CONTENTS

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SYNTHESIS OF EMERALDINE BASE – *PHRAGMITES AUSTRALIS* BIOCOMPOSITE AND ITS APPLICATION FOR Cu^{2+} IONS REMOVAL FROM AQUEOUS SOLUTIONS

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Abstract

In this paper the influence of *in situ* synthesized biocomposite of emeraldine base and *Phragmites australis* (Cav.) Trin. ex Steud (Common reed) on the removal of copper ions from aqueous media is discussed. Physico-chemical parameters such as initial copper ion concentration, composite dosage and contact time between composite and Cu^{2+} ions in the water solution were studied. The removal efficiency of copper by biocomposite of emeraldine base and Common reed was determined. Two different quantities of Common reed in the biocomposite (Biocomposite I and Biocomposite II) were used. The results show, that the longer contact time leads to the increased removal efficiency. Maximum removal efficiency of 99.9 % and 98.3 % was achieved using Biocomposite I and Biocomposite II, respectively.

Key words: Common reed, copper ions removal, Emeraldine base, *in situ* polymerization.

Introduction

The wastewaters generated during the non-ferrous metals production and their processing like electroplating and other industrial processes are the main sources of environmental pollution with heavy metals (Yong-hua et al. 2007). The most commonly found include copper, cadmium, chromium, arsenic, etc., all of which cause risks for the environment and humans (Lambet et al. 2000, Jaishankar et al. 2014). The heavy metals are difficult to remove from the environment and often cause secondary pollution (Pan et al. 2002). The most commonly used methods for removing them from wastewater are chemical precipitation, extraction, reverse osmosis, adsorption, ion exchange, membrane separation, electrodialysis, coagulation, biological treatment, etc. (Fu and Wang 2011). The high cost of most of these methods is a limitation for their widespread usage. Development of innovative and cost-effective processes for treating waters with low concentration of heavy metals is needed. Some new materials with polymeric nature, which are alternative to conventional sorbents, were used recently. In most cases, these substances are used in combination with other materials in order to increase their treatment efficiency. This effect is due to the enlarged surface area and greater electron donor

property, derived synergistically from both components of the composite material (Bhaumik et al. 2014). There are some composite materials such as polyaniline/polystyrene, aniline/sulfoanisidine, polyaniline/metallic iron nanoparticles, etc. (Gupta et al. 2004, Li et al. 2009, Sharma et al. 2014, Kang et al. 2015, Mittal et al. 2016, Naushad et al. 2016, Pathania et al. 2016, Sharma et al. 2016, Sharma et al. 2017, Sharma et al. 2015, Sharma et al. 2015). On the other hand it is well known that *Phragmites australis* (Cav.) Trin. ex Steud (Common reed) and other macrophytes play significant role in water purification as they accumulate in their tissue various organic and inorganic pollutants as well as metal compounds (Lavrova and Koumanova 2006, Lavrova and Koumanova 2008, Vymazal and Březinová 2016). The preparation of composites of polyaniline and vegetation waste, the so called biocomposites, is of interest for many researchers. In their scientific studies they use sawdust, rice husks, jute fibers, kapok fibers, etc. (Mansour et al. 2011, Ghorbani and Eisazadeh 2013, Kumar et al. 2008, Zheng et al. 2015). Such biocomposites showed high removal efficiency of heavy metal ions from aqueous media. Due to biocomposites selectivity and efficiency, easy preparation and cost effectiveness they are a good alternative to conventional sorbents which are used in practice.

The aim of this study was to investigate the possibility of *Phragmites australis*/Emeraldine base biocomposites to remove copper ions from a model aqueous media. The influence of the experimental conditions – metal ion concentration, polymer dosage and contact time on the Cu^{2+} ions removal from aqueous media was studied.

Material and Methods

Reagents

Aniline ($C_6H_5NH_2$), hydrochloric acid (HCl), ammonium persulfate ($(NH_4)_2S_2O_8$), sodium hydroxide (NaOH), copper sulphate pentahydrate ($CuSO_4.5H_2O$), sodium acetate ($C_2H_3NaO_2$) and acetic acid (CH_3COOH) pure for analysis were used in the experiments. Distilled water was also used. Leaves of *P. australis* from comparatively clean area in this study were used. After collection they were washed first with tap water, then with distilled water to remove any dust particles. The washed crops were dried for 2 h at 60 °C. The dried material was rinsed with acetone and then with 0.3 M NaOH and was again dried at the same temperature to constant weight (Mo et al. 2009). After its pre-treatment the dried material was finely grinded.

Preparation of the biocomposites

The preparation of the biocomposites was carried out in standard conditions and two different solid/solution ratios were used. In the first case *P. australis*/aniline ratio (Biocomposite I) was 1:15 and in the second case (Biocomposite II) it was 1:4. The preliminary weighed amounts of Common reed (leaves) were mixed with 1938.6 mL of 1 M HCl under continuous stirring at 800 rpm for 24 h. After that 61.4 mL of aniline was added to the reaction mixture and the suspension was stirred for 24 h at room temperature. The solution of the oxidant, which was necessary for the preparation of polyaniline from aniline monomer, was prepared by dilution of ammonium persulfate ($(NH_4)_2S_2O_8$) with distilled water to volume of 800 mL. This solution was add-

ed to the reaction mixture and the resulting suspension was stirred continuously for another 24 h. The suspension was filtered and washed with distilled water. In order to remove the impurities and possible residual monomers the suspension was washed several times with mixture of water and methanol at ratio 80:20. At the end of the process the polymerized aniline monomer in the biocomposites composition was under protonated form – emeraldine salt. It is well-known that it is not particularly efficient for removal of ions from an aqueous media and it has to be converted into more effective form – emeraldine base. The conversion of emeraldine salt into emeraldine base was carried out via washing the resulting precipitate with 1 M NaOH to pH 10.0–11.0. Thus deprotonation was achieved, consisting in the release of electron pairs on the nitrogen atoms in the amine groups of the emeraldine base polymer chain. These free electron pairs are the places where the metal ions, contained in the aqueous media, can be trapped. The washed precipitate was dried at 60 °C to a constant weight. Well-dried biocomposite was ground to a homogeneous powder.

Preparation of standard solutions of copper ions

Solution of copper ions with concentration of 1 g·L^{-1} was prepared, using 3.93 g $CuSO_4.5H_2O$ and distilled water. Then standard solutions with concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 30.0, and 50.0 mg·L^{-1} were prepared.

Experimental

In order to establish the influence of the contact time on the removal of copper ions from aqueous media by *P. australis*/Emeraldine base biocomposites, individual samples with initial copper ions concentration of 50.0 mg·L^{-1} (C_o) were prepared. The volume of each sample was 50 mL and certain amount of the biocomposites (0.1, 0.5, 1.0 and 1.5 g) was added to each of them. To adjust pH to 5.0, proper for the copper ions removal (Srithongkham et al. 2012), acetate buffer was added to each solution. The samples were placed in iodine flasks and were shaken with a plate shaker for 1, 3, 5, 7, 10, 15, 30, 60 and 360 min, respectively. Samples with volume 20 mL were taken and filtered through a blue ribbon filter paper to remove suspended biocomposite particles. The filtrates were analysed by ISP–OES ('Prodigy' High dispersion ICP-OES, Telledyne Leeman Labs) in order to determine copper ions concentration.

In order to establish the influence of initial copper ions concentration individual samples with certain initial Cu^{2+} concentration (C_o = 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 30.0, 50.0 mg·L^{-1}) were prepared. The volume of each sample was 50 mL. Certain amount of the biocomposites (0.1 g) was added to each of them. To adjust the pH to 5.0, acetate buffer was added to each solution. The samples were placed in iodine flasks and were shaken with a plate shaker for 30 min. Samples with volume 20 mL were taken and after filtration through a blue ribbon filter paper were analysed for Cu^{2+} ions concentration.

The removal efficiency was determined according to the formula (1):

$$RE = 100 - \left(\frac{C_t}{C_o}\right) \cdot 100 \qquad (1),$$

where: RE is removal efficiency (%), C_o is the initial concentration (mg·dm^{-3}), and C_t is the concentration at time t (mg·dm^{-3}).

Results and Discussion

Influence of the contact time and biocomposite dose on copper ions removal

The results show that with increasing of the contact time between biocomposites and the standard solutions, Cu^{2+} ions concentration is decreasing (Figs. 1 and 2). For example, using 0.1 g of Biocomposite I (Fig. 1) decreased initial copper ions concentration as follows: at 1st min – with 1.3 times, at 10th min – with 1.9 and at 360th min – with 2.2. When using the same amount of Biocomposite II the initial copper ions concentration was decreased as follows: at 1st min – 1.1 times, at the 10th min – 1.4, and at 360th min – 1.6.

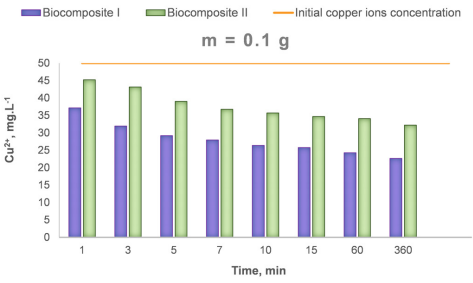

Fig. 1. Influence of contact time on copper ions removal by 0.1 g of biocomposites.

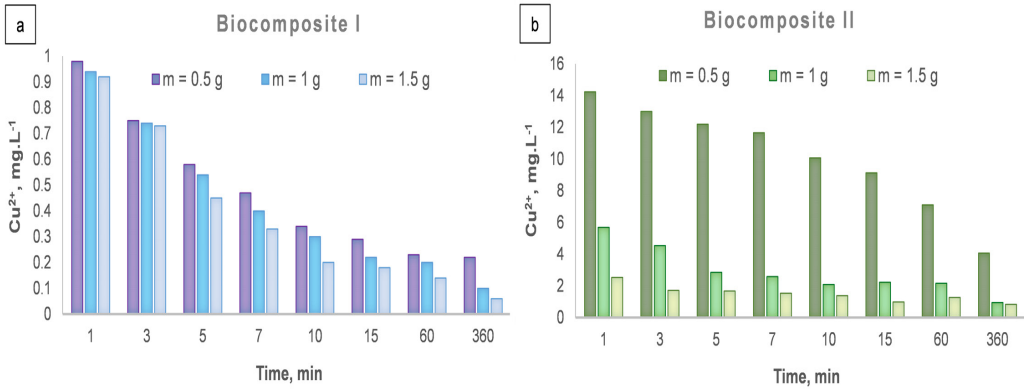

Fig. 2. Influence of the contact time on the copper ions removal at different Composite I (a) and Composite II dose (b).

Figure 2 (a, b) shows that the concentration of copper ions decreased with the time and the biocomposite dose increased toward their initial concentration. For example, for a period of one minute and usage of 0.5 g, 1 g and 1.5 g of Biocomposite I, the concentration of copper ions was reduced to 0.98 $mg \cdot L^{-1}$, 0.92 $mg \cdot L^{-1}$ and 0.94 $mg \cdot L^{-1}$, respectively. Using the same quantities of Biocomposite II and same period of time, it decreased up to 14.24 $mg \cdot L^{-1}$, 5.69 $mg \cdot L^{-1}$ and 2.53 $mg \cdot L^{-1}$, respectively.

The results show that the process of bonding between metal ions and biocomposite material occured immediately after mixing of the composite with the solution of copper ions. After that the process slowed down and at the 360th min the concentrations of Cu^{2+} which were achieved are 0.22 $mg \cdot L^{-1}$, 0.1 $mg \cdot L^{-1}$ and 0.06 $mg \cdot L^{-1}$, using 0.5 g, 1 g and 1.5 g of Biocomposite I and 4.06 $mg \cdot L^{-1}$, 0.94 $mg \cdot L^{-1}$ and 0.83 $mg \cdot L^{-1}$, using the same quantities of Biocomposite II. Fig. 2-b shows that usage of higher dose leads to better results.

A big difference was observed when 0.5 g Biocomposite II and the other two quantities of the same material were used (1 g and 1.5 g).

Based on the results obtained from the analysis, the removal efficiency of Cu^{2+} was calculated (Table 1).

Table 1. Summary of removal efficiency achieved.

Time, min	Removal efficiency, %			
	Dose, g			
	0.1	0.5	1.0	1.5
	Biocomposite I			
1	25.7	98.0	98.1	98.2
360	54.7	99.6	99.8	99.9
	Biocomposite II			
1	9.5	71.5	88.6	94.9
360	35.5	91.9	98.1	98.3

The results show that Biocomposite I was more effective in comparison with Biocomposite II and there was no significant difference when using 1 g or 1.5 g of them. Comparing the removal efficiencies which were achieved it was seen that in both cases a high degree of purification was achieved. Obviously, when greater doses of the composite material were used, better results were obtained. The larger content of emeraldine base in Biocomposite I provided more available free electron pairs on the nitrogen atom structure, where positively charged metal ions can be bonded. Although the percentage content of emeraldine base in Biocomposite II is less than that in Biocomposite I, and the content of plant matter is greater than that in Biocomposite I, respectively, high removal efficiencies were achieved. This means that the biosorbent *P. australis* also contributes to the successful purification process.

Identification of copper ions initial concentration on their removal with biocomposite

From the results obtained it was observed that with increasing of initial copper ions concentration, the removal efficiency of 0.1 g biocomposites was reduced (Fig. 3). The lowest one was observed in the samples containing 30 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$ copper ions. In the first case the removal efficiency was 82.8 %, using Biocomposite I and 58.3 % – using Biocomposite II and in the second case it was 49.4 %, using Biocomposite I and 31.0 % – using Biocomposite II. The higher removal efficiency at lower concentrations can be explained by existence of enough free nitrogen atoms in the polymer chain, which have higher electron density namely those involved in the complexing with copper ions. With increasing of metal ions concentration the possibility of their binding to polymer structure reduces.

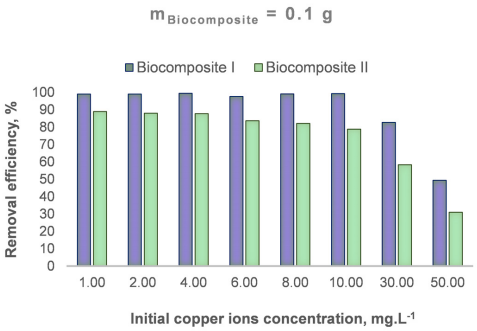

Fig. 3. Comparison between removal efficiency of 0.1 g Biocomposite I and Biocomposite II, using different initial copper ion concentrations.

Because of usage of $CuSO_4.5H_2O$ for standard solutions preparation, the sulphuric ions concentration was measured too. It is well-known that the polyaniline is not suitable for negatively charged ions removal. This fact was confirmed and the results are shown in Figure 4.

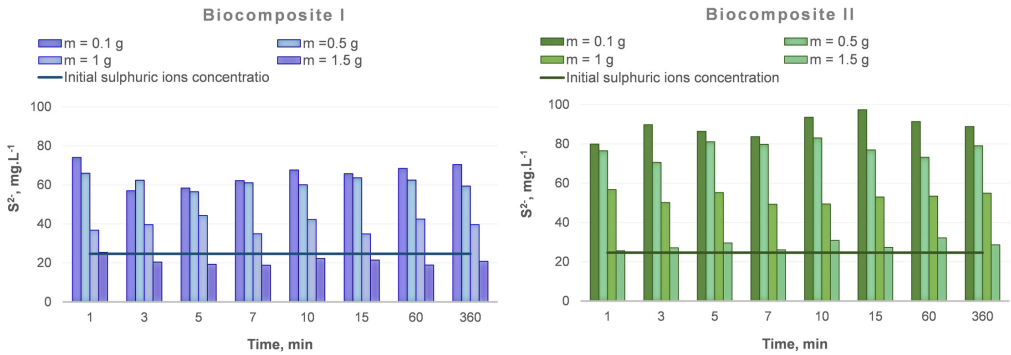

Fig. 4. Influence of the contact time on the concentration of sulphuric ions.

Their concentration was increased from 24.65 mg·L^{-1} to 97.38 mg·L^{-1} with time. Perhaps this increasing is due to the release of this element from *P. australis* joined in the biocomposite. Therefore, there was not observed water purification from sulphuric ions.

The mining industry causes aquatic environment contamination with sulphate ions in high concentrations, which are toxic for aquatic flora, influencing plants' growth and chlorophyll levels (Davies 2007). The animals are also susceptible to these ions and if young animals consume contaminated water they can get chronic diarrhea or in some cases it can cause their death. That is why effective removal of these ions from the wastewater is needed.

Conclusions

The possibility of *Phragmites australis*/Emeraldine base biocomposites to remove copper ions from model aqueous media was studied. For this purpose preparation of biocomposites consisted of emeraldine base and different quantities of *P. australis* was successfully performed by *in situ* polymerization of aniline. It was established that with increasing of the contact time greater removal efficiency was observed using both Biocomposites I and II. Maximum copper ions removal efficiency was achieved using 1.5 g of them for a period of 360 min. For Biocomposite I the obtained efficiency was 99.9 %, and for Biocomposite II – 98.3 %. The results show that Biocomposite I was more effective in comparison with Biocomposite II and there was no significant difference when using 1 g or 1.5 g of them. In the form that were used the synthesized *Phragmites australis*/Emeraldine base biocomposites are not suitable for removal of negatively charged ions, such as sulphuric ones.

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WATER QUALITY STATUS IN SACRED GROVES OF GARHWAL HIMALAYA, INDIA

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Abstract

Clean water is necessary to support the healthy living. Water is one of most important natural resources which supports several aspects of human development. Access to safe clean drinking water has a direct bearing on both quality and prosperity of human life. In Himalaya due to several natural and anthropogenic problems this precious natural resource is facing threat. The present study has attempted to form a water quality index (WQI) of three sacred groves located in Uttarakhand. Sacred grove is an age-old tradition where a forest patch or a water body is dedicated to local deity where cutting plants or killing animals are strictly prohibited, and is supposed to have high hydrological value. Water Quality Index is renowned effective method to express water quality in a simple, stable and reproducible unit of measure which communicates information of water quality to the concerned citizens and policy makers. Also a household survey was carried out in the year of 2015 in order to gather information regarding safe drinking water availability and sanitation. The results show that the water of all sacred groves is safe for different household purposes except for drinking as it needs treatment to make it perfect for this purpose.

Key words: correlation, sacred grove water, water quality index, water chemistry.

Introduction

Water is important in our daily life, used in several sectors like agriculture, industries and households. But worldwide consciously and unconsciously human being pollutes this precious natural resource on which its very existence is dependent. The problem is increasing day by day (Hidaka 2003, European communities 2002). In the last decade extensive decline in inland aquatic system's water quality has been recorded. In the 21st century anthropogenic activities are the topmost source for water quality deterioration. Health of human being is determined by easy availability of clean water. Water is supposed to be both source and support system for human development in every aspect. But today this resource even in Himalaya also faces several threats due to natural as well as anthropogenic issues. Sacred grove is an age-old tradition where a forest patch or a water body is dedicated to local deity and nobody is permitted to cut plants or kill animals or any form of life (Gaikwad et al. 2004). Sacred groves are associated with Sacred Natural Sites (SNS). IUCN defined SNS as ''Natural area of special spiritual significance to people and communities. They include natural areas recognized as sacred by indigenous and traditional people and institutionalized religions or faiths as places

for worship and remembrance" (Ray et al. 2008). Sacred groves are well preserved forests and have high hydrological value. Some workers define sacred grove as a typical of small scale societies which are largely practicing subsistence economies (Gokhale et al. 1998, Gadgil and Berkes 1991). Gokhale (1998) mentioned sacred groves are self-organized conservation systems. Several studies have confirmed their watershed value which is accompanied by presence of perennial streams and springs. As sacred grove is an intact forest and covered with litter and humus, therefore, it is supposed to have better water infiltration capacity than grassland, scrub and rocky areas (Ramachandra et al. 2007). In Garhwal Himalaya exists a rich tradition to conserve the nature by socio-religious constraints on sustainable use of common property resources (Anthwal et al. 2006). A study has been undertaken to find out the water quality status in different sacred groves located in Uttarakhnd. Also one Water Quality Index is prepared for easy understanding of its quality status in those places.

Materials and Method

Study area

The present study has been conducted in three sacred groves of Uttarakhand viz. Tarkeshwar sacred grove, Hariyali devi sacred grove and Ravigaon sacred grove (Fig. 1). Tarkeshwar sacred grove is situated in Pauri district (N29°50′30″ to E78°47′31.6″, elevation 1779 m – 1834 m above MSL). The sacred grove is covering 20 ha area and has social catchment of about 22 villages. The sacred grove is dedicated to Lord Shiva and conserved by local village community. Hariyali devi is situated at the boundary of Rudraprayag and Chamoli districts (N30°14′44.9″ to E79°02′12.6″, elevation 2861 m – 2377 m above MSL). The grove is dedicated to Goddess Hariyali mata. It is covering an area of 205.5 ha. Here about 25 ha out of total 205.5 ha area is considered as the residence of deity. Therefore, a very strict community based protection is observed in this sacred grove. It has a social

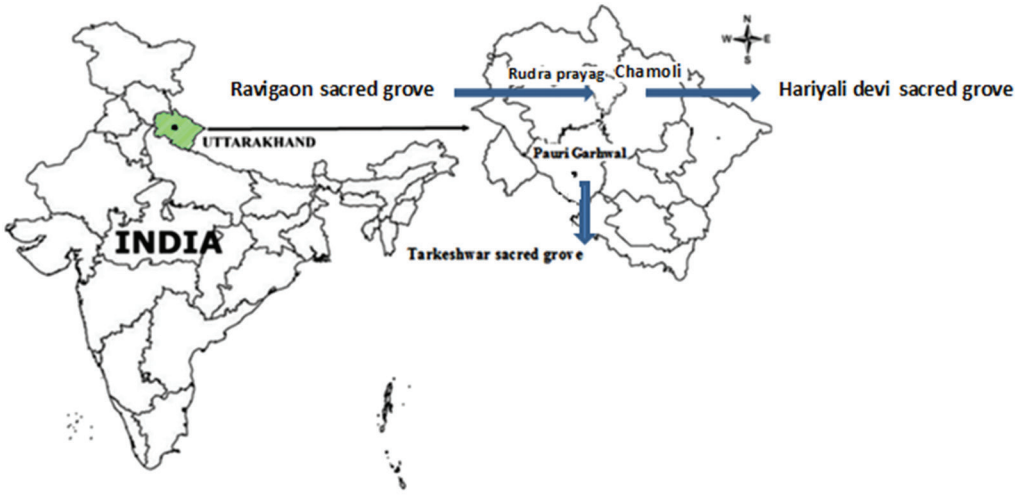

Fig. 1. Map of study area showing sample collection sites.

catchment of about 19 villages. Ravigaon sacred grove is situated in Rudraprayag district (N30°34′15.7″ to E79°02′43.9″, elevation 1727 m – 1834 m above MSL). Ravigaon covers an area of about 3.5 ha with a social catchment of about 20 villages. It is dedicated to Lord Triyugnarayan. All the three sacred groves are reserve forests but controlled mainly by village communities or temple authority.

