b
	Fumigant toxicity against *Callosobruchus maculatus* adults.	EBADOLLAHI *et al*., 2012
	Larvicidal against early fourth instar larvae of *Culex quinquefasciatus*.	RANA & RANA, 2012
	Contact and fumigant toxicity against adult male and female of *Blattella germanica*.	YEOM *et al*., 2012
Cymbocarpum erythraeum	Larvicidal effects on *Drosophila melanogaster*.	AKSAKAL *et al*., 2012.
Daucus carota	Larvicidal against *Aedes aegypti* and *Culex quinquefasciatus*.	LEE, 2006
Eleoselinum asclepium	Larvicidal against *Culex pipiens* third to fourth instar larvae.	EVERGETIS *et al*., 2009
Ferula gummosa	Larvicidal against third instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*.	AMER & MEHLHORN, 2006a,b
Ferulago angulata	Insecticide effects on *Tribolium castaneum*.	ATASHI *et al*., 2012
Ferulago nodosa	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Foeniculum vulare	Fumigant toxicity against adults of *Tribolium castaneum*.	LEE *et al*., 2002
	Contact and fumigant toxicity against adults of *Lasioderma serricorne*.	KIM *et al*., 2003a
	Adulticidal on *Sitophilus oryzae* and *Callosobruchus chinensis*.	KIM *et al*., 2003b
	Adulticidal effect on *Lipaphis pseudobrassicae*.	SAMPSON *et al*., 2005
	Fumigant toxicity against *Lycoriella mali* adults.	CHOI *et al*., 2006
	Larvicidal against *Anopheles dirus* and *Aedes aegypti*.	PITASAWAT *et al*., 2007
	Adulticidal activity on *callosobrucus chinensis*.	UPADHYAY *et al*., 2007
	Aphidicidial activity against *Brevicoryne brassicae.*	ISIK & GORUR, 2009
	Larvicidal activity against *Culex pipiens.*	MANOLAKOU *et al*., 2009
	Larvicidal activity against *Aedes albopictus*.	CONTI *et al*., 2010

	Fumigant activity against *Sitophilus oryzae* and *Sitophilus granarius* adults.	EBADOLLAHI, 2011c
	Adulticidal on *Meligethes aeneus*.	PAVELA, 2011
	Larvicidal activity against *Anopheles stephensi*.	SEDAGHAT *et al.*, 2011
	Fumigant toxicity on *Sitophilus granaries*.	ZOUBIRI & BAALIOUAMER, 2011a
	Fumigant toxicity against *Callosobruchus maculatus* adults.	EBADOLLAHI *et al.*, 2012
	Larvicidal against early fourth instar larvae of *Culex quinquefasciatus*.	RANA & RANA, 2012
	Fumigant toxicity on adults of *Callosobruchus maculatus*.	MANZOOMI *et al.*, 2010
Heracleum persicum	Adulticidal against *Plodia interpunctella*.	EBADOLLAHI & ASHOURI, 2011
	Larvicidal activity against *Anopheles stephensi*.	SEDAGHAT *et al.*, 2011
	Adulticidal against *Callosobruchus maculatus*.	IZAKMEHRI *et al.*, 2012
Heracleum sphondylium	Larvicidal against *Culex pipiens* larvae.	EVERGETIS *et al.*, 2009
Laserpitium pseudomeum	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
Ligusticum hultenii	Termiticidal activity against *Coptotermes formosanus*.	MEEPAGALA *et al.*, 2006
Ligusticum mutellina	Contact toxicity on third instar of *Pseudaletia unipuncta*.	PASSREITER *et al.*, 2005
Oenanthe pimpinelloides	Larvicidal against *Culex pipiens* larvae.	EVERGETIS *et al.*, 2009
Ostericum sieboldii	Contact and fumigant toxicity against *Tribolium castaneum* and *Sitophilus zeamais* adults.	LIU *et al.*, 2011a
Petroselinum sativum	Larvicidal activity against *Ochlerotatus caspius*.	KNIO *et al.*, 2008
	Larvicidal against *Culex pipiens*.	KHATER & SHALABY, 2008
	Ovicidal and larvicidal activity against *Plodia interpunctella*.	RAFIEI-KARAHROODI *et al.*, 2011
Peucedanum neumayeri	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
Peucedanum officinale	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
	Ovicidal activity against eggs of *Tribolium confusum* and *Ephestia kuehniella*.	TUNC *et al.*, 2000
	Fumigant toxicity against adults of *Tribolium castaneum*.	LEE *et al.*, 2002
Pimpinella anisum	Larvicidal, Adulticidal and ovicidal activities towards *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*.	PRAJAPATI *et al.*, 2005
	Adulticidal effect on *Lipaphis pseudobrassicae*.	SAMPSON *et al.*, 2005
	Fumigant toxicity against Lycoriella ingénue.	PARK *et al.*, 2006
	Larvicidal activity against *Ochlerotatus caspius*.	KNIO *et al.*, 2008
	Fumigant toxicity on *Pediculus humanus capitis* adults.	TOLOZA *et al.*, 2010
Pimpinella peregrina	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
Pimpinella rigidula	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
Pimpinella tragium	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012
Polylophium Involvucratum	Larvicidal against *Anopheles stephensi* and *Culex pipiens*	VERDIAN-RIZI & HADJIAKHOONDI, 2007
Prangos acaulis	Adulticidal and larvicidal against *Callosobruchus maculatus*.	TAGHIZADEH-SARIKOLAEI & MOHARAMIPOUR, 2010
Scaligeria cretica	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al.*, 2012

