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INVENTORIES OF CAUSES OF LEAF SCORCH OF LINDEN TREES UNDER ANTHROPOGENIC CONDITIONS IN KYIV

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Abstract

Linden species (*Tilia* spp.) are the most spread woody species in the street side plantings in Kyiv. It is determined that all linden species that grow along streets or sidewalks are in a poor state. The visual signs of scorch varied from severe to minimal damages (leaves collected in different parts of city). The control samples were collected in the conditions with minimal level of anthropogenic pressure (stands in the city outskirts). These were healthy leaves, without any visual signs of biotic or abiotic damages. In the leaves with maximal level of necrosis and chlorosis damages (to 90 % of leaf blade area) we found very high level of sodium ions and minimal level of potassium ions. Consequently, there is ionic imbalance in plant tissues – potassium ions are substituted for sodium ions. These chemical elements are monovalent and in the process of nutrient uptake the plant does not differentiate them. Accumulation of Na^+ ions and decrease of K^+ ions is typical to all the linden species growing along streets. Little-leaf linden is the most susceptible to salt contamination; however, it is an aboriginal species in Kyiv city conditions. Absence of biotic origin of necrosis and chlorosis on linden leaves is confirmed by luminescent analysis of photosynthetic apparatus – photo- and thermoinduction of chlorophyll fluorescence. Consequently, the results of conducted research allow to state that lindens are not suitable for growing in salt contaminated soils. In anthropogenically transformed (urban) conditions it is reasonable to select salt-tolerant wood species.

Key words: chlorosis, fluorescence, necrosis, photosynthesis, salt, urban environment.

Introduction

Planting in cities has a number of obvious advantages. Positive influence on the health of city residents is considered to be the main one. Species of linden tree genus (*Tilia*) have become common in urban landscaping in a lot of big cities within their natural area. Linden trees are widely spread in landscapes of large cities in Europe and the USA (Borowski and Pstrągowska 2010, Stravinskienė et al. 2015). Ukraine is of no exception and its capital city of Kyiv in particular (Nebesnyi and Grodzynska 2015, Karpyn and Zayika 2017).

Urban plantations decrease the level of air pollution, absorb large amounts of carbonic oxide, serve as a protective shield from noise and dust, cool down the surrounding areas, provide shade and coziness in hot seasons, make urban landscapes aesthetically attractive (Tyrväinen et al. 2005, Willis and Petrokofsky 2017). On the other hand, the growth and the development of a plant body depends on various environmental factors, which interact with each other as well as with the plant itself. The abnormality of physiological processes in a plant can be caused by a large number of stress factors that have a negative influence. They include both natural and anthropogenic factors (Nebesnyi and Grodzynska 2015). The first ones include the extremums in the conditions that influence plant life, namely temperature, humidity, oxygen and light. Nutritional deficiency or excess of nutrients (water, oxygen and mineral substances) negatively affects plants as well. The anthropogenic factors include air pollutants and soil contaminants. Unsatisfactory state of plant bodies, which grow in complicated urban environments, caused by abiotic factors can be further enhanced by biotic ones (Larher 1978).

Urban environment is quite complicated for the growth and the development of woody plants. Those plants that grow along the streets and pavements, particularly on paved territories, face both atmospheric pollution and unsatisfactory soil conditions, namely soil contamination and soil deficiency. Under such conditions woody plants often suffer from browning and death (necrosis) and yellowing (chlorosis) of leaf edges. International researches refer such damage to abiotic diseases and call it marginal leaf scorch (Appleton et al. 2009). There are several causes of it. Dehydration of leaf tissues is one of them. In a hot season plants that grow on paved and concreted surfaces are affected by solar radiation. Building walls and roadway pavements slowly give up heat, it is especially intensive from a south and a western side. This process continues even after sunset. This effect is called ‘urban heat island’ by urban ecologists (Kucheriavyi 1999, Appleton et al. 2009). High temperatures, which have a disastrous effect on leaves and roots, cause enhanced vegetal discharge. In order to decrease the temperature of leaf blades, a plant begins to cool down, that is to say, to transpire water through its stomas. It is known that one big tree can transpire 400 L of water into the environment. However, the same amount of water is not always available for a plant. It can cause the dieback of leaf blade edges, that is to say, marginal necrosis and chlorosis (Appleton et al. 2009). The above mentioned indicates the expediency of conducting a series of additional laboratory tests in order to prove the anthropogenic nature of linden leaf necrosis and chlorosis.

Accumulation of toxic elements is another reason for leaf blade damage. Under the conditions of heavy technogenic pollution in cities, street plants accumulate harmful phytotoxic elements in excess concentrations, which disturb cation-anion balance and the processes of mineral nutrition, suppress the activity of enzyme and hormonal systems, cause abnormalities in the permeability of cell membranes and inhibit photosynthetic processes

(Kondratyuk et al. 1980). Salt is one of such pollutants. In order to deal with icy roads and snow, sodium chloride and calcium dichloride are used (Douglas 2011). Although $CaCl_2$ is less harmful than NaCl, last is most commonly used both abroad and in Ukraine. There are two main ways of damaging woody plants by salt: transfer of salt grains from the air into plant tissues or saline spray and accumulation of ions rhizosphere area of soil (Douglas 2011). In the first case, salty water immediately contacts the leaves or the needles, salt penetrates into plant cells and intercellular area, and influences the vitality of buds and shoots. In a lot of cities in the USA, coniferous plantations along highways suffer from saline spray. It becomes evident as needle browning and scorch. Damage by salt can be usually seen from the road side and at 9–15 m from it. In the second case, the accumulated salt is dissolved in the soil and is broken down into Na^+ and Cl^- which influence both soil and plant. They change the structure chemically and, to a certain extent, physically, since sodium causes the adhesion of soil particles. The ions of Na^+ and Cl^-, which are dissolved in water, are easily absorbed by roots and are transported to plant active growth parts, namely to leaf blade edges and root tips, where they are accumulated in toxic concentrations (Delahaut and Hasselkus 1996, Craul 2010). This process results in ionic imbalance – sodium blocks the enrichment of a plant by necessary macro- and microelements, namely cations K^+, Ca^{2+} and Mg^{2+}. The displacement of potassium ions by sodium ions results in the so called potassium deficiency in a plant, the typical symptoms of which are necrosis and chlorosis of leaf blade edges and interveinal chlorosis (Douglas 2011). In addition, soil salinization results in the change of osmotic potential, which is caused by high concentrations of interstitial water and low water potential. There is a decrease in the amount of water, which is available for roots, therefore even the soil that is moist enough becomes dehydrated under the action of large amounts of salt (Slabu et al. 2009). It is obvious that salt affects plants at various times, but its use at the end of winter and the beginning of spring is more harmful, since there is less time left for washing out due to rainfall and snow melting compared to the use of salt at the beginning of winter.

Some researchers (Sinclair and Lyon 2005) think that browning of linden leaf edges can be microbially-derived. Having assumed that the possible cause of marginal necrosis and chlorosis of linden trees is bacterial affection, the detailed analytical survey of this problem has been considered as well. Thus, the bacterium called *Erwinia amylovora* (Burrill) Winslow et al., which causes bacterial blight, damages fruit trees only. This is a rather major illness that severely affects species of Rose family (Rosaceae; Juss.). Another bacterium called *Xylella fastidiosa* Wells et al. has been found on the leaves of ornamental woody plants in the southern and the eastern states of the USA (Gould and Lashomb 2007). It typically affects various species of oaks, elm trees and planes. Such leaf damage closely resembles the one that can be observed in the urban landscapes of Kyiv, namely brown edges and a compulsory yellow line between the brown died off part of a leaf and its green part. However, American scientists mentioned that *X. fastidiosa* has not been diagnosed on linden trees yet (Hartman et al. 1991).

The aim of the conducted research was to analyze the state of various linden tree species leaf apparatus and to determine possible reasons for its worsening.

Material and Methods

The paper presents the materials of the original investigation of photosynthetic apparatus of various species of *Tilia* genus, which was conducted in the plantations of Kyiv in 2010–2016. The subject of the research were model trees aged 10–40 of 4 species and 1 hybrid linden tree, namely *T. cordata* Mill, *T. platyphyllos* Scop, *T. begoniifolia* Stev., *T. tomentosa* Moench., and *T.* × *europaea* L. in three ecological-phytocoenotic zones or eco clines, according to Kucheriavyi classification (Kucheriavyi 1999) that represents the reduction of negative urban factors: I – suburban forests, II – garden squares and III – street plantations. The age of plants was determined according to the inventory data provided by a public utility company that manages community landscapes called Kyivzelenbud. The location of the model trees was plotted on a map. The plants, which grow in the landscapes where there is minimum anthropogenic impact, that is to say, in the plantations in the suburbs of the city (Fig. 1), served as control samples. The leaves were chosen from the southern-western side of a tree crown after a leaf blade finished its growth and after the end of a blossoming period of the trees aged 10–40-year-old.

The diagnostics of trees' functional state by the level of dechromatization or

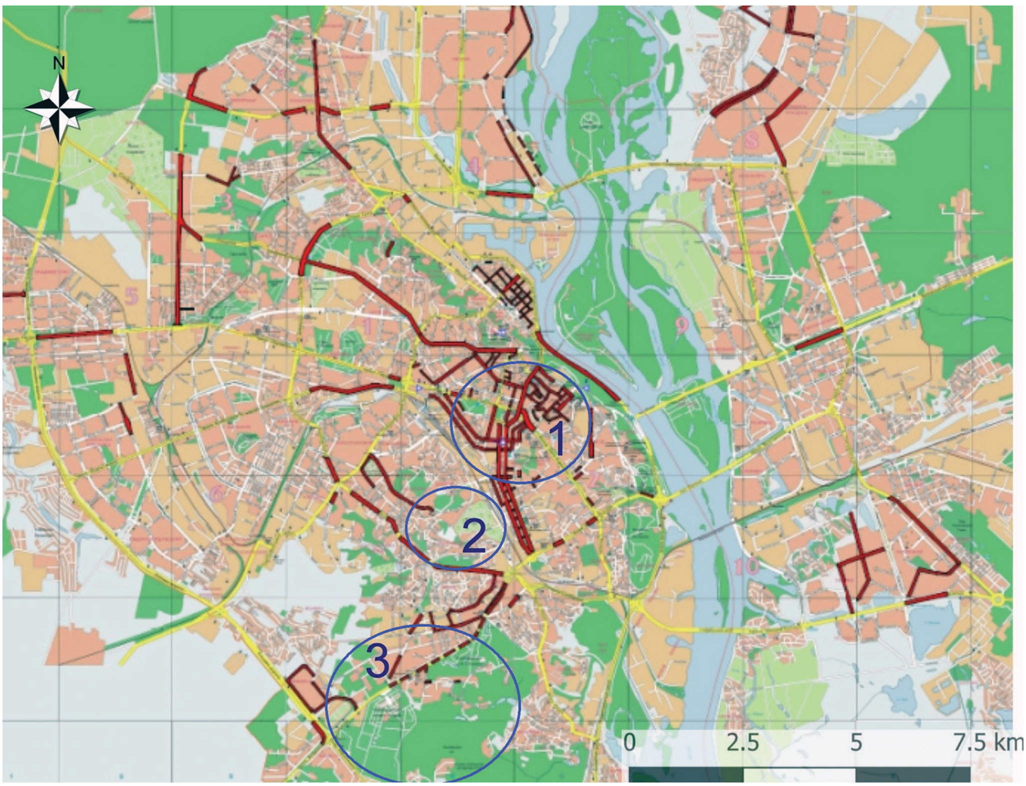


Fig. 1. Research places in Kyiv.

Note: Inspected street lining plants in Kyiv (marked in red); places of sampling (marked in blue): 1 – streets, 2 – parks, 3 – outside the city.

the change in colour included both field and laboratory investigations. During field studies were used: observation, analysis, synthesis, systemic approach scientific methods. The length of the streets according to the map on the Figure 1 is 80 km. The general information about observed trees is indicated in the Table 1.

Table 1. General characteristics of the observed linden plantings alongside Kyiv streets.

Length of observed streets, km	Average distance between trees, m	Number of trees	Number of trees per sample plot	Number of trees by species, %	Status / condition of them	Trees per km
80	4	20000	From 10 to 300	*T. cordata* – 40; *T. × europaea* – 58; *T. begoniifolia*, *T. platyphyllos*, *T. tomentosa* – 2	From 5 (wee tree) to 1 (dead tree)	250

Laboratory methods included analysis of macroelement content and biophysical or spectral research. Laboratory investigations were conducted with the equipment of the Laboratory of Plant Physiology and Microbiology of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine.

Since a definite indicator of the negative effect of the anthropogenic environment in plant bodies is a leaf blade, it has been decided to take leaves of woody plants as test objects. Various species of linden trees, which are the most common in the street plantations in Kyiv, have been chosen. In order to diagnose the cause of leaf tissue damage and dieback, it was necessary to verify the hypothesis about their abiotic nature. As opposed to the diseases caused by biotic factors, abiotic diseases cannot be passed from tree to tree, that is to say, they are noninfectious diseases. It is rather difficult to diagnose them, since there are various reasons for giving rise to the disease or the damage with similar manifestation markers.

The analysis of macroelements' content (Na^+ and K^+) in linden tree leaves was conducted according to modified Dekster procedure (Kirillov et al. 1981). The number of the free ions transferred into the solution, was determined using spectral micro fluorometer SMF-2. The functional state of photosynthetic apparatus was diagnosed with photo- and thermoinduction of chlorophyll fluorescence with the use of a luminescent image, according to modified procedure (Makarova and Kytaiev 2008). The measurement was conducted using laboratory scale spectral micro fluorometer SMF-2 (a luminescent microscope connected to a monochromator) in duplicate, according to the modified procedure (Makarova and Kytaiev 2008). The device made it possible to excite and collect fluorescent spectra at certain parts of a leaf surface, the individual changes in fluorescence, which are the results of light or heat effect. The emission of fluorescence was registered in 500–800 nm region of the spectrum. Luminous induction of chlorophyll fluorescence was measured at the length of waves being 680 and 740 nm with time interval from 0.5–1.0 s to 300 s. The changes in fluorescence induced by temperature were registered at the stationary level of luminous fluorescence induction when the leaves were heated from 20 to 80 ºC at a speed from 8–10 to 20–30 ºC per minute.

Luminous induction and thermoinduction of chlorophyll fluorescence of a leaf blade was excited in a continuous mode by a blue-violet light (λ_{max} = 435.8 nm). Exciting light of various intensity was used in the research (from 5 to 300–600 W/m^2). Thus, it was possible to reach the saturation of the light stages of photosynthetic process, which were responsible for producing luminous- and thermoinduction changes in chlorophyll fluorescence. Prior to the analysis, the leaves had been kept in darkness (darkness adaptation) during 30 min. Here, the experiment was replicated 10 times for every species under each condition (street, park and outside the city).

According to the results of the conducted investigation (Weinert et al. 1988), linden trees are intolerant to salinization and, in its turn, salt accumulation is the cause of marginal leaf injuries, which visually resemble similar problems with water supply or root damage. On the other hand, the above mentioned data could be outdated, thus, we set the task to verify the data.

In addition, we studied linden plantations under the condition of being loaded by the influence of urban environment and not in their natural ecosystem, in contrast to our predecessors. As for the other scientists (Borowski and Pstrągowska 2010, Douglas 2011), although they conducted their research under the condition of urban environment (Warsaw, Poland), they studied only 1 species of linden trees (*T. cordata*). Hence, the problem of salt accumulation and the state of other linden tree species that grow in urban environment remains uninvestigated. Thus, we aimed at investigating these processes through the example of 5 linden tree species, which are represented in Kyiv street plantations.

Results

The data of our survey in 2010–2016 has been verified the same results as the latest inventory in 1999, conducted in Kyiv street plantations, that linden trees are the most common among the woody plants that grow there. Having conducted the inventory of 300 streets (1/5 of their overall number in Kyiv) with the average number of trees 250 per kilometer. It has been determined that there are 4 species and 1 hybrid of linden trees that are common in street plantations – *T. cordata*, *T. platyphyllos*, *T. begoniifolia*, *T. tomentosa* and *T.* × *europaea*. *T. cordata* and *T.* × *europaea* prevail in terms of quantity and take up to 90 % of all the surveyed plantations. Most of the main streets are landscaped with trees planted in lines on paved territories, where every single tree is designed by a tree grill that is several square meters in area. Woody plants that are planted according to this principle suffer from insufficient watering and lack of micro- and macroelements. The soil near a tree trunk is very consolidated, especially if close to public transport stops, small architectural forms, etc. This inhibits plant breathing and moisture supply to the roots. Urban soil, which consists of sand, construction rubbish, etc. does not meet the needs of proper amount of nutrients. Besides, during tree planting, new fertile soil very often is not provided in planting holes, instead, a died out tree is substituted and nothing more. As a result, a tree can just stay in a hole without rooting, 30–40 % of newly planted linden plantings were this way. Another critical conclusion concerning the state of linden trees in street plantations is that fungal fruits of *Schizophyllum commune* Fr. could be seen on young trees everywhere. It is known that in the wild they are found only on decaying trees. Thus, the trees, which had been planted along the streets only several years ago, died out and should be replaced by the new ones.

Street plantations survey, which was conducted during three vegetative seasons, demonstrated the fact that leaf scorch causes serious damage to linden tree plantations. According to the state of a leaf blade, it can be said that marginal necrosis and chlorosis damages all the species. Linden trees that grow along the main streets are in the worst state. In some cases, necrosis and chlorosis covers up to 90 % of a leaf surface. The damage can be divided into interveinal chlorosis and interveinal necrosis, marginal chlorosis and marginal necrosis (Fig. 2). The browning of leaves begins in May just after their unfolding. The tree part, which is near a roadway or a pavement, is the most damaged, the same is stated by international researches as well (Delahaut and Hasselkus 1996, Craul 2010, Douglas 2011). The extension of marginal necrosis reaches its maximum in midsummer. Damaged leaves do not recover and remain during the whole vegetative season, which is curtailed in this case – the trees are characterized by premature defoliation. As a result, even at the end of the summer it is possible to find trees with completely fallen off leaves.