Sampling and data analysis

Sampling was done during 2015. The year was divided into 3 seasons viz. Pre-monsoon (March–June), Monsoon (July–October) and Post-monsoon (November to February). Water samples were collected from 3 randomly selected stream spots of 3 sacred groves (Tarkeshwar, Hariyali devi and Ravigaon – figures 2–4) and their adjacent areas during the three seasons of 2015.

Samples were stored in 1 L plastic bottles; for dissolved oxygen (DO) a 300 ml BOD bottle (a glass bottle with a 'turtleneck' and a ground glass stopper) was used. The samples were analysed following the standard methodology of APHA (2005) for their physico-chemical quality i.e. pH, total hardness, calcium hardness, total alkalinity, DO, total dissolved solids

Fig. 2. Views from Tarkeshwar sacred groves: a) Tarkeshwar Temple; b) Temple surrounded forest (photos by Purna Jana).

Fig. 3. View from Hariyali devi sacred grove (photos by Purna Jana).

Fig. 4. Views from Ravigaon sacred grove (photos by Purna Jana).

(TDS), nitrate and chloride. The methods for each parameter have been described below.

(a) pH

Method/Procedure: by using Hanna (USA) combined pH and ORP meter.

(b) Total dissolved solids (TDS)

Method: Gravimetrical method.

Procedure:

1. Take 100 ml of sample water into a pre-weighed clean, dry porcelain dish.

2. Evaporate the water in the dish to dryness over the water bath or hot air oven.

3. Remove the moisture, cool and weigh.

4. The difference between the weights is the weight of total soluble salts.

Calculation: TDS (mg/L) = $(B–A)/100 \cdot 10^6$, where: A = weight of empty porcelain dish in grams; B = weight of porcelain dish with residue; 100 = volume of sample taken.

(c) Dissolved oxygen (DO)

Method: Winkler method.

Reagent required:

1. Manganous sulfate solution: Dissolve 364 g $MnSO_4 \cdot H_2O$ in distilled water, filter through a 1.0 µm glass-fiber filter, and dilute to 1 L.

2. Alkali-iodide solution: Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or 150 g KI) in distilled water and dilute to 1 L.

3. Concentrated sulphuric acid.

4. Starch: Dissolve 0.5 g of starch powder in de-ionized water. Add this paste into 100 ml of boiling water. Allow to boil for few minutes. Cool and then use.

5. Standard sodium thio-sulphate (0.1 N): Dissolve 24.82 g $Na_2S_2O_3 \cdot 5H_2O$ in 1000 ml distilled water. Preserve by adding 5 ml of chloroform per litre.

Procedure:

1. Collect water sample in a BOD bottle using DO sampler.

2. Add 1 ml of manganous sulphate followed by 1 ml of alkaline iodide reagent immediately after sample collection.

3. The tip of the pipette should be below the liquid level while adding these reagents, and close with the stopper immediately.

4. Mix well by inverting the bottle 4–5 times and allow the precipitate to settle down, leaving 150 ml clear supernatant.

5. Then add 1 ml concentrated sulphuric acid. Mix well till precipitate goes into solution.

6. Transfer 25 ml of sample into a clean conical flask and add starch indication, then titrate against standard sodium

thio-sulphate.

7. Disappearance of blue colour is the end point.

Calculation: DO (mg/L) in sample = $(N \cdot E \cdot 1000 \cdot V_t)/V_2 \cdot (V_1 - V/V_1)$, where: N = Normality of sodium thio-sulphate used; E = equivalent weight of oxygen = 8; V_t = volume of sodium thio-sulphate used for titration; V_1 = volume of sample bottle after placing the stopper; V_2 = volume of the part of the contents titrated; V = volume of $MnSO_4$ and KI added.

(d) Total alkalinity (TA)

Reagents:

1. Standard sulphuric acid (0.02 N): Prepare 0.1 N H_2SO_4 by diluting 3 ml of concentrated H_2SO_4 to 1000 ml, Standardize against standard Na_2CO_3 0.1 N. Dilute appropriate volume of H_2SO_4 (approximately 0.1) to 1000 ml to obtain standard 0.02 N H_2SO_4.

2. Phenolphthalein indicator: Dissolve 0.5 g phenolphthalein in 1:1 95 % ethanol and distilled water. Add drop wise 0.02 N NaOH till faint pink colour appears.

3. Methyl orange indicator: Dissolve 0.5 g of methyl orange in 1000 ml CO_2 free distilled water.

Procedure:

1. Measure the suitable volume of sample in 250 ml conical flask.

2. Add 2–3 drops of phenolphthalein indicator.

3. If the pink colour develops titrate against 0.02 N H_2SO_4, till the colour disappears, which is the characteristic of pH 8.3. Note down the volume of H_2SO_4 consumed.

4. Add 2–3 drops of methyl orange and continue titration with H_2SO_4 till the yellow colour changes to orange, which is the characteristic of pH 4.5. Note down the additional amount of H_2SO_4 required.

5. In case pink colour does not appear after addition of phenolphthalein continue with adding methyl orange.

Calculation:

Phenolphthalein Alkalinity (mg/L as $CaCO_3$) = [($A \cdot N$) of $H_2SO_4 \cdot 1000 \cdot 50$]/ml of sample Total alkalinity (mg/L as $CaCO_3$) = [($B \cdot N$) of $H_2SO_4 \cdot 1000 \cdot 50$]/ml of sample, where: A = ml of H_2SO_4 required to raise pH up to 8.3; B = ml of H_2SO_4 required to raise pH up to 4.5; N = Normality of H_2SO_4.

(e) Total hardness (TH)

Method: EDTA titrimetric.

Reagents:

1. Standard EDTA indicator: (0.01 M) Weigh 3.723 g of EDTA and dissolve in 1000 ml of distilled water and standardize against standard calcium solution.

2. EBT Indicator: The powdery form of the Erichrome Black-T can be used for this purpose.

Procedure:

1. Take 25 ml of the sample (dilute it with distilled water if required) and add 1–2 ml of the buffer solution and 1–2 g of EBT indicator.

2. Then titrate against the EDTA, with continuous stirring; until the last reddish tinge disappears. At the end point the solution gives blue colour.

Calculation: Total hardness (EDTA) as mg $CaCO_3$/L = $A \cdot B \cdot 1000 \cdot$Dilution factor (if diluted) ml of sample, where: A = ml titrant required and B = mg $CaCO_3$ equivalent to 1 ml EDTA titrant.

(f) Calcium hardness (Ca-H)

Method: EDTA titrimetric.

Reagents:

1. Sodium hydroxide: (NaOH) 1 Normality.

2. Indicator: Murexide (Ammonium purpurate).

3. Standard EDTA indicator: (0.01 M) Weigh 3.723 g of EDTA and dissolve in 1000 ml of distilled water and standardise against standard calcium solution.

Procedure:

Sample preparation: Titrate immediately after adding alkali and indicator. Use 50 ml sample or a smaller portion diluted to 50 ml, so that the calcium content is about 5–10 mg. Analyse hard water with alkalinity higher than 300 mg $CaCO_3$/L by taking the smaller portion and diluting to 50 ml, or by neutralizing the alkalinity with acid, boiling 1 min and cooling before beginning the titration.

Titration:

1. Add 2 ml NaOH solution per volume sufficient to produce pH 12–13.
2. Stir well and add a pinch of indicator.
3. Add EDTA titrant slowly, with continuous stirring to the proper end point (pink to purple).
4. Run the blank along with the sample.

Calculation: Calcium hardness as $CaCO_3$ L^{-1} = $A \cdot B \cdot 1000 \cdot$Dilution factor (If diluted) ml of sample taken, where: A = ml titrant for sample and B = mg $CaCO_3$ equivalent to 1.0 ml EDTA titrant.

(g) Chlorides (Cl)

Method: Argentometric.

Reagents:

1. Potassium chromate indicator: Add 50 g K_2CrO_4 in little amount of water and dilute to 1000 ml.
2. Standard Silver nitrate (0.0141 N): Dilute 70.5 ml of 0.1 N $AgNO_3$ in distilled water to 500 mL (1 ml = 0.5 mg Cl = 500 µg Cl). Standardize $AgNO_3$ against standard NaCl.
3. Standard Sodium Chloride (0.0141 N): Dissolve 824.0 mg NaCl (dried at 140°C) and dilute to 1000 ml (1 ml = 0.5 mg Cl = 500 µg Cl).

Procedure:

1. Use a 50 ml of sample or a suitable portion diluted to 50 ml.
2. Directly titrate samples in the pH range 7–10.
3. Adjust sample pH to 7 to 10 with H_2SO_4 or NaOH if it is not in this range.
4. Add 2 ml of K_2CrO_4 indicator solution.
5. Titrate with standard $AgNO_3$ titrant to a pinkish yellow colour end point.
6. For better accuracy titrate distilled water (50 ml) in the same way to establish reagent blank.

Calculation: Chlorides mg/L = $(A–B) \cdot N \cdot 35.45 \cdot 1000$ ml sample, where: A = ml $AgNO_3$ required for sample; B = ml $AgNO_3$ required for blank; N = Normality of $AgNO_3$ used.

(h) Nitrates (NO_3^{-1})

Method: Phenol disulfonic acid method.

Reagents:

1. Phenol disulfonic acid: Dissolve 25 gm of white pure phenol in 150 ml of concentrated H_2SO_4 and add 75 ml of fuming H_2SO_4. Heat for two hours on a water bath and keep in a dark bottle. In place of fuming acid 85 ml of concentrated H_2SO_4 can also be added.
2. Silver sulphate solution: dissolve 4.4 g of Ag_2SO_4 in distilled water to prepare 1 L of solution.
3. Liquid ammonia 30 %.
4. Standard nitrate solution (1 mg N/L): 0.72183 g of KNO_3 in distilled water and make up the volume to 1 L. This solution contains 100 mg N/L. Dilute it to 100 times to prepare a solution having 1 mg N/L (10→1000 ml).

Procedure:

1. Take 50 ml of sample (filter if necessary), evaporate the sample in a porcelain basin to dryness.
2. Cool and dissolve the residue in 2 ml phenol disulfonic acid and dilute the contents to 50 ml.
3. Add 6 ml of liquid ammonia to develop a yellow colour.

4. Take the reading at 410 nm.

5. Calculate the concentration of nitrate from the standard curve.

6. Prepare the standard curve between concentration and absorbance from 0.0 mg N/L to 1.0 mg N/L at the interval of 0.1. Find absorbance of the standard solution using the same procedure described for the sample.

Water Quality Index has been formed based on Harkins (1974) and Pandey et al. (2014) methodology. It is well known and effective method to express water quality in a simple, stable and reproducible unit of measure which communicates information on water quality to the concerned citizens and policy makers.

Water Quality Index (*WQI*)

$$WQI = \sum q_i \cdot W_i \ ,$$

where $q_i = \frac{100.(V_a - V_i)}{(V_s - V_i)}$ is water quality rating, when V_a = actual value present in the water sample, V_i = ideal value (0 for all parameters except dissolved oxygen which is 14.6 mg/L), and V_s = standard value; —, $K = \frac{1}{\sum_{i=1}^{n} \frac{1}{S_i}}$, where K is constant, S_i = permissible limit for the i^{th} parameters, and n = number of standard value.

Data on water availability (*L*) per household per day, distance travelled to fetch the daily needed water, etc. were collected following PRA method (household questionnaire survey and group discussion) by visiting 18 villages reside surrounding the Tarkeshwar sacred grove area, 18 villages reside surrounding the Hariyali devi sacred grove and 18 villages reside surrounding the Ravigaon sacred grove. For statistical analysis MS Excel has been used.

Result and Discussion

Result

Mean water quality data of three sacred groves in different seasons of 2015

All the water quality parameters (pH, DO, hardness, alkalinity, nitrate, chloride, etc.) remain within standard limits of World Health Organization (1984, 2011) (Tables 1 and 2) except in case of DO (the amount was lower than the WHO limit at Hariyali devi and its adjacent area during pre-monsoon season), pH (higher at Tarkeshwar adjacent area during monsoon) and calcium hardness (concentration was higher at Tarkeshwar and its adjacent area during post-monsoon season).

Table 2. Permissible limit list by WHO for water quality parameters.

Parameter	WHO Standard (2011)
pH	6.5 to 8.5
Dissolved Oxygen, mg/L	5
Total Hardness, mg/L	500
Calcium-Hardness, mg/L	75
Total Dissolved Solids, mg/L	500
Chloride, mg/L	200
Total alkalinity, mg/L	200
Nitrate, mg/L	50

Note: WHO – World Health Organization.

WQI of three sacred groves by using specific physico-chemical parameters

As per the formed WQI the quality of water has then been compared with the table given by Tyagi et al. (2013). According to the index during pre-monsoon, monsoon and post-monsoon its value in all spots was <50 except at Tarkeshawar sacred grove during monsoon. The quality was good (<50) during pre-monsoon and the grade of water was B according to comparison table of Tyagi (Table 3). But during

Table 1. Mean water quality data.

Parameters	Season	Sampling site					
		TSG	TAD	HSG	HAD	RSG	RAD
		mg/L (Average ±SD)					
DO	1	9.73±0.25	9.27±0.97	4.83±0.91	3.61±0.24	8.51±0.21	8.10±0.00
	2	5.81±0.62	7.19±0.17	8.59±1.01	8.47±0.15	7.92±0.32	8.07±0.12
	3	11.29±0.83	11.71±1.04	8.17±0.06	8.13±0.23	8.33±0.58	9.00±0.00
pH	1	7.69±0.72	8.47±0.15	7.79±0.44	7.71±0.36	7.74±0.29	7.95±0.26
	2	7.68±0.57	8.58±0.11	7.93±0.51	7.82±0.24	7.70±0.10	7.96±0.30
	3	8.33±0.09	8.45±0.08	7.97±0.52	7.87±0.35	7.93±0.58	8.16±0.14
Cl	1	4.99±1.00	3.66±0.58	1.22±0.69	1.78±0.39	6.33±3.59	5.32±1.16
	2	2.83±1.89	2.16±0.76	1.70±0.61	1.55±0.69	2.38±0.58	1.71±0.24
	3	2.99±0.00	2.67±0.58	2.44±0.49	2.71±0.41	2.44±0.49	2.16±0.17
TH	1	71.22±52.03	95.67±8.50	32.42±21.73	48.99±32.34	42.67±6.03	37.00±1.00
	2	112.44±102.33	103.33±3.06	27.33±6.81	55.33±15.95	37.11±6.00	29.00±2.00
	3	156±100.41	124.11±18.55	36.33±2.52	60.33±18.58	43.78±5.01	36.33±2.96
Ca-H	1	42.33±24.38	70.33±2.08	17.98±11.56	22.99±15.30	23.33±3.51	20.00±2.00
	2	72.67±63.45	69.67±19.35	17.32±2.68	35.00±1.73	18.22±3.02	16.05±1.13
	3	91.5±68.59	93±24.33	18.67±2.52	36.67±4.04	25.11±2.01	17.56±2.04
NO_3^{-1}	1	0.08±0.02	0.09±0.015	0.85±0.085	0.80±0.06	0.59±0.41	0.13±0.005
	2	0.14±0.02	0.095±0.06	0.112±0.06	0.08±0.01	0.106±0.06	0.09±0.03
	3	0.06±0.001	0.08±0.008	0.12±0.07	0.08±0.02	0.05±0.03	0.07±0.017
TA	1	24.78±11.31	23.017±0.015	6.107±5.13	9.10±5.00	9.67±2.31	6.33±0.58
	2	12.67±6.43	14.07±4.31	11.33±0.33	19.23±2.01	9.22±2.16	8.83±1.61
	3	28.00±5.66	34.00±7.21	8.00±1.00	14.67±1.53	9.53±1.73	7.25±1.00
TDS	1	123±39.66	182.33±22.74	67.67±76.69	132.67±0.36	125.23±25.92	102.33±15.28
	2	276.67±75.88	275.00±31.22	117.33±12.58	130.67±17.56	132.67±50.52	158.33±36.89
	3	233.50±133.64	145.33±16.50	114.00±5.00	129.67±9.02	80.33±1.53	143.00±27.73

Note: TSG – Tarkeshwar sacred grove, TAD – Tarkeshwar adjacent area, HSG – Hariyali devi sacred grove, HAD – Hariyali devi adjacent area, RSG – Ravigaon sacred grove, RAD – Ravigaon adjacent area, SD – Standard deviation; Seasons: 1 – Pre-monsoon, 2 – Monsoon, 3 – Post-monsoon.

Table 4. WQI of three sacred groves by using specific physico-chemical parameters.

SA	Season	Parameters								WQI	Status*
		DO	Cl	TH	NO_3^{-1}	TDS	TA	Ca-H	pH		
TSG	1	12.53	0.02	0.04	0.00	6.08	0.08	0.35	14.20	33.29	Good
	2	22.63	0.01	0.06	0.01	13.68	0.04	0.60	14.19	51.20	Poor/Mod. poll
	3	8.52	0.01	0.08	0.00	11.54	0.09	0.75	15.38	36.37	Good
TAD	1	13.73	0.01	0.05	0.00	9.01	0.07	0.58	15.64	39.10	Good
	2	19.06	0.01	0.05	0.00	13.60	0.04	0.57	15.85	49.19	Good
	3	7.44	0.01	0.06	0.00	7.19	0.11	0.76	15.61	31.18	Good
HSG	1	25.15	0.00	0.02	0.04	3.35	0.02	0.15	14.39	43.12	Good
	2	15.48	0.01	0.01	0.01	5.80	0.04	0.14	14.65	36.14	Good
	3	16.57	0.01	0.02	0.01	5.64	0.02	0.15	14.73	37.14	Good
HAD	1	28.31	0.01	0.02	0.04	6.56	0.03	0.19	14.24	49.39	Good
	2	15.78	0.00	0.03	0.00	6.46	0.06	0.29	14.45	37.07	Good
	3	16.65	0.01	0.03	0.00	6.41	0.05	0.30	14.53	37.98	Good
RSG	1	15.68	0.02	0.02	0.03	6.19	0.03	0.19	14.30	36.46	Good
	2	17.19	0.01	0.02	0.01	6.56	0.03	0.15	14.22	38.18	Good
	3	16.14	0.01	0.02	0.00	3.97	0.03	0.21	14.65	35.03	Good
RAD	1	16.74	0.02	0.02	0.01	5.06	0.02	0.16	14.68	36.71	Good
	2	16.82	0.01	0.01	0.00	7.83	0.03	0.13	14.71	39.55	Good
	3	14.42	0.01	0.02	0.00	7.07	0.02	0.14	15.08	36.76	Good

Note: SA – Sampling area, TSG – Tarkeshwar sacred grove, TAD – Tarkeshwar adjacent area, HSG – Hariyali devi sacred grove, HAD – Hariyali devi adjacent area, RSG – Ravigaon sacred grove, RAD – Ravigaon adjacent area; Seasons: 1 – Pre-monsoon, 2 – Monsoon, 3 – Post-monsoon; Status* – Status as per Tyagi et al. (2013).

monsoon, at Tarkeshwar sacred grove the water quality deteriorated slightly (>50) (Tables 3 and 4).

Table 3. Standard rating of water quality as per Weight Arithmetic WQI method (Tyagi et al. 2013).

WQI scale	Water quality rating (WQR)	Grading
0–25	Excellent water quality	A
26–50	Good water quality	B
51–75	Poor water quality	C
76–100	Very poor water quality	D
>100	Unsuitable water quality	E

Dependency of villagers on water from various sources and distance travelled to collect water

Dependency on natural water source was higher in Hariyali devi sacred grove (64.03 %) than in the other two ones. In Tarkeshwar sacred grove area water scarcity was severe than in the other two places. In Tarkeshwar sacred grove average distance travelled by villagers to fetch daily needed water was 0.5 km which increased during lean season and become 3–4 km in order to get daily needed water (Table 5).

Table 5. Status of household based on water availability.

A	B	C	D	E	F
Tarkeshwar	28.70	73.15	13.89	0.9 (max 3–4 km)	0.32
Hariyali devi	64.03	35.97	0.00	0.6 (max 1 km)	0.58
Ravigaon	26.89	73.10	0.00	0.3 (max 1 km)	0.26

Note: A – Name of Sacred grove, B – Percentage dependency on natural source of water, C – Percentage dependency on Government supply, D – Percentage dependency on tube-well, E – Average distance travelled for water collection during lean season (km), F – Average distance travelled for water collection during other seasons (km).

Correlation coefficient values of various physico-chemical parameters of streams from both sacred groves and their adjacent areas:

Karl Pearson correlation (*r*-values) estimated to quantify the relationship between various physical and chemical parameters which revealed that alkalinity was positively correlated with calcium. Hardness showed significant correlations with pH, calcium and DO. pH showed positive correlation with alkalinity, chloride, TDS, DO. Alkalinity showed correlation with DO. DO showed negative correlation with nitrate (Table 6).

Table 6. Correlation coefficient values (*r*-values) of physico-chemical parameters of streams from three sacred groves and their adjacent areas.

	DO	Cl	TH	Ca-H	NO_3^{-1}	TA	TDS	pH
DO	1							
Cl	0.306*	1						
TH	0.345*	0.103	1					
Ca-H	0.368#	0.085	0.966#	1				
NO_3^{-1}	-0.506#	0.103	-0.162	-0.203	1			
TA	0.599#	0.182	0.700#	0.759#	-0.26	1		
TDS	0.14	0.114	0.665#	0.653#	-0.182	0.384#	1	
pH	0.553#	0.208	0.356#	0.339*	0.026	0.347*	0.393#	1

Note: Level of significance *p = 0.05, #p = 0.01 (Pearson's rank correlation).

Discussion

Almost all water quality parameters at three sacred groves during all three seasons were within permissible limit as per WHO (1984, 2011) except DO (lower at Hariyali devi sacred grove and its adjacent area during pre-monsoon season), pH (shows alkaline pH all over but higher at Tarkeshwar adjacent area during monsoon) and calcium hardness (higher at Tarkeshwar and its adjacent area during post-monsoon season). Kumar et al. (2010) and Madan et al. (2013) have found that the rivers of Uttarakhand show alkaline pH. According to WQI the quality of water at Tarkeshwar was good during pre-monsoon but it gets deteriorated during monsoon. During monsoon several festivals, rituals, ceremonies take place in Tarkeshwar sacred grove to please Lord Shiva. For this reason a number of pilgrims come to visit it. Most of them are somehow related to this grove by birth but migrated long time ago. During these festivals lots of waste related to rituals, festivals are generated which is discarded directly to the water body. That is the reason of water quality deterioration during monsoon but the quality is revived during post-monsoon and turned into good one. Madan et al. (2013) also mentioned that water body plays central role in lots of religious activities all through year in India, even rivers of Uttarakhand in this regards are not untouched. Central Pollution Control Board (2005) also recorded and stated several hazardous effects on aquatic life due to religious and cultural activities. Therefore, during monsoon the water needs treatment before consumption. Apart from Tarkeshwar sacred grove the other two sacred groves have good water quality during all three seasons.