Seseli montanum	Larvicidal against *Culex pipiens* third to fourth instar larvae.	EVERGETIS *et al*., 2009
Seseli parnassicum	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Smyrnium rotundifolium	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Thamnosciadium junceum	Larvicidal against third to fourth instar larvae of *Culex pipiens*.	EVERGETIS *et al*., 2012
Trachyspermum ammi	Fumigant toxicity against *Anopheles stephensi*.	PANDEY *et al*., 2009
	Fumigant antitermitic activity against *Reticulitermes speratus*.	SEO *et al*., 2009
	Contact and fumigant toxicity against adult male and female of *Blattella germanica*.	YEOM *et al*., 2012
	Fumigant toxicity against adults of *Callosobruchus chinensis*.	CHAUBEY, 2011a

Table 2. Summary of reports on main components in the introduced Apiaceae essential oils as insecticides.

Plant species	Main constituents	Reference
Ammi visnaga	Isobutyrate (14.0%), 2,2-dimethylbutanoic acid (30.1%), croweacin (12.2%) and linalool (12.1%).	KHALFALLAH *et al*., 2011
Anethum graveolense	Carvone (57.3%) and Limonene (33.2%).	SEFIDKON, 2001
Angelica archangelica	α-Pinene (19.1%), δ-3-carene (16.0%), β-limonene (8.0%) and osthol (3.6%).	NIVINSKIENCE *et al*., 2003
Angelica dahurica	3-Carene (12.7%), beta-elemene (6.2%), beta-terpinene (3.5%) and beta-myrcene (1.9%).	ZHAO *et al*., 2011
Angelica sylvestris	β-Phellandrene (42.9%), α-pinene (24.6%), myrcene (4.7%) and germacrene D (4.4%).	EVERGETIS *et al*., 2012
Apium graveolens	(Z)-3-Butylidenephthalide (27.8%), 3-butyl-4,5-dihydrophthalide (34.2%) and α-thujene (7.9%).	SELLAMIA *et al*., 2012
Athamanta densa	β-Bisabolene (12.7%), β-pinene (8.8%), trans-ocimene (5.1%) and Myrcene (4.3%).	EVERGETIS *et al*., 2012
Azilia eryngioides	α-Pinene (63.8%), bornyl acetate (18.9%), β-pinene (2.6%) and linalool (2.1%).	EBADOLLAHI & MAHBOUBI, 2011
Azorella cryptantha	α-pinene, α-thujene, sabinene and δ-cadinene.	LÓPEZ *et al*., 2012
Bifora radians	(E)-2-tridecenal (47.2%) and (E)-2-tetradecenal (23.4%).	BASERA *et al*., 1998
Bunium persicum	ρ-Cuminaldehyde (16.9%), γ-terpinen-7-al (10.5), ρ-cymene (8%) and γ-terpinene (4.2%).	AZIZI *et al*., 2009
Bupleurum fruticosum	α-Pinene (37.8%), β-pinene (28.5%), β-phellandrene (21.6%) and cis-ocimene (5.4%).	EVERGETIS *et al*., 2009
Carum carvi	(R)-Carvone (37.9%), D-limonene (26.5%), α-pinene (5.2%) and cis-carveol (5.0%).	FANG *et al*., 2010
Carum copticum	Thymol (41.3%), α-terpinolene (17.4%) and ρ-cymene (11.7%).	SAHAF *et al*., 2007
Centella asiatica	α-Humulene (21.0%), β-caryophyllene (19.0%), bicyclogermacrene (11.2%) and germacrene B (6.2%).	OYEDEJI & AFOLAYAN, 2005
Chaerophyllum heldreichii	Sabinene (71.76%), β-Phellandrene (10.86%), α-terpineol (3.35%) and γ-terpinene (2.54%).	EVERGETIS *et al*., 2012
Cnidium officinale	cis-Butylidene phthalide (33.2%), 3-butyl phthalide (21.1%), cis-3-isobutylidene phthalide (10.1%) and terpinen-4-ol (8.5%).	CHOI *et al*., 2002
Conopodium capillifolium	α-Pinene (37.8%), Sabinene (29.1%), p-Cymene (4.6%) and Limonene (4.1%).	EVERGETIS *et al*., 2009
Coriandrum sativum	Linalool (57.1%), trans-anethol (19.8%), c-terpinene (3.8%) and geranyl acetate (3.2%).	KNIO *et al*., 2008
Cuminum cyminum	Caryophyllene oxide (6.1%), α-pinene (4.8%), geranyl acetate (4.1%) and âcaryophyllene (3.4%).	ROMEILAH *et al*., 2010