Our research shows, that all the species, the leaf blades of which had visual signs of necrosis and chlorosis damage (50–90 % of a leaf area), contained phytotoxic sodium in the amount from 1.0 % (*T. begoniifolia*) to 3.6 % (*T. cordata*) (Fig. 3). In control trees without any signs ofleaf damage those indices reached 0.01–0.02 %. The highest level of Na^{+} in the leaves was found in June. Obvious monthly reduction in the content of sodium ions in linden tree leaves was observed.

So, the large damage of linden leaves by necrosis and chlorosis occurs in the middle or at the end of the summer and the initial disease signs, which are evident at the beginning of the vegetation period, are intensified. In midsummer this process is expanded by insufficient moisture

a

b

Fig. 2. Condition of *T. cordata* leaf blades in the central streets: a) marginal necrosis and interveinal chlorosis in midsummer; b) marginal and interveinal necrosis and interveinal chlorosis in the beginning of autumn.

and high temperatures of soil and air.

It has been determined that all the species of linden trees with damaged leaves are characterized by displacement of potassium ions by sodium ones (Fig. 4).

The lowest potassium content in the

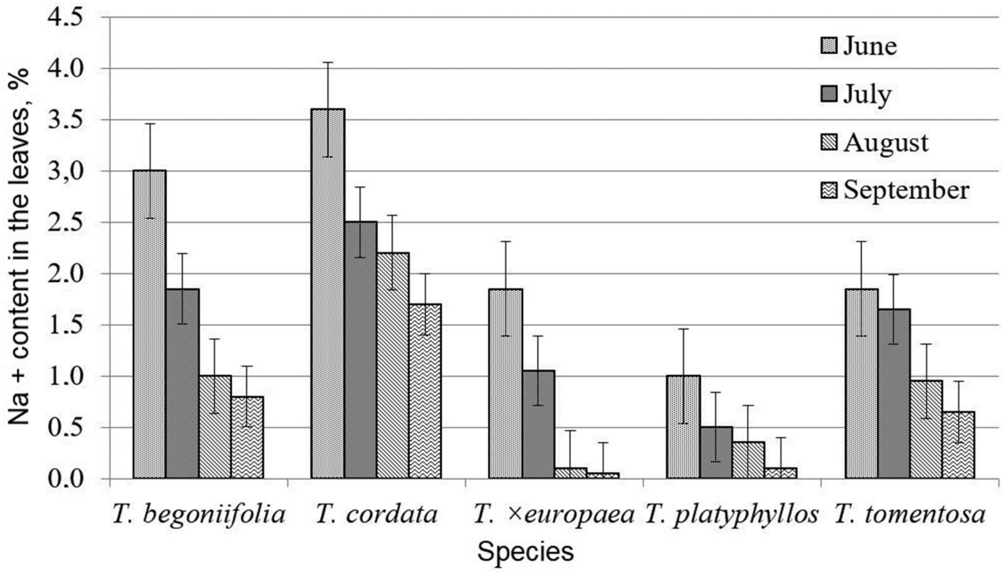
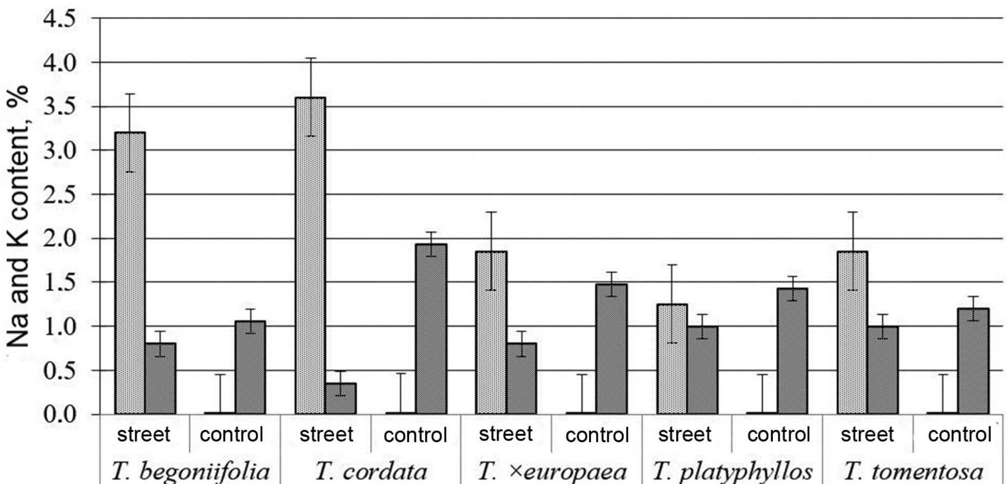


Fig. 3. Seasonal dynamics of sodium content.



	T. begoniifolia street	*T. begoniifolia* control	*T. cordata* street	*T. cordata* control	*T. ×europaea* street	*T. ×europaea* control	*T. platyphyllos* street	*T. platyphyllos* control	*T. tomentosa* street	*T. tomentosa* control
Na	3.2	0.01	3.6	0.02	1.85	0.01	1.25	0.01	1.85	0.01
K	0.8	1.06	0.35	1.93	0.8	1.48	1	1.43	1	1.2

Species / conditions of growth

Fig. 4. Ionic imbalance in leaves of lindens from different ecological phytocoenotic belts.

leaves of *T. cordata* was found when the level of sodium was the maximum compared to other species. It is to be noted that young trees aged 10–40 prevail in street plantations and they are highly sensitive to salinization, since salt resistance improves with aging (Udovenko 1977). The ‘potassium deficiency’ in plants can be visually identified by necrotization of leaf edges. The small-leaved linden tree (*T. cordata*), namely its leaves as the center of variability and flexibility of a plant body, can be used as a test-object for indication of saline environmental pollution.

By means of fluorescence photoinduction, which is widely used in ecological monitoring for the assessment of the toxic influence of anthropogenic pollutants on plant test-organisms, the disruption of photosynthetic reactions has been recorded in the damaged plants of all the objects under study. The shape of the induction curve depends on the state of the photosynthetic apparatus, which is greatly affected by environmental factors (Krajicek and Vrbova 1994, Makarova and Kytaiev 2008). For example, Figure 5 presents the obtained data concerning the most widespread linden tree species – small-leaved.

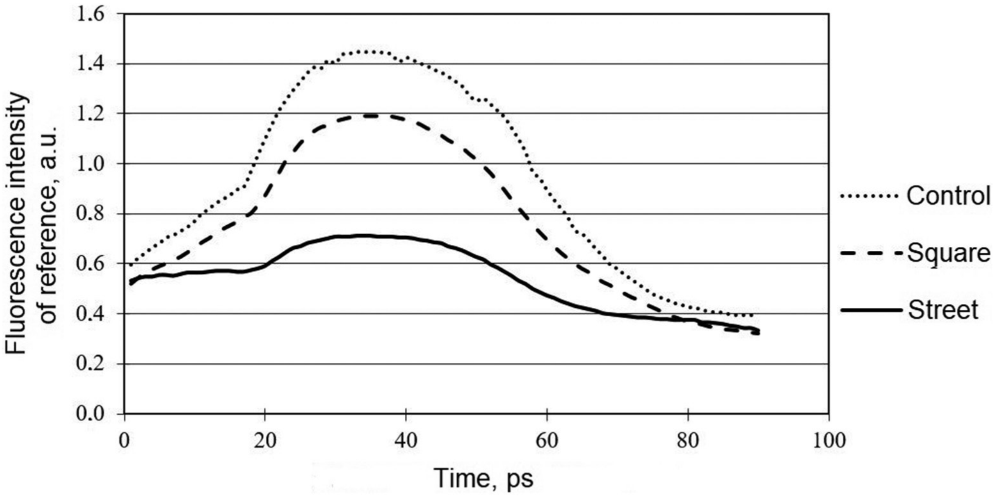


Fig. 5. Changes in fluorescence intensity of *T. cordata* leaves.

Comparative analysis of the functioning of leaf chloroplasts has been conducted and the results have been compared to the ones obtained under control conditions.

It is to the point to note that a linden tree forms its root system gradually and older trees have roots in deeper soil layers that are less saline. Young trees with roots in a shallow soil layer risk to receive larger amounts of sodium. Newly planted trees are at greater risk, which can be traced in rapid leaf blade decay and can be evident in their dechromatization and premature defoliation.

Discussion

It has been determined that under street conditions linden plants show greater changes in photosynthetic processes, which are more apparent is case of a dark photosynthesis phase and are caused by negative urban environmental factors (Fig. 5). The obtained data are prove the investigations of other scientists (Oleksiichenko et al. 2009, Hallik et al. 2012). It is worth mentioning that, according to indices of the rapid changes in chlorophyll fluorescence at the so called plateau level, virus infection has not been found in the experimental samples.

By means of fluorescence thermoinduction it has been determined that dark photochemical processes are the most intolerant of salt contamination. It has become evident in the increase of the stationary level of chlorophyll fluorescence induction as a result of the decrease in ribulose bisphosphate carboxylase activity and general attractive capacity of a plant body due to salinity stress. With the help of a microspectral analysis, the increase of yellow-green leaf fluorescence (λ_{max} = 530 nm) has been determined to be several times higher even in the green parts of damaged leaves compared to the healthy leaves. It is necessary to note that in yellow leaf parts fluorescence intensity increases by ten folds at the wave length being λ_{max} = 530 nm. The latter is caused by the accumulation of oxidized lipids in cell membranes and is followed by the increase of their permeability, which is also controlled by the changes in the conductance of plant cells. According to instrumental control data, it has been determined that salinity stress causes catabolic changes in plant cells, which are permanent and are followed by premature plant defoliation.

Besides the determination of virus plant damage, microspectral express-methods make it possible to conduct bacterial damage testing. Thus, the determination of bacterial infection of the leaf samples with large leaf blade areas damaged by chlorosis and necrosis, as well as of dark green healthy leaves gathered in control environments has been conducted. Having analyzed the samples, no signs of bacterial damage has been found.

Conclusions

Our research demonstrated the fact that leaf scorch of linden trees causes serious damage to plantations under anthropogenic conditions along the streets of Kyiv. The results of inventory survey determined that the most of them are in an unsatisfactory state. That is to say, they are characterized by drying of tree branches, top drying, crown density loss, leaf damage in the form of marginal and interveinal necrosis and chlorosis and abnormal refrondescence. Constant man-made load breaks the physiological resistance of woody plants. The main factor of unsatisfactory state of various species of linden trees is saline soil contamination.

The accumulation of sodium ions in linden leaves causes the displacement of the elements that are necessary for a plant, namely, the displacement of potassium. Dark photochemical processes have proved to be the most sensitive to saline contamination, which is caused by the decrease in ribulose biphosphate carboxylase activity and general attractive capacity of a plant body due to salinity stress. It is to the point to choose drought- and salt-resistant species of wooden plants in order to create street plantations.

If linden trees are continued to be planted, we propose to use species such as *T. begoniifolia* or *T. caucasica* and *T. tomentosa* as more drought resistant. Express and informative biophysical methods, which include photo- and thermoinduction of chlorophyll fluorescence, can be used for ecological monitoring of environmental conditions.

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COMPARATIVE CHARACTERISTICS OF THE RATE OF RETURN ON INVESTMENTS IN A FOREST PLANTATION AND OTHER REAL ESTATES IN BULGARIA

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Abstract

A comparative analysis of the rate of return on investments in a fast-growing poplar plantation in South Bulgaria and in two-room residential apartments in Sofia (average area 68 sq.m.) (including a room and a bedroom), differentiated by districts has been made. The investigation is based on the concept accepted in the normative basis, which determined these kinds of estates as real. It is motivated by the necessity to reveal suitable and perspective variants for investments, according to the utilities and abilities of a wide range of potential investors. Both static and dynamic methods have been applied for assessment of the rate of return on investments in a medium-period perspective. As a result of the carried out investigations it is determined that the rate of return is 4.01 % and it is expected to raise along with raising of the demand of poplar wood material. The advantage of this kind of investment is that it does not require a large amount of capital; it is ecological and allows using lands in rural areas. The rate of return of investment in residential real estates varies from 6.27 % to 15.26 % depending on the location and the result is always positive.

Key words: fast-growing tree species, financial efficiency, real estates, two-room residential apartments, profit.

Introduction

The investments in real estates are well-studied field of research and are of real interest for contractors from all over the world. In a study based on 121 sources covering the markets in Europe, Asia, North America and Australia, Benjamin et al. (2001) pay attention to the following key points: the expected risk and return on investments in real estates, compared to other assets and to inflation; benefits from optimization of investment portfolios through including of real estates; results from the society activities with specific investment purpose. The obtained results reveal that:

- The return in real estates is more easily to be prognosticated and higher than the rate of return in other kinds of assets.

- Precence of real estates makes the investment portfolios less vulnerable from inflation in long-term aspect. The reason is the high correlation with real inflation.

- Investment portfolios, including real estates, are less risky in long-term aspect from investments only in stocks and bonds.

According to Djaparidze (2013) the share of real estates in the total amount of investments in Russia is 21 %.

Some authors share their point of view that the investments in residential real estates bring better return than the stocks and at the same time are poorly volatile as the bonds (Jordà et al. 2017). They come to this conclusion on the basis of numerous analyses of the annual return from residential estates, stocks, bonds and depositary bonds from 1870 to 2015 in 16 economicaly developed countries, eg France, Norway, Great Britain, USA, etc.

In a research Kopf (2018) establishes that in developed countries the annual return from lodgings during the last two decades has been about 7 % (corrected with the inflation), and the return from the stocks has been a little under 7 %. Iman et al. (2012) consider that in the process of selection and purchase of estate one should assess and take into account suitable characteristics of the estate to maximize the expected benefit. In this case it is particularly valuable to know the characteristics and range of the real estates market.

Summarising the national and international experience in the determination of this term, Iliev et al. (2007) present a classification of real estate markets, depending on the kind of the target (commodity), in which they include the markets of: 1) land (land estates); 2) buildings and single targets in buildings; 3) facilities and networks; 4) complexes; 5) perennial plant stands; 6) laws of estate; 7) other targets. This classification allows revealing the range of the term ‘real estate’ and to look for possibilities for including of all its varieties in an economic turnover, some of them until now being underestimated.

Usually, the investment in a real estate in Bulgaria is related to the purchase of a land or estate with various functions (lodging, office, shop, etc.). Less studied is the investment in perennial stands and forest plantations as a form of immovable property. It gives the opportunity to obtain economic benefit from the territories of rural regions, as well, where the land is the basic source of income. During the last 25 years, the world market shows deficit of forest products (Glauner et al. 2012, Indufor 2012). It stimulates the investments in forestry activities and investors can differentiate into two groups – institutional and small- and medium-sized enterprises (SME). The first ones invest mainly in plantations of fast-growing tree species in the southern hemisphere and the USA. These investments are attractive because have enough high rate of return. In this relation it should be mentioned that the average return on investements in forest plantations in some countries is as follows: Argentina – 14.8 %; Brazil – 21.6 %, Uruguay – 18.0 %; USA – 14.9 % (Campanale 2009, Macqueen 2013, Brotto 2015).

In contrast to more authors, who focus on the financial result from the investment in a forest plantation, others pay attention to ecological and social contributions of plantation forestry, underlining its significance for some branches of the national economy, like agriculture, water management, transport, tourism, etc. (Milev et al. 2017).

As a result of their research (Campanale 2009, Macqueen 2013, Brotto 2015) come to the conclusion that investments in forest plantations are characterized with lower level of investment risk, compared to investments in other assets, providing same rate of return.

The lower risk of the investments in forest plantations is a consequence of the positive correlation subordination between return and inflation and the absence of correlation between the return on investments in forest plantations and return on investments in financial instruments.

All this determines the preferences of institutional investors to investment in forest plantations in times of financial uncertainty for diversification and reduction of the total risk of the portfolios, which they possess (Brotto 2015). The investments of SME in the field of forestry provide wood and non-wood products, and employment in poorly developed regions. The significance of the topic provokes numerous investigations (Elson 2012, Macqueen 2012, Davies et al. 2015). All they distinguish two categories – investments in real assets and enabling investments. The aim of the first ones is to establish real assets, which would accumulate profit and increase the value for SME owners. The aim of the second ones is to establish conditions for financially efficient investments in real assets. The conditions are related to clearly defined rights of ownership on forest resources, proper management and positive net money flows, covering the investment expences. The so-called 'enabling investments' are provided by non-governmental organizations, philanthropists, governments, etc. The combination of investments in real assets and enabling investments should provide a balance between financial results, social fairness for local inhabitants and environmental protection (Macqueen 2013).

Most of the investments in real assets, quoted in the above-mentioned articles, contribute to the sustainable management of forest territories and provide profits for SME, producing wood and non-wood products. In the forest sector of Bulgaria the institutional investors are not present. Meanwhile investments in SME in real assets are insufficient and are predominantly in wood processing equipment, because the main forest product in the country is round wood (Kolev 2017).

One of the explanations about the absence of enough investments in real assets in forestry in Bulgaria is the low return (Kolev 2017). As far as the return of investments in forest plantations in Bulgaria is concerned, by the time being it is still insufficiently investigated.

It is known that poplars (*Populus* spp.) are recognized as the fastest-growing forest tree species at temperate continental climate. They possess many other valuable characteristics, such as easy propagation, aptitude to hybridize, pleasing appearance and many uses (Isebrands and Richardson 2014).