The regression equation with significant correlation coefficients is well-thought-out to be very suitable to estimate the concentration of other constituents' availability. Significant correlation was shown by alkalinity with calcium which revealed that the ground water is alkaline in nature chiefly due to the presence of calcium salts. Rao et al. (2011) also found alkalinity having significant correlation with calcium. It also showed positive correlation with DO. This result is supported by Khatoon et al. (2013). pH is positively correlated with alkalinity and TDS. Khatoon et al. (2013) also recorded positive correlation of pH and total alkalinity. Ishaq and Khan (2013) recorded positive correlation between pH and TDS. pH showed positive correlation with hardness and dissolved solids. Bhandari and Nayal (2008) also recorded positive correlation between pH and hardness. Matta and Kumar (2015) recorded positive correlation between pH and dissolved solid. Chloride is positively correlated with pH which is supported by Bhandari and Nayal (2008). Hardness showed positive correlation with DO. Matta and Kumar (2015) also recorded similar result. Hardness showed positive correlation with calcium. This suggested that total hardness of water samples is mainly due to the presence of calcium salt. DO is negatively correlated with nitrate. Khatoon et al. (2013) also found negative correlation of DO and nitrate.

Based on availability of water daily life also gets changed. Higher dependency on water source enhances the chance of water body to get polluted (Anonymous 2014, 2017). Water availability has effect on lavatory system. For example, each household of Tarkeshwar sacred grove area possesses lavatory facility but due to non-availability of water it has become store room and the villagers use open defecation. This is a real concerned issue

and a matter of hygiene too for this area which has also adverse effect on local surface water as in most cases surface water is used in daily domestic use.

Conclusion

In all the three sacred groves water quality was good according to Water Quality Index. The overall water quality in all three sacred groves was safe for use in daily life except for drinking purpose; there is a need for treatment. In Uttarakhand during summer prominent water scarcity condition is prevailed although this state is the origin of several main rivers of India. In these sacred grove areas, particularly during pre-monsoon season (summer), also acute water scarcity is felt. However, due to presence of this sacred grove, though the supply is meagre but the villagers never run out of daily needed water supply which is also good in quality. Proper care should be taken to manage the sacred grove which can maintain further the water quality and quantity, flowing through it. To save the water of sacred grove strong rules must be implemented by government or local management committee during festivals. At this time people come from different parts of country but simply used to forget all the issues arise during post festival duration and keep on polluting the local water by simply throwing all the waste generated during the festival time. Even to support the huge amount of pilgrims during festivals lots of construction works are also undertaken. These activities also keep on polluting the sacred grove environment. Even the transport through which the pilgrims come to visit the place pollute the local atmosphere. Awareness is needed to protect the sacred grove. Therefore input of government, some NGOs or even local management committee is strongly recommended. In this regards some awareness programmes must be organized to save the sacred grove ecosystem.

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PRINCIPLES FOR THE FORMATION OF SPACES ALONG WALKING TRAILS IN FOREST LANDSCAPES

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Abstract

Some conditions and opportunities for improving the aesthetic qualities of forest landscapes and strengthen their visual effect are the focus of discussion in this paper. The panoramic views and attractive landscape fragments and landscape paintings are often main focus in the construction of trails for hiking, cycling, mountain biking and horse riding. Most of them use the existing pedestrian routes that do not always provide landscape attractiveness. The aim of study is to define principles for the formation of spaces along the tourist trails considering to increase the emotional influence of forest landscapes. The result of the analysis shows the great importance of vegetation as a primary component of the visual space and the importance of ably trails tracing for the visual perception of forest landscapes. The article discusses the application of the principles of landscape architecture for environmental friendliness, logical paths, optimal visual quality and landscape influence on tourists. Particular attention was paid to the role of eco-trails as a prerequisite for creating unique landscape attractions, enhance cognitive nature of outdoor recreation and suggesting respect to nature by tourists.

Key words: forest landscapes, panoramic views, pedestrian routes, perceptions, recreation, visual quality.

Introduction

The main objective of this study is to define some principles of forest landscape design the implementation of which would improve aesthetic qualities and visual attractiveness of forest landscapes. Although it is not possible to choose absolutely objective measure of aesthetic assessment, there are many theoretical formulations for indicators and criteria for this. The article has attempted a pragmatic approach on forest landscape design. The research objects are selected from different parts of Bulgaria, and are preferred territories with high tourist potential. It is very well known that most tourists gather and keep their attention for a long time in places with long panoramic views towards vast territories. This usually happens at these points of the walking trails where they pass through ridges, protruding parts of the slopes or edges of rock slopes, and due to the sharp displacement, a wide view is revealed to areas located away from the observer. In almost all other cases, tree vegetation in forest landscapes obstructs visibility and tourists see only nearby. In these cases, the small detail in the landscape is important – it could be a tree or a rock piece, a mountain stream, etc. In this logic, the present study

focuses on relief and tree vegetation as the most significant factors in generating visual landscape attractiveness. The use of these two factors is at the heart of the general principles defined in the end of the present study. Therefore, the methods of natural survey and landscape inventory have been used jointly in this study.

Material and Methods

Open spaces along walking trails provide best opportunities for visual perceptions of tourists and modeling of forest landscapes. Therefore, the field of surveillance is the most spacious and gives a good choice of perspective. Since each level of monitoring we have a different horizon and a different distance to viewable objects. This determines as important different plans in space examined in great detail in Bell (2004).

It is very difficult to summarize and systematize all scientific views on the issue of forest landscape perceptions and especially of scenic beauty.

Several models of scenic quality have been researched and investigated (e.g., Gurkova 2016, Stoycheva 2016). They are based on combining parts of reconstructive methods, park perspective, spatial composition and painting techniques of imagery. They can make design process much easier and the resulting landscape “picture” – applicable to planning process, respective to forest landscape design. Simple and effective guidelines for forest landscape design are published in Kosarevskiy (1977). Although visual complexity and aesthetic quality are stretchable concepts, the whole picture of a perfect landscape comes from the well-thought out and successful park design drawing and is responsible for the subsequent realization in real space. That is why the landscape planning itself, as a pictorial space, is essential for the panoramic views recovery. The theoretical considerations regarding problems studied in this paper was influenced by similar publications in ecology, especially in Anonimous (2000) and in van Oosten et al. (2014), which proposed that increasing ecological diversity is correlated with increasing stability, and hence with environmental quality. Other researchers (Forestry Commission 2011, Watts and Tolland 2005) have explicitly linked these two ideas, proposing that ecologically stable, i.e., healthy, landscapes are intrinsically beautiful. This paper takes into account scenic quality aspects that have been examined so far and tries to implement them in landscape design.

It is always difficult and very relative to postulate the principles of aesthetic qualities of landscapes and, in particular, forest landscapes. It is even more difficult to typify human perceptions of landscape attractiveness. Whether or not, research is being done in this direction by Lovell and Johnston (2009), SAEFL (2003) and many others.

Results and Discussion

It is very difficult to summarize all visual situations that predispose to stop the movement and static observation. Given previous research on the subject we can subtract some general principles or requirements for forest landscape design along walking trails. These requirements generally relate to the design decision of volume-spatial composition of the vegetation and to a less extent – to the formation of trails.

As a general rule may be indicated

the necessity of compliance of the design solution with natural conditions. Accordance with the most significant landscape components (relief, rock formations, lakes, rivers, streams, vegetation, etc.) always is a leading factor that ensures an adequate design solution.

Complying a rate in accent saturation is very important aesthetic rule in forest landscape design. Each recreational environment requires a correct accent dosage. This is particularly important in forest landscapes, to preserve their natural appearance and charm.

Another important condition for a positive artistic and emotional effect in forest environment is the proper proportioning of main highlights.

Regarding the planned structure of the trails on the first place we must be careful that they should not be like axes of symmetry in open spaces. Viewed in plan, they have to pass closer to a periphery of meadows and forest's border and not in the middle of the open spaces.

The peripheries of forest massifs and tree groups themselves have to be designed by schemes that provide dynamic stirred outlines. Global indentation in the shape of the periphery of forest massifs provides comfortable seating "boxes" for the deployment of recreational facilities. Small finely indentation of forest edge knurling in turn gives them a natural environmental friendly appearance.

In terms of landscape diversity and attractiveness it is very desirable for tourist trails to pass through glades, meadows and other open spaces. When tourists come out of closed spaces in wood massifs and enter in the open meadows and lawns, trails would be traced in a way that allow a full overview of landscape "paintings" into smaller lawns or provide a smooth and gradual change of nature scenic views during the transition from one to another part of the larger lawns. For larger meadows it is normal to create internal secondary paths inside them, too. They allow tourists a continuous stay in open sunny space and support a dynamic rotation of sights and assist for static observation of attractive landscapes. Generally these internal trails have to follow the peripheral parts of the lawn as occasionally may enter through the trees in the surrounding forest stands and again go to the open spaces forming a closed circular route. With a view to preserve the integrity of grasslands, it is not appropriate for these lanes to cross the lawn in the middle unless there is a special compositional design goal. The main route is not suitable to traverse through the middle of the open spaces and should not be an axis of symmetry in them. Within each open space it would be traced so as to reveal merits of the landscape to tourists.

A right balance between open and closed spaces creates valuable and attractive dynamics of volume-spatial composition and creates the expression of views (Fig. 1). The massifs of trees and shrubs build peculiar green "walls" that limit the open spaces and separate them to close-up (30–100 m) and deep or distant (300–400 m) perspective views.

A great pursuit of every designer is creating multilayered landscape views. These views are biggest "prize" for tourists after many time transitions. The creation and maintenance need proper stewardship of forest vegetation, which provokes the eyes to focus but also close the beautiful panoramic views.

When settling forest landscapes as recreation environment it is very important to position correctly points of accents. The example in Figure 2 shows properly defined place in the bend of a river as a

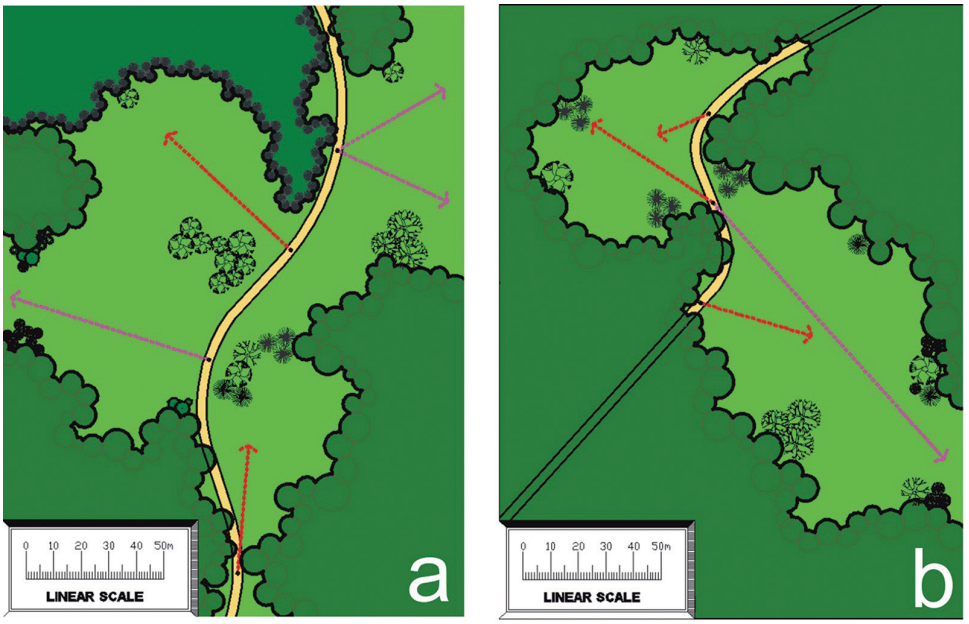

Fig. 1. Dynamics of perception of landscape views: a – slow alternation of close and deep perspective views; b – quick attention "switching" from close to deep perspective views.

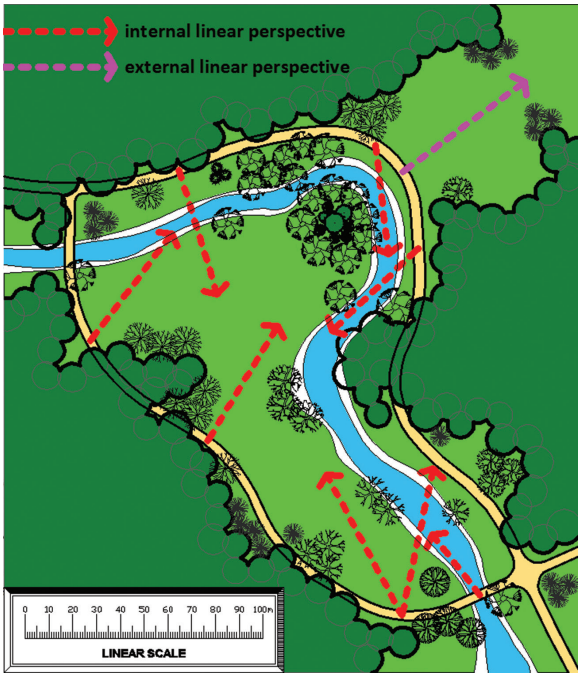

Fig. 2. Landscape design project of a space along a walking trail parallel to a stream.

focus on composition. There are several secondary accent groups of trees and several internal linear perspectives along the promenade are also designed.

These favourable aesthetic effects are also achieved in the spatial design of the coastal promenade in Figure 3. There are even deep and external linear perspectives to the opposite shores of the water area.

Among all landscape components, woody vegetation plays to greatest extent the strongest role in shaping the visual perspective views. These views are internal when the visibility is within the meadow or external when the visibility goes beyond the open space where the observer is.

Figure 4 illustrates how the vegetation determines the depth of

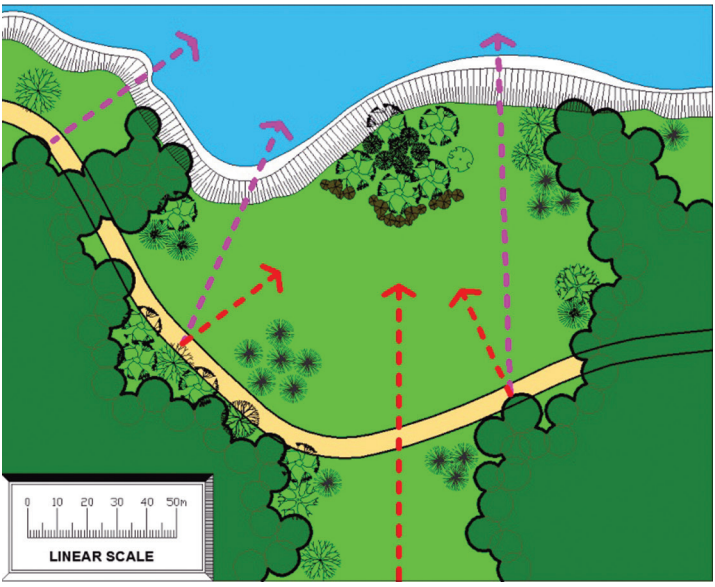

Fig. 3. Landscape design project for space planning along a coastal walking trail near the shore of a water area.

the view and serves as a barrier to short perspectives or as wings for external perspectives. Even greater is the impact of vegetation in shaping the visual frames, which exhibit perspective views.

As shown in Figure 5, visual frames can be on both sides of landscape views, but in some cases these frames can be of the three and even of the four sides of the natural picture. It may even represent an openwork screen that transmits the eyes of the observer beyond.

Conclusions

Artistic process during landscape planning requires creating spectacular views until we get the whole picture of a recreational forest landscape. Unlike urban environment where we have to comply strictly with the architectural framework, in forest areas we have to make much less intervention and to take into account main environmental features. In order to make the design project more realistic and to ensure its subsequent implementation in real space it is advisable to use existing tree groups and massifs, which immediately or after a partial reconstruction could assume the functions of the relevant part of the perspective view.

In this case, some essential principles, combining parts of park perspective methods and spatial composition can make design process much meaningful and the resulting natural landscape more picturesque and attractive. Such principles must be sufficiently simple, fast and effective to provide an aesthetic forest landscape design.

As a result of the comparison of different objects, the following basic landscape modelling requirements for the formation of spaces along walking trails in forest landscapes can be derived:

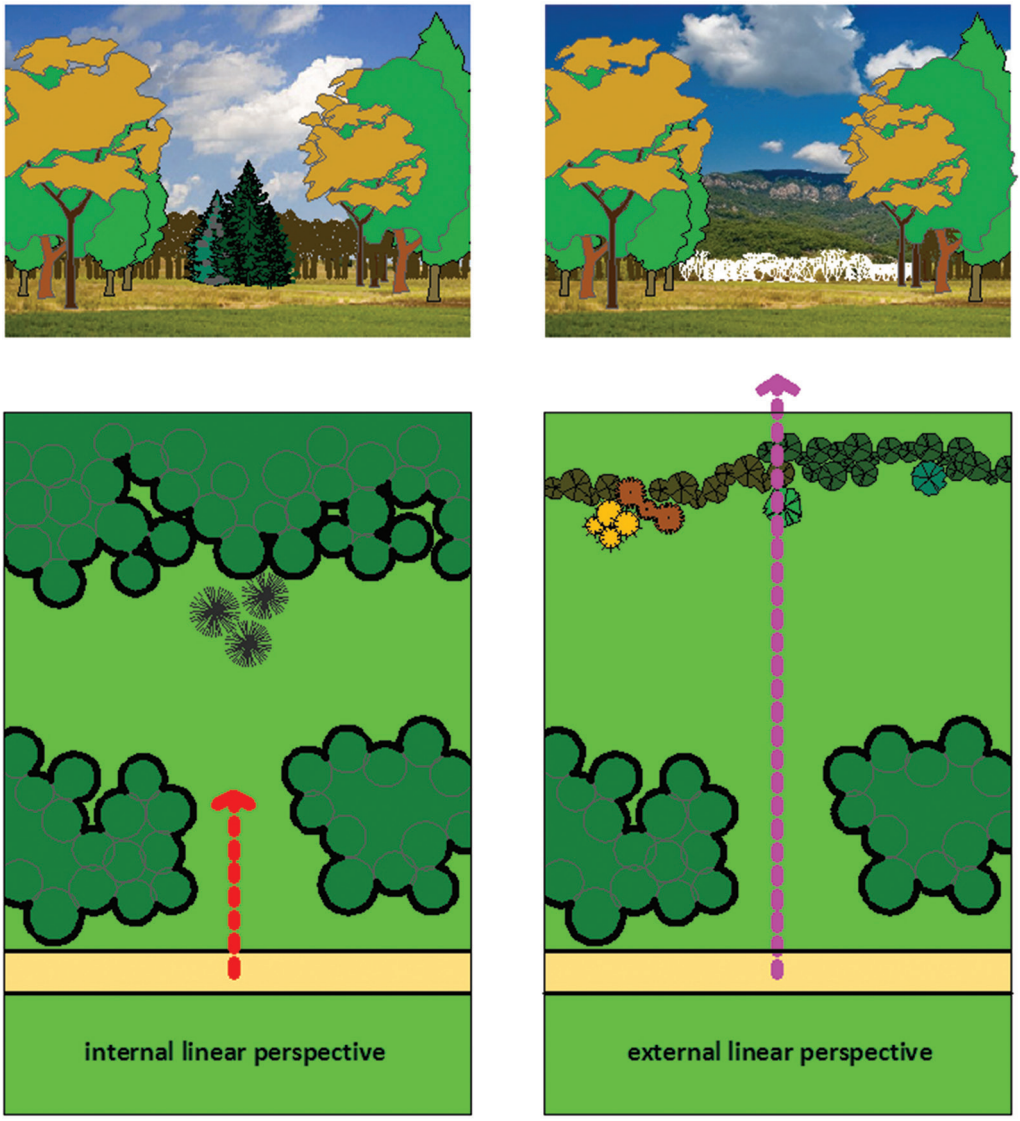

Fig. 4. Internal and external linear perspectives.

- consistency with natural conditions;
- creating maximum internal and external linear perspectives;
- maintain a measure of saturation with accents;
- correctly align the main accents;
- alleys are not axes of symmetry in open spaces (forest meadows);
- perimeters of tree arrays and tree groups themselves should be constructed according to schemes that provide dynamic agility.

Nevertheless, each design solution must have its own logic, whatever it is. For

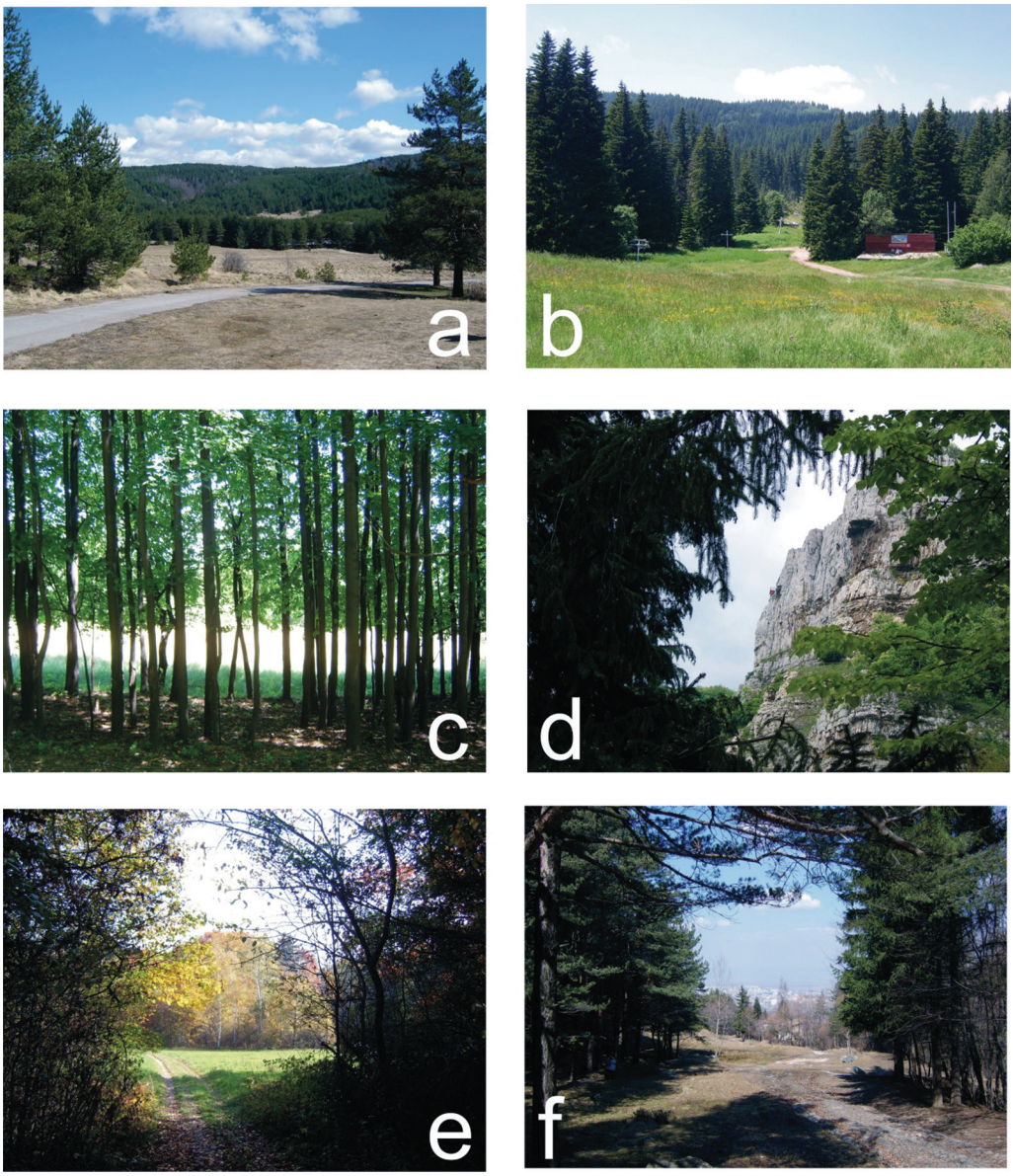

Fig. 5. Visual frames: a – wide visual frame to a landscape fragment without finishing accent; b – narrow visual frame; c – openwork barrier in front of the observer's eye; d – visual frame from all sides; e – narrow visual frame side and top without finishing accent; f – narrow visual frame side and top with finishing accent.

most general reasons, in most cases, the logic is chosen to provide most panoramic landscape views (external linear prospects). For internal perspectives, however, it would be better to bear in mind the last 4 of the listed above principles.