Cymbocarpum erythraeum	(E)-2-Decenal (52.2%), (2E)-dodecenal (15.7%), 8S.14-cedranediol (8.5%) and ntetradecenal (5.5%).	AksakAl *et al*., 2012.
Daucus carota	Carotol (66.7%), daucene (8.7%), (Z,Z)-α farnesene (5.8%) and germacrene D (2.3%).	ÖZCAN & CHALCHAT, 2007
Eleoselinum asclepium	Sabinene (35.3%), α-Pinene (27.4%), Myrcene (5.9%) and β-Pinene (5.2%).	EVERGETIS *et al*., 2009
Ferula gummosa	Sabinene (40.1%), α-pinene (14.3%), β-pinene (14.1%) and p-cymene (8.46%).	ABEDI *et al*., 2008
Ferulago angulate	α-Pinene (27٪), cis-ocimene (22٪), and bornyl acetate (8.5٪) and trans-verbenol (5.8%).	GHASEMPOUR *et al*., 2007
Ferulago nodosa	α-Pinene (30.8٪), β-Phellandrene (10.2%), myrcene (6.6%) and camphene (4.3%).	EVERGETIS *et al*., 2012
Foeniculum vulare	Methyl clavicol (43.5%), α-phellandrene (16.0%) and fenchone (11.8%) .	CONTI *et al*., 2010
Heracleum persicum	(E)-Anethole (47.0%), terpinolene (20.0%), γ-terpinene (11.6%) and Limonene (11.5%).	SEFIDKON *et al*., 2004
Heracleum sphondylium	Octyl acetate (17.4%), limonene (13.1%), trans-β-farnesene (6.3%) and germacrene-D (5.0%).	EVERGETIS *et al*., 2009
Laserpitium pseudomeum	α-Pinene (49.5%), sabinene (24.7%), β-pinene (8.5%) and α-Phellandrene (6.7%).	EVERGETIS *et al*., 2012
Ligusticum mutellina	Myristicin (39.3%) and alpha-phellandrene (23.4%).	BRANDT & SCHULTZE, 1995
Oenanthe pimpinelloides	γ-Terpinene (43.2%), o-Cymene (14.4%), β-Sesquiphellandrene (8.2%) and β-Pinene (6.7%).	EVERGETIS *et al*., 2009
Ostericum sieboldii	Myristicin (30.3%), α-terpineol (9.9%), α-cadinol (7.2%) and β-farnesene (6.2%).	LIU *et al*., 2011a
Petroselinum sativum	Apiol (18.2%), α-pinene (16.1%) and β-pinene (11.1%).	ROMEILAH *et al*., 2010
Peucedanum neumayeri	γ-Terpinene (32.2%), α-pinene (21.2%), β-phellandrene (12.7%) and cis-ocimene (4.7%).	EVERGETIS *et al*., 2012
Peucedanum officinale	1-Bornyl acetate (81.1%), 2,3,4-trimethyl bezaldehyde (4.6%) and liminene (2.7%).	EVERGETIS *et al*., 2012
Pimpinella anisum	trans-Anethol (76.7%), anisalacetone (7.1%), estragol (6.1%) and anisaldehyd (1.5%).	KNIO *et al*., 2008
Pimpinella peregrina	α-Brgamontene (62.1%), aristolene (19.9%), β-selinene (3.7%) and calaren (3.4%).	EVERGETIS *et al*., 2012
Pimpinella rigidula	β-Selinene (23.2%), trans-isomiristicin (7.7%), α-zingiberene (7.7%) and miristicin (6.7%).	EVERGETIS *et al*., 2012
Pimpinella tragium	Germacrene (23.3%), germacrene B (19.2%), geigerene (10.2%) and pregeigerene (5.1%).	EVERGETIS *et al*., 2012
Polylophium Involvucratum	Limonene (60.3%), perillaldehyde (25.8%), α-pinene (7.1%) and perillalcohol (6.6%).	VERDIAN-RIZI & HADJIAKHOONDI, 2007
Prangos acaulis	δ-3-Carene (25.5%), α-terpinolene (14.7%), α-pinene (13.%) and limonene (12.9%).	MESHKATALSADAT *et al*., 2010
Scaligeria cretica	β-Farnesene (29.2%), germacrene D (28.3%), sabinene (13.7%) and α-pinene (8.7%).	EVERGETIS *et al*., 2012
Seseli montanum	α-Pinene (32.2%), Sabinene (16.9%), β-Phellandrene (19.0%) and Myrcene (4.9%).	EVERGETIS *et al*., 2009
Seseli parnassicum	β-Sesquiphellandrene (30.3%), germacrene D (13.0%), germacrene B (10.6%) and β-elemene (10.8%).	EVERGETIS *et al*., 2012
Smyrnium rotundifolium	Myrcene (11.2%), furanodiene (11.8%), germacrone (5.6%) and α-selinene (5.2%).	EVERGETIS *et al*., 2012
Thamnosciadium junceum	Limonene (40.7%), cis-ocimene (18.5%), terpinolene (12.9 %), trans-isomirticisin (10.1%).	EVERGETIS *et al*., 2012
Trachyspermum ammi	Thymol (41.7%), γ-terpinene (27.7%), *p*-cymene (24.40%) and β-pinene (1.4%).	PARK *et al*., 2007