Wang et al. (2014) present results of their study in China, which shows that the management of poplar plantations is twice more profitable compared to natural forests. It is based on the possible varying of the average annual increment, inflation percentage and rate of interest. The calculated net present value (*NPV*) is from 1024 to 6925 $USD{\cdot}ha^{-1}$, the equivalent annual income is from 120 to 623 $USD{\cdot}ha^{-1}{\cdot}year^{-1}$ and the internal rate of return (*IRR*) is from 13.2 to 29.3 %.

The aim of the present study is to compare the rate of return of investments in two different kinds of real estates – buildings and single targets (namely two-room residential apartments) and perennial stands (in this particular case – forest plantation of fast-growing tree species).

Although a study like this has not been carried out until now, the two types of real estates are methodologically included in the term 'real estate'. Venedikov (1995) defines the immovable things (often called real estates) as distinct parts of ground surface and everything, which is related to this surface and cannot be moved without destroying this correlation.

Objects

Object of investigation is a plantation established in 2017 year on the territory of the municipality Bratya Daskalovi, Stara Zagora district, with co-ordinates 42º10'0'' N and 25º8'0'' E. Economic characteristics are calculated for an area 1 ha. Afforestation has been carried out with *Populus* × *canadensis* (*Populus* × *euroamericana*) cv. 'I-214' at planting spacing 5×5 m and density 40 plants/dka. The selection of the object is determined by the fact that 'I-214' cultivar is the mostly used for afforestations with poplars in Bulgaria. In the period 2012–2019, the plantations with it occupy 44 % (2012) to 64 % (2014) of the total area of poplar plantations in Bulgaria (EFA 2012–2019).

The study on the investment in two-room residential apartments on the territory of the city of Sofia is determined by the fact that, according to the data from the National Statistical Institute in 2018, they represent biggest relative share of the housing in this city, grouped according to the number of rooms.

This allows forming of a large-scale totality, which is necessary for the correct calculation of compendious statistical indices. Basic source of information about the prices of residential real estates is the statistical heading of one of the biggest websites for sale-trade and rent (IMOT.BG 2019). The used data are according to months about the average offer prices for sale and rent for 2017 and 2018 to the end of the relevant month.

Methods

For the carrying out of the analysis, the following quantitative methods were used:

1. For calculation of the typical and regular annual levels of prices of residential real estates and the expected income from rent, the method of average quantities was used. Average annual chronological current individual quantity was used on the basis of the current prices to the end of the relevant months after formula (1) (Kaloyanov and Petrov 2014).

$$\overleftarrow{Y} = \frac{\sum_{i=2}^{N-1} Y_i + \frac{Y_1 + Y_N}{2}}{N-1}, \tag{1}$$

where: N is the number of elements in the dynamic row (N=12 in this case), and Y_i are the values of elements in the dynamic row.

2. To calculate the average annual rate of return (*AARR*) on investment in two-room residential apartments, the average annual expected income is compared through a static method (net cash flow). The correlation is presented through formula (2) (Stefanov 2018).

$$AARR = \frac{\overline{CF}}{IC} = \frac{\frac{\sum_{t=1}^{T} CF_t}{T}}{\sum_{t=0}^{T} I_t}, \tag{2}$$

where: $\overline{CF}$ is average annual net cash flow; IC is invested capital; CF_t is net cash flow in future period t; T is the number of periods within the investment term; I_t is the investment expense in period t.

We divide the expected average 10-monthly rental income from 1 m^2 of living area by the average purchase price of 1 m^2 of living area in the same neighbourhood. The rental income for the two remaining months of the year is deducted for taxes, routine repairs and absence of occupancy.

3. The variation of the return indices (V_σ) is calculated with the help coefficient of variation based on the standard deviation. Formula (3) was used (Stoenchev 2013):

$$V_\sigma = \frac{\sigma}{\mu}.100\ , \qquad (3)$$

where: σ is standard deviation and μ is arithmetic mean quantity.

In order to assess in a long-term period the economic efficiency of a potential investment project, involving the purchase and renting out of a residential property, and management of fast-growing tree species, some of the dynamic methods for return assessment have been used, and in particular:

1. The method of *NPV* as it has a number of advantages over the other methods (Stefanov 2018). The most significant is that it allows comparison between projects with different amounts of investment, net cash flow and periods of economic life. When applying the NPV, we calculate the sum of the discounted at the current moment, investment net and terminal cash flows, which were gained as a result of the investment by formula (4) (Tsoklinova 2017).

$$NPV = -\sum_{t=0}^{m}\frac{I_t}{(1+r)^t} + \sum_{t=m+1}^{n}\frac{CF_t}{(1+r)^t} + \frac{L_n}{(1+r)^n}, \qquad (4)$$

where: I_t is the investment cash flow in year *t*, in leva; L_n is the future net income from possible sale of the assets at the end of the economic life of the project, in BGN; *n* is the number of years of the economic life of the project, in number of years; *m* is the number of years of the economic life of the project during which investment cash flows are produced, in number of years; *r* is the required discount rate, part of 1.0; CF_t is the net cash flow during year *t* (positive or negative). In this particular case it is estimated by subtracting the expenses for year *t* from the income for the same year.

In the current study, as far as the investment in a poplar plantation is concerned, *NPV* is calculated at a discount rate 0.06 (6 %) and is conformable to the investigation of Cubbage et al. (2014).

The net cash flow from the investment is calculated through formula (5). It is the preferred in the present study since it represents the contractor's disposable income after all the expenses related to the project activities have been covered and the tax on the profits have been paid to the state budget (Georgiev et al. 2013).

$$CF_t = (R_t - C_t - A_t)(1 - T_t) + A_t\ , \qquad (5)$$

where: R_t is net income from sales, in BGN; C_t is total expenses (not including the depreciation) during year *t* from the economic life of the project, in BGN; A_t is the depreciation expenses officially acknowledged by the tax authorities in year *t* from the economic life of the project, in BGN; T_t is the rate of the corporate income tax, part of 1.0.

2. The method of the internal rate of return *(IRR)* is calculated through formula (6) (Tsoklinova 2017).

$$0 = -\sum_{t=0}^{m}\frac{I_t}{(1+IRR)^t} + \sum_{t=m+1}^{n}\frac{CF_t}{(1+IRR)^t} + \frac{L_n}{(1+IRR)^n}, \qquad (6)$$

IRR determines the level of discount rate, at which it is possible to invest resources in the project without losses for the investor.

The assessment of the financial efficiency of the investment in 1 ha of poplar plantation is made under the following limiting conditions:

1. All expenses are calculated based on the current prices towards 2017, VAT not included, prolonged with the inflation for 2017, which amounts to 2.06 % (NSI 2017).

The time necessary for manual activities is determined on the basis of administrative labour norms for afforestation activities (EFA 2008), and the time stake for manual operations amounts to 3.37 BGN/h. The prices of mechanized activities are determined as an average value of contracts signed for the relevant activity for 2017, carried out by the regional branch where the poplar plantation is situated, as follows: rooting out of logs – 650 BGN/ha; clearing logs – 600 BGN/ha; ploughing the soil in a depth of 60 cm – 1200 BGN/ha; levelling – 400 BGN/ha. The price of the planting material is 720 BGN (400 items × 1.80 BGN/item).

2. The rotation is 15 years.

3. The sale prices of the poplar wood material for 2017 are prolonged by the rotation of the trees with the updated prices of 2017, compared to 2016, for economic activity ‘Production of timber and timber products, without furniture‘, which, according to data from the National Statistics Institute, amounts to 2.8 % (NSI 2017).

Based on the above-mentioned, the following statement presents the investment expenses, the net cash flow at the end of a 15th year and the financial efficiency of the investment.

Under investment expences are considered the expences for establishment of 1 ha of poplar plantation at planting scheme 5×5 m. They include:

I. Purchase of uncultivated agricultural land.

II. Preparation of the soil:

- preliminary preparation of the area – rooting out and clering logs out of the site;
- ploughing the soil on a depth 60 cm.

III. Afforestation:

- purchase of 1-year-old saplings;
- excavation of holes 60/60/60 cm with a tractor drill and planting of saplings;
- whitewashing of the saplings a single time after planting.

IV. Maintenance of the plantation:

- expences for completion during the second year (forecasted completion is 15 %);
- digging up of the saplings in plots and disc ploughing between the tree rows in two directions – three times in the first and second year and twice in the third year;
- watering – during the first three years – 3 times each year;
- nourishing with ammonium nitrate, norm 250 kg/ha, a single time during first, second and third year;
- whitewashing of the saplings a single time during second and third year;
- prunning a single time during second and third year;
- cutting a single time during fourth, fifth and sixth year;
- painting of the cutted branches a single time during fourth, fifth and sixth year.

V. Expences for organization, implementation and control.

Results and Analyses

Financial dimensions of an investment in a poplar plantation

The amount of the investment expences is presented in Table 1, according to years of implementation.

Table 1. Amount of the investments for the establishment of 1 ha of poplar plantation, BGN.

Expences according to the activities	1st year	2nd year	3rd year	4th year	5th year	6th year
I. Expences for purchase of uncultivated agricultural land	5380					
II. bExpences for the preparation of the soil						
2.1. Rooting out of logs	650.00					
2.2. Clearing logs	600.00					
2.3. Ploughing the soil in a depth 60 cm	1200					
2.4. Levelling	400.00					
Total for group II	2850					
III. Expences for purchase of 1-year-old poplar saplings	720.00					
IV. Expences for afforestation	1260					
V. Expences for completion		340.2				
VI. Expences for the maintenance						
6.1. Expences for disc ploughing between the tree rows	360	367,42	250			
6.2. Digging up of the saplings in plots	194.43	198.44	135.01			
6.3. Whitewashing of saplings		121.37	123.87			
6.4. Watering	148.65	151.71	154.83			
6.5. Nourishing with mineral fertilizers	71.35	72.82	74.31			
6.6. Pruning		151.70	154.83			
6.7. Cutting				303.20	477.34	671.96
6.8. Painting the cutted branches				322.55	493.80	839.96
Total for group VI	774.43	1063.46	892.85	625.75	971.14	1511.92
VII. Total (I+II+III+IV+V+VI)	10,984.43	1403.66	892.85	625.75	971.14	1511.92
VIII. Expences for organization, implementation and control – 5 % from the amount of the above-mentioned expences	549.22	70.18	44.64	31.29	48.56	75.60
IX. Total (VII+VIII)	11,533.65	1473.84	937.49	657.04	1019.70	1587.52

Net cash flow in the year of felling

The expenses for felling and initial processing of 1 m^3 of poplar wood towards 2017 amounts to 7 BGN, and the wholesale prices of wood are as follows: big-sized – 70 BGN/m^3; medium-sized – 45 BGN/m^3; small-sized – 45 BGN/m^3; firewood – 45 BGN/m^3. The quantity of wood expected to be obtained during roation is 299 m^3 and is with the following assortment structure: big-sized – 204 m^3; medium-sized – 61 m^3; small-sized – 7 m^3; firewood – 27 m^3. In the year of felling it is forecasted to sale the agricultural land, which value is determined through prolongation of its initial price with the inflation rate for the year 2017. On the basis of specifications made and applying formula (5) it was established that the amount of the net cash flow in the year of felling is 29,286.46 BGN.

Financial efficiency of the investment

At a discount rate 0.06, *NPV* of the assessed investment in a poplar plantation is negative (-4083.86 BGN), i.e. it is financially inefficient. In the same time *IRR* of the investment in the plantation is 0.0401 (4.01 %), which in fact is the unsatisfactory rate of return in comparison with the rate of return on market portfolio in Bulgaria and one of the reasons that currently forest plantations in Bulgaria are still not in an industrial stage of exploitation.

The above-mentioned obtained results are in conformity with the results of a similar investigation carried out by Pra and Pettenella (2017) in the river Po valley (Northern Italy). Authors establish that the investment in a poplar plantation provides *IRR*, which values vary from 4.4 % to 11 %. They underline that the investments in poplar stands provide interesting opportunities for income only when average prices of the wood are high. In other cases the investments are at the limit of economical viability. To our opinion, it is realistic to expect increasing of the average prices of wood in medium-term and long-term perspective. Reason for this are the perspectives for replacement of some plastic products, which disintegrate slowly in nature and significantly pollute the environment, by wood products. In support of this vision is also the point of view of McEwan et al. (2019), who claim that the demand of round wood towards 2050 is forecasted to reach 6 billion m^3, which would stimulate the investments in forest plantations.

Analysis of the return of the investment in two-room residential apartments in Sofia according to residential districts

On the basis of the monthly data in euro until now in BGN and in single residential areas, the annual average chronological price was calculated for purchase and rent of a square meter residential area in euro. The analysis was made according to homogenous bulks of lodgings taking into account factors like number of rooms and location, because there are significant differences in the return in the varieties of these factors.

Table 2 shows the average annual norm of return of the investment in two-room residential apartments in some residential areas in Sofia in 2017 and 2018 for the first five and last five residential areas, arranged according to the amount of the return norm in 2017.

Table 2. Average annual return of the investment.

Residential areas with highest return	Avegare annual return, %		Residential areas with lowest return	Average annual return, %	
	2017	2018		2017	2018
Gorublyane	6.69	4.42	Nadezhda 3	3.44	4.48
Karpuzitsa	5.67	3.54	Strelbishte	3.44	4.11
Lyulin 2	5.53	3.84	Kremikovtsi	3.34	4.23
Lyulin – centre	5.52	5.09	Doktorski pametnik	3.29	4.59
Lyulin 8	5.50	4.33	Yavorov	3.00	2.73

The average return for all residential areas decreases from 4.46 % in 2017 to 4.38 % in 2018. Slump was also recorded in the variation coefficient of the index of return, calculated according to residential areas. From 15.06 % in 2017 it decreases to 13.19 % in 2018.

The conclusion is made that market mechanisms very quickly neutralize the significant differences in investment profitability according to residential areas, revealed in 2017.

Residential areas like Hipodruma, Ivan Vazov, Belite brezi, Doktorski pametnik, Strelbishte, Oborishte, Iztok, which are traditionally preferred for living, are not on the top of the charts according to return of investments due to the too high prices for purchase of a real estate, which could not be compensated by the amount of the rents.

In the application of dynamic methods for calculation of the expected efficiency of investment in two-room residential apartments in Sofia, the following economic parameters were used:

- As investment cash flow (value of the investment), the average offer price for purchase of a two-room lodging according to residential areas in 2018 is set.

- For covering of the average annual cash expences during the implementatin of the project, the two-month expected rent is forecasted according to average data about 2018 (expences for maintenance, taxes and insurances, lost profits by changing tenats, etc.).

- Cash income from the investment is the expected yearly 10-month rent according to average data about 2018 and the expected income from sale of the lodging at the end of the planned 5-year period of the project.

The rate of the used norm of discount is determined on the basis of the achieved average annual return for 2018 for the relevant residential area.

The expected selling price of the lodging at the end of the project's duration is obtained on the basis of the hypothesis that the prices of the two-room residential apartments lodgings in various residential areas will change over the next 5 years following the same trend as during the previous 5 years, namely the period 2013–2018. The forecasted prices in various areas are estimated via multiplying the average offer prices of the two-room lodgings by residential area for 2018 by the coefficient of increase of the prices during the previous basic period.

The results obtained from the investment and represented by the net present value of the income and internal rate of return after a 5-year period of utilisation and sale of the property are presented in Table 3. The residential areas in the table are listed in descending order of the investment's net present value. The arrangement of the residential areas according to the internal rate of return is identical.