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NEST-SUPPORTING TREES USED BY TURTLE DOVE (*STREPTOPELIA TURTUR*) IN THE SAKAR MOUNTAINS, SOUTHEAST BULGARIA

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Abstract

Thirty-seven nests of Turtle Doves were found in the Sakar Mountains in 2014–2016. The average height of their situation was 5.3±1.8 (2.1–10) m. The average height of the trees-substrates was 7.9±2.5 (3–12) m. The earliest nesting was observed at the beginning of May. First laid eggs were found in the second half of May. Groups of flying juvenile Turtle Doves appeared in early June to the end of the research period. The first nests were observed on 01.05., eggs – on 21.05., and well-flying juveniles on 04.06. The largest number of nests was found in Oak (*Quercus* spp.) forests (*n*=12) and Austrian Pine (*Pinus nigra*) plantations (*n*=9). Breeding in small villages of the turtle dove in Bulgaria was established.

Key words: nest locations, nest height, nesting habitats, columbiform birds, Sakar Mountain.

Introduction

Turtle Dove (*Streptopelia turtur* Linnaeus, 1758) is a widespread migrant breeder in Central and Southern Europe, Asia, Middle East and North Africa (Baptista et al. 2015). The population declines in Europe and Turtle Dove is included in the Red List of IUCN as vulnerable species (IUCN 2017).

Turtle Dove is widely spread in Bulgaria, most often up to about 1200 m s.l. (Simeonov et al. 1990). Its density is unevenly, and reaches its highest in loose forests or mosaics of trees and shrubs adjacent to open areas. The trends of distribution and population size, according to last ornithological studies, are probably stable (Stoychev and Mitev 2007). Until the end of the 20th century, the Turtle Dove was a common breeding species in the plains of Bulgaria (Simeonov et al. 1990, Nankinov 1994). However, some data indicate its decline between 1970 and 1990 (Stoychev and Mitev 2007).

The purpose of this study is to present data on the nesting of the Turtle Dove in the Sakar Mountain, as well as nest distribution, habitats, height, and brood size.

Material and Methods

The researched area is a part of MG-14, 10 km-square of the Universal Transverse Mercator grid (Fig. 1). It falls within the

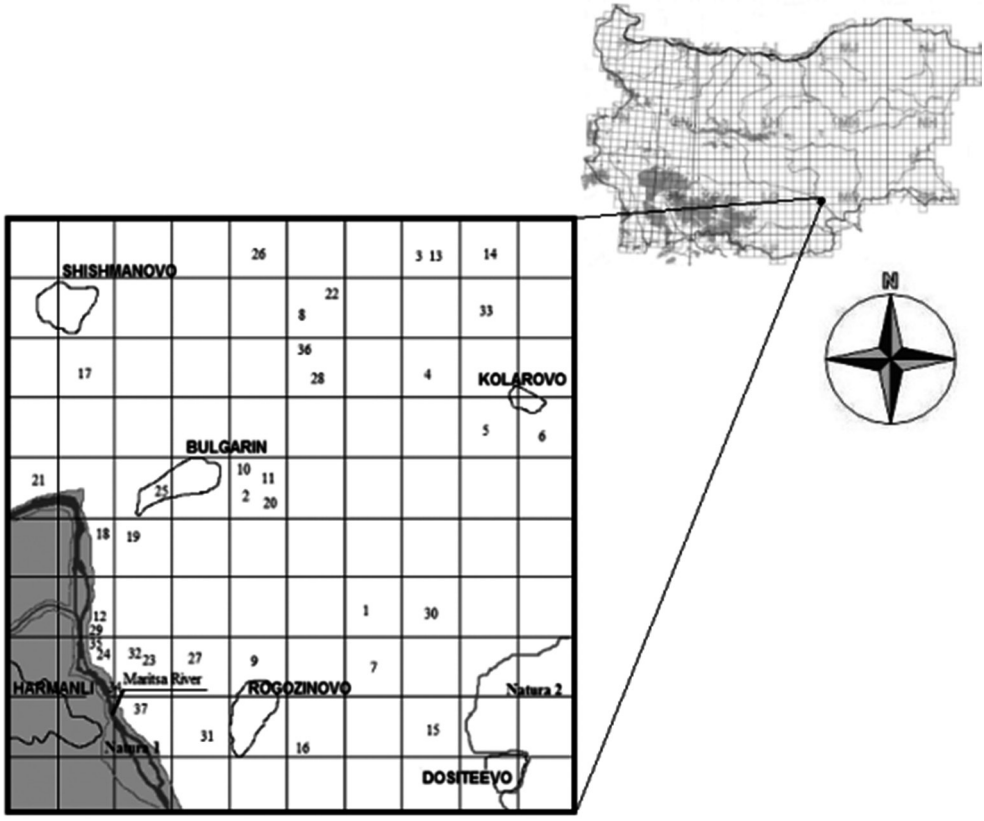

Fig. 1. Study area and nest location in MG 14 UTM.
Note: The numbers show the location of found nests, their number are the numbers of the nests in Table 1. Natura 1 – Nature zone Reka Maritsa BG 0000578; Natura 2 – Nature zone Radinchevo BG 0002020. Gray squares are outside the surveyed territory.

boundaries of the Sakar Mountain, where mixed Oak forests predominate. Large forest massifs are preserved only in the high parts of the mountain. Forests are fragmented into separate spots within the studied area. Plantations of Austrian Pine (*Pinus nigra* Arnold) and Cedar (*Cedrus* sp.) have been created in many places. A large part of the area is occupied by arable land and vineyards divided by many narrow stripes of deciduous trees and shrubs. Separate scattered spots with Jerusalem thorn (*Paliurus spina-christi* Miller) with single trees occur throughout the researched area. Numerous small artificial ponds determine the presence of wetlands, as from the west the territory is limited by the Maritsa River (Natura 2000 – Reka Maritsa BG 0000578).

Within 2014–2016, each 1 km square was visited 7 times a year: 18.04–24.08.2014; 15.04–03.08.2015 and 23.04–20.08.2016. Potential nesting sites were searched around the observations of singing Turtle Doves. Each found nest was determined according to: its height; the height of the tree; tree species; number of eggs, when possible. Tree heights and

distances from the base of the stem to the lowest part of each found nest were measured with altimeter “SUUNTO-PM 5/1520”. The number of eggs was determined when there was visibility of the nest, viewed from below (the Columbiform nests are made of thin layer of twigs and often the eggs are visible (Simeonov et al. 1990)).

During the study, 16 crops of adult Turtle Doves were analysed: 2014 – 7; 2015 – 5; 2016 – 4. They were collected during hunting trips for hunting associations for the surveyed territory.

Results

A total of thirty-seven nests were detected and observed (9 in 2014; 18 in 2015 and 10 in 2016) (Table 1, Fig. 1).

Table 1. Nest distribution by plant species, nest height and height of tree/shrub by data 2014–2016.

No	Species of tree/shrub	Nest height, m	Plant species height, m	Data	Eggs, number
1	*Quercus cerris* L.	unind.	12	02.05.2014	unind.
2	*Quercus cerris* L.	unind.	10	23.05.2014	2
3	*Pinus nigra* Arn.	6.5	9	23.05.2014	2
4	*Quercus cerris* L.	6	9	23.05.2014	unind.
5	*Ulmus minor* Mill.	4.5	6	24.05.2014	unind.
6	*Acer negundo* L.	6	11	24.05.2014	2
7	*Pinus nigra* Arnold	8.5	11	26.05.2014	unind.
8	*Quercus pubescens* Willd.	6.5	8	14.07.2014	1
9	*Paliurus spina-christi* Mill.	2.8	3.1	18.07.2014	2
10	*Salix fragilis* L.	4.5	6	06.05.2015	0
11	*Quercus pubescens* Willd.	6.3	9	06.05.2015	0
12	*Robinia pseudoacacia* L.	4.5	9	21.05.2015	1
13	*Pinus nigra* Arn.	8	10	21.05.2015	2
14	*Quercus pubescens* Willd.	4	5.5	23.05.2015	unind.
15	*Pyrus* L. sp.	3.2	4	26.05.2015	2
16	*Quercus pubescens* Willd.	4.5	6	27.05.2015	2
17	*Paliurus spina-christi* Mill.	2.6	3.1	26.05.2015	unind.
18	*Acer negundo* L.	6	8.5	26.05.2015	unind.
19	*Quercus cerris* L.	4	7	26.06.2015	unind.
20	*Quercus cerris* L.	5	9	26.06.2015	unind.
21	*Salix alba* L.	6	9	26.06.2015	unind.
22	*Robinia pseudoacacia* L.	6	8	28.06.2015	unind.
23	*Pinus nigra* Arn.	6	9	28.06.2015	2
24	*Acer negundo* L.	8	11	02.07.2015	unind.
25	*Salix alba* L.	4.5	7	11.07.2015	unind.
26	*Quercus pubescens* Willd.	5	7	11.07.2015	1
27	*Pinus nigra* Arn.	10	11	12.07.2015	unind.
28	*Pyrus* L. sp.	3.5	5	01.05.2016	0
29	*Ulmus minor* Mill.	2.6	3	01.05.2016	unind.
30	*Paliurus spina-christi* Mill.	2.1	3	02.05.2016	unind.
31	*Quercus cerris* L.	3.5	8	21.05.2016	unind.
32	*Pinus nigra* Arn.	7	9.5	23.05.2016	unind.

No	Species of tree/shrub	Nest height, m	Plant species height, m	Data	Eggs, number
33	*Quercus* L. sp.	4.5		03.06.2016	unind.
34	*Pinus nigra* Arn.	6.5	10	04.06.2016	unind.
35	*Ulmus* L. sp.	4	6.5	30.06.2016	unind.
36	*Pinus nigra* Arn.	8	10	01.07.2016	unind.
37	*Pinus nigra* Arn.	6	9	02.07.2016	unind.

The average height of the nests was 5.3±1.8 (2.1–10) m (mean±SD (min–max)). The average height of the trees or shrubs on which nests were located was 7.9±2.5 (3–12) m. Most nests were found on deciduous trees – *Quercus* sp. (Fig. 2).

The first observed nests were on 01.05.2016, 02.05.2014 and 06.05.2015. The earliest eggs were observed first on 21.05.2015 (Table 1). The second clutch was probably formed in the end of June. A nest of two eggs on Austrian Pine was observed on 28.06.2015. Copulation of Turtle Doves was observed on 29.06.2015. Groups of well-flying juveniles were detected at the earliest on 14.06.2014; 26.06.2015 and 04.06.2016.

Singing Turtle Doves were observed on 23.05.2014 in Kolarovo village, and on 02.05 and 22.05.2016 in Rogozinovo village. On 11.07.2015 a Turtle Dove nest was found at a height of 4.5 m on White Willow (*Salix alba* L.) with a height of 7 m at 25 meters in a straight line from the closest house within the village of Bulgarin (N4157.302; E2556.823). This was the only confirmed breeding of the species within human settlement found during this study.

Five crops of Turtle Doves were analyzed. Analysis showed the presence of developed epithelial tissue in four of

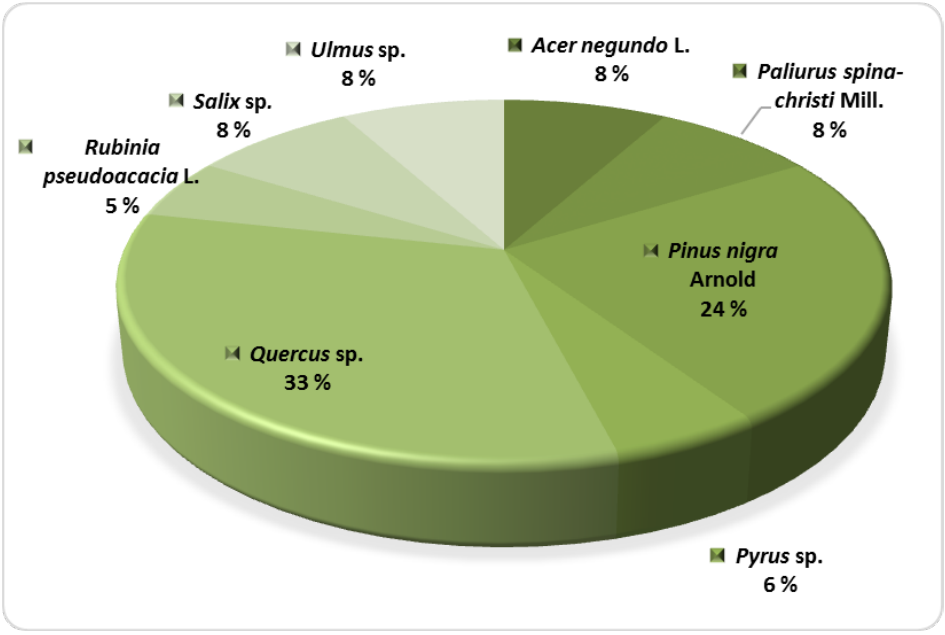

Fig. 2. Distribution of observed nests according to tree and shrub species.

them. The crops were from the hunted turtle doves found on 08.08.2015 and 15.08.2015. This fact causes suggestions for a third or replacement breeding of the species in 2015.

Discussion

In the present study, the number of nests found in Oak forests (*n*=120) predominates. Turtle Dove is one of the dominant species in similar forests in Bulgaria (Simeonov and Petrov 1978, Petrov 1981, Nankinov 1994). It is a high density species in the belts of Dobrudzha, where the oaks predominate (Karaivanov 2003), in the forests of Kermes Oak (*Quercus coccifera* L.), and the low forests in southern Dobrudzha (Karaivanov 2005, Karaivanov et al. 2006). The large number of nest found in Austrian Pine conifers (*n*=9) is reported for the first time in Bulgaria. Previous studies do not mention nests in conifers (Nankinov 1994), but are known to be found in conifers in Europe (Browne et al. 2005). In the present study, the prevailing height of found nests is between 2.1 and 6 m, similar to other studies (Patev 1950, Nankinov 1994, Browne and Aebisher 2004, Browne et al. 2005). But unlike them, we did not find nests at height below two meters. Half (*n*=17) of established nests are between 6.1 and 10 m high. The greater height of the nests is due to their location – the nests built on trees predominate. The high nesting location could be due to the larger hunting press by the predators or the decontamination of pastures and the lack of sufficient nesting places at a small height. In the habitats where the shrubs of the Jerusalem Thorn are preserved, they are 3.1 m high and the nests were always located at the top of the bushes (Table 1). High mountain nests in Bulgaria are reported for Stara Planina Mountain (Donchev 1970).

The first nests were observed on 01.05., eggs on 21.05., and well-flying juveniles on 04.06. Similar data are available for Turtle Dove in other studies (Simeonov et al. 1990, Miltchev 1991, Nankinov 1994, Browne et al. 2005, Baptista et al. 2015). This study indicates the end of June as the probability of the second clutch. This information coincides with the indicative deadlines in such studies (Simeonov et al. 1990, Milchev 1991, Nankinov 1994, Browne and Aebisher 2004). The average brood size in this study is 1.7 (*n*=11). Although the obtained result is similar to that of other studies (Browne and Aebisher 2004, Browne et al. 2005, Baptista et al. 2015), the sample is very small to be representative.

Established crops with developed epithelial tissue in August suggest a third or replacement breeding attempt of the species in Bulgaria. Milchev and Kovachev (1998) report the possible third clutch. The same authors found a nest of Oriental Hornbeam (*Carpinus orientalis* Miller), and on 06.08.1994 nestlings about one week old (Milchev and Kovachev 1998).
The established nesting of the Turtle Dove in the settlements was predicted by a number of observations of singing birds – on a lamp from street lighting in the village of Valchi Izvor, Bolyarovo municipality, on 01.06.2013, as well as a couple with a singing bird in the village of Dolno Yabalkovo on 02.05.2016 (Milchev unpubl. data). Nankinov (1981) reports the occupation of breeding territories on the periphery of settlements. In the period 1996–2005 singing Turtle Doves were observed in scattered mountainous neighbourhoods, but no breeding was established (Stoychev and Mitev 2007). The nest found in this study establishes

the possibility of the single nesting of the Turtle Dove in settlements and indicates a confirmed breeding of the species in small towns and villages in Bulgaria. Turtle Dove is known as a breeding species in parkland in Europe (Paton et al. 2012).

Conclusion

The average height of the nests was 5.3 m and the average height of the trees or shrubs on which nests were located was 7.9 m. The first observed nests were on 01, 02 and 06 May. The earliest eggs were first observed on 21.05.2015. A nest of two eggs on Austrian Pine was observed on 28.06.2015. Copulation of Turtle doves was observed on 29.06.2015. Groups of well-flying young turtle doves were detected at the earliest on 14.06.2014; 26.06.2015 and 04.06.2016. Analysis of crops of turtle doves showed the presence of developed epithelial tissue in four of them.

Singing turtle doves were observed on 23.05.2014 in Kolarovo village, and on 02.05. and 22.05.2016 in Rogozinovo village. On 11.07.2015 a turtle dove nest was found at a height of 4.5 m on Willow tree with a height of 7 m at 25 meters in a straight line from the closest house within the village of Bulgarian (N4157.302; E 2556.823). This was the only confirmed breeding of the species found during this study.

This study determines Oak forests, coniferous plantations, and single-tree in Jerusalem Thorn communities as important for the nesting of the Turtle Dove in the Sakar Mountains. Broadleaved forests are the habitats with the most found nests. Their continued fragmentation, as well as periodic burning over the years, can aggravate the Turtle Dove nesting habitats. As an alternative, coniferous crops appear in the absence of sufficient nesting habitats. *Paliurus* scraps with single trees in them also provide suitable nesting sites, but this should be taken into account when decontamination the pastures in the research area. Full decontamination may reduce the habitat of the species. It is necessary to establish the reasons for the high nesting location. Providing more nesting places could increase nesting density of the species in the Sakar Mountain.

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PERFORMANCE OF BLACK PINE (*PINUS NIGRA* ARN.) PROVENANCES IN WEST BULGARIA

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Abstract

Fourteen provenances of *Pinus nigra* Arn. from the best autochthonous populations in Bulgaria were tested on two sites in West Bulgaria. Data of the 36–37th year of the plantations on tree diameter, height, survival and stem quality show varying results per site but provide grounds for recommending Goleshevo (Petrodan) and Razlog provenances and avoiding the use of Hristo Danovo provenance in similar conditions. Significant site-provenance interactions are confirmed. The source for the seeds – be it from mother plus trees or seed aggregations seems to matter and plus tress shall be preferred. Showing of different ecotypes and edophotypes is also discussed indicating better performance of the low-mountain ecotype and the calcareous edaphotype for similar conditions although not statistically substantiated for the two sites.

Key words: performance, productivity, provenance trials, site-provenance interactions.

Introduction

Black pine (*Pinus nigra* Arn.) has been extensively used for reforestation and land restoration in South-Eastern Europe, the Mediterranean basin and other parts of the world since the late 19th century. The species presently covers more than 3.5 million hectares (Isajev et al. 2004) making it one of the most widespread conifer species in the Balkans and Asia Minor (Enescu et al. 2016). For example, Black pine is the most used species in Anatolia for afforestration (Öner et. al. 2016). Within Bulgaria, Black pine occupies circa 275,000 ha with a growing stock of around 74 million m^3 and annual harvesting levels of about 700,000 m^3 per annum (EFA 2016) with potential for further increase of the area (Kostov and Rafailova 2009). Natural Black pine forests have been historically reduced to approximately 20,000 ha occupying scattered patches (Zlatanov et al. 2010) as is the case throughout the natural range of distribution of the species, thus leaving a considerably larger proportion to artificially established plantations. Some of the latter have been established exclusively for erosion control purposes on dry and poor sites while others targeted purely commercial objectives replacing natural broadleaved vegetation. In both cases, advantages of some important features of the species were sought which, as summarized by Matziris (1985) include:

- it is well adapted to a wide range of environmental conditions, forming different ecotypes;
- it is highly tolerant and grows well even on degraded lands;
- plantations are easily established re-

quiring minimum cultivation;

● grows fast, producing high quality and resistant timber.

Given the increasing demand for timber and other forest products and the resulting pressure on natural forests, commercial plantations will continue playing an important role in satisfying the needs of timber (Kanowski 1997; Savill et al. 1997; Evans 1999, 2001; Milev 2013). Taking into account Black pine's ecological plasticity and above all drought resistance (Mataruga et al. 2010, 2012), the species can well serve this role in the afforestations and reforestations in part of the lower and the middle (in terms of altitude) forest belt in South-Eastern Europe.

Bulgarian afforestation practices however, have not always taken into consideration the site conditions, the influence of the origin and the hereditary qualities of the sowing materials. As a result, coupled to a large extent with the untimely implementation of the tending and thinning operations, part of the established Black pine plantations deteriorate in general, have reduced growth characteristics, unfavourable environmental and commercial effect, which places serious questions over their future use (Bardarov and Milev 2015), although not as serious as for other species that face similar challenges such as the Scots pine.

Provenance trials provide good opportunity to actively seek answers to some of the most pressing issues related to sustainability and improving the production potential of plantations. Experience within Europe is already generated but Black pine provenance trials are not unknown even at places far from the natural range of distribution of the species including USA (Wheeler at al. 1976) and New Zealand (Wilcox and Miller 1975). While the purpose of some of the trials was to further clarify the taxonomy of the species or its morpho-anatomical features (Arbez and Millier 1971; Matziris 1984; Matziris 1989a, 1989b; Matziris 1994; Sıvacıoglu and Ayan 2010; Ayan et al. 2011) others serve for obtaining data on provenance performance in nursery production (Ivetić and Škorić 2013) and on-site (von Röhrig 1966, Wheeler et al. 1976, Varelides et al. 2001, Gökdemir et al. 2012) although data on the productivity of the provenances within the trials is scarce.