Sublethal toxicity

Investigations in several countries confirm that some plant *EO*s not only have lethal toxicity, but possess repellency against insect pests as well as exhibited feeding inhibition or harmful effects on the reproductive system and growth of insects. In the following, repellent, Antifeedant, oviposition deterrent, growth inhibition and progeny production effects of some EOs from Apiaceae family that recently published will be discussed as sublethal toxicityies;

a- Repellent: The repellents are desirable chemicals as they offer protection with minimal impact on the ecosystem, as they drive away the insect pest from the treated materials by stimulating olfactory or other receptors. Concern over health implications from the use of residual and broad insecticidal spray treatments has been impetus for research on alternative methods. Repellents from plant origins are considered safe in pest control for minimize pesticide residue; ensure safety of the people, and environment. Repellents may play a very important role in some situations or in some special space where the insecticides are not able to use. Many plant EOs and their components have been shown to have good repellent activity against insect pests. Insect repellent activity of some EOs from Apiaceae family summarized in Tables 3. The major promising uses for EOs in the human health are for repelling biting flies. The anti-mosquito activities of some of *EO*s from Apiaceae plants are shown in table 1 (lethal toxicity) and table 3 (repellency). In 2007 RAJKUMAR & JEBANESAN, investigated the repellent effect of *Centella asiatica* (L.) Urb. EO against the malaria fever mosquito *Anopheles stephensi* Liston in mosquito cages. The oil was tested at three concentrations: 2, 4 and 6%. In general, a dose-dependent effect was noticed. The highest concentration (6%) led to the highest repellency effect. The results showed repellency effect at the highest concentration (6%) lasted up to 150 min whereas ethanol (as a control) showed only 8 min repellency. The effect of thymol from the EO of *Tachyspermum ammi* against *Anopheles stephensi* was investigated by PANDEY *et al*. (2009). The larvicidal, oviposition deterrent, vapour toxicity and repellent activity against the malarial vector were evaluated. Thymol (major component in *Trachyspermum ammi* oil) showed an LD_{50} value of 48.88 mg/ml toward fourth-instar larvae of *A. stephensi*. So it was 1.6-fold more toxic than the oil, which showed an LD_{50} value of 80.77 mg/ml. After treatment with vapours of thymol the egg laying by female adults of this fly was significantly more reduced compared to the treatment with the EO. The evaluation of the egg hatching and larval survival showed similar results. The vapour toxicity assay exhibited an LC_{50} value of 185.4 mg/mat for the crude oil against adults of *A. stephensi*, whereas thymol showed an LC_{50} value of 79.5 mg/mat. After 1 h, the treatment of adult flies with 25.0 mg/mat of thymol demonstrated complete repellency. The same degree of repellency was obtained by the oil of *Trachyspermum ammi* at the dose of 55.0 mg/mat. This indicates that thymol possesses two-fold activity. Moreover, the repellency of *a*-pinene, myrcene, carvacrol, thymol and caryophyllene oxide was found 2, 4, 6, and 24 h after treatment against *Tribolium castaneum* adults. In addition, caryophyllene oxide and *a*-pinene gave 85 and 82% at 0.001 mg/cm^2, respectively and hydrogenated monoterpenoids such as thymol, carvacrol, and myrcene also showed more than 77% at 0.03 and 0.006 mg/cm^2 repellent activity (KIM *et al*., 2012). These monoterpenoids are major constituents in the many Apiaceae EOs. Results emphasize the performance of EOs and components isolated from Apiaceae family as repellents. The repellent properties of the tested EOs are not unexpected given that EO products are generally considered broad spectrum because of multiple active ingredients and modes of action. In conclusion, the identification of these potential repellent plants from the local flora will generate local employment and stimulate local efforts to enhance public health.