Table 3. ***NPV*** **and** ***IRR*** **of the investment in two-room lodging in Sofia for a 5-year period from 2018 to 2023.**

Residential areas	*NPV*, euro	*IRR*, %	Residential areas	*NPV*, euro	*IRR*, %
Yavorov	87,102.85	15.26	Slatina	16,676.53	10.89
Medical academy	67,173.82	15.03	Nadezhda 2	17,242.99	10.83
Poligona	47,917.20	14.54	Gotse Delchev	25,151.21	10.79
Doktorski pametnik	82,880.42	14.25	Ovcha kupel	18,118.64	10.68
Ivan Vazov	63,721.88	14.25	Levski G	16,842.64	10.68
Krasna polyana 1	28,790.44	14.24	Malinova dolina	17,454.67	10.66
Vrabnitsa 1	23,222.78	13.94	Lyulin 2	18,941.97	10.65
Obelya 2	25,218.90	13.67	Nedezhda 3	17,288.26	10.59
Iztok	53,394.05	13.51	Lyulin 1	11,368.45	10.44
Sveta Troitsa	33,699.08	13.49	Zapaden park	15,706.86	10.38
Mladost 2	37,525.14	13.45	Krasno selo	20,899.68	10.25
Lyulin 4	25,001.21	13.40	Mladost 3	18,585.96	10.19
Tolstoy	24,643.79	13.34	Serdika	17,141.08	10.09
Gorublyane	21,365.15	13.26	Lyulin 7	12,623.95	10.01
Lyulin 9	23,946.99	13.12	Geo Milev	23,369.40	9.93
Levski V	26,393.00	13.07	Knyazhevo	15,911.22	9.88
Mladost 1	29,986.07	12.72	Lyulin 6	12,438.87	9.82
Hadzhi Dimitar	23,481.85	12.67	Lyulin 3	13,800.80	9.82
Lyulin 5	16,153.70	12.66	Nadezhda 4	10,661.51	9.77
Krasna polyana 2	21,745.58	12.64	Nadezhda 1	10,216.97	9.77
Svoboda	18,228.14	12.53	Hladilnika	19,523.55	9.76
Krastova vada	35,720.43	12.52	Slavia	15,176.35	9.73
Lyulin 8	23,146.72	12.49	Zona B-19	16,212.52	9.70
Obelya 1	20,786.38	12.44	Buxton	16,512.25	9.59
Izgrev	41,340.58	12.12	Gevgeliyski	15,766.77	9.53
Lozenets	40,758.57	12.06	Dianabad	20,391.64	9.46
Moderno predgradie	21,396.80	12.03	Razsadnika	11,532.87	9.34
Lagera	32,394.00	11.99	Lyulin – centre	11,747.79	9.32
Karpuzitsa	24,636.39	11.91	Pavlovo	13,015.06	9.30
Hipodruma	27,553.25	11.88	Darvenitsa	14,280.03	9.26
Lyulin 10	21,386.09	11.86	Belite brezi	19,536.89	9.23
Orlandovtsi	15,065.55	11.75	Reduta	13,871.19	9.19
Mladost 4	25,135.97	11.73	Gorna banya	10,143.76	9.03
Druzhba 1	23,677.34	11.71	Musagenitsa	10,901.88	8.47
Manastirski livadi	36,967.20	11.67	Ovcha kupel 1	9890.91	8.39
Zona B-5	23,464.31	11.62	Zona B-18	12,176.85	8.38
Druzhba 2	23,495.94	11.62	Poduyane	11,175.50	8.28
Simeonovo	31,415.30	11.52	Studentski grad	5822.52	7.82
Oborishte	39,293.45	11.48	Vitosha	8794.82	7.75
Borovo	27,293.27	11.40	Banishora	10,659.89	7.55
Boyana	27,982.85	11.35	Mladost 1A	12,335.58	7.32

Residential areas	*NPV*, euro	*IRR*, %	Residential areas	*NPV*, euro	*IRR*, %
Ilinden	22,768.55	11.34	Strelbishte	12,593.06	7.26
Zaharna fabrika	17,266.37	11.24	Levski	5542.27	6.89
Suhata reka	16,694.98	11.20	Zona B-5-3	5350.40	6.80
Ovcha kupel 2	21,357.11	11.19	Dragalevtsi	8096.01	6.76
Krasna polyana 3	17,281.39	11.14	Obelya	5693.35	6.59
Centre	26,818.57	11.10	Vrabnitsa 2	3512.60	6.27
Fondovi zhilishta	19,122.76	11.05	Kremikovtsi	-348.36	3.83

It was determined that the result from the investment period depends mostly on the expected change of prices of two-room lodgings in the relevant residential area and not that much on the expected rent. According to the parameters set in the investment project, only Kremikovtsi shows negative result. The residential areas with norm of return calculated according to the static method based on expected income from rent, which is lowest in a long-term aspect, appear to have highest profitability.

It could be concluded that the investment in two-room lodging in Sofia in a 5-year outlook provides high return but its optimization requires precise selection of lodging's location.

Conclusions

Both types of investments in real estates provide various advantages, which gives the opportunity for optimal choice on the side of potential investors. They are as follows:

The advantage of an investment in the establishment of a poplar plantation is the necessity of less initial capital. The nominal amount of the investment expences is relatively low and amounts to 17,209.24BGN. At the expense of this, the indices *NPV* and *IRR* are unsatisfactory. At prices of the poplar wood as of 2017, prolongated to the rotation with the inflation rate for 2017, which is 2.8 %, it turns to be inefficient. At discount norm 0.06, *NPV* of the assessed investment in the poplar plantations is -4083.86 BGN, i.e. it is financially inefficient. At the same time *IRR* of the investment in the plantation is 0.0401 (4.01 %), which in fact is unsatisfactory norm of return in comparison with rate of return of market portfolio in Bulgaria. Besides, the expected final result is with 15 years perspective. This outlook, however, could be an opportunity for improving of the final economic results, if there are prerequisits for increasing of the demand of wood. In this sense, to our opinion, within the investment in poplar stands there are characteristics like foresight, ecology and innovation on the territory of the country and it deserves the attention of economic subjects.

The advantage of the investment in a two-room lodging is that if the location is well-selected, it provides high and stable return in spite of the dynamics of rents. For a 5-year outlook, for which realistic prognosis of change in prices could be done, the investment in a two-room lodging in Sofia could be profitable in all investigated residential areas with the exception of Kremikovtsi. It is financially efficient by discount rate – the achieved average annual return in 2018 for the relevant residential area. The *IRR* varies within the range from 15.26 % in Yavorov to 6.27 % in Vrabnitsa 2. However, it is necessary to invest considerably more financial resources compared to the investment in fast-growing poplar stands. The minimal value of the necessary capital is 40,027 EUR for the purchase of a two-room lodging in Lyulin 5 with *IRR* 12.66 %. For the purchase of two-room lodging in Yavorov, where highest *IRR* would be expected to be achieved, would be needed average 117,467 EUR according to prices in 2018.

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THE GROUND BEETLES (COLEOPTERA: CARABIDAE) FROM A SIGNIFICANT, BUT POORLY STUDIED REGION IN NW BULGARIA. PART 1: TAXONOMIC, FAUNISTIC AND ZOOGEOGRAPHIC NOTES

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Abstract

The paper presents results of the first study on the ground beetle fauna in the region of the Zlatiya Plateau. It aimed at clarifying the species composition and analyzing the zoogeographical structure of the carabid fauna followed by a subsequent assessment of the environmental trends and the extent of anthropogenic impact. Field work was carried out in the period March – October 2008. Ground beetles were collected with pitfall traps in 8 sampling sites representing the different types of habitats in the area. A total of 6598 adult carabid specimens were captured. They belonged to 138 species, 49 genera, and 20 tribes, representing almost 19 % of the species, 39 % of the genera and 56 % of the tribes included in the list of carabids of Bulgaria. The most species-rich was the genus *Harpalus* (25 species), followed by the genera *Amara* (14 species), *Ophonus* (13 species) and *Pterostichus* (11 species). The most abundant in specimens was the genus *Harpalus* (2461 ex.). The most abundant species were: *Harpalus tardus* (908 ex.), *Harpalus rufipes* (829 ex.), *Calathus fuscipes* (567 ex.), *Brachinus crepitans* (414 ex.), *Pterostichus melas* (355 ex.), *Abax carinatus* (348 ex.), *Ophonus laticollis* (199 ex.), *Anchomenus dorsalis* (168 ex.), *Carabus coriaceus* (168 ex.). For *Ophonus convexicollis*, a second certain location for Bulgaria was reported. Six species were new for the whole Bulgarian Danubian Plain: *Leistus ferrugineus*, *Amara sabulosa*, *Harpalus melancholicus*, *Pangus scaritides*, *Microlestes fulvibasis*, *Microlestes minutulus*. Remarkably, 120 species were new for the Western Danubian Plain, where the studied site is located. Most of the species were European-Neareastern (18 %), European and Central Asian (18 %), and Palearctic (12 %).

Key words: carabids, new species records, Western Danubian Plain, Zlatiya Plateau.

Introduction

Zlatiya or Zlatiyata is a loess plateau in the Bulgarian western Danubian Plain (Northwestern Bulgaria), situated between the Danube River and the town of Kozloduy in the north, the road connecting the town of Valchedram and the village of Hayredin to the south, and the currents of the Tsibritsa and Ogosta Rivers to the west and east, respectively. The territory includes mainly arable lands with intensively cultivated crops – predominantly wheat, sunflower and maize, and less barley, rapeseed and alfalfa. Natural vegetation is almost destroyed, with small patches preserved only along the Ogosta River and on the steep slopes around it. The territory is cut by many eroded gullies, overgrown with secondary (herbaceous) pseudo steppe vegetation, shrubs, and single trees. In separate places, the slopes of gullies are forested with black locust or are naturally grown with shoots of indigenous woody vegetation. Some of the deep ravines are intercepted and pooled by spring waters and small pools. In a similar way, but on a larger area Shishmanov Val Dam was built, once used as a leveller and fire facility of 'Kozloduy' Nuclear Power Plant, and subsequently used for irrigation purposes. Fragmented and scattered on relatively large distances, some pastures, orchards, vineyards, protective belts, and anti-erosion shafts are located near the settlements. Soils are chernozems characterized by high natural fertility and degraded to varying degrees (Galabov 1973, Zhelyazkov et al. 2004).

The depopulation of the region and the lack of grazing animals initially led to the overgrowing of most pastoral systems with ruderal nitrophilous vegetation. Subsequently, with the absorption of organic manure nitrogen and the reduction of soil fertility, a secondary derivative system formed on the eroded loess slopes. It is different from the pasture and is visually perceived as a steppe-like habitat.

The area of the plateau is 600 km^2 with an average altitude of about 100 m a.s.l. In 2008, with Order No RD-548 of 5 September, on 43,498.73 ha of the territory of the Zlatiyata two Natura 2000 zones are pronounced: SPA Zlatiyata (BG0002009), included in EU Directive 79/409, and SAC Zlatiya (BG0000336) of Directive 92/43. In 2005, BirdLife International pronounced the plateau as an Important Bird Area with code BG009 (Criteria B2, B3, and C6) and an area of 43,494.44 ha.

For territories similar to Zlatiya Plateau, the most typical are the representatives of the openly living beetles from the tribes Harpalini, Amarini and Sphodrini. In contrast, in less exposed and transitional habitats with increasing humidity, a greater proportion of the tribes Carabini, Pterostichini, Platynini and Nebriini is observed (Teofilova 2013, Kodzhabashev 2016). In biotopes with different soil and vegetation cover on highly urbanized territory of Dnipropetrovsk region with a similar environment, a rich carabid species composition of 280 species was found in a 10-year period of research (Brigadirenko 2003). During a research of Carabidae of the Danube River delta, 190 species were registered (Bašta 2002). However, the types of habitats in the mentioned studies were very different from those of Zlatiyata, so we cannot refer correctly to those data for comparison with our results. In a study of open habitat ground beetles in Belarus, using pitfall traps, 169 species were collected, many of which were macropterous steppe beetles from the tribes Harpalini and Amarini (Aleksandrowicz 2011). In habitats of the city of Kaluga, 64 ground beetle species were found, of which some Harpalini and Amarini species formed the stable, resident and most abundant part of the taxocoenoses, along with highly eurytopic species, like *Pterostichus melanarius* (Illiger, 1798) and *Limodromus assimilis* (Paykull, 1790) (Aleksanov et al. 2019).

So far, the area of Zlatiyata, and the Western part of the Bulgarian Danubian Plain has not been subjected to detailed investigations of the carabid fauna. Such studies are missing even in the adjacent Danube areas in Romania. The lack of reliable faunistic studies impedes the overall assessment of the species abundance, population size, nature of their spatial distribution, biodiversity and extent of anthropogenic impact. The present study aims to add new data to the list of Carabidae species from NW Bulgaria and to focus on clarifying the species composition and analyzing the zoogeographical structure of the carabid fauna followed by a subsequent assessment of the environmental trends and the extent of anthropogenic impact.

Materials and Methods

Field work was carried out in the period 1 March – 24 October 2008. Ground beetles were collected with pitfall traps. The traps were made of cut plastic bottles with 2 l volume and diameter of the enter hole about 12 cm, buried at the level of the substrate. As a fixation fluid, a 4 % solution of formaldehyde was used. In all sampling sites 12 traps were set with a distance of about 15 m between them. This method is suitable for ecological research on adult beetles, and mainly reflects their activity (Lövei and Sunderland 1996); there are no reasonable alternatives to this type of traps in the study of epigeic arthropod communities (Spence and Niemela 1994); it is considered that the application of this method allows approximately 95% of the species active in a radius of 50 m around the traps to be caught (Baars and Van Dijk 1984). The periods of the setting of the traps and collections were: from 01–04 March to 15–19 April 2008 (sampling period 1), from 15–19 April to 15–19 July 2008 (sampling period 2) and from 15–19 July to 20–24 October 2008 (sampling period 3). The investigations were performed at 8 different sampling sites (Fig. 1, Table 1).

Fig. 1. Location of Zlatiya Plateau in NW Bulgaria and an indication on the sampling sites.

Table 1. List and description of the studied sampling sites.

Code	Location	Sampling site description	Coordinates	Alt., m a.s.l.	Period
I	2 km SE of Zlatiya vill.	Harvested, not plowed wheat field, overgrown with nitrophilous weeds and ruderal vegetation.	N 43°43’46” E 23°31‘18“	141	1, 2
II	~ 5 km E of Zlatiya vill.	Overgrown with marsh hygrophilous vegetation shore of a micro dam, built through subdivision of a gully with a small natural water source. The reservoir was heavily overgrown with reeds, bulrush (*Typha* L.) and willows (*Salix* L.), and the steep banks were afforested with Black locust (*Robinia pseudoacacia* L.).	N 43°44’03” E 23°35‘06“	105	1, 2, 3
III	~ 5 km E of Zlatiya vill.	Pseudo-steppe-like habitat, located along the eroded slope of the ravine around the same micro dam, near the Black locust plantation. The territory was overgrown with xerothermic herbaceous vegetation, in places with shrubs and single trees.	N 43°44’01” E 23°35‘15“	111	1, 2, 3
IV	1.4 km NE of Daneva melnitsa Site, near Hayredin vill.	The habitat was in the floodplain of the Ogosta River and was occupied by riparian woody vegetation – white poplar (*Populus alba* L.), alder (*Alnus* spp.), mulberry (*Morus* spp.) and elderberry (*Sambucus nigra* L.). The undergrowth consisted of *Amorpha fruticosa* L., elderberry, nettle and other meso-hygrophilic climbing and creeping plant species.	N 43°37’06” E 23°41‘49“	55	1, 2, 3
V	NE Hayredin vill.	Old mesophilic mixed deciduous forest on an eastern slope, dominated by European oak (*Quercus robur* L.). Traps were located on the slope and reached the ridge part, which was an abandoned field.	N 43°37’04” E 23°41‘49“	65	1, 2, 3
VI	SW coast of the Shishmanov Val Dam	Pannonian loess steppe grass habitat (code 6250/EUNIS code E1.2C1) situated on a loess hilly base and probably formed on an abandoned pasture after its degradation. At the time of research, it was a mesoxerophilic grassland with a pronounced gradient of humidity from the shore of the dam to the hill. As a result of the felling of the woody vegetation in the distant past, the superficial soil layer eroded, and in places were formed erosive furrows and small ravines.	N 43°44’27” E 23°36‘54“	100	2, 3
VII	W Kozloduy, near the coast of the Danube River	Loess steppe habitat similar to the previous, but located between a loess hill and a vertical loess bank of the Danube River. Traps were located on a steep, landslide loess shaft, formed by the erosion processes of the shoreline of the river. The vegetation was similar to that of the previous habitat.	N 43°47’08” E 23°37‘34“	65	2, 3
VIII	E Hayredin vill.	Actively grazed pasture with bushes and an active colony of sousliks (*Spermophilus citellus* L.). The terrain was well drained due to its ridge location. The vegetation was of pasture grass communities that withstand high-intensity grazing and prolonged trampling by livestock.	N 43°35’48” E 23°40‘26“	67	2, 3

Captured animals were determined with the help of several main literature sources: Trautner and Geigenmüller (1987), Hůrka (1996), Arndt et al. (2011), Kryzhanovskij (Fauna Bulgarica – Carabidae, manuscript), and were deposited in the first author's collection in the Institute of Biodiversity and Ecosystem Research (Bulgarian Academy of Sciences, Sofia).

The systematic list follows Kryzhanovskij et al. (1995).

According to their zoogeographical affiliation, the ground beetle species were classified in zoogeographical categories and complexes according to Kryzhanovskij (1965, 1983, 2002), Vigna Taglianti et al. (1999), Casale and Vigna Taglianti (1999) and Kodzhabashev and Penev (2006), with modifications, done by the authors of the present study. The used zoogeographical complexes were: Northern Holarctic and European-Siberian complex, including species distributed mainly in the northern regions of the Holarctic, mostly in Europe and Siberia; European complex, including mostly forest dwelling species connected to the middle and southern parts of Europe; European-Asiatic complex, including species whose ranges fall between the Eurosiberian and Mediterranean zones; Mediterranean (*sensu lato*) complex, including species distributed in the so-called region of the 'Ancient Mediterraneum' (Popov 1927; Kryzhanovskij 1965, 1983, 2002); Endemic complex, including species with limited ranges.

Results

Taxonomic structure and ground beetle diversity

During the study, a total of 6598 adult carabid specimens were captured. Beetles belonged to 138 species, classified into 49 genera and 20 tribes. This figure represents almost 19 % of the species, 39 % of the genera and 56 % of the tribes included in the full list of Carabidae of Bulgaria, and 37 % of the species, 58 % of the genera and 65 % of the tribes established in the Danubian Plain (Teofilova and Guéorguiev, unpublished results). The complete check list of the established species with their full name, author and year of description, zoogeographic category, life form, state of wings development, and ecological group in the relation of the humidity is presented in the Appendix. All ecological data will be discussed in the forthcoming second part of this study.

Our investigation presents some new data about carabid diversity in Bulgaria. For *Ophonus convexicollis*, a second certain location for the country was given (for more details see Teofilova et al. 2020). Six species were new for the fauna of the Bulgarian Danubian Plain: *Leistus ferrugineus*, *Amara sabulosa*, *Harpalus melancholicus*, *Pangus scaritides*, *Microlestes fulvibasis* and *Microlestes minutulus*. Except for *L. ferrugineus*, all other species are characteristic for open xerothermic habitats, covered with herbaceous vegetation, and can be assigned to the group of the steppe species.