Through the methods of selection, some 650 ha (EFA 2013) of Black pine provenance trials have been established in Bulgaria since the beginning of XX century. These encompass all known national natural populations as well as some foreign. Subject to testing are both distinct ecotypes – low-mountainous (occurring at altitudes of up to 800–1000 m) and mid-mountainous (above 1000 m) (Zahariev 1977) as well as the three known edaphotypes (depending on the parent material) – "silicate", "calcareous" and "rocky" (Dobrinov et al. 1982). The studies conducted in these trials cover mainly the early stages of the plantations including initial survival, canopy formation and differentiation as well as the stages of the intensive growth and culmination in height increment. Thus, the current study is to build-up this existing knowledge by adding information related to a more mature stage for two of the trials, where differentiation in diameter is more distinct.

Materials and Methods

Site characteristics

Subject to research are two plantations, which are part of series of provenance

trials from Bulgaria's main coniferous species, established in the 1970s. The first plantation (Site 1 – provenance trial "Petrohan") is located in the Training and Experimental Forestry "Petrohan" of the University of Forestry – Sofia (N43°11'38", E23°08'22"), while the second on the territory of State Forestry Unit "Ihtiman" (Site 2 – provenance trial "Ihtiman"; N42°29'05", E23°42'01") (Fig. 1). Both trials were established in 1978 (except for two of the provenances in Site 2 which were introduced 2 years earlier) with a main objective to study the hereditary potential of selected offspring and provenances of the most valuable natural Black pine populations in Bulgaria at the given environmental conditions. The initial concept also included the idea, that after some additional selection, plantations may also be used as a source of reproductive material with improved qualities (Bogdanov and Dakev 1982) – from the categories "selected" and "qualified" as per national regulations.

For the establishment of the provenance trials, three year old seedlings were produced from seeds derived from individual pre-selected plus trees or from provenance aggregations from the best autochthonous Black pine stands in Bulgaria. Thus, provenance trial "Petrohan" holds 11 provenances represented by the generative offspring of 28 plus trees and 12 provenance aggregations, while provenance trial "Ihtiman" tests 13 provenances represented by the offspring of 34 plus trees and 15 aggregations (one of the provenances represented in the two sites is derived from two different altitudes). In

Fig. 1. Location of the provenance trials and used provenances.

addition, one provenance from Adana, Turkey was introduced in 1975 but this is not subject to the current study. Eleven of the provenances are common for both sites. The original location of the provenances used, sorted down per altitude and the features of the mother plus trees, are presented in Table 1.

Table 1. Provenances used in the trial.

Provenance		Altitude, m	Longitude	Latitude	Represented at	
Name*	Code**				"Petrohan"	"Ihtiman"
Melnik	Mk	280	23°24'	41°31'		✓
Hristo Danovo – lower	HD (d)	400	24°35'	42°43'	✓	✓
Borika (Haskovo)	Has	400	25°09'	41°54'	✓	✓
Vodenicharsko (Kardjali)	Vd (Kj)	420	25°15'	41°29'		✓
Borovo	Br	590	23°43'	41°35'	✓	✓
Ploski	Pl	700	24°51'	41°39'	✓	✓
Zhenda (Kardjali)	Zh (Kj)	740	25°10'	41°47'	✓	✓
Tzarvaritza (Gabra)	Gbr	980	22°50'	42°01'	✓	
Gorno Izvorovo	Gl	750	25°28'	42°39'	✓	✓
Teshel	Ts	1000	24°23'	41°38'		✓
Razlog	Rz	1050	23°24'	41°53'	✓	✓
Momchilovci	Mc	1000	24°45'	41°37'	✓	✓
Orehovo	O	1100	24°35'	41°52'	✓	✓
Goleshevo (Petrodan)	Pd (Gol)	1100	23°35'	41°24'	✓	✓
Hristo Danovo – upper	HD (g)	1200	24°35'	42°45'	✓	✓

Note: * The popular names of the geographic provenances are given in brackets.
**The ID of the mother plus tree is annotated as a number to the code; aggregations are annotated as OS in the text.

The trial layouts are of block design where each treatment represents a rectangular plot – cells (Fig. 2). Site 1 is composed of 26 cells most of which consist of 12 rows each with 13–14 seedlings, making 156–168 seedlings in total per cell. Site 2 has 35 cells composed, in the general case, of 15 rows with 14 seedlings each. Depending on the availability of planting material, some of the variants were reduced and formed smaller cells, while for others the offspring of different plus trees, but from one and the same provenance, were combined into a single cell. For clear distinction of the plots, isolation rows of *Picea abies* (L.) Karst. were planted among them, using the same interval of 2 m. This has guaranteed good clarity of the adopted scheme and avoids mistakes during observations and measurements. Permanent 2×2 m planting intervals are used in both sites allowing for adequate comparison. However, no replication of each variant is available.

The altitudes of the provenance trials are 600 m (Site 1) and 750 m (Site 2) respectively, both falling within lower part of the mid-mountain belt. Locations of sites are selected to have uniform conditions throughout the site. Both plantations have north-eastern aspect and clay-sandy soils – *Cambisols* and *Leptosols* respectively, with average depth. The mean annual temperature for both site is 8–9 °C the highest being in July (around 15 °C) and lowest in January (2–3 °C). Annual precipitation is 500–700 mm, higher for "Petrohan". Natural vegetation for the lat-

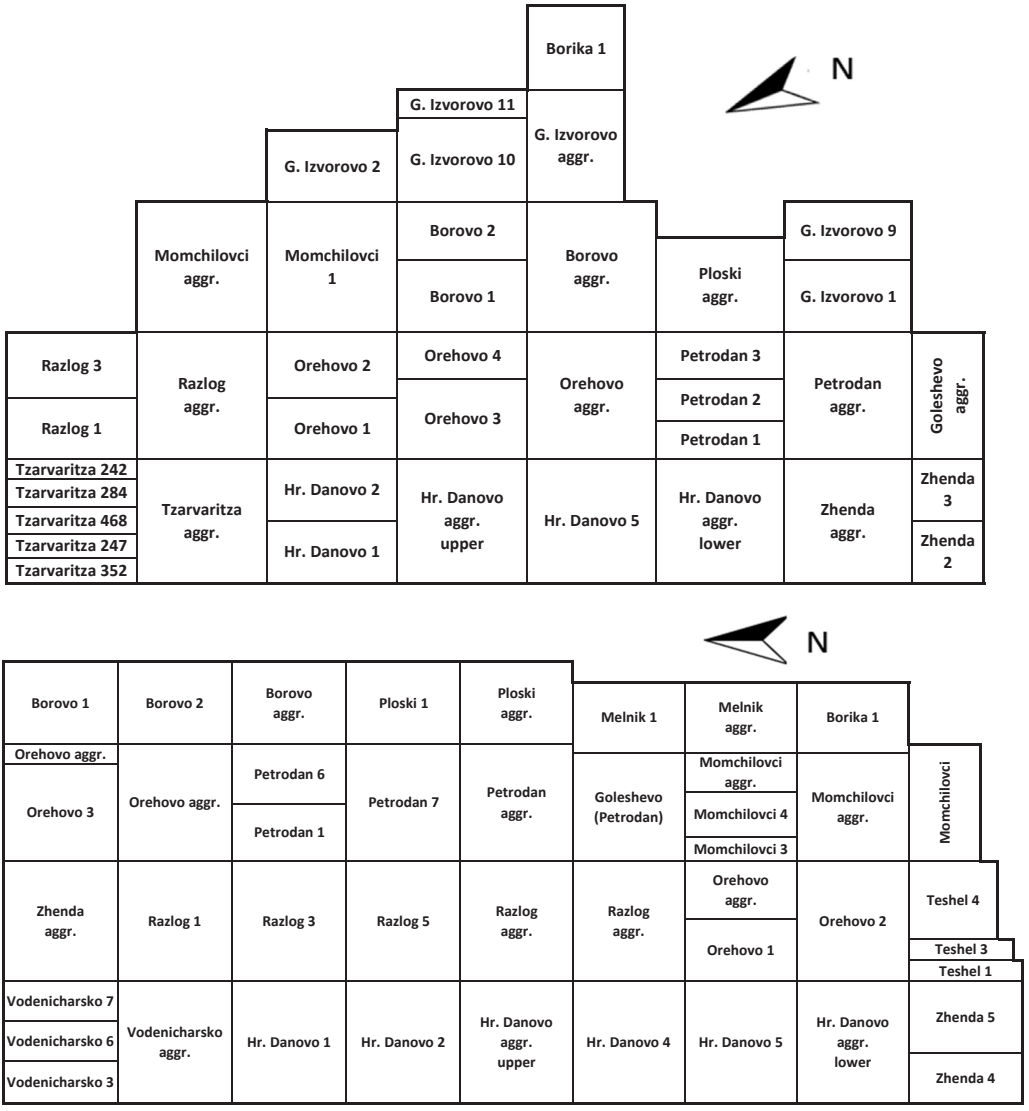

Fig. 2. Layout of the experimental plots – "Petrohan" (top) and "Ihtiman" (bottom).

ter is represented by *Carpinus betulus* L. and *Fagus sylvatica* L. while in "Ihtiman" by *Quercus frainetto* Ten.*, Quercuss cerris* L. *and Carpinus betulus* L. Both sites are considered favourable for the Black pine.

Methods

Field measurements were carried out in the autumn of 2013 ("Petrohan") and in spring of 2015 ("Ihtiman") using an adaptation of the methodology for analysis

of forest plantations (Milev 2013). A detailed inventory of the site was carried out, where presence (taking note of normal, dry, died out and cut trees) and condition (including curvatures, bifurcation, damages) of every individual tree was evaluated. Diameter at breast height (DBH) and height were measured.

Collected field data was introduced to MS Excel environment, where some basic indicators of each trial were calculated including: survival; share of normal, curved, bifurcated and bent trees or trees with highly developed side-shooting; site index, basal area, basal area per hectare, mean and dominant diameter, increment.

Based on initial comparative analysis of the DBH, survival and percentage of quality trees within each trial, best performing variants were determined and a 'model' tree for these was selected on the field. Each model tree selected was felled, measured in length and cut to sections (1 cross-section disc from base, breast height and every 2 m taken for processing). Stem analysis for each model tree was subsequently carried out using the specialized "STA.13" software (Poryazov and Radulov 2007). Twelve model trees for each site were analyzed.

Data were analyzed statistically using the free R Software (R Development Core Team 2008). Given the age of the plantations and the stage of differentiation, diameter was considered a better indicator for the growth and productivity of the plantations than height (as opposed to young stands when height is a more secure indicator). Thus, the statistical analysis took the diameter as the main variable.

Data were tested for homogeneity of variances using the Fligner-Killeen test (Conover et al. 1981) from R's 'car' library. Variances for "Petrohan" proved homogenous but those for "Ihtiman" heteroscedastic which was also confirmed using Levene's test (Fox and Weisberg 2011). This is most probably due to the large number of observations. In order to further proceed with the application of ANOVA for "Ihtiman", power transformation of data was tested for stabilizing the variance, returning an estimated transformation parameter close to 1 (1.034653) meaning unchanged data and possibility for application of the analysis of variance. On a similar note, the variance using medians through a non-parametric Kruskal-Wallis rank sum test (Myles and Wolfe 1973) was applied (p-value < 2.2e-16).

Subsequently, ANOVA was applied to test: the influence of provenance including testing for significant differences among each variant; the influence of the origin of the reproductive material – plus trees versus provenance aggregations; the influence of the ecotype – low versus mid altitude; as well as the influence of the edaphotype – provenances originally growing on silicate parent material versus calcareous versus rocks.

Where statistically significant differences were returned, a post-hoc pair comparison to evaluate pair means was conducted using the LSD test (Steel et al. 1997) from R's 'agricolae' library.

Data on survival and implications on sustainability of the "Petrohan" trial were reported by the authors in a separate study (Bardarov and Milev 2015).

Results

The plots included in "Petrohan" trial recorded an average survival of 68.4 %, varying between 38.1 % (Zhenda 2) and 91.9 % (Gorno Izvorovo 11) for the different plots. Most preserved are the

variants of G. Izvorovo 11, Momchilovci aggr. and Hristo Danovo 2, while highest mortality is revealed for Zhenda 2, Hristo Danovo 1 and 5. Generalizing data for the represented provenances shows that the most sustainable, if sustainability is measured through survival, are the provenances Momchilovci, Petrodan, Borovo and Razlog. A similar status is observed for "Ihtiman" trial, where of 7508 seedlings initially planted, 5247 have survived, making an average survival of 68.0 %, varying between 33.3 % (Vodenicharsko 6) and 87.7 % (Borika aggr.). In terms of provenance generalization, 6 provenances manage a survival above the 70 % mark (Borika, Razlog, G. Izvorovo, Ploski, Petrodan and Hristo Danovo) while Melnik provenance ranks lowest with 57.3 %. Comparison between survival rates generalized per provenance is presented on Fig. 3.

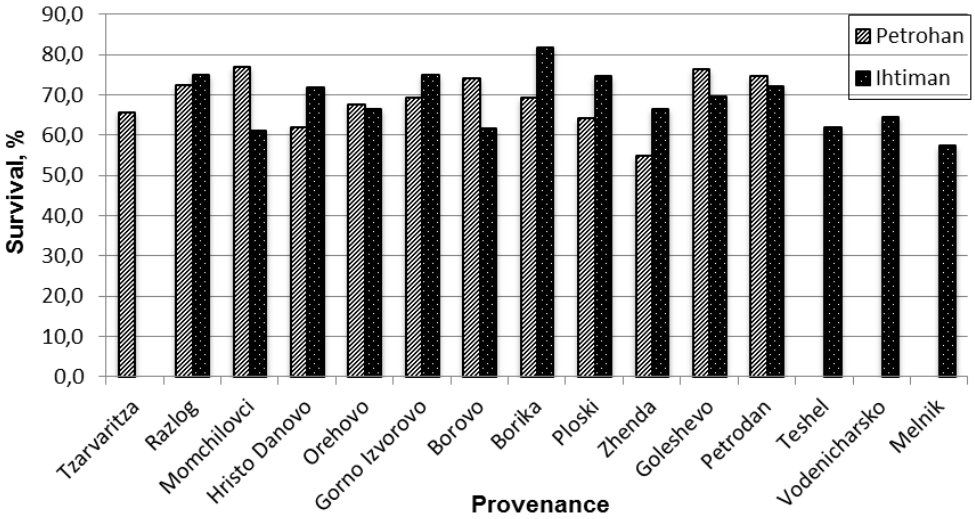

Fig. 3. Survival rates generalized per provenance for the two trials.

Survival percentages could have been even higher if trees had not been deliberately cut as result of illegal activities or for the purposes of previous studies. Taking this into account, the theoretic survival could have been as high as 79 % in average or between 66 and 90 % for the different provenances (94.8 % as the case for Razlog aggr. plot).

The site indexes assessment reveal good growth showing of the offspring and provenances in both trials favored by the unified environmental conditions of the site. The height was measured as mean for each plot and the average for "Petrohan" is calculated to 18 m, varying from 17.4 m (Petrodan) to 19.0 m (Momchilovci and Ploski). Looking at the comparison between the different variants, an interesting fact represents the difference in the mean height for Goleshevo aggr. (19.5 m) and Petrodan aggr. (17.0 m) which are in fact one and the same provenance but from different locations. Given the data on the mean height, all plots record grade I of the site index (as compared to the results of a study carried out in 2007 where 4 variants recorded grade II). The mean

annual increment in height is 47–54 cm/annum, or 51 cm/annum.

The mean height of the trees in "Ihtiman" trial is calculated to 15.0 m, varying from 13.3 m for Teshel provenance and 16.8 m for Vodenicharsko (in more absolute terms the variance of the mean height among the plots is between 12.8 m – G. Izvorovo aggr. and 17.0 m – Vodenicharsko 6 and 7). The average site index is grade II, with only one provenance (G. Izvorovo) graded III. Here, the mean annual increment is 38 cm/annum, varying between 33 and 43 cm/annum taking the aggregated data for the provenances.

The dynamics of the periodic annual increments, reconstructed through the stem analysis of model trees from the two trials is presented on Fig. 4. Similar trends are observed for the two sites, with relatively

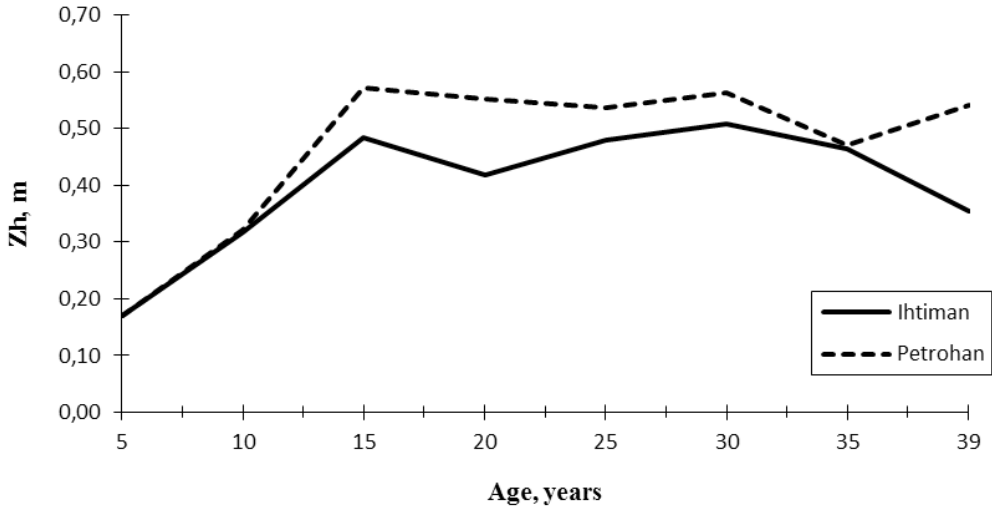

Fig. 4. Periodic annual increments in height for the two provenance trials.

steady increment rates between year 15 and 30, with good signs that growth in height slows down afterwards.

The mean diameter, calculated through the basal area, in "Petrohan" trial is estimated to 21.1 cm, varying between 17.9 cm (Tzarvaritza 352) and 22.9 cm (Borovo 2) or between 20.0 cm (Hristo Danovo) and 21.9 cm (Razlog) when aggregating the data per provenance. As for the diameter of the dominant trees this varies between 28.6 cm (Hristo Danovo) and 33.4 cm (Goleshevo) or 30.3 cm on average. The mean annual increment in diameter is estimated to 0.59 cm/annum (0.56–0.61 cm/annum). "Ihtiman" provenance trial shows visibly lower figures, with mean diameter estimated to 19.0 cm, highest for Borika (20.6 cm) and lowest for Hristo Danovo (17.6 cm) provenances. The mean annual increment in diameter for "Ihtiman" is 0.48 cm/annum (0.43–0.53 cm/annum). The dynamics of the periodic annual increments in diameter, reconstructed through the stem analysis of model trees from the two trials is presented on Fig. 5.

Within "Petrohan" trial, we determined significant variation for the basal areas given that no thinnings have been carried out so far: between 32.1 m^2/ha (Zhenda

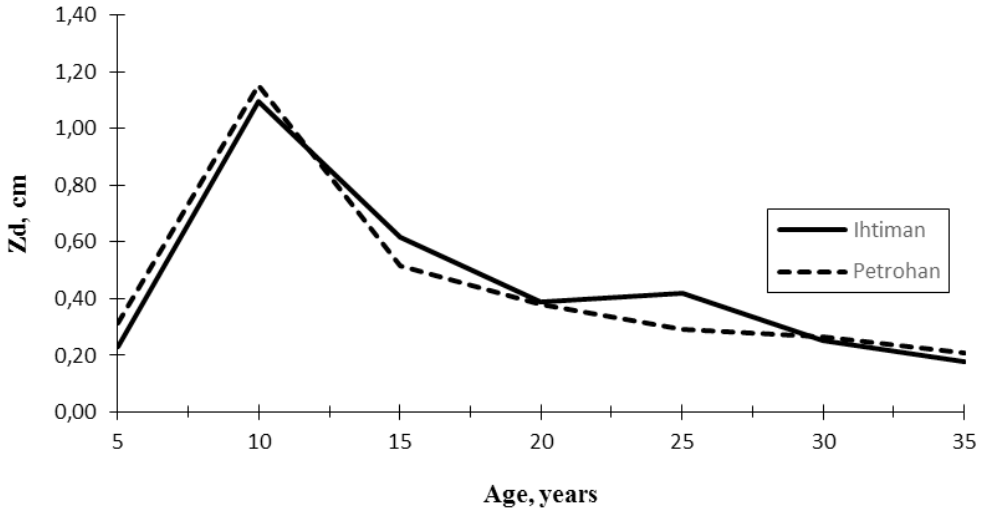

Fig. 5. Periodic annual increments in diameter for the two provenance trials.

2) and 92.9 m²/ha (G. Izvorovo 11) or 60.9 m²/ha on average. When aggregating the data per provenance highest ranked are Goleshevo (69.2 m²/ha) and Razlog (68.2 m²/ha). In comparison, the average value on this indicator for "Ihtiman" trial is 48.7 m²/ha, varying between 35.2 m²/ha (Momchilovci 4) and 58.0 m²/ha (Razlog 5). Overall, Razlog, Zhenda and Borika provenances rank highest.

Using the standard growth models for determining the growing stock revealed differences from the data obtained from the stem analysis. In order to avoid distortion of the results, productivity in terms of volumes was calculated only for the variants from which model trees were taken thus indicating the potential of the plantations. For "Petrohan" this potential could be as high as 683 m³/ha (Goleshevo aggr.) or around 450 m³/ha on average (12.6 m³/ha). Given the lower values for the mean height and diameter, the growing stock for "Ihtiman" is lower as expected but still in the region of 340 m³/ha (highest for Razlog 5 – 423 m³/ha).

General grouping of provenances per "regions of provenance" for "Petrohan" trial shows that the influence of the provenance on performance (diameter) is a trend (*p*-value=0.0542) and statistically significant variances could be expected. The application of the LSD test for grouping of the means, shows that data are clustered in 3 groups where highest ranked are Rila mountain provenances and lowest are the provenances from Stara Planina Mountain. This was further confirmed when testing the performance of the 11 provenances included in the trial where the applied ANOVA revealed highly significant influence of the provenance on performance (*p*-value=0.000195) – Fig. 6. The post-hoc analysis identifies 3 groups of equivalent performance where Razlog (Rz) provenance ranks highest while Momchilovci (Mc) and Hristo Danovo (HD) rank lowest.