Table 3. Summary of reports indicating repellent of essential oils isolated from Apiaceae family.

Plant species	Tested insect	Reference
Anethum graveolens	*Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*	AMER & MEHLHORN, 2006c
	Adults of *Tribolium castaneum*	CHAUBEY, 2007
	Adults of *Plodia Interpunctella*	RAFIEI-KARAHROODI *et al.*, 2009b
Angelica sinensis	*Blattella germanica*	LIU *et al.*, 2011b
Apium graveolens	Adults of *Acanthoscelides obtectus*	PAPACHRISTOS & STAMOPOULOS, 2002
	Aedes aegypti	KUMAR *et al.*, 2012
Carum carvi	female *Culex pipiens* adults	KANG *et al.*, 2009
	Adults of *Plodia Interpunctella*	RAFIEI-KARAHROODI *et al.*, 2009b
	Blattella germanica, *Periplaneta americana* and *Periplaneta fuliginosa*	YOON *et al.*, 2009
	Adults of *Meligethes aeneus*.	PAVELA, 2011
Centella asiatica	*Anopheles stephensi*	RAJKUMAR & JEBANESAN, 2007
Coriandrum sativum	*Tribolium castaneum*	ISLAM *et al.*, 2009
	female *Culex pipiens* adults	KANG *et al.*, 2009
	Blattella germanica, *Periplaneta americana* and *Periplaneta fuliginosa*	YOON *et al.*, 2009
	Sitophilous oryzae and *Tribolium castaneum*	MISHRA & TRIPATHI, 2011
Cuminum cyminum	Adults of *Diaphorina citri*	MANN *et al.*, 2012
	Sitophilus oryzae adults	CHAUBEY, 2011b
Foeniculum vulgare	Adults of *Sitophilous zeamais*, *Cryptolestes ferrugineus* and larvae of *Tenebrio molitor*	COSIMI *et al.*, 2009
	female *Culex pipiens* adults	KANG *et al.*, 2009
	Adults of *Plodia Interpunctella*	RAFIEI-KARAHROODI *et al.*, 2009b
	Adults of *Meligethes aeneus*.	PAVELA, 2011
Ferula assa-foetida	Adults of *Ectomyelois ceratoniae*	PEYROVI *et al.*, 2011
Ferula galbaniflua	*Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus*	AMER & MEHLHORN, 2006c
Petroselinum sativum	Adults of *Plodia Interpunctella*	RAFIEI-KARAHROODI *et al.*, 2009b
Pimpinella anisum	*Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*	PRAJAPATI *et al.*, 2005
	Culex pipiens	ERLER *et al.*, 2006
Pturanths tortosus	larvae and moths of *Phthorimaea operculella*	SHARABY *et al.*, 2009
Tachyspermum ammi	Adults of *Tribolium castaneum*	CHAUBEY, 2007
	Anopheles stephensi	PANDEY *et al.*, 2009