Remarkably, 120 species were ascertained to be new for the fauna of the Western Bulgarian Danubian Plain (calculations are according to the catalogue of Bulgarian carabids – Teofilova and Guéorguiev, unpublished results), where the studied site is located. Only *Calosoma auropunctatum*, *Carabus ullrichi*, *C. coriaceus*, *Asaphidion flavipes*, *Bembidion quadrimaculatum*, *Dolichus halensis*, *Amara aenea*, *A. anthobia*, *A. consularis* and *Chlaenius festivus* were previously reported in the literature about this region.

Representatives of 20 tribes were collected (tables 2 and 3). The most species-rich was the tribe Harpalini, probably due to the high presence of inhabitants of the open habitats (Fig. 2). The same pattern was also noticed with the abundance of the ground beetles – the largest number of specimens was established for the same tribe (Fig. 3).

Table 2. Comparison between the carabid faunas of Bulgaria, the whole Danubian Plain, and the present research.

Tribe	Bulgaria		Danubian Plain		Zlatiya	
	No sp.	%	No sp.	%	No sp.	%
Cicindelini	15	2.0	9	2.4	2	1.4
Omophronini	1	0.1	1	0.3	-	-
Nebriini	13	1.7	3	0.8	3	2.2
Notiophilini	10	1.3	3	0.8	1	0.7
Carabini	28	3.7	19	5.1	5	3.6
Cychrini	1	0.1	1	0.3	-	-
Elaphrini	4	0.5	3	0.8	1	0.7
Loricerini	1	0.1	-	-	-	-
Siagonini	1	0.1	-	-	-	-
Scaritini	2	0.3	2	0.5	-	-
Clivinini	4	0.5	4	1.1	-	-
Dyschiriini	31	4.1	13	3.5	1	0.7
Broscini	3	0.4	-	-	-	-
Apotomini	1	0.1	-	-	-	-
Trechini	55	7.4	7	1.9	1	0.7
Tachyini	19	2.5	9	2.4	1	0.7
Lovriciini	1	0.1	-	-	-	-
Bembidiini	98	13.1	47	12.7	4	2.9
Pogonini	13	1.7	2	0.5	-	-
Patrobini	1	0.1	1	0.3	-	-
Pterostichini	56	7.5	27	7.3	15	10.9
Sphodrini	23	3.1	15	4.0	9	6.5
Platynini	37	5.0	17	4.6	3	2.2
Zabrini	58	7.8	33	8.9	16	11.6
Harpalini	156	20.9	97	26.1	51	40.0
Perigonini	1	0.1	1	0.3	-	-
Panagaeini	2	0.3	2	0.5	2	1.4
Callistini	15	2.0	10	2.7	4	2.9
Oodini	2	0.3	1	0.3	-	-
Licinini	11	1.5	8	2.2	4	2.9
Masoreini	1	0.1	1	0.3	-	-
Odacanthini	1	0.1	-	-	-	-
Lebiini	56	7.5	26	7.0	11	8.0
Dryptini	1	0.1	1	0.3	1	0.7

Tribe	Bulgaria		Danubian Plain		Zlatiya	
	No sp.	%	No sp.	%	No sp.	%
Zuphiini	5	0.7	1	0.3	-	-
Brachinini	18	2.4	7	1.9	3	2.2
Paussini	1	0.1	-	-	-	-
Total No sp.	746		371		138	

Table 3. Number of specimens and species in the different tribes of Carabidae collected in the different sampling sites (I–VIII, Table 1).

Tribe	I		II		III		IV		V		VI		VII		VIII		Total	
	N	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*
Cicindelini	2	5	-	-	-	-	-	-	-	-	2	20	2	7	-	-	2	32
Nebriini	-	-	1	5	1	7	1	22	2	25	-	-	-	-	-	-	3	59
Notiophilini	-	-	-	-	-	-	1	26	1	3	-	-	-	-	-	-	1	29
Carabini	1	7	3	53	2	44	4	101	3	92	1	5	1	10	1	4	5	316
Elaphrini	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Dyschiriini	-	-	1	3	-	-	-	-	-	-	-	-	-	-	-	-	1	3
Trechini	-	-	1	9	1	31	1	7	1	55	-	-	-	-	1	1	1	103
Tachyini	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Bembidiini	-	-	2	8	-	-	2	13	-	-	-	-	-	-	-	-	4	21
Pterostichini	-	-	11	61	3	4	5	254	5	462	-	-	1	1	-	-	15	782
Sphodrini	4	10	1	1	4	4	5	66	4	123	5	628	5	23	5	31	9	886
Agonini	-	-	3	13	1	1	2	163	2	20	-	-	-	-	-	-	3	197
Amarini	3	3	7	75	8	44	9	166	6	23	2	28	4	14	3	10	16	363
Harpalini	11	152	25	342	19	713	18	582	12	492	32	458	22	252	21	233	51	3224
Panagaenini	-	-	2	24	2	7	-	-	-	-	-	-	-	-	-	-	2	31
Callistini	1	2	1	2	1	1	-	-	-	-	1	3	-	-	-	-	4	8
Licinini	-	-	3	21	3	6	2	2	1	2	1	1	1	4	-	-	4	36
Lebiini	1	1	5	8	4	8	3	14	1	1	7	28	-	-	1	2	11	62
Dryptini	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	1	1
Brachinini	1	2	2	10	1	73	3	179	2	161	2	15	1	2	1	1	3	443
Total	24	182	70	637	51	944	56	1595	40	1459	53	1186	37	313	33	282	138	6598

Note: *N* – number of species, *n* – number of specimens.

The taxonomic structure of the carabid complex showed a clear qualitative and quantitative predominance of the openly living species from the tribes Harpalini, Amarini and Sphodrini (totally over 60 %) over the predominantly forest or extrazonal coastal species from the tribes Agonini, Pterostichini, Nebriini, and Carabini.

The results of the quantitative data of the taxonomic structure (Fig. 3) showed even more convincingly the predominance of the xero-thermophilic faunistic elements from open habitats. Similarly to the qualitative data, the proportions of the quantitative significance of the tribes Pterostichini, Carabini, and Agonini can be attributed to the relatively good preservation of the forest fauna in refugia located around the river of Ogosta, regardless their relatively small size. Similarly, with relatively identical qualitative and quantitative proportions, intra-and extra-zonal

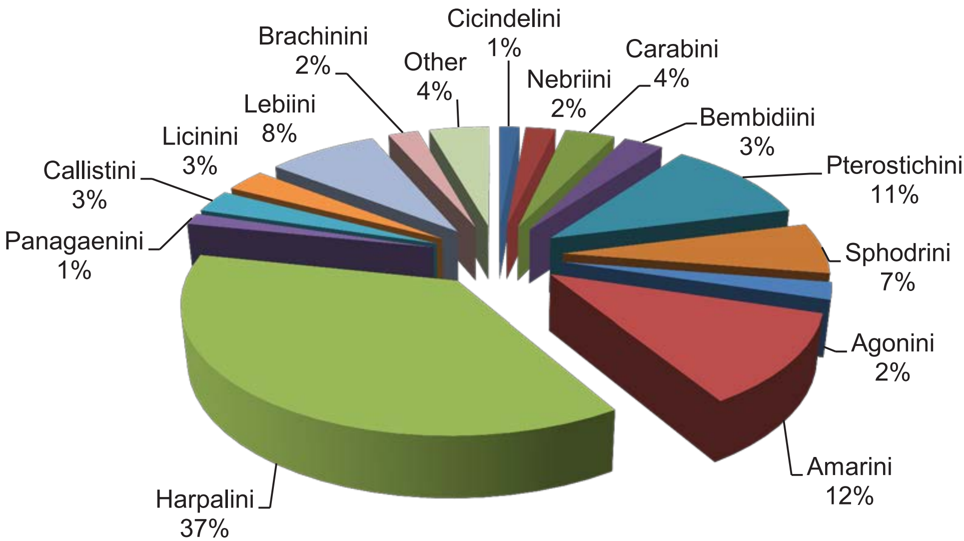

Fig. 2. Number of species in the different tribes.

Note: Section 'Other' includes tribes Notiophilini, Elaphrini, Dyschiriini, Trechini, Tachyini, Dryptini, each one represented by a single species.

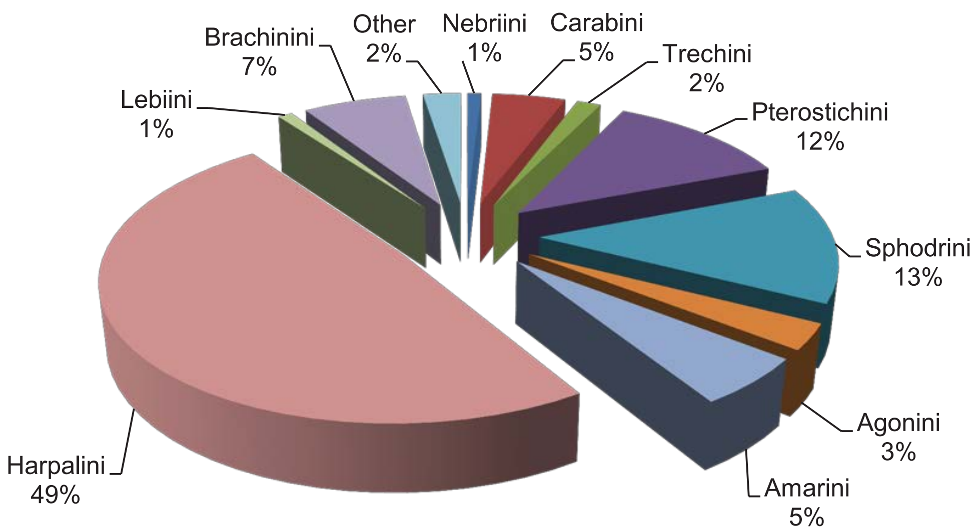

Fig. 3. Number of specimens in the different tribes.

Note: Section 'Other' includes all species, represented by less than 50 specimens (tribes Cicindelini, Notiophilini, Elaphrini, Dyschiriini, Tachyini, Bembidiini, Panagaenini, Callistini, Licinini, Dryptini).

coastal species were represented. They have survived in the preserved coastal habitats of rivers and artificial ponds.

The results obtained for the entire carabid complex showed that the most species-rich was the genus *Harpalus* (25 species), followed by the genera *Amara* (14 species), *Ophonus* (13 species), *Pterostichus* (11 species). Genera *Microlestes* and *Calathus* were represented by six species, and the genus *Carabus* – by four. Three species were found both in the genera *Bembidion* and *Brachinus*. With two species were presented the genera *Leistus*, *Zabrus*, *Stenolophus*, *Acinopus*, *Dixus*, *Panagaeus*, *Chlaenius*, *Licinus*, *Badister*, *Lebia*, *Synthomus*. Only one species was established for each of the genera *Cylindera, Cicindela*, *Nebria*, *Notiophilus*, *Calosoma*, *Elaphrus*, *Dyschiriodes*, *Trechus*, *Tachys*, *Asaphidion*, *Stomis*, *Myas*, *Poecilus*, *Abax*, *Dolichus, Laemostenus*, *Platyderus*, *Agonum*, *Limodromus*, *Anchomenus*, *Anisodactylus*, *Acupalpus*, *Anthracus, Parophonus*, *Pangus, Ablystomus, Callistus*, *Ditomus*, *Cymindis* and *Drypta*.

The largest number of specimens was also found in genus *Harpalus* (2461 ex.). Highly abundant were also the genera *Calathus* (782 ex.), *Ophonus* (583 ex.), *Brachinus* (443 ex.), *Pterostichus* (389 ex.), *Amara* (350 ex.), *Abax* (348 ex.) and *Carabus* (309 ex.). It is interesting to note that the number of specimens caught was not directly dependent on the number of species established in the genus concerned.

The most abundant species were: *Harpalus tardus* (908 ex.), *Harpalus rufipes* (829 ex.), *Carabus coriaceus* (168 ex.), *Brachinus crepitans* (414 ex.), *Pterostichus melas* (355 ex.), *Abax carinatus* (348 ex.), *Calathus fuscipes* (567 ex.), *Harpalus rubripes* (135 ex.), *Anchomenus dorsalis* (168 ex.), *Trechus quadristriatus* (103 ex.), *Carabus ullrichi* (120 ex.), *Calathus ambiguus* (127 ex.), *Ophonus laticollis* (199 ex.), *Amara anthobia* (118 ex.), *Harpalus subcylindricus* (110 ex.), *Harpalus caspius* (93 ex.). Twenty four species (17 %) were represented by a single specimen.

Zoogeographic structure

The zoogeographical analysis showed that the European and the Northern Holarctic and European-Siberian complexes prevailed, encompassing, respectively 39 (28%) and 37 (27%) of all established carabid species (Fig. 4). European-Asiatic complex consisted 31 species (22%), and the Mediterranean complex had 30 species (22 %). Endemic complex was represented only by one subendemic species (1 %).

The greatest number of species had the European-Neareastern, European-Central Asian, and Palaearctic zoogeographic categories (Table 4). These three categories were also the most abundant, as Palaearctic species were represented by one-third of all specimens (more than 2000) and European-Central Asian and European-Neareastern had, respectively, 26 % and 16 % of all specimens.

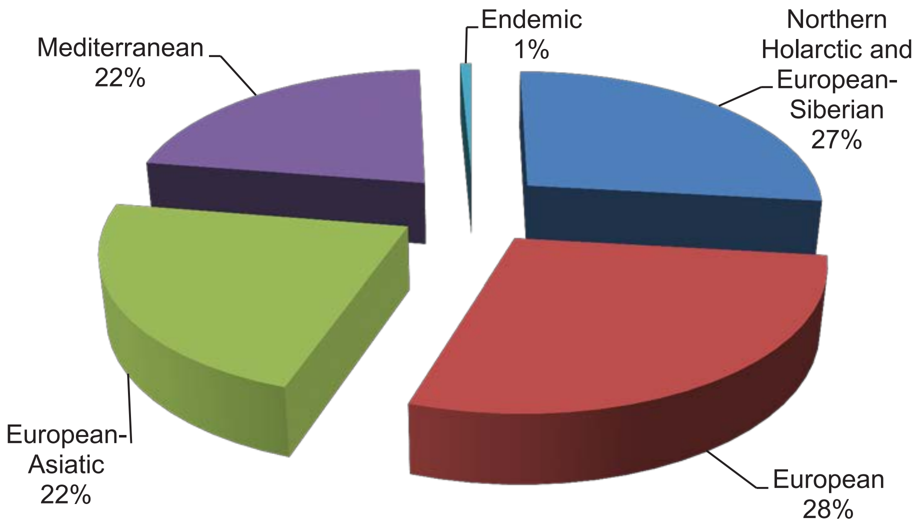

Fig. 4. Number of species in the different zoogeographic complexes.

Table 4. Zoogeographical categories of the ground beetles in the Zlatiya Plateau

Zoogeographical complex	Code	Zoogeographical element	Species No	Species %
Northern Holarctic and European-Siberian	OLA	Holarctic	6	4
	PAL	Palaearctic	14	10
	WPAL	Western Palaearctic	6	4
	E-SI	European-Siberian	9	7
	E-WSI	European and West Siberian	2	1
European	E-PAS	European-Neareastern	25	18
	EUR	European	1	1
	CE-PAS	Central European and Neareastern	5	4
	CEE-PA	Central and Eastern European and Neareastern	2	1
	CE-E	Central and Eastern European	6	4
Euroasiatic	E-AS	Euroasiatic steppe and forest-steppe complex	7	5
	E-CAS	European and Central Asian	24	17
Mediterranean	E-CA-M	European-Central Asian-Mediterranean	9	7
	E-PA-M	European-Neareastern-Mediterranean	10	7
	CA-MED	Central Asian-Mediterranean	2	1
	MED-PA	Mediterranean-Neareastern	2	1
	NM-CAS	Northmediterranean-Central Asian	2	1
	NMED	Northmediterranean	3	2
	B-PAS	Balkan-Neareastern	2	1
Endemic	BAL-K	Balkan subendemic (Balkan-Carpathian)	1	1

Discussion

For the territory of Bulgaria the collection of 138 species for only one year and in a relatively small and compact territory, such as Zlatiya Plateau, indicates that the carabid fauna there can be evaluated as very rich. This fact is probably resulting from the landscape diversity (Petrov 1985, 1997) and the strongly expressed humidity gradient caused by the rivers Ogosta, Tsibritsa, and the Danube, on the one side, and the local xero-mesophilous habitats, on the other.

Similar to our results about the number and composition of the established carabids, using the method of collecting with pitfall traps, were also obtained in other regions of northern Bulgaria (Kodzhabashev and Penev 1998, Popov and Krusteva 1999, Teofilova et al. 2015, Kodzhabashev 2016). This method is widely used in ecological studies, despite the critics on the need for lengthy research periods and its unsuitability for catching small-sized species. In the analysis of the concrete carabid fauna, we will use as a comparison the data from similar studies in northern Bulgaria carried out with the same method.

Considering the full species compositions of the carabid fauna of Bulgaria and the Western Danubian Plain (Teofilova and Guéorguiev, unpublished results) (Table 2) and the fact that with this method for one year of sampling approximately 65 % of the species occurring in the given territory could be captured (Teofilova 2013, Kodzhabashev 2016), the probable species composition of the carabid fauna in the region in case of complete, long-term studies would grow up to 320–330 species and would be comparable to that of well researched regional faunas such as that of the Southern Dobrudzha (Kodzhabashev and Penev 1998, 2006), or even the fauna of large regions such as the whole Danubian Plain. Similar calculations were done about the carabid fauna of Dnipropetrovsk region, suggesting 180 additional species not found so far (Brigadirenko 2003). Table 2 demonstrates the differences in the proportions of the larger taxa, which is relevant to our analysis and conclusions.