ANOVA "Petrohan"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Provenance	10	741	74,14	3,404	0,000195 ***
Residuals	2490	54232	21,78		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

LSD test "Petrohan"

	Mean	CV	Mserror
	20,45902	22,89893	21,77978

$parameters

	Df	ntr	bonferroni	alpha	test	name.t
	2499	11	3.32125	0,05	bonferroni	Prov

$groups

trt	means	M
Rz	21.36596	a
Has	21.01852	ab
Gl	20.92938	ab
Pl	20.78667	ab
O	20.67192	ab
Kj	20.64000	ab
Pd	20.49653	ab
Gbr	20.40000	ab
Br	20.38095	ab
Mc	19.85417	b
HD	19.52713	b

Note: Means annotated with the same letter do not differ at p<0.05

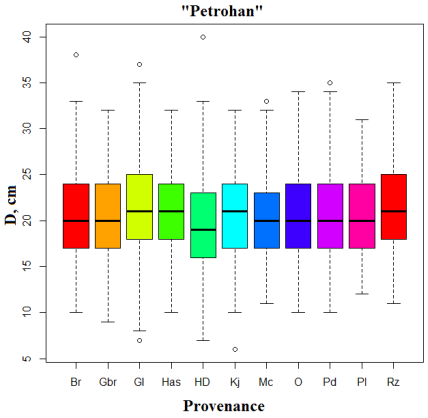

Fig. 6. Analysis of variance and pos-hoc test for tree diameter (D) for 11 provenances in "Petrohan" trial.

Similar procedure was applied at the level of a single variant, where the LSD test excels Razlog 3 and Borovo 2 trials, and ranks lowest Hristo Danovo aggr.-low and Tzarvaritza 352 (which however is represented by only 24 trees).

The analysis of the data for "Ihtiman" also reveals highly significant differences resulting from the influence of the provenance on performance. Here, data are separated into five groups: group 1 comprising the provenances of Teshel, Momchilovci, Kardjali and Orehovo (all from the Rhodopi mountain); group 2 – Borovo, Razlog, Melnik, Haskovo and Petrodan; group 3 – Gorno Izvorovo; group 4 – Ploski; and group 5 – Hristo Danovo (Fig. 7).

The results of the statistical analysis on the influence of the origin of the reproductive material – plus trees versus provenance aggregations, are presented on Fig. 8 and Table 2. Significant differences were identified only for "Petrohan" trial (*p*-value=0.0174) where variants growing from seeds derived from pre-selected individual plus trees show better performance in diameter than trees originating from provenance seed aggregations.

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ANOVA "Ihtiman"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Provenance	11	2825	256,79	22,44	<2e-16 ***
Residuals	5263	60218	11,44		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

LSD test "Ihtiman"

	Mean	CV	Mserror
	18,44152	18,34213	11,44176

$parameters

	Df	ntr	bonferroni	alpha	test	name.t
	5263	12	3,369823	0,05	bonferroni	Prov

$groups

trt	means	M
Ts	19,50467	a
Mc	19,43131	a
Kj	19,34964	a
O	19,21655	a
Br	18,6426	ab
Rz	18,6114	ab
Mk	18,43448	ab
Has	18,41841	ab
Pd	18,40099	ab
Gl	17,94821	b
Pl	17,84332	bc
HD	17,22851	c

Note: Means annotated with the same letter do not differ at p<0.05

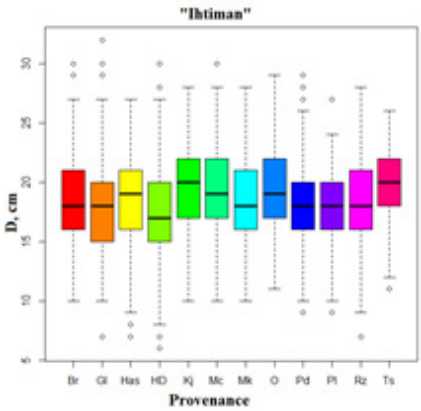

Fig. 7. Analysis of variance and pos-hoc test for tree diameter (D) for 12 provenances in "Ihtiman" trial.

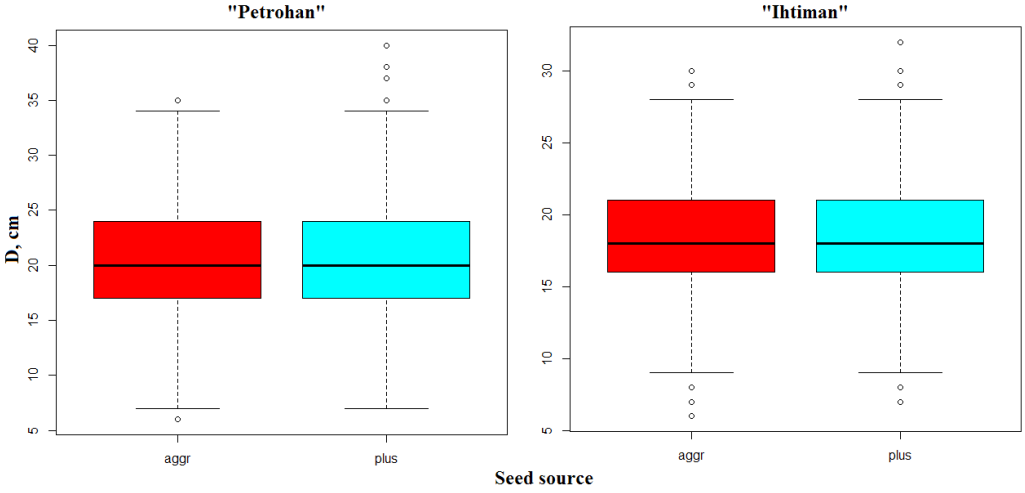

Fig. 8. Box-plots on the growth in diameter depending on the seed source.

Table 2. Analysis of variance and post-hoc test on the influence of the seed source.

ANOVA "Petrohan"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	1	124	124,37	5,667	0,0174
Residuals	2499	54849	21,95		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA "Ihtiman"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	1	1	0,823	0,069	0,973
Residuals	5273	63042	11,956		

LSD test "Petrohan"

	Mean	CV	Mserror			
	20,45902	22,89893	21,94825			
$parameters						
	Df	ntr	bonferroni	alpha	test	name.t
	2499	2	1,960914	0,05	bonferroni	Seed source
$means						
	Diam	std	r	LCL	UCL	Min	Max
aggr	20,21867	4.705853	1157	19,94859	20,48875	6	35
plus	20,66592	4.666782	1344	20,41534	20,91651	7	40
$groups							
trt	means	M					
1 plus	20,66592	a					
2 aggr.	20,21867	b					

Trials were also grouped per ecotypes of origin – low-mountain (occurring at altitudes of up to 800–1000 m) and mid-mountain (above 1000 m) (Zahariev 1977). Applied analysis of variance reveals that significant differences (*p*-value=0.0308), although close to the critical value, are observed only in "Ihtiman" plantation where provenances representing the low-mountain ecotype perform slightly better (Fig. 9 and Table 3).

An interesting case represents the results of the statistical analysis on the influence of the soil of origin (in broader terms depending on the parent material) on growth in diameter or in other terms, the performance of the different edaphotypes in both sites. While the "silicate" and "calcareous" provenances only are represented in the "Petrohan" plantation, "Ihtiman"

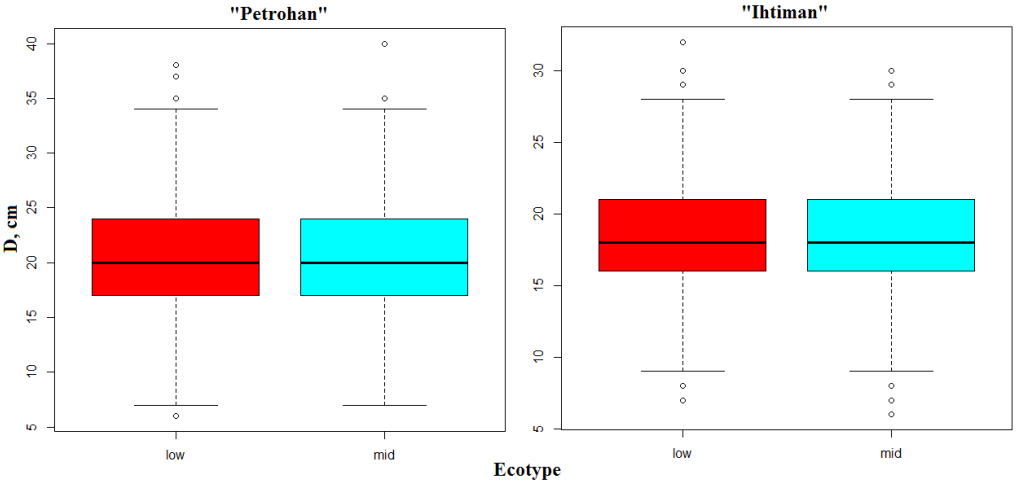

Fig. 9. Box-plots on the growth in diameter depending on the ecotype of origin.

Table 3. Analysis of variance and post-hoc test on the influence of the ecotype of origin.

ANOVA "Petrohan"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	1	3	2,921	0,133	0,716
Residuals	2499	54970	21,997		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA "Ihtiman"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	1	56	55,76	4,668	0,0308
Residuals	5273	62987	11,95		

LSD test "Ihtiman"

	Mean	CV	Mserror			
	18,44152	18,7413	11,94518			
$parameters						
	Df	ntr	bonferroni	alpha	test	name.t
	5273	2	1,960414	0,05	bonferroni	Ecotype
$means						
	Diam	std	r	LCL	UCL	Min	Max
low	18,5664	3,586579	2131	18,41963	18,71318	7	32
mid	18,35687	3,364938	3144	18,23603	18,47771	6	30
$groups							
trt	means	M					
1 low	18,5664	a					
2 mid	18,35687	b					

holds all three types including the "rocky" edaphotype, although in actual terms this is only one provenance – Teshel. No significant differences on performance were revealed in Site 1, but highly significant variance was determined for "Ihtiman". There, "rocky" and "calcareous" soil types show significantly better performance than the "silicate" soil type, having also in mind that the "rocky" type is in fact also "calcareous" in its essence (Fig. 10 and Table 4).

The complex picture for the offspring showing shall be completed with the data on stem quality and the occurrence of different deficiencies resulting from various biotic and abiotic factors. These deficiencies include among all bifurcation and cur-

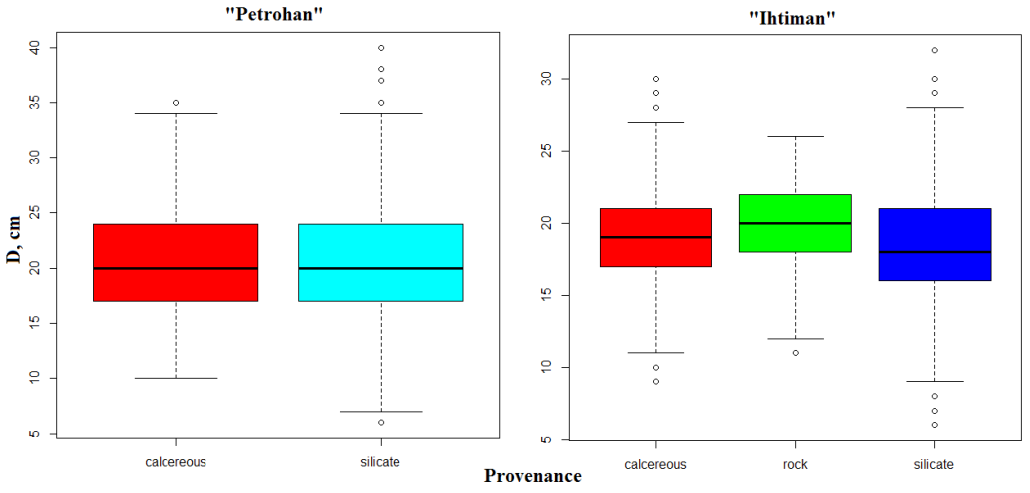

Fig. 10. Box-plots on the growth in diameter depending on the parent material of origin.

Table 4. Analysis of variance and post-hoc test on the influence of the parent material of origin.

ANOVA "Petrohan"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	1	3	3,076	0,14	0,708
Residuals	2499	54970	21,997		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

ANOVA "Ihtiman"

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Seed source	2	474	237,22	19,99	2.25e-09 ***
Residuals	5272	62568	11,87		

LSD test "Ihtiman"

	Mean	CV	Mserror				
	18,44152	18,68068	11,86803				
$parameters							
	Df	ntr	bonferroni	alpha	test	name.t	
	5272	3	2,394744	0,05	bonferroni	Soil	
$means							
	Diam	std	r	LCL	UCL	Min	Max
calcereous	18,79108	3,220022	1704	18,62747	18,95469	9	30
rock	19,50467	3,291997	107	18,85178	20,15757	11	26
silicate	18,23672	3,554854	3464	18,12197	18,35147	6	32
$groups							
trt	means	M					
rock	19,50467	a					
calcereous	18,79108	a					
silicate	18,23672	b					

vatures but also breakages and bending. Data for "Petrohan" trial and the influence of wet snowfalls have been reported by the authors in a previous survey (Bardarov and Milev 2015). "Ihtiman" trial records better percentages of the normally developed trees – 74 % (as opposed to "Petrohan" where the share is 66 %), highest for Teshel 3 plot – 100 % and lowest for Hristo Danovo 4 plot – 38 %. Aggregated per provenance, best showing have Teshel and Vodenicharsko while Ploski, Orehovo and Momchilovci the worst – just short of 70 %. Observed shortcomings are also common for the "Ihtiman" trial but again with better values than for Site 1. Bifurcation, attributed to attacks of *Rhyacionia* (*Evetria*) *buoliana* Schiff. in previous studies of the plantation is common, with around 15 % on average for the different provenances, mostly occurring in Hristo Danovo provenance – 21.5 % and least for Teshel – 8.4 %. Curvatures are around 16 % which is common for both trials. Of the other observed indicators it is worth mentioning the occurrence of trees with strongly developed side shooting – mostly common for the provenances of Orehovo (6.0 %), G. Izvorovo (4.8 %), Hristo Danovo and Ploski (4.6 %).

Discussion

Differences in growth, mainly early, among *Pinus nigra* provenances have been almost always observed in trials (Wheeler et al. 1976, Zahariev 1983, Alptekin 1986, Varelides et al. 2001, Gökdemir et al. 2012), with different results depending on the sites. Consequently, judging by early growth, different provenances should be selected for different environments, depending on the soil and climatic conditions of the site (Varelides et al. 2001). Statistically significant differences in growth (judged by the growth in diameter) were also observed in the two trials subject to our research even at an age higher than the reported in literature. Three and five

groups of behavior are formed in the respective sites but provenances doing statistically better in one site do not maintain their better performance in the other trial. One exception is Hristo Danovo provenance which is lowest ranked in both plantations. The varying results per site, in general confirms that the site-provenance interactions are prevailing, which has often been attributed to the broad natural distribution of the species in a wide range of environments, leading to distinct races. Evidences for this are also apparent within the sites as for example with the difference in the mean height for Goleshevo aggr. (19.5 m) and Petrodan aggr. (17.0 m) which are in fact one and the same provenance but from different locations. Our results come also in support of the suggestion of Nicolic and Tucic (1983) that the differentiation of *Pinus nigra* provenances is possibly due to heterogeneity of environments over the areas of its natural distribution, where gene frequencies are controlled by the local environment through selection.

The existence of clear track record for both trials on the process of selection of provenances and their progenies to be included: from mother trees/ stands selection and their passportization to seed collection, seedlings production and planting, presents some unique opportunities to study the influence of different factors on the offspring showing. One such question is what the influence of the seed source is – be it mother plus trees with desired qualities or seed aggregations from a whole stand but also with targeted quantitative and qualitative features. References on the issue were not found to make more substantiated conclusions, but our study shows that the offspring grown from seeds from mother plus trees show better performance than the offspring derived from seed mixtures and aggregations from a whole stand or forest. This however, shall be taken as a trend as statistically significant differences were recorded only for the “Petrohan”. Taking into consideration the existing information, one area that could be further studied is to reveal the extent to which the characteristic of the mother plants are inherited by the offspring given that the source of the pollen is not known but very likely from the same or nearby stands.

Better performance in early growth of the low-mountain ecotype has already been reported by Zahariev (1983) in another provenance trial, and our research could confirm that in both studied cases, variants representing the low-mountain ecotype show better performance in general than the mid-mountain ecotype. This again shall be taken with some cautious as statistically significant differences are revealed only for “Ihtiman” trial and the fact that there are some distinct exceptions as is the case with Goleshevo (Petrodan) provenance.

The existence of soil ecotypes has been suggested in other provenance trials (Nicolic and Tucic 1983, Varelides et al. 2001) and that it might be possible that performance of the provenances is influenced by the site soil factors and the inherited ability of certain provenances to exploit those factors (Varelides et al. 2001). The analysis of the performance of the three edophotypes described in Bulgarian literature is not straightforward. There, “rocky” and “calcareous” soil types show significantly better (statistically proven) performance than the “silicate” soil type in “Ihtiman”, while the “silicate” type is slightly better but not statistically different for “Petrohan” (where the “rocky” type was not tested). Taking into consideration that both trials are established on a “silicate”

parent material, it could be suggested that "calcareous" type is more prominent in harsher conditions as are the conditions within natural populations where this type occurs.

Conclusions

The complex assessment of the provenance showing in the two trials taking into consideration survival, share of normally developed trees and growth in height and diameter, reveals that no recommendations on the choice of provenance can be safely made, because of the strong site-provenance interaction. However, Goleshevo and Razlog provenances show good performance in both trials and could be recommended. Momchilovci and Gorno Izvorovo also record reasonable values for "Petrohan" as are Kardjali and Borika for "Ihtiman" but these results are strictly specific to the respective site. One thing that is statistically proven is the modest performance of Hristo Danovo provenance so its use should be avoided in plantations in similar conditions.

Observed growth trends reveal that culmination in growth – both in height and diameter comes around the age of 10–15, maintaining relatively good increment until the age of 30, showing signs of decrease afterwards. So this age could be cautiously considered for obtaining reasonable volumes if plantations are to be managed more intensively in shorter rotations in similar conditions.

Proper selection of provenance taking into account the seed source, ecotype and edaphotype may grant of up to 3 times larger growing stock per hectare in more favorable conditions and 1.6 times more in less favorable ones but still not within the limits for survival.

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ROOTING OF NORWAY MAPLE (*ACER PLATANOIDES* L.) CUTTINGS

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Abstract

Four-year-old container Norway maple seedlings were forced in a glasshouse and were shaded. Etiolation of the shoots, as well as banding their base with black electric tape, was used in order to increase the rooting ability of the softwood cuttings. IBA as a 0.8 % powder was used for root stimulation. Both methods and the combination of them resulted in high percentage of rooted cuttings, but those which were only etiolated stroke faster and developed a better rooting system. Further research with mature genotypes can be included in future studies.

Key words: etiolation, banding, donor plants treatment, shading.

Introduction

Numerous techniques of vegetative propagation are described and commonly used in order to provide trees from different species for afforestation or landscaping. It is well known that vegetative propagation has some advantages over sexual reproduction, such as reproduction of the exact same genotype with its preferable qualities (Kolev 1977, Kolarov 1979, Bonga 1982) or faster growth of young plants (Komisarov 1964, Bonga 1982). Propagation by cuttings is an inexpensive, rapid and simple method for improving desirable genotypes of plants, and does not require the special techniques necessary in grafting, layering, budding or micropropagation (Iliev et al. 2010).

The rooting capacity of cuttings is influenced by many factors such as genotype, age, phenological stage, temperature and light intensity etc. (Hartmann et al. 1997, Scaltsoyiannes et al. 2009, Osterc and Štampar 2011). A vital one is the plant species or genotype. It's known that some woody plants are difficult to root, such as those from genus *Acer, Quercus, Carpinus, Syringa, Olea, Prunus* etc. (Maynard and Bassuk 1985, Petridou and Voyiatzis 2002, Scaltsoyiannes et al. 2009). Etiolation and banding could improve the rooting percentage of softwood cuttings from such species (Amissah and Bassuk 2007; Maynard and Bassuk 1985, 1987, 1990; Maynard et al. 1996; Podaras and Bassuk 1996; Sun and Bassuk 1991; Miske and Bassuk 1985). Furthermore, etiolation of stock plants before the harvest of cuttings is claimed to be one of the simplest and the cheapest methods to increase the effectiveness of rooting in stem cuttings (Pacholczak et al. 2016).

Acer platanoides L. is a popular orna-

mental tree with a lot of ornamental cultivars (Krüssman 1984, Dirr 1998, Tomov 2015). It is easily propagated from seeds, but as seedlings show a wide variability in phenotypic characteristics, vegetatively propagated cultivars are preferable in urban areas. Norway maple cuttings are considered difficult to root (Chong and Daigneault 1986, Maynard and Bassuk 1987). For that reason most of the cultivars are grafted onto seedlings origin rootstock (Iliev and Tomov 2017). Furthermore, production of large quantities of grafts is limited by the season, the period for rootstock production and the success depends on the method of grafting and climatic conditions in the field (Krüssman 1984, Dirr and Heuser 1987, Iliev and Tomov 2017). The growing market for ornamental plants with unique ornamental features that are often not transmitted via sexual reproduction and thus have to be propagated vegetatively imposes on growers the need to increase the effectiveness of their production (Pacholczak et al. 2017). The low rooting capacity of *Acer platanoides* makes the propagation by stem cuttings an uncommon method. In order to root cuttings from Norway maple, unpopular techniques such as etiolation and banding need to be applied. The aim of the study is to investigate the effect of those techniques on the rooting ability of Norway maple softwood cuttings.

Materials and Methods

Four-year-old Norway maple seedlings in containers were placed in a heated glasshouse in February and were fertilized with 5 g of Osmocote per plant. After the first signs of bud development were noticed, the plants were covered with PPX foil (letting frequency of the photosynthetic photon flux no more than 0.0925 $\mu mol \cdot m^{-2} \cdot s^{-1}$), until the new shoots reached 5 cm. After that they were gradually uncovered, starting from the north side (Fig. 1). Parallel with the removal of

Fig. 1. Unfolding of the shaded plants, starting from the north side.

the cover the base of the new shoots was wrapped with black electric tape, dipped in 0.8 % IBA powder. In 4 weeks the shoots were cut below the tape, the tape was removed and 10–15 cm long cuttings (Fig. 2) were placed for rooting. The experiment was conducted through the following ways of treatment of softwood cuttings:

Fig. 2. Cuttings from taped shoots after 4 weeks of 0.8 % IBA amplification, just before placement in the substrate.

- Control 1 (non-etiolated, non-taped, without IBA);
- Control 2 (non-etiolated, non-taped, with IBA);
- Etiolated and taped;
- Etiolated, non-taped;
- Non-etiolated, taped.

Within the non-taped treatments the IBA was applied by dipping the base of the cutting in the powder.

All cuttings were placed in a substrate of peat and perlite 1:1 (v/v), following a net scheme 3×3 cm. The experiment was conducted in a glasshouse, at a temperature of 22–24 ºC and relative humidity of 85–95 %, maintained by an automatic mist irrigation system.

The percentage of rooted cuttings, as well as the number and length of the roots were evaluated after 4 weeks. The non-rooted cuttings were placed back in the substrate and evaluated after 4 more weeks.