b- Antifeedant: Feeding deterrents or antifeedants are materials that inhibit feeding but do not kill the insect directly. However, the insect may remain close to the plant but will die from starvation or dehydration rather than feeding from it. Such deterrents can be found among all of the major classes of secondary metabolites. Many *EO*s and their components have been known to exhibit antifeedant properties against insects (HUMMELBRUNNER & ISMAN, 2001; TRIPATHI *et al.*, 2001a; KIRAN *et al.*, 2007; BENZI *et al.*, 2009; EBADOLLAHI, 2011a; AKHTAR *et al.*, 2012). In the following, antifeedant activity of some EOs from Apiaceae family that recently published will be discussed. The effect of *Petroselinum sativum*, *Foeniculum vulgare*, *Carum carvi* and

Anethum graveolens EOs on nutritional indices of 15 days old larvae of *Plodia interpunctella* demonstrated by RAFIEI-KARAHROODI *et al*. (2009a). In other study, the EO extracted from *Carum copticum* was tested against *Tribolium castaneum*, for antifeedant activity (SAHAF & MOHARAMIPOUR, 2009). In this study, several experiments were designed to measure the nutritional indices such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI). Results indicated that nutritional indices were significantly varied as EO concentrations increased and *Carum copticum* decreased RGR, RCR and ECI significantly. *Carum copticum EO* increased FDI as the oil concentration was increased, showing high feeding deterrence activity against *Tribolium castaneum*. In the study of KOSTIĆA *et al*. (2013) ethanol solutions of EO obtained from *Athamanta haynaldii* (Borbás & Uechtr.) Tutin. was tested for their toxicity and antifeedant activity against the second instar gypsy moth larvae. Tested oil showed low to moderate larvicidal effect in both residual toxicity test and in chronic larval mortality bioassay. However, antifeedant index achieved by application of tested solutions in feeding choice assay was significantly higher in comparison to control. They stated that low toxic and high antifeedant properties (antifeedant index 85-90%) make these EOs suitable for integrated pest management programs. Accordingly, exploration of the influence of chemical complexity of EOs on feeding behavior of insects can assist in the development of new crop protection products for use in integrated pest management systems. Understanding the role of each constituent in the efficacy of the oil renders an opportunity to create artificial blends of different constituents on the basis of their activity and efficacy against different pests. Feeding inhibitors have several advantages in plant protection, compared to traditional chemical methods. The host choice of generalists and specialists may be modified when inhibitors are used. If an insect species can feed on other plants than its targeted host, it can be easier to direct away than if it is highly specialized in one host. The range of insect species targeted may be chosen by either the chemical structure of the inhibitor or by the composition of a mixture of inhibitors, if different inhibitors are active against different species within the range. The practice of using feeding inhibition allows us to develop and exploit naturally occurring plant defense mechanisms, thereby reducing the use of traditional pest management chemicals.