Similar results about the taxonomic structure demonstrating the prevalence of tribe Harpalini were obtained for Southern Dobrudzha (Kodzhabashev and Penev 2006), Bulgarian Black Sea coast (Popov and Krusteva 1999, Teofilova et al. 2012, Teofilova et al. 2015) and Eastern Rhodope Mts. (Teofilova and Kodzhabashev 2020). Such taxonomic structure is typical for the forest-steppe zone in northern Bulgaria (Kodzhabashev and Penev 2006), where the natural forest-steppe landscape has gradually merged into a landscape of the open agricultural territories – fields and pastures. With the emergence of new habitats, a new faunistic complex has been formed and many xero-thermophilic species have expanded from south to north and from east to west, colonizing the newly deforested open spaces. The original taxonomic structure has probably undergone changes related to the transformation of the landscape, the increase of open areas, soil erosion, the felling of the vast forests and the droughting due to the reduced flows of the rivers of Ogosta and Tsibritsa, and the transition to more intensive land use. These changes have catalysed the expansion of invasive steppe Asian and thermophilic Mediterranean fauna, having enhanced taxonomic participation in the structure of the carabid fauna of the Zlatiya Plateau.

Similar to our results, the most species rich were the genera *Harpalus* (8 species) and *Calathus* (4 species) in an ecological study of the carabid communities in a fodder's mixture field in North Poland (Aleksandrowicz et al. 2009). The most species-rich was the genus *Harpalus* (17 species) in a study in Cefa Nature Park (Romania) too (Dehelean et al. 2012). Also, according to our research, *Calathus fuscipes* was among the most abundant species in suburban areas near Skopje (Gorgievska et al. 2009). Large numbers of specimens have been established for *Trechus quadristriatus* and *Anchomenus dorsalis* too by Dehelean et al. (2012).

The large percentage of species represented by a single specimen doesn't seem unusual, as it was also established in other studies (Coddington et al. 2009, Ferro et al. 2012, Teofilova 2013). Explanations of the presence of species represented by single specimens may be different – an insufficient number of samples or inappropriate collecting methods, as well as peculiarities in phenology or actual rarity of the species concerned (Novotný and Basset 2000, Coddington et al. 2009). On the other hand, some species that usually fall in the pitfall traps had very low significance in our research. Such species very often have an indicative value and conservation significance or may indicate the beginning of a species expansion and invasion. Exceptionally interesting is the complete absence of some species that are very common in neighboring areas. For example, in an intensive agricultural area near the village of Grivitsa (near Pleven) Popov and Krusteva (1988) established some widely spread species in Bulgaria, such as *Calosoma inquisitor* (Linnaeus, 1758), *Carabus montivagus* Palliardi, 1825, *Carabus scabriusculus* Olivier, 1795, and *Anisodactylus signatus* (Panzer, 1796), not recorded in our study.

One of the frequent disputes about the naturalness of the habitats in Zlatiyata includes the contemporary steppe-like habitats. The probable causes of their appearance are two: degradation of forest habitats after the felling of autochthonous forest vegetation or transformation of the primary steppe and subsequent secondary succession after cessation of almost all agricultural and livestock husbandry activities. The current steppe vegetation is mainly developed on unsuitable for agriculture inclined and eroded terrains or abandoned pastures, developing on a heavily drained and eroded loess base. Such are the loess landslide shafts on the Danube river coast, near the town of Kozloduy, and the steep coastal surroundings of Shishmanov Val Dam, which probably represent abandoned xerophilic pastures. Only in these habitats we found some rare steppe species, such as the newly established *Ophonus convexicollis, Harpalus melancholicus, Pangus scaritides*, *Microlestes fulvibasis*, as well as some stenotopic thermophilic xerobionts, as *O. subquadratus, O. melleti, H. tenebrosus, Acinopus ammophilus, A. picipes, Dytomus calydonius, Dixus clypeatus, D. obscurus, Chlaenius decipens*, *M. corticalis, M. negrita*, and *Cymindis lineata*. Most of these species have low relative significance and belong to the groups of the single, occasional or sporadic species. Pseudosteppe plant communities have been formed a long time ago and have acquired a specific appearance that predetermines specific micro-habitats inhabited by rare and extrazonal ground beetles, which are considered to be of conservation significance and a subject to strict protection.

Specific mesophilous forest species established in the forest refugia in Zlatiyata were: *Leistus rufomarginatus, Carabus ullrichi, C. convexus, Myas chalibaeus,* *Pterostichus melas, Abax carinatus, Platyderus rufus*. Most of these species were presented with single specimens, but as they were only found in this habitat type, they can be considered qualitative indicators. Riparian biotopes are inhabited by specific complexes of extrazonal coastal hygrophilous carabid species, many of which are rare due to special requirements for the conditions of the environment (e.g. *Elaphrus uliginosus*, *Dyschiriodes globosus*, *Pterostichus leonisi*, *P. elongatus*, *P. ovoideus*, *Agonum viduum*, *Stenolophus* ssp., etc.).

In the agrocoenoses we found mainly ecologically plastic eurytoptic species. The specific microclimate shaped by the land use regime, cereal monocultures and constant tempering also supports some stenotopic thermophilic xerobionts. Typical eurybionts (*Harpalus rufipes*, *H. rubripes*, *H. caspius*), usually eudominants, coexist with steppic stenobionts, some of which represented by a very small number of specimens (*Ophonus diffinis*, *H. fuscicornis*, *H. pygmaeus*, *H. hospes*, *Chlaenuis decipiens*).

It is probable that cultural and grassy agrocoenoses have long ago evolved into expansion corridors and peculiar agro-refugia for Asian steppe and Medterranean xero-thermophilic species, very often wrongly considered as conservationally significant. Given their survival in an aggressive environment, we consider that this type of secondary generated fauna should be treated as invasive and a threat to the indigenous faunistic complexes and elements. Similar observations were discussed in a study of steppe carabids in Belarus (Aleksandrowicz 2011).

The species newly established for the region have specific habitat requirements and six of them (*Ophonus convexicollis*, *O. schaubergerianus*, *Harpalus melancholicus*, *Pangus scaritides*, *Microlestes minutulus*, and *M. fulvibarbis*), along with 11 other species (e.g. *Calatus distinguendus*, *Acinopus ammophilus*, *Ditomus calydonius*, *Dixus clypeatus*, *D. obscurus*, *Chlaenius decipiens*, *Microlestes negrita*, *M. plagiatus*, *Cymindis lineata*) can be considered as indicative for the secondary loess steppe communities. Most of these species are xerotermophiles originally established for the southern regions of Bulgaria (Popov and Krusteva 1999). Their recent distribution is probably a consequence of global landscape and climate changes. During our investigation these species were recorded at least in one of the three steppe-like habitats and in none of the others except the pasture.

Zoogeographic structure of the species from Zlatiyata demonstrated some common features with that from the Eastern Rhodope Mts. (Teofilova and Kodzhabashev 2020), where the most species-rich were also the European-Neareastern, European-Central Asian and Palaearctic categories. The established zoogeographical qualitative and quantitative structures were somewhat similar, indicating a relatively homogeneous distribution of species and specimens in the study area. Combined qualitative values of the four zoogeographical complexes two by two, showed that the northern biota dominated over the southern by ~55 % to ~45 %, and in quantitative terms the percentages were exactly reversed. Probably many of the expanding thermophilic species are in process of colonization and the great variety of open habitat beetles is due to suitable agro-habitats. The loess steppes differ from the cultivated fields in species composition and specificity, with the prevailing of the Balkan-Neareastern and Mediterranean-Central Asian zoogeographical forms.

The quantitative zoogeographical structure was characterized by a relatively high percentage of the Palaearctic elements (33%), due to the great abundance of some eurybiont species, as well as some of the intrazonal species occurring in the waterside areas, and some forest species preserved in forest refugia located on steep river banks and slopes. From the group of the southern biota, relatively abundant were the species with European-Central Asian and European-Neareastern distribution, most of which are plastic open habitat forms. A similar distribution of the zoogeographic complexes of the carabids was also found in the eastern part of the Danubian Plain (Kodzhabashev and Penev 2006, Kodzhabashev 2016).

Conclusions

The analyses of the established carabid fauna in the region of Zlatiya showed the changes that have occurred due to anthropogenic impacts related to changes in landscape and land-use regime. As autochthonous and primary the forest and intrazonal riparian fauna can be considered. Due to the relatively long period of existence of the loess steppes, developed as a result of the felling of large forest areas and their subsequent use for pasture farming and intensive agriculture, a specific carabid complex of mainly xerophilous thermophiles was formed there. Some of them expand their ranges to the north, as a consequence of global climatic and landscape changes. Probably due to their initial stage of colonization, these newly established species are rare, being recorded with single findings.

It is difficult to assess whether a residual indigenousness or an onset of invasive expansion is the reason for the low density of most xero-thermophilic species. The lack of previous studies and recent data on the habitats' genesis in the western part of the Danubian Plain makes it difficult to objectively determine the conservation significance of the rare species. Their assessment can only be based on the modern distribution and analysis of data from similar studies in neighbouring or similar territories.

Following the principle of the ecological requirements to specific environmental factors, we will consider that all rare species are of conservation significance and require priority protection of their habitats.

Among the most drastic changes in the environment in the region, the felling of lowland mesophilic forests, ploughing of land for agricultural purposes and changes in the natural hydrologic regime of the rivers can be noted. Priority in protection of the conservationally significant habitats in the region should have forest refugia (remnants of mesophilic and floodplain forests), secondary steppe refugia (formed probably after the felling of forests and subsequent soil erosion) and riparian habitats.

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Appendix

List of Carabidae species established in the Zlatiya plateau region.

No	Species	Range type	Life form	WD	HP	Number of specimens in the different sampling sites								(n_i/N). 100, %	F, %
						I	II	III	IV	V	VI	VII	VIII		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Tribe Cicindellini														
1	*Cylindera* (*Cylindera*) *germanica* (Linnaeus, 1758)	E-PAS	1.2.4	m	M	1					18	1		0.3	37.5
2	*Cicindela* (*Cicindela*) *campestris* Linnaeus, 1758	PAL	1.2.4	m	MX	4					2	6		0.18	37.5
	Tribe Nebriini														
3	*Leistus* (*Pogonophorus*) *rufomarginatus* (Duftschmid, 1812)	EUR	1.3(1).1	D	M					1				0.015	12.5
4	**Leistus* (*Leistus*) *ferrugineus* (Linnaeus, 1758)	E-SI	1.3(1).2	D	M		5	7						0.18	25
5	*Nebria* (*Nebria*) *brevicollis* (Fabricius, 1792)	E-PAS	1.3(1).1	D	MH				22	24				0.7	25
	Tribe Notiophilini														
6	*Notiophilus rufipes* Curtis, 1829	E-PAS	1.3(1).1	m	M				26	3				0.44	25
	Tribe Carabini														
7	*Calosoma* (*Campalita*) *auropunctatum* (Herbst, 1784)	E-CAS	1.2.2	m	MX	7								0.11	12.5
8	*Carabus* (*Eucarabus*) *ullrichi* Germar, 1824	CE-E	1.2.2	b	M		2	6	82	30				1.82	50
9	*Carabus* (*Carabus*) *granulatus* Linnaeus, 1758	E-SI	1.2.2	D	MH		8		1					0.14	25
10	*Carabus* (*Tomocarabus*) *convexus* Fabricius, 1775	E-PAS	1.2.2	b	MX				9	3				0.18	25
11	*Carabus* (*Procrustes*) *coriaceus* Linnaeus, 1758	E-PAS	1.2.2	b	E		43	38	11	57	5	10	4	2.55	87.5
	Tribe Elaphrini														
12	*Elaphrus* (*Neoelaphrus*) *uliginosus* Fabricius, 1792	E-CAS	1.2.3	m	H		1							0.015	12.5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Tribe Dyschiriini														
13	*Dyschirius* (*Eudyschirius*) *globosus* Herbst, 1784	PAL	1.4.2(1)	D	MH		3							0.045	12.5
	Tribe Trechini														
14	*Trechus* (*Trechus*) *quadristriatus* (Schrank, 1781)	E-CA-M	1.3(1).2	m	E		9	31	17	45			1	1.56	62.5
	Tribe Tachyini														
15	*Tachys* (*Paratachys*) *bistriatus* (Duftschmid, 1812)	E-PA-M	1.3(1).4	m	MH		1							0.015	12.5
	Tribe Bembidiini														
16	*Asaphidion flavipes* (Linnaeus, 1760)	WPAL	1.2.3	m	MH				12					0.18	12.5
17	*Bembidion* (*Metallina*) *lampros* (Herbst, 1784)	OLA	1.3(1).2	D	M				1					0.015	12.5
18	*Bembidion* (*Philochthus*) *inoptatum* Schaum, 1857	CE-PAS	1.3(1).1	m	H		1							0.015	12.5
19	*B.* (*Bembidion*) *quadrimaculatum* (Linnaeus, 1761)	PAL	1.3(1).1	m	MH		7							0.11	12.5
	Tribe Pterostichini														
20	*Stomis* (*Stomis*) *pumicatus* (Panzer, 1796)	E-PAS	1.3(1).2	D	MH		10	1	5	2				0.27	50
21	*Myas* (*Myas*) *chalybaeus* (Palliardi, 1825)	BAL-K	1.3(2).1	b	M					1				0.015	12.5
22	*Poecilus* (*Poecilus*) *cupreus* (Linnaeus, 1758)	E-AS	1.3(2).1	m	E		24	1	1					0.39	37.5
23	*Pterostichus* (*Platysma*) *niger* (Schaller, 1783)	E-AS	1.3(2).1	D	MH		1	2		3				0.09	37.5
24	*Pterostichus* (*Argutor*) *cursor* (Dejean, 1828)	E-CAS	1.3(1).2	m	H		2							0.03	12.5
25	*Pterostichus* (*Argutor*) *leonisi* Apfelbeck, 1904	CE-E	1.3(1).2	m	H		1							0.015	12.5
26	*Pterostichus* (*Adelosia*) *macer* (Marsham, 1802)	E-CAS	1.3(2).1	m	M		1							0.015	12.5
27	*Pt.* (*Pseudomaseus*) *anthracinus* (Illiger, 1798)	E-PAS	1.3(2).1	D	H		8							0.12	12.5
28	*Pt.* (*Pseudomaseus*) *nigrita* (Paykull, 1790)	PAL	1.3(2).1	D	H				2					0.03	12.5
29	*Pterostichus* (*Phonias*) *strenuus* (Panzer, 1796)	E-SI	1.3(1).2	D	MH		6							0.09	12.5
30	*Pterostichus* (*Phonias*) *ovoideus* (Sturm, 1824)	E-SI	1.3(2).1	D	MH		5							0.08	12.5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
31	*Pt.* (*Melanius*) *elongatus* (Duftschmid, 1812)	E-CA-M	1.3(2).1	m	H		2							0.03	12.5
32	*Pt.* (*Petrophilus*) *melanarius* (Illiger, 1798)	E-SI	1.3(2).1	D	E		1							0.015	12.5
33	*Pterostichus* (*Feronidius*) *melas* (Creutzer, 1799)	CEE-PA	1.3(2).1	b	MX				77	278				5.38	25
34	*Abax* (*Abacopercus*) *carinatus* (Duftschmid, 1812)	CE-E	1.3(2).1	b	M				188	159		1		5.27	37.5
	Tribe Sphodrini														
35	*Calathus* (*Calathus*) *distinguendus* Chaudoir, 1846	B-PAS	1.3(1).2	D	MX						2		3	0.08	25
36	*Calathus* (*Calathus*) *fuscipes* (Goeze, 1777)	PAL	1.3(1).2	D	E			1	4	104	429	7	22	8.59	75
37	*Calathus* (*Neocalathus*) *ambiguus* (Paykull, 1790)	E-CAS	1.3(1).2	m	MX	1					125	1		1.92	37.5
38	*C.* (*Neocalathus*) *erratus* C. R. Sahlberg, 1827	E-AS	1.3(1).2	D	MX						50			0.76	12.5
39	*Calathus* (*Neocalathus*) *mollis* (Marsham, 1802)	E-PA-M	1.3(1).2	D	X	1			1					0.03	25
40	*Calathus* (*Neocalathus*) *cinctus* Motschulsky, 1850	E-PAS	1.3(1).2	D	MX	2	1	1	1		22	1	3	0.47	87.5
41	*Dolichus halensis* (Schaller, 1783)	PAL	1.3(1).2	m	MX			1		3		2	1	0.11	50
42	*Laemostenus* (*Pristonychus*) *terricola* (Herbst, 1783)	E-PAS	1.3(1).6	D	M	6		1	1	2		12	2	0.36	75
43	*Platyderus* (*Platyderus*) *rufus* (Duftschmid, 1812)	CE-E	1.3(1).2	b	M				60	13				1.11	25
	Tribe Agonini														
44	*Agonum* (*Olisares*) *viduum* (Panzer, 1796)	E-SI	1.3(1).1	m	H		3			1				0.06	25
45	*Limodromus assimilis* (Paykull, 1790)	E-SI	1.3(1).2	m	MH		2		23					0.38	25
46	*Anchomenus dorsalis* (Pontoppidan, 1763)	PAL	1.3(1).1	m	E		8	1	140	19				2.55	50
	Tribe Zabrini														
47	*Amara* (*Zezea*) *tricuspidata* Dejean, 1831	E-CA-M	2.2.1	m	M		1							0.015	12.5
48	*Amara* (*Amara*) *aenea* (De Geer, 1774)	OLA	2.3.1	m	E	1	2	7	5		27	10	1	0.8	87.5
49	*A.* (*Amara*) *anthobia* A. Villa et G. B. Villa, 1833	E-PAS	2.1.1	m	MX				114	3			1	1.79	37.5
50	*Amara* (*Amara*) *convexior* Stephens, 1828	E-CAS	2.3.1	m	MX		58	22	9	7				1.45	50