All methods of treatment variants were done in 3 replications of 15 cuttings. All results were analyzed by Analysis of Variance (ANOVA) and Post Hoc LSD test using SPSS 19.0 (SPSS for Windows 7). Percentage values were transformed using arcsine square root ($\sqrt{p}$) (Compton 1994) to normalize error distribution prior variance analysis.

Results and Discussion

The rooting capacity of the Norway maple softwood cuttings was generally in-

creased by all treatment variants of the donor plants. Moreover, after 56 days there was no significant difference of the rooting percentage between different treatment techniques. The highest percentage (84.4 %) of rooted cuttings was obtained by using non-etiolated taped shoots, however it didn't differ significantly and the number of shoots was lower, as well as no secondary roots were formed (Table 1). Furthermore, the rooting of the taped cuttings was slower than the rooting of the etiolated ones. Probably these differences are associated with the hormone amplification which was done in deferent parts of the cuttings and lasted substantially longer for the taped cuttings. The amplification of high concentration of auxins for a short period of time resulted in faster root formation for other species such as *Syringa vulgaris* L. The beginning of rooting process *in vitro* for all pulse treatments has been observed earlier in comparison with experiments without pulse treatment (Lyubomirova and Iliev 2013).

Table 1. Rooting of *Acer platanoides* cuttings, in relation with treatments of the donor plants and duration of the process.

Treatment variant	Rooted cuttings, %			Number of primary roots	Length of the primary roots, mm	Cuttings with secondary roots, %
Duration of rooting	28 days	56 days	total	28 days	28 days	28 days
Control 1	15.5 ± 2.3 a	28.4 ± 4.5 bc	44.5 ± 2.2 b	2.9 ± 0.6 a	34.2 ± 2.9 bc	27.8 ± 14.7 ab
Control 2 (IBA applied)	4.4 ± 4.4 a	9.2 ± 2.1 a	13.3 ± 3.9 a	3.5 ± 2.5 a	33.3 ± 6.8 abc	0.0 ± 0.0 a
Etiolated, non-taped	62.0 ± 5.9 c	18.0 ± 9.7 ab	80.0 ± 3.9 c	10.9 ± 1.0 b	21.2 ± 1.0 a	47.5 ± 18.4 b
Etiolated, taped	42.3 ± 2.3 b	37.7 ± 6.0 c	80.0 ± 3.9 c	4.3 ± 0.5 a	36.5 ± 3.0 c	53.1 ± 15.3 b
Non-etiolated, taped	51.3 ± 8.1 bc	33.1 ± 4.0 bc	84.4 ± 8.0 c	2.7 ± 0.4 a	29.8 ± 2.9 b	0.0 ± 0.0 a

Note: Mean values ± standard error of mean, followed by the same letter are not significantly different with level of significance ($p \leq 0.05$).

The etiolated non-taped cuttings (Fig. 3) formed highest number of roots (10.9 ±1.0). All the other ways treatments, including the controls, resulted in number of primary roots between 2.7 and 4.3, and didn't differ significantly from each other (Table 1).

The roots of etiolated and taped cuttings (Figs. 3B and 4) were longest. However, this index did not differ significantly from the controls (Table 1).

Girdling the shoots and auxin amplification is a similar rooting technique to the taping, which also gave good results with difficult to root species such as olive, walnut, wild cherry (Petridou and Vogiatzis

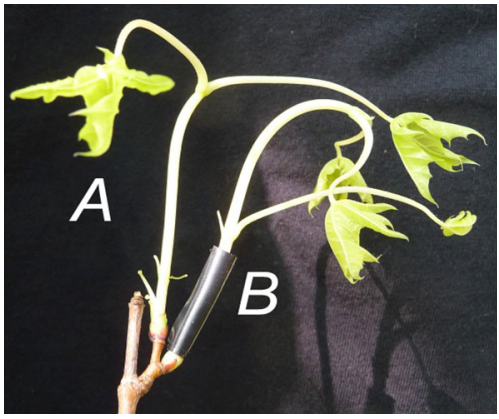

Fig. 3. Etiolated non-taped (A) and etiolated taped (B) shoots (cuttings) before placement in the substrate.

Fig. 4. Rooted cutting from etiolated and taped shoots.

1994, Tsoulpha and Scaltsoyiannes 1998, Scaltsoyiannes et al. 2009). Root initiation is promoted by root promoting substances, e.g. carbohydrates, amino acids, hormones, and various phenolic metabolites produced at the apex, which are accumulated above each girdling point, as suggested by many researchers (Stoltz and Hess 1966, Kozlowski 1971, Tsoulpha and Scaltsoyiannes 1998). Taping and the amplification of auxin caused similar changes at the taped point, which resulted in callus formation and subsequently to root formation (Fig. 2).

The effect of etiolation on the rhizogenic ability of the shoots has been proved for some tree species such as *Carpinus betulus* L. (96 %), *Castanea mollissima* Bl. (100 %), *Quercus coccinea* Muenchh. (46 %), *Quercus palustris* Muenchh. (50 %) and *Quercus rubra* L. (50 %) (Maynard and Bassuk 1985). Furthermore, shading of dogwood (*Cornus alba* L.) cultivars stock plants before harvest led to a statistically significant increase of rooted cuttings by 6–8 % (Pacholczak et al. 2017).

For *Acer platanoides* L. the best reported result is 75 % rooting of etiolated cuttings (Bassuk et al. 1986). The results of this experiment are identical with the findings of Bassuk et al. (1986) and confirm the positive effect of complete shading of the donor plants or different parts of the shoots (taping) on the rooting ability of the cuttings.

It is considered that this effect is caused by the amount of endogenous auxin in the etiolated tissues, but the conclusions are contradictorily (Bertram 1992). Tillberg (1974) reported that concentration of endogenous auxins in the *Phaseolus vulgaris* L. plants grown in a normal light is higher than in the etiolated plants. But there wasn't any change in the levels of concentration after exposure of the etiolated plants to light. However, Kawase and Matsui (1980) argue that etiolation doesn't affect the levels of endogenous auxin in the *Phaseolus vulgaris* tissues.

Some authors announce the hypothesis that the reason for increase in rhizogenic ability of the etiolated plants is not higher level of auxin, but higher sensibility of the cells in the etiolated tissues (Eliasson 1980, Maynard and Bassuk 1988). Anatomical investigations proved high presence of unidentified parenchymal

cells in the etiolated tissues. Those cells are considered transient in formation of adventive roots (Maynard and Bassuk 1988). Although many studies proved the role of endogenous phytohormones in physiological stress response, still little is known about the absorption of exogenous phytohormones in plant tissues, how they affect cell differentiation, and the correlation between the effects of exogenous phytohormones in organogenesis and the changes in endogenous phytohormone levels (Golovatskaya and Karnachuk 2007, Zhang et al. 2012, Djilianov et al. 2013, Koike et al. 2017).

Conclusions

Etiolation combined with taping the base of the shoot with black tape dipped in 0.8 % IBA powder, only etiolation and only taping are good methods for increasing the rooting ability of softwood Norway maple cuttings. All these techniques give opportunities for successful vegetative propagation of the species. The investigation of the effect of the used methods and some others (girdling for example) on mature genotypes or cultivars of Norway maple can be subject of further studies.

Concerning practice, the combined treatment of the donor plants is not a lucrative choice, because it's more labour-intensive and expensive. Therefore, etiolation and application of IBA by dipping the bottom of the cutting in powder is the preferable technique. It provides high rooting percentage in shorter period of time and better root system. If it is difficult to apply that method due to the size of the donor plant, taping the base of the shoots could be applied instead.

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SEED PRODUCTION AND MASTING BEHAVIOUR IN ORIENTAL BEECH (*FAGUS ORIENTALIS* LIPSKY) FORESTS OF NORTHERN IRAN

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Abstract

Mast-seeding behaviour of tree species has a key role in regeneration of plant populations. Seed production data were collected from 360 sampling plots to identify environmental factors influencing beech masting in the old-growth beech stands in the north of Iran. The mean seed number per m^2 was 4687 ± 164 in the study sites. Seed production significantly correlated with tree diameter, height and crown diameter. In relation to the site properties the highest seeding recorded was close to the parent in the lower altitude and north facing slopes. The canonical correspondence analysis revealed that seed production correlated with first canonical axis, reflecting the soil nutrition including N, C, Cu, Ca, Fe, and Br. Results revealed that beech masting is affected by the amount of organic C, P, Fe, and Ca content of soil. Seed production varied from tree to tree, and site to site, and quantitative analyses in relation to both environmental factors and seed tree dimension showed a highly complex interplay of factors dictating seed production across the forest stands.

Key words: beech nuts, natural regeneration, *Fagus orientalis*, seed size.

Introduction

Masting (mast seeding), defined as the episodic production of large seed crops by a plant population at intervals of years (Kelly 1994), is observed in many tree species. It is expressed synchronously over the subcontinent (Western Asia), in a pattern occurring only once every few years (Koenig and Knops 2000, Fischer et al. 2016). Several hypotheses have been proposed to explain the evolution of large seed crop production. These hypotheses include increased pollination efficiency, seed predator satiation and potential impact of selection on seed size (Nilsson and Wastljung 1987, Sork 1993). Nut-producing trees (e.g. Fagaceae) produce abundant and highly nutritious seeds, which are an important food source for many forest vertebrates (Jensen and Nielsen 1986, Ouden et al. 2005, Övergaard et al. 2007). Nuts are also heavy seeds that need biotic agents (animals) to be dispersed, and, thus, have developed certain traits to attract seed-dispersing animals (Vander Wall 2001). Successful seedling recruitment following mast year is critical for some species (Negi et al. 1996, Akashi 1997, Alvarez-Aquino and Williams-Linera

2002, Wagner et al. 2011).

In most forest trees the regeneration potential is determined by the frequency of mast years (Taylor and Aarssen 1989). The species of Fagaceae are well known for their irregular production of nuts (Matthews 1955, Nilsson and Wästljung 1987, Hilton and Packham 1997). Masting and production of large seed crop is common in *Fagus grandifolia* Ehrh. in North America (Canham 1990, Walters and Reich 1996, Alvarez-Aquino and Williams-Linera 2002), *F. sylvatica* L. in Europe (Nilsson and Wastljung 1987, Peltier et al. 1997, Övergaard et al. 2007, Drobyshev et al. 2010), *F. crenata* Blume in Japan (Igarashi and Kamata 1997, Suzuki et al. 2005), and *F. orientalis* Lipsky in Iran (Etemad and Marvi Mohajer 2004).

Fagus orientalis Lipsky is the dominant species in the Caspian forest region in Northern Iran and covers about 1,000,000 ha (Knapp 2005). Masting behaviour had been reported previously for this species (Etemad and Marvi Mohajer 2004). Beech forests as productive and commercial were far from human manipulation in long-term history, so have a high ecological and conservation value.

Studies in Oriental beech forests indicated that the structure of such undisturbed beech stands are more or less irregular and uneven-sized (Sagheb-Talebi and Schütz 2002, Sefidi 2012) and that natural regeneration normally occurs in small gaps (Delfan Abazari et al. 2004, Esmailzadeh et al. 2011, Sefidi et al. 2011). The relic forests it in the north of Iran remain relatively stable and far from large scale disturbances since Tertiary (Knapp 2005). These remaining areas provide a valuable reference for studying temperate deciduous forest ecosystem dynamics, and the silvicultural options for beech forests in general.

Nowadays, management of old-growth forest is a crucial challenge for forest managers in Iran and in other countries.. The successful establishment of commercially valuable beech trees is important their successful long-term forest management. In forest dominated by shade tolerant species much of this regeneration will be recruited in canopy gaps (Drößler and Von Lüpke 2005, Nagel et al. 2010, Sefidi et al. 2011). Knowledge of seed production, dispersal, phenology, and conditions needed for successful regeneration is fragmentary or lacking for oriental beech. Information about masting behaviour and seed production of mature beech trees can be considered as most important for beech stands in close to nature forestry in the north of Iran.

To address this knowledge gap and to advance our understanding of beech masting and seed production in a temperate deciduous forest of Northern Iran, we analysed records of regional masting from three study areas in beech stands (Fig. 1). We formulated the following objectives: (1) to identify whether seed tree variables are related to seed production; (2) to identify main environmental variables controlling seed production of beech.

Material and Methods

Study area

This study was conducted in the forest of Mazandran province in the north of Iran. It is located at latitude from 36°58’ N to 35°35’ N and longitude from 54°8’ E to 51°21’ E. Three study sites were selected in this region, including: Band, Bon forest (BS) in the western part of this province, Patom forest (PS) in its center, which is

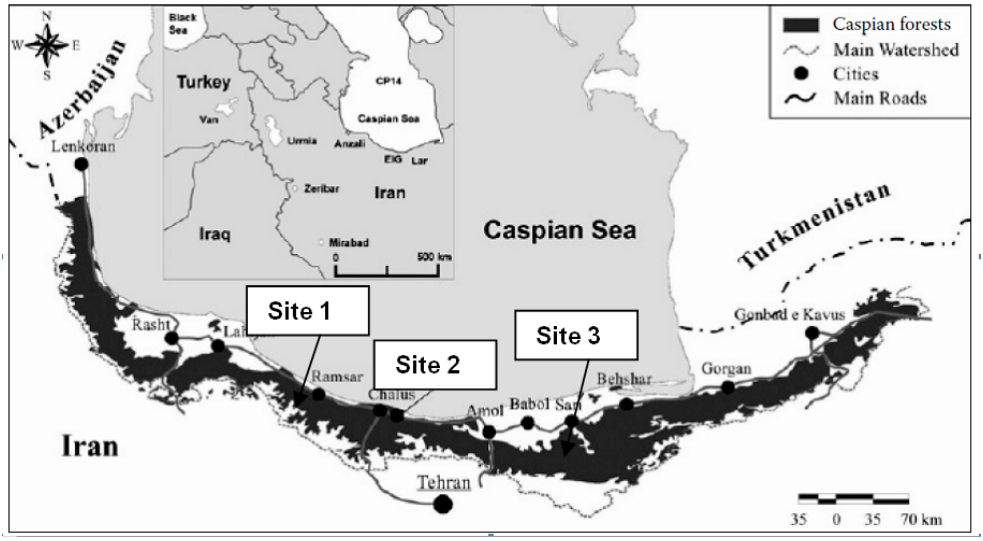

Fig. 1. Distribution of Caspian forest in the north of Iran (Modified according to Knapp 2005) and three selected study sites.

managed by the University of Tehran as an Experimental forest, and the third one was Tolenchal forests (TS) located in the east of the province (Table 1). PS has a long term management history, but BS and TS are unmanaged and undisturbed area.

Table 1. Location, dominant overstory species, and canopy height of the three *F. orientalis* – dominated forest stands from Northern Iran.

Study Site	Site code	Location	Dominant trees	Management history
Patom	PS	36°38' N, 34°51' E	*Acer velutinum – Carpinus betulus*	Managed
Bandbon	BS	36°52' N, 50°37' E	*Fagus orientalis – Carpinus betulus*	Unmanaged
Tolanchal	TS	36°22' N, 53°38' E	*Fagus orientalis – Carpinus betulus*	Unmanaged

The climate is sub-Mediterranean with a mean annual temperature of 9 °C and total annual precipitation of 1380 mm. Mature forests are dominated by beech, which has an average volume of 187 $m^3 \cdot ha^{-1}$, representing 74 % of the total stand volume. Structurally, these forests have high volumes of coarse woody debris with mature forests averaging 51 $m^3 \cdot ha^{-1}$ (Sefidi et al. 2013). According to the Habibi Kasseb (1992), the most important soil types in the Hyrcanian region are Brown, Alluvial, Rendzina, Colluvial, Rankers and Lithosols.

Each location plot was selected to satisfy the following conditions: (1) the plot should be placed in the central part of the forest to prevent edge effects; (2) the plot should be located in sufficient distance from former wind throw and managed area; and (3) the plot should be homogenous regarding the slope.

Field sampling and sample preparation

Seed collection of the samples was done

in September 2002 and seedling emergence and survival was recorded from April 2003 to June 2004.

In the study area, in order to recognize seed production and related environmental factors, three parallel transects were established in east-west direction within each selected site, starting from randomly chosen points. All Oriental beech seed trees with diameter at breast height (DBH) >50 cm that intersected transect were selected. Transects were separated by 50 m interval to avoid duplicate sampling. Their length varied depending on the extent on homogeneous forest structure and stand density. Measurement continued by recording 30 seed trees in each transect.

Intersected trees in a transects were considered as a sampling plot. Around the sample trees four 1 m^2 wide rectangular micro plots were laid out in different directions, as illustrated in Figure 2. The length of seed traps varied depending on productive tree's crown length and area covered by crown. Micro plots were separated into two equal sections in order to record seed production and germination. Data collected were from 360 micro plots in each study site. We also recorded density of beech nesting in different distances from seed trees and found different patterns in altitudinal categories.

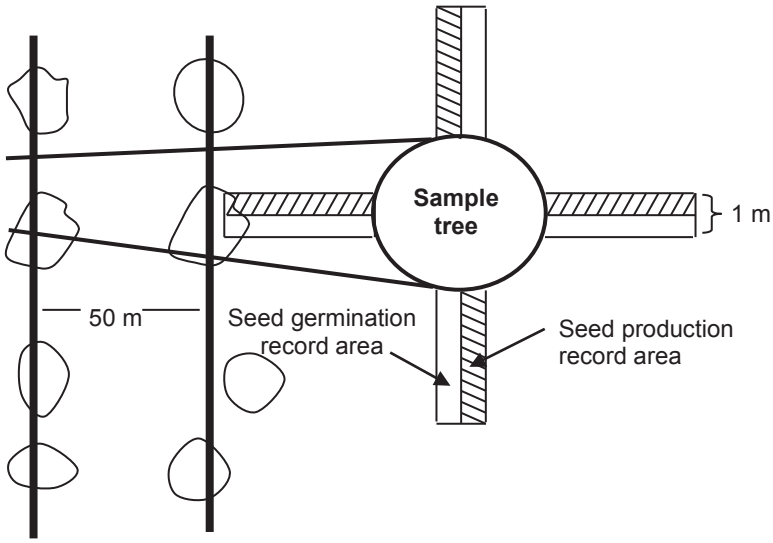

Fig. 2. Seed traps experiment and sampling design of seed traps around productive trees.

In the laboratory, these samples were divided into flowers, seeds, leaves, branches and other material. After identification of beech seeds, they were counted and classified into five categories: (1) viable, having sound viable cotyledons; (2) immature, having no or undeveloped cotyledon; (3) insect damaged, suffering damage by insect larvae; (4) mouse damaged, suffering damage by rodents or birds; and (5) decayed. The term 'seed' is used throughout this paper in a broad sense as being a single dispersal unit usually containing a single embryo.

In laboratory after seed blowing some seed properties were measured including: seed moisture content, weight of 1000 seeds, physical purity, seed size (length),

germination and vigour. Moreover, in sampling plots other important environmental factors influencing seed production such as dominant tree species, crown canopy coverage, slope steps and aspect were recorded. Seed germination analysis was conducted in the laboratory. Four hundred seeds forming four replications were planted in recommended substratum for germination after dormancy breaking using vernalization treatment. Number of seedlings emerging daily was counted from the day of planting. Also to assess seed dispersal, we sampled seeds in seed traps located in 1 m intervals in different aspects and slope steps.

Soil samples were collected in the study sites. The soil texture and proportion of clay silt and loam were measured by hygrometry method. Large live plant material (root and shoots) and pebbles in each sample were removed by hand in the laboratory and discarded. Then, air-dried soil samples were sifted with aggregates broken up to pass through a 2 mm sieve and roots removed prior to chemical analysis. Samples were dried at 608 °C to 708 °C for 18 h, and organic C concentration at 0–15, 15–30 and 30–45 cm soil depths using a core soil sampler with an 81 cm^2 cross section was determined (Rahmani and Zare Maivan 2004). Total N was determined using the Kjeldahl method. The kinetics of nitrogen mineralization for 100 g of each soil sample was measured using a laboratory incubation procedure under controlled conditions (Robertson et al. 1999). In order to quantify organic P that becomes soluble by extraction with 1 M HCl (which is expected to remove Al and Fe) we used a second extraction with 0.1 M NaOH to obtain 'occluded' P (Condron et al. 1996).

Analysis

Data followed a normal distribution (Shapiro–Wilk W test, $P > 0.05$) and therefore, differences in seed production between the quantitative properties of seed trees such as diameter at breast height (DBH), tree height, crown diameter and height, and some environmental factors were examined by one-way Analysis of Variance (ANOVA).

Spearman's rank correlation coefficient was used to test the relationship between seed production and seed tree DBH, tree height and crown height. The statistical tests were conducted at a significance level of $\alpha = 0.05$.

To investigate the relationships between seed production and environmental parameters, we used a multivariate approach. Multivariate analyses were performed using CANOCO version 4.5 (ter Braak and Smilauer 1998). Principal component analysis (PCA) was used to assess the gradient length of seed crop data for each of the environmental factors. It is mostly used as a tool in exploratory data analysis and for making predictive models. PCA is a mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linear uncorrelated variables called principal components.

Results

The mean of seed production and 1000-seed weight in the study sites were 4688 ±164 and 182.6 ±1.3 g, respectively. Descriptive statistics of seed production is shown in Table 2.

Table 2. Descriptive statistics of beech seed production, n·m^{-2}.

Seed type	Mean	Median	Max	Min
Viable	493.93 ±26	166	6554	0
Immature	452.9 ±26	200	7652	0
Insect predation	182.3 ±10	75.5	3272	0
Other predation	2081.2 ±105	601	2470	6
Total	4687.7 ±164	2395	33472	82

Seed production significantly varies within three study sites (P < 0.000, F = 58.86). The highest number of seeds was counted in TS (914.2 m^{-2}), the lowest in PS (416.6 m^{-2}) and intermediate one – in BS (424.28 m^{-2}). Figure 3 illustrates the number of seeds per square meter among study sites. The mean of DBH and height of seed trees was 81.53 cm and 33.6 m, respectively. Tree diameter (r = 0.488, P < 0.000) and height (r = 0.468, P < 0.000) were significantly correlated with the seed production. Also, the crown diameter as third variable was significantly correlated with seed production (r = 0.378, P < 0.000).

In relation to the site properties the highest number of seed production was recorded in the lower altitude and north facing slopes. The mean weight (of 1000 seeds) also significantly varied among study sites (F = 26.281, P < 0.000). The highest seed weight was recorded in TS (195.45 ±2.57) and the lowest one – in PS (172.12 ±1.94). BS had middle value of seed weight (178 ±1.95). The mean length of Oriental beech seeds was 14.5 ± 0.05 mm. The results showed that the seed size significantly differed among sites (F = 19.25, P < 0.000, Figs. 3 and 5). Results of ANOVA indicated that seed production in beech trees significantly differs among crown diameter (F = 5.193, P > 0.000, Fig. 4) crown height (F = 9.025, P > 0.000) and crown area (F = 4.044, P > 0.000) classes. Also DBH (F = 6.248, P > 0.000) and the height of seed trees (F = 11.057, P > 0.000) had significant impact on seed production.