c- Oviposition deterrent, growth inhibition and progeny production effects: Many Apiaceae EOs and their constituents were evaluated against insect pests for their efficiency on oviposition, egg hatching, growth inhibition and fecundity and progeny production. In the following, these effects from recent studies will be discussed. PAPACHRISTOS & STAMOPOULOS (2002) revealed that along with Adulticidal fumigant toxicity and repellent effect of *Apium graveolens*, this oil had a reduce fecundity, decrease egg hatchability, increase neonate larval mortality and adversely influence offspring emergence. Toxic and developmental inhibitory activity of the EOs from *Anethum graveolens* and *Trachyspermum ammi* against *Tribolium castaneum* were tested. The EOs reduced the oviposition potential and increased the developmental period of the insect. Fumigation of these EOs inhibited development of larvae to pupae and the pupae to adults and also resulted in the deformities in the different developmental stages of the insect (CHAUBEY, 2007). In similar study, along with insecticidal and oviposition effects, egg hatching and developmental inhibitory activities of *Anethum graveolens*, *Cuminum cyminum* and *Trachyspermum ammi* were determined against *Callosobruchus chinensis*. These EOs reduced the oviposition potential, egg hatching rate, pupal formation and emergence of adults of F1 progeny on the insect with fumigated by sublethal doses. Furthermore, these oils caused chronic toxicity as the fumigated insects caused less damage to the grains (CHAUBEY, 2008). Most of the EOs tested seem to have no effect upon the eggs hatchability, but increase the first instar larval mortality before penetration into the seeds. Those results must be due either to direct toxicity towards larvae or to an indirect effect such as repellency and/or antifeedant activity. Oviposition deterrence of EOs from dry seeds of *Carum copticum* with six concentrations (0.02-0.5l oil per one gram seed) was determined against *Callosobruchus maculatus* by SAHAF & MOHARAMIPOUR, 2008b. At the highest concentration (0.5 l per one gram seed) oviposition deterrence was reached to 100%. ISIK & GORUR (2009) studied the aphidicidial activity of *Foeniculum vulgare EO* against cabbage aphid, *Brevicoryne brassicae* L., under laboratory conditions. Applications of *Foeniculum vulgare EO* significantly reduced the reproduction potential of the cabbage aphid and resulted in higher mortality. The biological activity of EO extracted from *Coriandrum sativum* against eggs, larvae and adults of *Tribolium castaneum* was reported by ISLAM *et al*. (2009). On the developmental inhibition, individuals fumigated at the larval stage confirmed that the percentage of larvae reaching to pupal stage and pupae to adult stage, decreased significantly ($P < 0.001$) with increasing dosage concentration. Effect of the EO from *Ferula assa-foetida* was investigated on some reproductive behaviors of *Ectoyelois ceratoniae* (Zeller) under field and laboratory conditions. The EO can prevent pheromone release in the females and/or disrupt male searching behavior. Preliminary results from laboratorial data showed interference in pheromone production in females. Time period and number of pheromone production was decreased in the presence of the EO (KAMELSHAHI *et al*., 2010**)**. In the other study, fumigation of *Tribolium castaneum* adults with two sublethal concentrations of α-pinene, β-caryophyllene, main component of several members of Apiaceae family, and its binary combination reduced oviposition potential of insect. Oviposition was reduced to 71.91% and 58.54%, and 68.20% and 48.09% of the control when *Tribolium castaneum* adults were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone. Similarly, oviposition was reduced to 56.4% and 36.52% of control when *T. castaneum* adults were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene binary combination. The percentage of larvae transformed into the pupae and percentage of pupae transformed into adult were decreased when fumigated with two sublethal concentrations of α-pinene and β-caryophyllene alone or in binary combination. Pupation in treated larvae was reduced to 70.8% and 55%, 85% and 64.2%, and 64.2% and 34.2% of control when *Tribolium castaneum* larvae were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone or in binary combination respectively. Adult emergence was reduced to 50.8% and 39.2%, 66.7% and 45.8%, and 47.5% and 15.8% of control when *Tribolium castaneum* larvae were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone or in binary combination respectively (CHAUBEY, 2012). In the study of IZAKMEHRI *et al*. (2012), lethal and sublethal effects of EO from *Heracleum persicum* were evaluated on the adults of *Callosobruchus maculates*. The LC_{50} value of *Heracleum persicum* in fumigant toxicity was 136.36 µl/l air after 24 hours. The results showed that sublethal concentration of EO (78.78 µl/l air) negatively affected the longevity and fecundity of female adults. The sex ratio of *Callosobruchus maculatus* offspring was not significantly affected by EO. Inhabitation of egg hatching or ovicidal effect of EO extracted from seed of Parsley, *Petroselinum sativum*, on the eggs of *Ephestia kuehniella* was studied by SALAHI *et al*. (2012). The LC_{50} value of this oil for ovicidal effect on eggs of *Ephestia kuehniella* was assessed as 860 µl/l air. In another study, the EO and extracts obtained from the seeds of *Angelica archangelica* were used to determine the efficacy in terms of antifeedancy and growth inhibition of *Spodoptera littoralis* Boisd. larvae. Significant acute toxicity was caused only by the EO (LD_{50} 96 µg/larva). Significantly higher chronic toxicity was found for the extracts obtained using organic solvents (LD_{50} was estimated at 0.32, 0.82 and 0.52 mg/g for benzene, acetone and methanol, respectively), compared to the EO (LD_{50} = 7.53 mg/g). All the tested extracts and the EO caused growth inhibition. The highest larval growth inhibition was caused by the benzene extract, with ED_{50} estimated at 2.4 µg/g. All extracts and the EO also showed antifeedant activity. However, the benzene extract showed the highest efficacy (ED_{50} = 0.31 µg/cm^2), while the least efficacy was shown by the water extract (ED_{50} = 1.92 µg/cm) (PAVELA & VRCHOTOVÁ, 2013). EOs have been reported to have low vapour density than fatty oils. Hence, they are readily volatilized. This could be the reason why most of the eggs that might have hatched could not survive. These studies revealed good results for utilizing sublethal doses of EOs and its constituents (as antifeedant, repellent, reduction of egg laying, and hatching, progeny production and growth inhibitors) along with lethal dose for insect pest management.