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
51	*Amara* (*Amara*) *eurynota* (Panzer, 1796)	OLA	2.3.1	m	M		1							0.015	12.5
52	*Amara* (*Amara*) *communis* (Panzer, 1797)	E-SI	2.3.1	m	M			3	3					0.09	25
53	*Amara* (*Amara*) *familiaris* (Duftschmid, 1812)	OLA	2.1.1	m	E	1		1	1					0.045	37.5
54	*Amara* (*Amara*) *ovata* (Fabricius, 1792)	PAL	2.3.1	m	E				4					0.06	12.5
55	*Amara* (*Amara*) *saphyrea* Dejean, 1828	CE-E	2.3.1	m	MX				25	10				0.53	25
56	*Amara* (*Amara*) *similata* (Gyllenhal, 1810)	E-CA-M	2.3.1	m	E		7	2	3					0.18	37.5
57	*Amara* (*Celia*) *bifrons* (Gyllenhal, 1810)	E-CAS	2.3.1	m	MX		5	5		1	1	2		0.21	62.5
58	**Amara* (*Celia*) *sabulosa* (Audinet-Serville, 1821)	E-PAS	2.3.1	m	M			3						0.045	12.5
59	*Amara* (*Bradytus*) *consularis* (Duftschmid, 1812)	E-CAS	2.3.1(1)	m	MX					1				0.015	12.5
60	*Amara* (*Curtonotus*) *aulica* (Panzer, 1797)	E-AS	2.3.1	m	MX		1	1				1		0.045	37.5
61	*Zabrus* (*Zabrus*) *tenebrioides* (Goeze, 1777)	E-CAS	2.3.2	m	MX				1				8	0.14	25
62	*Zabrus* (*Pelor*) *spinipes* (Fabricius, 1798)	NMED	2.3.2	b	MX	1			2			1		0.06	37.5
	Tribe Harpalini														
63	*Anisodactylus* (*Anisodactylus*) *binotatus* (Fabricius, 1787)	E-AS	2.3.1	m	MH		3		1					0.06	25
64	*Stenolophus* (*Stenolophus*) *skrimshiranus* Stephens, 1828	WPAL	2.1.1	m	H		3							0.045	12.5
65	*Stenolophus* (*Stenolophus*) *mixtus* (Herbst, 1784)	PAL	2.1.1	m	H		69	1			1			1.08	37.5
66	*Acupalpus* (*Acupalpus*) *flavicollis* (Sturm, 1825)	E-CAS	2.1.1	m	MH		3							0.045	12.5
67	*Anthracus longicornis* (Schaum, 1857)	CE-PAS	2.1.1	m	H		1							0.015	12.5
68	*Parophonus* (*Parophonus*) *maculicornis* (Duftschmid, 1812)	E-PAS	2.2.1	m	M				4		1		1	0.09	37.5
69	*Ophonus* (*Metophonus*) *laticollis* Mannerheim, 1825	E-CAS	2.2.1	D	MX		1	6	99	90		3		3.02	62.5
70	*O.* (*Metophonus*) *cordatus* (Duftschmid, 1812)	E-PA-M	2.2.1	m	X				11	3	4		74	1.39	50
71	*Ophonus* (*Metophonus*) *puncticeps* Stephens, 1828	E-PA-M	2.2.1	m	MX		4	4	2	17	1	6	2	0.55	87.5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
72	*Ophonus* (*Metophonus*) *rufibarbis* (Fabricius, 1792)	WPAL	2.2.1	m	M		2		21			1		0.36	37.5
73	*O.* (*Metophonus*) *schaubergerianus* (Puel, 1937)	CEE-PA	2.2.1	m	M		1	3						0.06	25
74	*Ophonus* (*Metophonus*) *melleti* (Heer, 1837)	E-PAS	2.2.1	m	MX		1						1	0.03	25
75	*Ophonus* (*Metophonus*) *subsinuatus* Rey, 1886	E-PA-M	2.2.1	m	MX		1							0.015	12.5
76	***O.* (*Hesperophonus*) *convexicollis* Ménétriés, 1832	B-PAS	2.2.1	n.a.	MX						1			0.015	12.5
77	*O.* (*Hesperophonus*) *azureus* (Fabricius, 1775)	E-CA-M	2.2.1	D	MX		7	42	1	1	27	1	11	1.36	87.5
78	*O.* (*Hesperophonus*) *subquadratus* (Dejean, 1829)	E-CA-M	2.2.1	m	MX							1		0.015	12.5
79	*O.* (*Hesperophonus*) *cribricollis* (Dejean, 1829)	E-CAS	2.2.1	m	MX			2	2		35	20	6	0.98	62.5
80	*Ophonus* (*Ophonus*) *diffinis* (Dejean, 1829)	E-PAS	2.2.1	m	X	1								0.015	12.5
81	*Ophonus* (*Ophonus*) *sabulicola* (Panzer, 1796)	E-PAS	2.2.1	m	MX		1	5				41	20	1.02	50
82	*Harpalus* (*Semiophonus*) *signaticornis* (Duftschmid, 1812)	E-CAS	2.2.1	m	MX						20		15	0.53	25
83	*Harpalus* (*Pseudophonus*) *rufipes* (Degeer, 1774)	PAL	2.2.1	m	E	117	101	80	226	185	33	85	2	12.56	100
84	*Harpalus* (*Pseudophonus*) *griseus* (Panzer, 1797)	PAL	2.2.1	m	MX	4	3	2		2	2	2	2	0.26	87.5
85	*H.* (*Pseudophonus*) *calceatus* (Duftschmid, 1812)	PAL	2.3.1	m	X	2	4	2			5		1	0.21	62.5
86	*Harpalus* (*Cryptophonus*) *tenebrosus* Dejean, 1829	E-CA-M	2.3.1	m	MX							1	6	0.11	25
87	**H.* (*Cryptophonus*) *melancholicus* Dejean, 1829	E-PAS	2.3.1	m	MX						5			0.08	12.5
88	*Harpalus* (*Harpalus*) *rubripes* (Duftschmid, 1812)	OLA	2.3.1	m	E	4	2	20	2	1	66	22	18	2.05	100

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
89	*Harpalus* (*Harpalus*) *attenuatus* Stephens, 1828	E-PA-M	2.3.1	m	MX						1	1		0.03	25
90	*Harpalus* (*Harpalus*) *atratus* Latreille, 1804	E-CAS	2.3.1	D	MX				26	66		1		1.41	37.5
91	*Harpalus* (*Harpalus*) *serripes* (Quensel, 1806)	WPAL	2.3.1	m	MX		1				3		6	0.15	37.5
92	*Harpalus* (*Harpalus*) *flavicornis* Dejean, 1829	CE-PAS	2.3.1	D	MX			27	2		25	9	11	1.12	62.5
93	*Harpalus* (*Harpalus*) *pumilus* Sturm, 1818	E-CAS	2.3.1	D	MX			6			18	1		0.38	37.5
94	*Harpalus* (*Harpalus*) *subcylindricus* Dejean, 1829	E-CAS	2.3.1	m	X		2	1			106	1		1.67	50
95	*Harpalus* (*Harpalus*) *zabroides* Dejean, 1829	E-SI	2.3.2	m	X			1						0.015	12.5
96	*Harpalus* (*Harpalus*) *tardus* (Panzer, 1796)	E-CAS	2.3.1	m	E		115	457	186	111	16	7	16	13.76	87.5
97	*Harpalus* (*Harpalus*) *albanicus* Reitter, 1900	E-PAS	2.3.1	m	X		8	23	4		18	2		0.83	62.5
98	*Harpalus* (*Harpalus*) *latus* (Linnaeus, 1758)	E-AS	2.3.1	m	E		1		2	1				0.06	37.5
99	*Harpalus* (*Harpalus*) *fuscicornis* Ménétriés, 1832	WPAL	2.3.1	m	MX	1								0.015	12.5
100	*H.* (*Harpalus*) *smaragdinus* (Duftschmid, 1812)	E-AS	2.3.1	m	X						15			0.23	12.5
101	*Harpalus* (*Harpalus*) *dimidiatus* (P. Rossi, 1790)	E-PAS	2.3.1	m	MX		1		1		1			0.045	37.5
102	*Harpalus* (*Harpalus*) *caspius* (Steven, 1806)	E-PAS	2.3.1	m	X	5	6	28	2	3	8	38	3	1.41	100
103	*Harpalus* (*Harpalus*) *pygmaeus* Dejean, 1829	NMED	2.3.1	m	MX	1					1			0.03	25
104	*Harpalus* (*Harpalus*) *hospes* Sturm, 1818	CE-PAS	2.3.1	m	X	1					1			0.03	25
105	*Harpalus* (*Harpalus*) *affinis* (Schrank, 1781)	E-CAS	2.3.1	m	MX						3			0.045	12.5
106	*H.* (*Harpalus*) *distinguendus* (Duftschmid, 1812)	PAL	2.3.1	m	E	11	1		1	1	3	1		0.27	75
107	**Pangus scaritides* (Sturm, 1818)	E-PA-M	2.3.1	m	MX						1		1	0.03	25
108	*Acinopus* (*Acinopus*) *picipes* (Olivier, 1795)	NMED	2.3.2	D	MX	5					1	4	21	0.47	50
109	*Acinopus* (*Osimus*) *ammophilus* Dejean, 1829	CE-PAS	2.3.2	b	X						8		14	0.33	25
110	*Ditomus calydonius* (P. Rossi, 1790)	NM-CAS	2.3.3	m	MX							4		0.06	12.5
111	*Dixus clypeatus* (P. Rossi, 1790)	E-PA-M	2.3.3	m	X						18		2	0.3	25
112	*Dixus obscurus* (Dejean, 1825)	NM-CAS	2.3.3	m	X						10			0.15	12.5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
113	*Ablystomus metallescens* (Dejean, 1829)	MED-PA	2.1.1	m	MH			3						0.045	12.5
	Tribe Panagaeini														
114	*Panagaeus* (*Panagaeus*) *bipustulatus* (Fabricius, 1775)	E-PAS	1.3(1).1	m	MX		14	4						0.27	25
115	*P.* (*Panagaeus*) *cruxmajor* (Linnaeus, 1758)	E-WSI	1.3(1).1	m	MH		10	3						0.2	25
	Tribe Callistini														
116	*Callistus lunatus* (Fabricius, 1775)	E-CAS	1.3(1).1	m	MH	2								0.03	12.5
117	*Chlaenius* (*Dinodes*) *decipiens* (L. Dufour, 1820)	E-PA-M	1.3(1).1	m	MX						3			0.045	12.5
118	*Chlaenius* (*Chlaenius*) *festivus* (Panzer, 1796)	E-CAS	1.3(1).1	m	H		2							0.03	12.5
119	*Chl.* (*Chlaeniellus*) *nigricornis* (Fabricius, 1787)	E-PAS	1.3(1).1	m	H			1						0.015	12.5
	Tribe Licinini														
120	*Licinus* (*Licinus*) *depressus* (Paykull, 1790)	E-WSI	1.3(1).1	D	M		14	2						0.24	25
121	*Licinus* (*Licinus*) *cassideus* (Fabricius, 1792)	E-PAS	1.3(1).1	b	X			2	2		1	4		0.14	50
122	*Badister* (*Badister*) *bullatus* (Schrank, 1798)	E-PAS	1.3(1).1	m	M		4	2	1					0.11	37.5
123	*Badister* (*Badister*) *lacertosus* Sturm, 1815	E-PAS	1.3(1).2	m	MH		3		1					0.06	25
	Tribe Lebiini														
124	*Lebia* (*Lamprias*) *chlorocephala* (J. J. Hoffman, 1803)	E-CAS	1.1.3	m	M		1	1						0.03	25
125	*Lebia* (*Lebia*) *humeralis* Dejean, 1825	CE-E	1.1.3	m	M		1							0.015	12.5
126	*Syntomus obscuroguttatus* (Duftschmid, 1812)	E-PA-M	1.3(1).3	m	M		3	1	7	1				0.18	50
127	*Syntomus pallipes* (Dejean, 1825)	E-CA-M	1.3(1).3	D	MX		1		6					0.11	25
128	*Microlestes corticalis* (L. Dufour, 1820)	CA-MED	1.3(1).3	m	M						12			0.18	12.5
129	*Microlestes fissuralis* (Reitter, 1901)	E-CAS	1.3(1).3	D	M	1		2	1		2			0.09	50
130	**Microlestes fulvibasis* (Reitter, 1901)	CA-MED	1.3(1).3	b	M						2			0.03	12.5
131	**Microlestes minutulus* (Goeze, 1777)	OLA	1.3(1).3	D	MX		2	4			6		2	0.21	50

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
132	*Microlestes negrita* (Wollaston, 1854)	MED-PA	1.3(1).3	D	MX						1			0.015	12.5
133	*Microlestes plagiatus* (Duftschmid, 1812)	E-CAS	1.3(1).3	m	M						1			0.015	12.5
134	*Cymindis* (*Cymindis*) *lineata* (Quensel in Schonherr, 1806)	E-PAS	1.3(1).2	m	MX						4			0.06	12.5
	Tribe Dryptini														
135	*Drypta* (*Drypta*) *dentata* (P. Rossi, 1790)	WPAL	1.1.2	m	H			1						0.015	12.5
	Tribe Brachinini														
136	*Brachinus* (*Brachinus*) *crepitans* (Linnaeus, 1758)	PAL	1.3(1).3	D	MX	2	8	73	167	160	2	2		6.28	87.5
137	*Br.* (*Brachinus*) *psophia* Audinet-Serville, 1821	E-CA-M	1.3(1).3	m	MX				1					0.42	12.5
138	*Br.* (*Brachynidius*) *explodens* Duftschmid, 1812	E-CAS	1.3(1).3	m	MX		2		11	1	13		1	0.015	62.5
	Total:					182	637	944	1641	1413	1186	313	282		

Notes:

* – new for the Danube Plain; ** – second record for the Bulgarian fauna.

Column No 1. Consecutive number.

Column No 2. List of the species recorded in the Zlatiya plateau.

Column No 3. Zoogeographical belonging. Explanation to the codes are given in Table 4 in the Results section of the paper.

Column No 4. Life forms. Explanation to the indexes and discussion of the life forms will be presented in the next part of the paper (Part 2. Ecology).

Column No 5. WD – wing development (m – macropterous, D – di(poly)morphic, b – brachypterous, n.a. – no data). Discussion will be presented in the next part of the paper (Part 2. Ecology).

Column No 6. HP – humidity preference (H – hygrophilous, MH – mesohygrophilous; M – mesophilous, MX – mesoxerophilous, X – xerophilous, E – eurybiont). Discussion will be presented in the next part of the paper (Part 2. Ecology).

Column No 7–14. Numbers of collected ground beetles in the different sampling sites (I–VIII, Table 1).

Column No 15. Degree of dominance [(n_i/N).100]. Discussion of the dominance structure will be presented in the next part of the paper (Part 2. Ecology).

Column No 16. Frequency of occurrence [F, %]. Discussion of the occurrence will be presented in the next part of the paper (Part 2. Ecology).

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ASSESSMENT OF THE METAL CONCENTRATION IN YANTRA RIVER WITHIN AN AREA WITH ACTIVE ANTHROPOGENIC INFLUENCE

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Abstract

The anthropogenic pollution of the Yantra River was assessed during six year period (2013–2018) in terms of metals in key sites along the river, pointed near to industrial activities, municipal effluents and pollution from tributaries in the catchment area. The assessment of metal concentrations in the water samples was made in accordance with Directive 2000/60/EC – Water Framework Directive and its equivalent criteria transposed into the Water Law in Bulgaria. The results show that the concentrations of metals, such as Hg and Cd, were above the permissible limits of the Bulgarian surface water standard especially in S1 point (the Yantra River near Debeletz). The application of hierarchical cluster analysis for the interpretation of a large and complex dataset obtained during a monitoring program of surface water in the Yantra River is presented in this study. The hierarchical clustering of data shows a correlation between Fe, Mn, Cr, Mg, Ca, Zn, which proves that the increase of Fe concentration could be mainly related to the increased number of the landfills and the unregulated pollution of the catchment area. The overall quality of the Yantra river water corresponds to the descriptive indicator of ‘very good’ water despite the numerous populated places and industrial activities on the territory of the Yantra River basin. The obtained results would be useful in an in-depth future assessment of the general condition of the Yantra River system.

Key words: metal ions, pollution, statistical treatment, surface water quality.