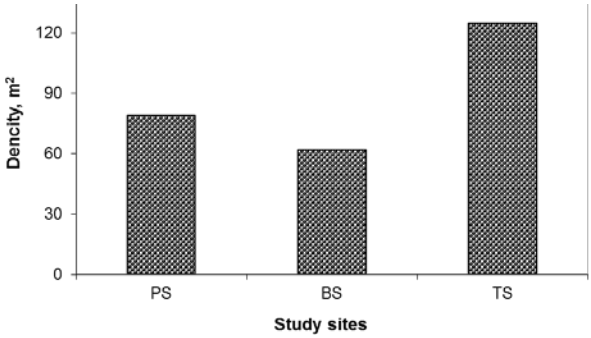

Fig. 3. Seed production in the study sites.

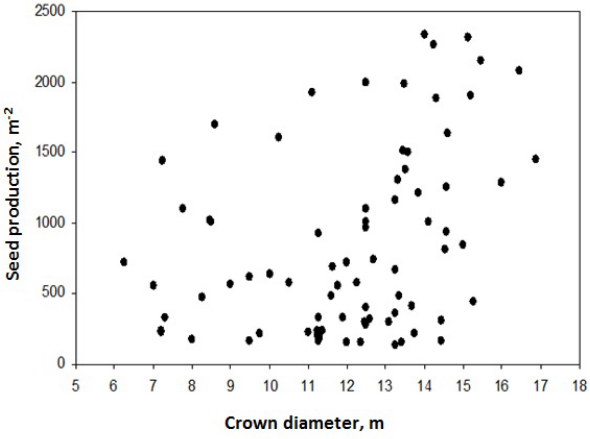

Fig. 4. Relationship between seed production and tree crown diameter.

Seed quality and dispersal

The quality of seed was analysed in three study sites. The mean number of viable seeds in study sites was 493.93 ±26 per

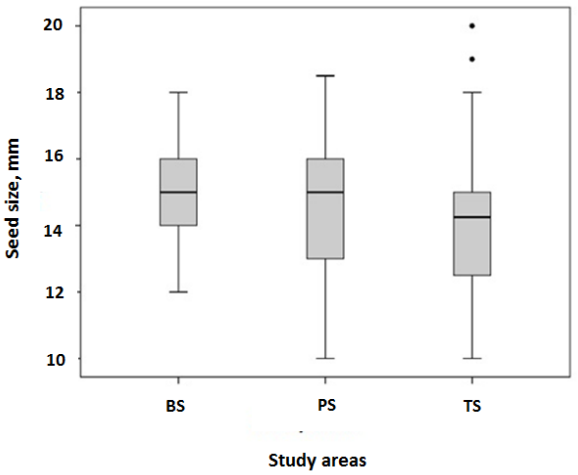

Fig. 5. Box plot of seed size in three study sites.

square m. Table 3 show results of ANOVA for the three study sites. The highest amount of viable seeds was recorded in BS.

Table 3. Results of one way ANOVA of study sites.

Source of variation	df	*F*	*P*
Viable	2	69.85	< 0.000
Immature	2	45.48	< 0.000
Insect predation	2	40.35	< 0.000
Other predation	2	13.55	< 0.000
Total	2	58.82	< 0.000

Results of seed germination analysis revealed significant difference between study sites. The highest amount of germination was recorded for seeds from the middle elevation (around 1500 m). The results of ANOVA showed that elevation categories had a consistent significant effect on seed germination ($F = 164.31$). In BS 36 % of the seeds germinated. In PS and TS this amount was 40 and 41 %, respectively. Mean number of seedlings in TS (9.8 m^{-2}) was significantly higher than in the other two sites.

We also recorded density of beach nesting in different distances from seed trees; according to our results we have different patterns in altitude categories. Beech seeds in uplands are found close to the seed trees. Increasing the distance from seed trees caused decrease of the amount of beech seeds in all study sites (Fig. 6).

Soil properties

Seed production moderately correlated with the soil C ($r = 0.519$, $P > 0.021$), N ($r = 0.686$, $P > 0.021$), Cu ($r = 0.321$, $P > 0.061$) and Fe ($r = 0.121$, $P > 0.012$).

Multivariate PCA showed that the first axis (which accounted for 24.0 % of the total variation) was represented in horizontal structure and heterogeneity of vegetation. The second component (43.4 %) represented increasing vertical heterogeneity.

Of micro- and macroelements studied, percent content of K in the soil showed different impact on the seed production in comparison to N, C, Cu, Ca, Fe, and Br. Results revealed that beech masting was affected by the amount of C in soil, soil depth and P, Fe, Br, and Ca content (Fig. 7).

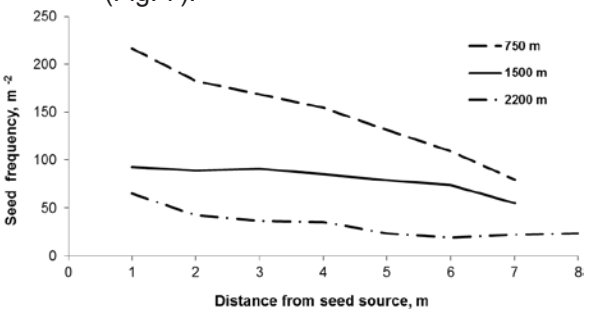

Fig. 6. Change in the seed frequency by distance from seed trees in different elevation.

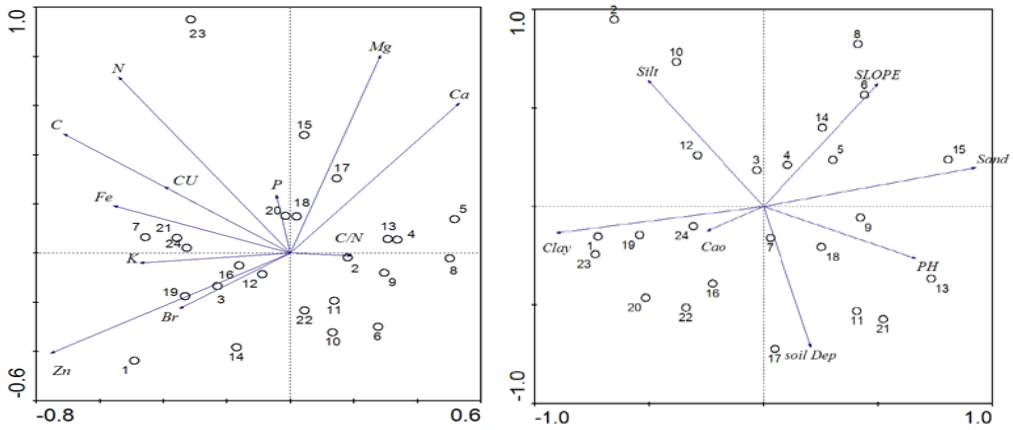

Fig. 7. Seed production-soil properties biplot diagram from PCA with soil related variables including nutrients (left) and other soil properties (right).

Discussion

This research highlighted key parameters influencing masting of *F. orientalis* in the unmanaged and managed natural forests in the north of Iran. Our analysis of the most regional dataset of beech seed production suggested that tree dimension, including height and diameter, crown diameter and some environmental variables such as altitude and nutrient availability in the soil were the most important factors influencing beech seed production.

Quantitative analyses of seed production showed that 10.5 % of nuts were viable and it seems this percent cannot provide suitable seed bank for successful regeneration. Total 4687.7 ±164 seed per unit of ground area were counted in the study sites, but 20,263 seeds were predated that equals to 48.2 % of total seed production and is higher than the values reported for *Fagus crenata* stands (Kon et al 2005; 48 %). However, in some studies 70–100 seeds per m^2 were reported as sufficient seed bank for the sustainable regeneration of beech (Osward 1983). Beech, as shade tolerant species produces few and heavy seeds that disperse locally by gravity. This can be advantageous for seedling growth in vicinity of the parent trees. Cutini et al. (2013) reported significantly lower seed masting in beech than in the other tree species like Turkey Oak (*Quercus cerris*) and Chestnut (*Castanea sativa*) in Italian broadleaved forests. The widely accepted evolutionary advantages of masting behaviour are associated with seed-predator satiation and improved pollination (e.g. Kelly 1994, Shibata et al. 1998, Koenig and Knops 2000). High immature seed record in the study area could be due to unsuccessful pollination in the spring. Late spring frost observed in the study area affected pollination and damaged seed quality. Male and female flowers of beech are vulnerable to spring frosts (Young and Young 1992).

Piovesan and Adams (2001) reported that sufficient seed production in three beech species depends on climatic conditions during two consecutive years before masting year. The best predictors were the unusually moist and cold summer two years before masting, favouring carbohydrate build-up, and the early sum-

mer drought in the next year. According to the authors (Piovesan and Adams 2001) there is a close relationship between masting (mast year) and preceding growing season climate events in Eastern North America and Europe. The high temperature seems to be a climatic factor controlling the seed crop in Oriental beech forests – this fact was emphasized also by similar study in Japan (Masaki et al. 2008). They demonstrated that mainly two types of climatic signals cause mast seeding of *F. crenata* in the subsequent year: higher temperature in summer and lower temperature in spring.

As we expected, in the study area tree dimensions, including diameter, height and crown diameter were significantly correlated with the seed production. Ammer et al. (2002) reported that seeding of beech was directly influenced by crown canopy. Forest managers often know about tree age effect on the seeding that is caused by some physiological changes. Seed production occurs during the mature phase in which flowering occurs. Maturation or adult phase first appears at the top of crown as tree gets older and increases crown size (Barnes et al. 1998), so existence of positive and significant relation among tree crown and seed production is expectable. In the same way, *F. sylvatica* studies showed seed production relates to tree diameter (Wagner 1999). Seed production and flowering begins at about the age of 40 to 50 for *F. sylvtica* (Young and Young 1992, Kon et al. 2005) and about at the age of 60 in *F. orientalis* with masting every 3 to 18 years (Mirbadin and Namiranian 2005). In the same study in the north of Iran, some evidence was reported about strong dependence of flowering and fruiting on climate and site conditions (Etemad and Marvi Mohajer 2004).

The highest number of seed production was recorded in the lower altitude, and north facing slopes. *F. orientalis* dominates in the northern aspects of Elborz mountain, so high number of seeds was recorded in the north facing slopes. The highest amount of germination was recorded for seeds from the middle elevation (around 1500 m). Moreover, seed germination significantly varied among different elevation classes. In the same study within mixed beech stands seed production correlated with soil nutrition, northern aspects, and an altitude of around 1500 m. Seeds also had greater size and weight in stands of 750 to 1500 m (Etemad and Marvi Mohajer 2004). Several studies have reported a relationship between mast occurrence and physiographical characteristics of site in other types of forests (Masaki et al. 2008, Ayari et al. 2011). Topographic gradient, particularly in mountain ecosystems, is an important factor influencing plant growth. Site condition and resource availability varies across topographic gradients, so forest sites with different topographical gradients support different life history characteristics for individual tree species. Moreover, moisture availability and daily insolation are hypothesized to be major factors affecting plant distribution and growth. These variables are affected by several topographic characteristics such as slope aspect, position, and steepness. North-facing slopes, lower-slope positions, and low degree of slope often characterize mesic site conditions, while southern aspects, upper-slope or ridgetop positions and steep slopes often support xeric sites.

Soil analysis indicates that beech masting is affected by the amount of organic C in soil, soil depth, P, Fe, Br, and Ca content. Soil nutrition effect on seed production and density of regeneration was shown in the European and Hyrcanian

beech forests (Modry et al. 2004, Etemad and Marvi Mohajer 2004). In terms of dry weight, most seeds consist of more than 90 % storage reserves. The nutrient reserves contain carbohydrates, lipids (usually triacyl glycerides) and specialized storage proteins. These storage reserves are of critical importance for germination and especially, when combined with different micro- and macronutrient. In the pine forest results showed that the density was the highest whereas soil clay content and pH was lowest (Puhlick et al. 2012). Effects of soil P on pollen production was demonstrated previously (Lau and Stephenson 1994). This study showed that growing conditions such as soil P availability can influence the size of a pollen grain and its chemical composition, which, in turn, can affect its ability for successful pollination and mature seed production. Agnes and Partick (1997) showed that deficiency of Fe and Br cause decrease of the transmission of hydrocarbons and delay in flowering, which resulted in poor seed crop. Furthermore, ability of seed production is affected by some physiological processes, which are dependent on soil nutrient availability. We can conclude that seed production varies from tree to tree and site to site. Quantitative analyses of seed production in beech trees showed relation to both environmental factors and seed tree dimension, which reflects a highly complex interplay of factors shaping the seed production in the studied forest stands.

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DISTRIBUTION AND DENSITY OF COYPU (*MYOCASTOR COYPUS* (MOLINA, 1782)) IN DOWNSTREAM OF MARITSA RIVER SOUTHEAST BULGARIA

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Abstract

The distribution and density of Coypu were researched in the downstream of the Maritsa River. Twenty-four new localities were found, twenty of them are situated along the Maritsa River. Three more new localities were found in ponds near the researched area. The average density was 3.4±2.3 (0–8 ind./9.6 km) – 0.35 ind./km. The average size of the social groups was 1.6±0.9 (1–4 ind.; *n*=42) from the Maritsa River and 2.4±0.99 (1–4 ind.; *n*=12) in the other three new localities in the ponds. An invasion of Coypu in the downstream of the Maritsa River is observed.

Key words: aquatic habitat, aquatic rodents, density, invasion species, nutria, size of social groups.

Introduction

The Coypu (*Myocastor coypus* (Molina, 1782)) is a large semi-aquatic rodent that lives along rivers, lakes, marshes, and other wetlands. Coypu is native in South America, but is tolerant to different aquatic habitats and has a high capability of dispersion and colonization (Carter and Leonard 2002, Bertolino et al. 2005). One of the reasons for the prevalence is the valuable fur and control of aquatic vegetation (Carter and Leonard 2002). It is included in the list of the 100 World's Worst Invasive Alien species (Bertolino 2009). Nutria is common in many countries in Europe, where it was also farmed. The species were introduced in the most European countries in the middle of last century (Aliev 1967, Velatta and Ragni 1991, Herrero and Couto 2002, Carter and Leonard 2002). During the period 1948–1968 Nutria is established outside in farms in Greece (Aliev 1967). Subsequently, there are numerous sights of the species in the wild in northern Greece (Mitchell-Jones et al. 1999). In the European part of Turkey it was established in 1994 (Özkan and Kurtonur 1994) and the distribution in the downstream of the Maritsa and the Tundzha Rivers increased until 1999 (Özkan 1999).

Coypu was introduced in Bulgaria in lake Mandrensko and Arkutino Natural Reserve, at the southern Black Sea Coast in 1953 (Peshev et al. 2004). Inland, it was reported only from the Maritsa River, near the city of Plovdiv (Popov and Sedefchev 2003), and from the river of Sazliyka, at the drainage basin of the Maritsa,

near Radnevo town (Myhajlov and Stoyanov 2001). A new locality was found in the downstream of the Maritsa River by Gruychev (2012). In 2015–2016, increasing of distribution of Coypu was reported in many parts of Bulgaria and downstream of the Maritsa River (Tsekova and Georgiev 2016).

The aims of this study are to present new information about the distribution of Nutria along the downstream of the Maritsa River (Natura 2000, BG 0000578), as well as the density and the size of the social groups in that area.

Materials and Methods

Study area

The researched area is localized in the south-eastern part of Bulgaria (Fig. 1). It covers the banks of the Maritsa River, between the towns of Simeonovgrad and Svilengrad. To the south, it touches the state border between Bulgaria and Turkey. The average annual temperatures are between 8 and 13.5 °C. The annual rainfall is between 500 and 800 mm, with

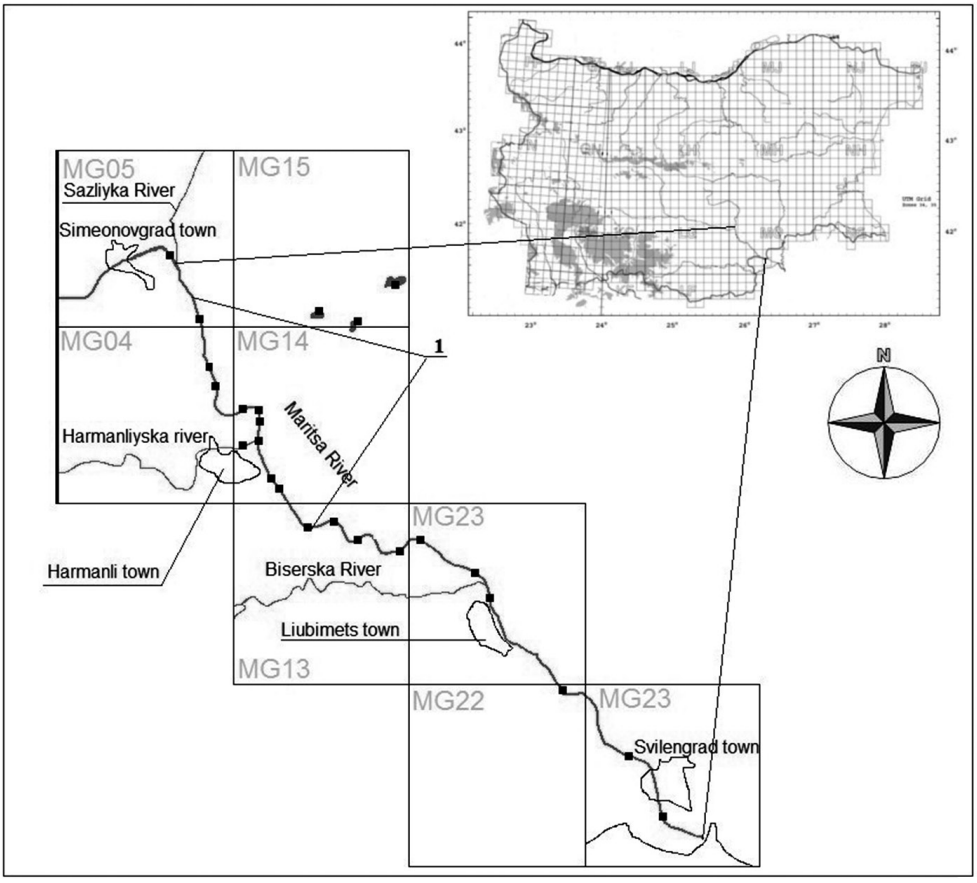

Fig. 1. Study area, new locality and distribution of Coypu (*Myocastor coypus*) in downstream of Maritsa River SE Bulgaria (1 – sample area, including transects to determine the density and size of social groups).

maximum winter and summer-autumn minimum. The snow cover lasts least compared to all other areas in Bulgaria. The average annual flow of the Maritsa River in Svilengrad is 110 m^3/s (Kopralev 2002). That study area is included in the protected zones of Natura 2000 (BG 0000578) by Directive 09/147/EEC on the protection of natural habitats.

Field methods

The distribution of Coypu in the research area was established by standard methods for aquatic mammals adapted for study area (Krebs 2004). The banks of the Maritsa River were observed during the period 2014–2016 between Svilengrad and Simeonovgrad. Searching implemented a walkthrough on the banks of the river. All data from monitored individuals, trails, footprints and burrows were used to determine the distribution of Coypu (Krebs 2004). To increase the success of detection, a dog was used (typical German wirehaired pointer).

A model section of the river banks was chosen to determine the density of Nutria (Fig. 1, 1). It contains transects with a total length of 9.6 km, including the two banks of the river. Each transect was at length of 500 m and 30 transects were randomly selected. Each transect was visited 12 times a year during the months of October till March 2014–2016. During the rest of the year, the vegetation is high and the coypu cannot be found because the visibility is reduced. Index of relative density was then given (number of individuals per km) (Krebs 2004, Marini et al. 2011). The sizes of the social groups are given with average ±StDev. (min–max) individuals. Differences in density of Coypus over the years tested with Kruskal-Wallis test were observed.

Results

Twenty-four localities were found, twenty of them were in the researched section of the Maritsa River. Another three localities were in the ponds at a minimum distance of 6 km from the Maritsa River. One locality was found in the Harmanliyska River (Fig. 1).

The average density was 3.4±2.3 (0–8 ind./9.6 km) – 0.35 ind./km. In 2014 it was 3.2±1.9 (0–6 ind./9.6 km) – 0.3 ind./km; 2015 – 3.7±2.3 (0-8 ind./9.6 km) – 0.4 ind./km; 2016 – 3.4±2.7 (0–8 ind./9.6 km) – 0.3 ind./km. There are no significant differences in the density of Coypu during the researched period (Kruskal-Wallis chi-square = 0.37, p=0.82), as well as in the density in different months (Kruskal-Wallis chi-square = 3.1, p=0.68). The average size of the social groups was 1.6±0.9 (1–4 ind.; n=42) of the Maritsa River and 2.4±0.99 (1–4 ind.; n=12) in the three new localities in the ponds (Fig. 1). Most commonly single Coypus were seen.

Discussion

This study establishes the increase of localities and distribution of Coypu along the reaches of the Maritsa River. There were three new localities into the ponds. Perhaps they were a result of immigration from the Maritsa River. The Coypu inhabited ranges of 2–5 ha, exceptionally to 12 ha according to Jouventin (1996). Other studies reported home range of about 3–82 ha (Doncaster and Micol 1989). Usually Coypus does not depart at distance more then 200–400 m from water (exceptionally up to 1.25 km); they have small size territories (Adams 1956, Woods et al. 1992, Popov and Sedefchev 2003), but it can overcome a distance of the order of

40–50 km (Aliev 1965). We assume two ways of occupying the new territories of Coypu in the researched area: migration from locality near Simeonovgrad (Tsekova and Georgiev 2016) or the Sazliyka River (from the locality found by Myhajlov and Stoyanov 2001), and occupying new northern localities and migration from the south of the known localities, situated in the Republic of Turkey (Özkan and Kurtonur 1994, Özkan 1999).

The density of Coypu (0.35 ind./km) is lower than that one, shown in other similar studies – between 1.4 and 5.7 ind./100 m (Salsamendi et al. 2009, Marini et al. 2011); 1–8 ind./ha (Guićhon and Cassini 2005) and 2.4–9.1 ind./ha (Doncaster and Micol 1989). The size of social groups is also considerably smaller than established in other regions (Guićhon et al. 2003). This is probably due to the recently occupied habitat or the season in which the observation was done. Usually, the density of Coypus in autumn – winter seasons decreases due to high mortality of individuals in severe winters (Doncaster and Micol 1989, 1990, Velatta and Ragni 1991, Reggiani et al. 1995, Bertolino et al. 2005).

Conclusion

Early studies in the United States reported minor damage, as subsequently reported that Coypu can cause damage by destroying marsh habitats, destroyed dikes, destroyed crops and can be a reservoir of diseases (Howerth et al. 1994, Mouton et al. 2001, Carter and Leonard 2002). This study demonstrates an increase in the distribution of Coypu in Southeastern Bulgaria. The density of Coypus in the researched area is still too low to cause any damage, but such may occur in future. Further studies are needed to determine the impact on aquatic habitats.

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