Conclusion

The most attractive aspect of using EOs and/or their constituents for pest control is their favorable mammalian toxicity because many EOs and their constituents are commonly used as culinary herbs and spices and as medicines. It is found that the use of biopesticides will help in preventing the discarding of thousands of tons of pesticides on the earth and provide the residue free food and a safe environment to live (DEVI & MAJI, 2011). The present review shows a range of EOs and phytochemicals from Apiaceae family that exhibit interesting insecticidal properties against several inst pests.

Elucidation of the mode of action of oils and their constituents is of practical importance for insect control because it may give useful information on the most appropriate formulation and delivery means. Volatile oil can disrupt communication in mating behavior of insect by blocking the function of antennal sensilla and unsuccessful mating could lead to a lower fecundity and ultimately lower the population of insect pest (AHMED *et al.*, 2001). Rapid action of EOs or its constituents against insect pests is an indicative of neurotoxic actions. Treatments the insects with natural compounds such as *EO*s or pure compounds may cause symptoms that indicate neurotoxic activity including hyperactivity, seizures, and tremors followed by paralysis (knock down), which are very similar to those produced by the insecticides pyrethroids. ENAN (2001) suggested that toxicity of constituents of EO is related to the octopaminergic nervous system of insects. Relatively few studies have been done on insecticidal activity or fumigant toxicity of caryophyllene oxide. Its high toxicity may result from the inhibition of the mitochondrial electron transport system because changes in the concentration of oxygen or carbon dioxide may affect respiration rate of insect, thus eliciting fumigant toxicity effects (EMEKCI *et al.*, 2004). Several reports indicate that EOs and monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity. Effects of furanocoumarins and pthalides isolated from *Angelica acutiloba* Kitagawa var. *sugiyame* Hikino against *Droshophila melanogaster* revealed the hypothesis that the insecticidal properties of the plant extracts are connected with the AChE (Acetylcholinesterase) inhibition (MIYAZAWA *et al.*, 2004). Thymol binds to GABA receptors associated with chloride channels located on the membrane of postsynaptic neurons and disrupts the functioning of GABA synapses (PRIESTLEY *et al.*, 2003).Further studies on cultured cells of *Periplaneta americana* (L.) and brains of *Drosophila melanogaster* demonstrated that eugenol mimics the action of octopamine and increases intracellular calcium levels (Enan, 2005). Ethanolic extract from the fruits of *Pimpinella anisoides* V Brig. exhibited activity against AChE and BChE (Butyrylcholinesterase), with IC_{50} values of 227.5 and 362.1 µg/ml, respectively. The most abundant constituents of the extract were *trans*-anethole that exhibited the high activity against AChE and BChE with IC_{50} values of 134.7 and 209.6 µg/ml, respectively (Menichini *et al*., 2009). It is confirmed that the insecticidal activity of *EO*s and/or monoterpenes is due to several mechanisms that affect multiple targets.

The physiological pattern of plants varies with the seasons and that within the same plant species different varieties may produce different chemical types having different effects on insects. From this point of view, it is very difficult to establish a clear relation between botanicals and insects because different varieties or even different parts of the same plant species give different effects. Accordingly, analytical studies should be carried out to determine the exact chemical structure of the extracted EOs and to clarify how each of their constituents influences insect physiology and behaviour. Moreover, EOs are complex mixtures of various molecules. Their biological effects might be either the result of a synergism of all molecules or could reflect only those of the main molecules. Almost literature cases analyses only the main constituents of EOs. In that sense, for biological purposes, it could be more informative to study the entire oil rather than some of its components because the concept of synergism seems to be important.

In conclusion, the development of natural or biological insecticides will help to decrease the negative effects of synthetic chemicals. Negative effects refer to residues in products and insect resistance. I believe that the present study with the utility of Apiaceae EOs and phytochemicals together with the previous studies on others support the biopesticidal nature of the plant derived EOs. These oils can be used as a cheap, safe and efficient alternative as well as a supplement, in the developing countries to protect the crops against the various plant pathogens. For all these reasons, we can infer that the EOs could be considered as a natural alternative in the control insects. However, for the practical application of the EOs as novel insecticides, further studies on the safety of the oils to humans and on development of formulations are necessary to improve the efficacy and stability and to reduce cost.

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