Introduction

In the European Union (EU), a number of legislative and policy processes have imposed more integrated way of urban rivers management by linking water quality improvements to ecosystem protection (Directive 2000/60/EC; Gunatilaka et al. 2007; Bird et al. 2010a, 2010b; Poórová and Vranayová 2019). The Water Framework Directive (WFD) requires the achievement of good ecological and chemical status for all European surface waters, including rivers. The river water quality depends on a number of interrelated factors like climate, topography, geology, and hydrology (Khatri and Tyagi 2015). The knowledge and understanding of chemical composition of surface water is very important because the accumulation of toxic elements in higher concentration can modify the activity of halophilic microorganisms (i.e. algae, diatomee, *Artemia* sp., *Halobacterium* sp., etc.), which have a vital role in C, N, P and S circuit elements, in order to maintaining the rivers as healthy ecosystems (Radulescu et al. 2014). Surface water plays a very important role in water supply. Rivers provide water for public use, recreation, irrigation, as well as for hydropower production. Many rivers and streams are significantly polluted due to the industry, agriculture and household activity (Aytas et al. 2009). Since ancient times, populated places and industries have been located along the rivers because they provide easy transport and are a convenient place for waste discharge. The industrial effluents contain a variety of pollutants like CN^-, Zn, Pb, Cu, Cd, and Hg, some of them being above permissible limits and may affect different components of the aquatic ecosystem, such as micro- and macro-organisms, as well as the sediments (Demirak et al. 2006; Bird et al. 2010a, 2010b; Karbassi and Pazoki 2015; Vladkova 2016; Vladkova et al. 2018; Hazrat at al. 2019; Yotova et al. 2019). The metals are toxic and may contain carcinogenic metalloids that cause cancer of the skin, lungs and urinary tracts; cardiovascular disease; neurotoxicity; and diabetes (Järup 2003, Björkman et al. 2007, Choong et al. 2007, WHO 2007, Akpor and Muchie 2010, Acosta et al. 2015). It is known that the metals can occur in aquatic environment in various forms like complex compounds, ions adsorbed on the surface of the suspended solids, and as a free ion – the most toxic to living organisms (Manahan 2000, Uyguner and Bekbolet 2005, Koumanova 2006, Singh et al. 2011, Başyiğit and Tekin-Özan 2013, Todorova et al. 2016, Gyosheva et al. 2017). Several physicochemical factors, such as salinity, pH, conductivity, temperature, dissolved oxygen, redox potential, and ionic strength influence the concentrations of metals in river water and sediments (Huang et al. 2017). Many methods for metal ions determination in water samples have been known in the literature (Sanfilippo et al. 2007), e.g. HPLC, electrochemical, spectrochemical methods, but ICP-OES and ICP-MS are methods, allowing multi-element and highly sensitive analyses.

Pollution of water bodies is directly related to the need for continuous monitoring of water on various indicators. In this regard, the present study aims to present an assess of some metal concentration in a short area from Yantra River, Bulgaria, by (i) statistical treatment of data for water quality in the period 2013–2018; (ii) Own monitoring of the surface river waters by collecting samples from ‘hot-points‘ of River Yantra. The obtained information could be useful for making decisions on appropriate management actions from resource managers.

Study Area

This study has been carried out along the Yantra River (Fig. 1), which is one of the main river bodies in the Danube region of Bulgaria and is a right tributary of the Danube River (Gerassimov and Bojilova 2008; RBMP-DR 2010–2015, 2016–2021). It is 285.5 km long and has a wa-

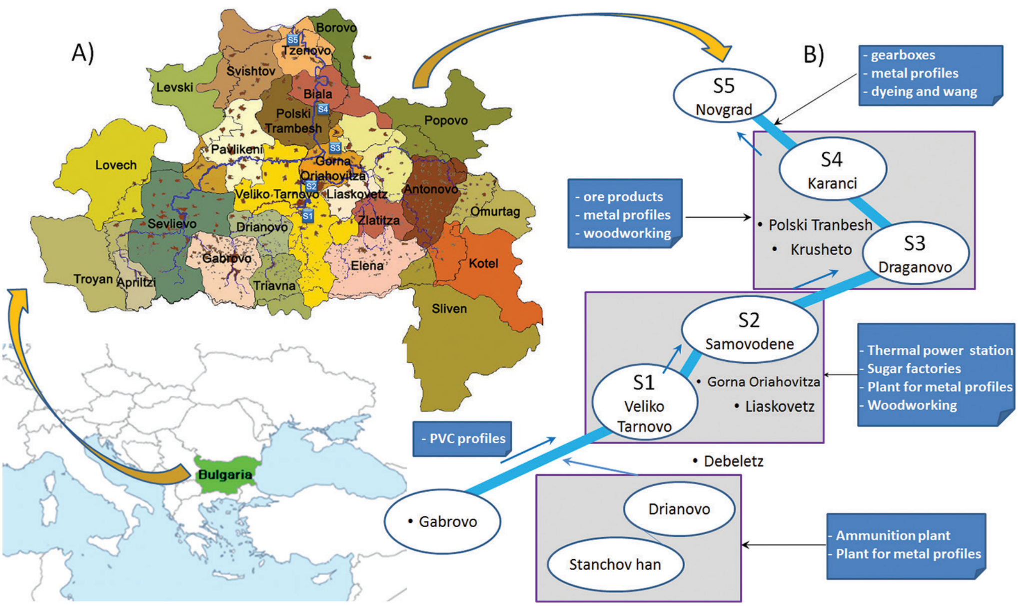

Fig. 1. Study area.

Note: A) The Yantra River basin and the sampling stations; B) Some major pollution zones near the Yantra River Basin.

tershed of 7862 km^2. The river begins from the northern foot of Hadzhi Dimitar peak in Central Stara Planina Mountain, at an altitude of 1340 m and flows into the Danube River near to Svishtov. 783 populated places with approximately 519,000 inhabitants are situated within the Yantra River catchment, representing 6.1 % of the Bulgarian population. Major cities along the river are Gabrovo, Veliko Tarnovo, Gorna Oryahovitsa, Polski Trambesh and Byala. The Yantra River drainage basin is located in one of the economically most important regions of the northern part of Bulgaria. The river water is used for water supply, irrigation and hydropower production. The municipalities are extremely industrialized and negatively influence the ecological state of the environment (Hristov and Ioncheva 2006, Raynova 2015). According to the River Basin Management Plan in the Danube Region (RBMP-DR 2010–2015, 2016–2021), the anthropogenic impact on the Yantra catchment area is complex and it is due to both point and diffuse sources. There are over 100 point sources of water pollution and agriculture is responsible for diffuse pollution. In this way, the contemporary ecological principles of sustainable use of water resources are violated (Namieśnik and Rabajczyk 2010).

Within the Yantra River catchment area the point sources of pollution are different industrial activities like plastic pipes manufacturing, processing of metal surfaces, including galvanizing, wood processing, wastewater treatment plant, thermal power plant, extraction and processing of non-metallic mineral raw materials, industrial laundry and dyeing proposing, which are the main sources of metals in the river basin (RBMP-DR 2010–2015, 2016–2021).

Materials and Methods

Regarding numerous and various anthropogenic activities, this survey takes into consideration the surface water in the Yantra River watershed, considering the spatial and temporal variations in heavy metal content and also evaluated the status of the river water quality with respect to its use for different purposes. The study is based on Ordinance on characterization of surface water (Ordinance No H-4 2012) and Ordinance on environmental quality standards for priority substances and some other pollutant (Ordinance EQSPSSOP 2015).

Sampling and sample locations

In this study, water quality data for certain indicators as metal concentration of Fe, Mn, Mg, Ca, Cd, Hg, As, Cu, Cr, Zn, Pb and Ni, provided by the Executive Environment Agency (ExEA), were used for selected points of the national monitoring network for five year period (2013–2017). On the other hand, during 2018 samples were collected four times per year (once every three months). In order to collect the samples, the basic rules for sampling from water sources were used (DW-GWA 2009). All sampling points selected were situated near suspected sources of industrial, urban or agricultural pollution (Table 1). At least three samples per sampling point were collected. A representative sample (1l plastic bottles, previously washed with HNO_3 solution) from each point was used for analysis. The sampled surface water was put in the acid washed plastic container and acidified with 3 ml nitric acid to avoid unpredictable changes in the water characteristics.

Each of the samples was analyzed for 12 metal ions, such as Fe, Mn, Mg, Ca, Cd, Hg, As, Cu, Cr, Zn, Pb and Ni.

Table 1. The sampling stations of the Yantra River basin.

Sampling station	Location	Water body code	Water catchment area, m^2
S1 (BG1YN00079MS200)	GPS: N 43.06411 E 25.63358 (the Yantra River near to Debeletz)	BG1YN700R1017	330.2
S2 (BG1YN00079MS190)	GPS: N 43.13631 E 25.61356 (the Yantra River near to Samovodene)	BG1YN700R1017	330.2
S3 (BG1YN00059MS130)	GPS: N 43.20484 E 25.74409 (the Yantra River near to Draganovo)	BG1YN307R1127	117.9
S4 (BG1YN00319MS030)	GPS: N 43.38694 E 25.66812 (the Yantra River near to Karanci)	BG1YN130R1029	572.1
S5 (BG1YN00001MS010)	GPS: N 43.61241 E 25.58934 (the Yantra River near to Novgrad)	BG1YN130R1029	572.1

Methods for metals determination

For metal determination, a standard ICP-OES method (Inductively Coupled Plasma Optical Emission Spectroscopy, method: ISO 011885) was used. The main analytic characteristics of the equipment were: Prodigy High Dispersion ICP-OES spectrometer, Teledyne Leeman Labs, USA equipped with a dual view torch, cyclonic spray chamber, and concentric nebulizer with following conditions: coolant gas 18 L·min^{-1}, auxiliary gas 0.5 L·min^{-1}, nebulizer gas 34 psi, RF power 1.2 kW, pump rate 1.2 mL·min^{-1}, sample uptake time 30 s, integration time 40 s. High purity Ar 99.999 % was used to sustain plasma and as a carrier gas (Ilieva et al. 2018, Iacoban et al. 2019). A multi element standard solution (‘Ultra scientific’, Lot: P00332) was used for calibration (Ilieva et al. 2018). Each solution was scanned at least three times and a mean analytical signal was calculated (Ilieva et al. 2018).

Statistic methods for data analysis

In total, a dataset for water quality of the Yantra River was used for further multivariate analysis. The 56 samples were characterized by 6 variables. Hierarchical cluster analysis was used to reveal similarities between variables and sampling sites. HCA was performed only on the basis of major metal ion concentrations using STATISTICA 8 Software. The Euclidean distance as a similarity measure and Ward’s method as a linkage method gave the most efficient results for analysing surface water chemical composition (Retike et al. 2016). Trace elements (here should be listed) were not included in multivariate statistical analysis because complete dataset is required but most of the measurements were made at different times and locations (Surinaidu 2016).

Results and Discussion

The concentrations of analyzed metals in surface water of the Yantra River collected for the period from 2013 to 2018 are presented in Fig. 2. Several so-called ‘hot’ spots during analyses period were detected. From the results, it has been observed that concentrations of metals such as Fe, As, Mn, Mg, Ca, Cu, Cr and Zn were well below the permissible limits of Bulgarian surface water standard in the last observation period. The mean annual concentrations (mean value from max and min concentration measurement in the each season per year) of Fe, Mn, Mg, Ca, Cd, Hg, As, Cu, Cr, Zn, Pb and Ni are shown on figures 2, 3 and 4 each point of which include total five times sampling for the four seasons of the year (Fig. 2). From the data for the single results by seasons, a tendency increase of the metal concentrations was observed at all points during the summer months.

The values of twelve metals assessed indicated that in sampling station S1, mercury and cadmium detected during 2013–2016 were observed to be higher than the permissible limits for waters with different purpose according to the Bulgarian regulations (Table 2), with mean values of concentrations as follows: 20.10 μg·L^{-1}, 10.80 μg·L^{-1}, 15.60 μg·L^{-1}, 13.00 μg·L^{-1} (for Cd) and 71.80 μg·L^{-1}; 62.70 μg·L^{-1}; 70.20 μg·L^{-1}; 67.90 μg·L^{-1} (for Hg). In the years of observation (2015–2017), the mercury standards remain relatively high at this sampling point, while those of cadmium are lowered to the required minimum (Fig. 2). A value of cadmium (30.10 μg·L^{-1}) was

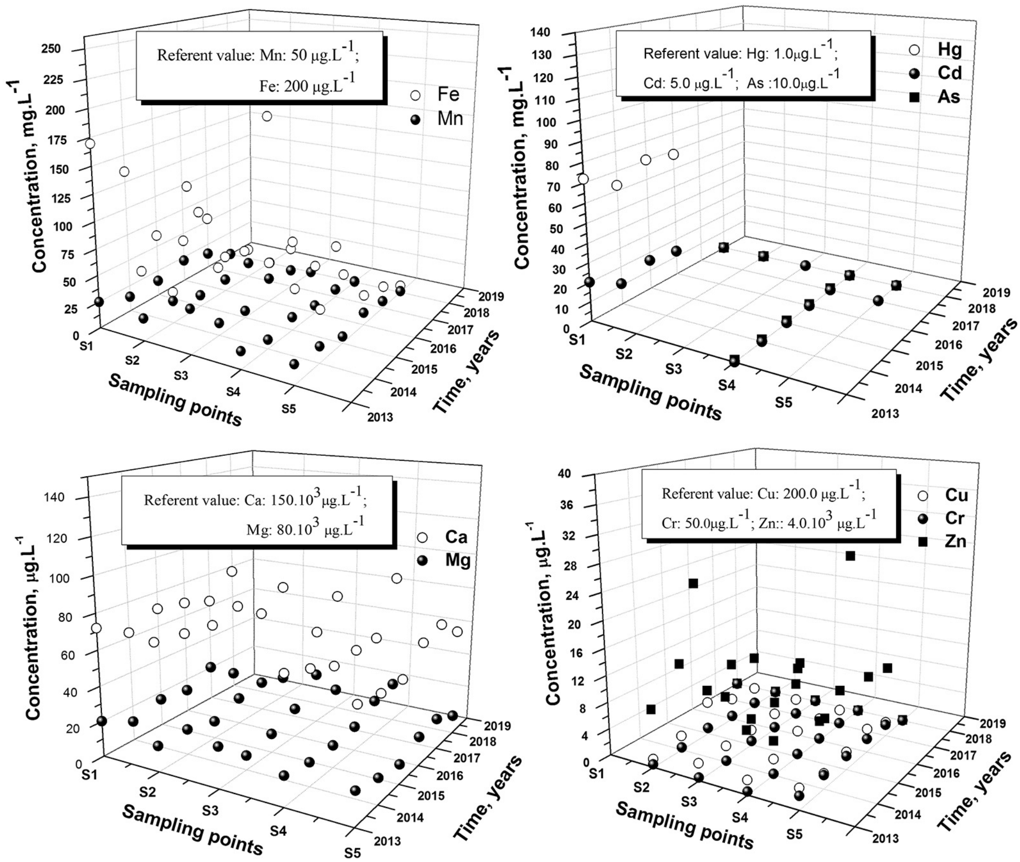

Fig. 2. Distribution of metal content in studied period (2013–2018).

Note: The results are mean value from least three parallel determinations. The referent values according Ordinance No 9 (2001) are given in the boxes of the each graphic plot.

found to be highest during February 2013. The highest concentration (78.80 μg·L^{-1}) that exceeded the maximum permissible concentration of mercury was observed during May 2013. Information on mercury and cadmium content in other samples is scarce. The concentrations of mercury (Hg) and cadmium (Cd) in S1 during almost the whole analysed period are above the permissible limits and this part of the river is determined as the most polluted by heavy toxic metals. Yantra River passes through various urbanized territories, which are local pollutants of the river. One of the major pollutants is the town of Gorna Oryahovitsa, a hot spot of national significance, located between the two main sampling points Draganovo (S3) and Debeletz (S1). The main pollution during this period could be attributed to the existence of factories with poor sewage treatment. The main polluter in this area is the sugar producing plant discharging at the town of Gorna Oryakhovitsa. Basically, water is used as an ingredient, for cleaning and transportation of raw materials, as well as for the cleaning of machines, facilities and work areas. Despite the mentioned

Table 2. The maximum permissible concentration of the investigated metal ions for waters with different purpose according to the Bulgarian regulations.

Bulgarian ordinances \ Metal		Ca	Mg	Mn	Fe	Cu	Zn	Pb	Hg	Cd	As	Cr	Ni
		mg·L^{-1}											
No H-4 (2012)		-	-	0.05	0.1	0.001÷ 0.022*	0.008÷ 0.1*	-	-	-	0.025	0.008	-
No 9 (2001)		150	80	0.050	0.200	2.0	4.0	0.010	0.0010	0.005	0.010	0.050	0.02
No 11 (2002)		-	-	-	-	-	-	0.02	0.001	0.005	0.05	0.02	-
No 12 (2002)	Categories A1	-	-	0.05	0.3	0.05	3.0	0.05	0.001	0.005	0.05	0.05	0.02
	A2	-	-	0.1	2.0	0.05	5.0	0.05	0.001	0.005	0.05	0.05	-
	A3	-	-	1.0	1.0	1.0	5	0.05	0.001	0.005	0.1	0.05	-
No 18 (2009)		400	300	0.2	5.0	0.2	2.0	0.05	0.001	0.01	0.1	0.05	0.2
EQSPSSOP (2015)		-	-	-	-	-	-	0.0012	7.10^{-5}	0.0002	-	-	0.0086

Note: * the value depends on the hardness of the water.

above, the analysis of the water samples during 2018 did not show an excess of the permissible limits of the Cd and Hg concentrations.

The concentrations of Mn and Fe were also determinate. Highest mean values of Fe in 2016 and 2017 were observed for S1 (169.50 μg·L^{-1} and 134.70 μg·L^{-1}) (Table 2 and Fig. 2) which are in permissible level of Fe (Table 2). The manganese in the surface water can be found as dissolved Mn^{2+} or as particulate manganese oxides, hyroxides and carbonates. One can see that the total content of manganese in studied water areas is lower than permissible limit. This may be due to the fact that generally, manganese is more prevalent in groundwater than in surface water (Palmucci et al. 2016).

An assessment of the Pb content during the period was also made. Lead is considered to be the most widely used toxic metal due to its widespread use in industry both in the light and heavy industries. A serious increase of the metal norms compared to other sampling points is observed for S5 during 2017 (Fig. 3). However, its content is well below the critical standards for different nature waters (Table 2).

The change in the nickel concentration was also monitored for the period 2013–2018. Nickel was found in some from samples (S4 and S5) (Fig. 4). Despite that the Nickel content is in permission limit it total concentration in all investigated sampling points could be arranged in the following order: S4 (for all period) > S5 (for all period) > S2 (for 2018) > S1 (for 2018) > S3 (for 2018) (Fig. 4). Nickel is biologically important metal

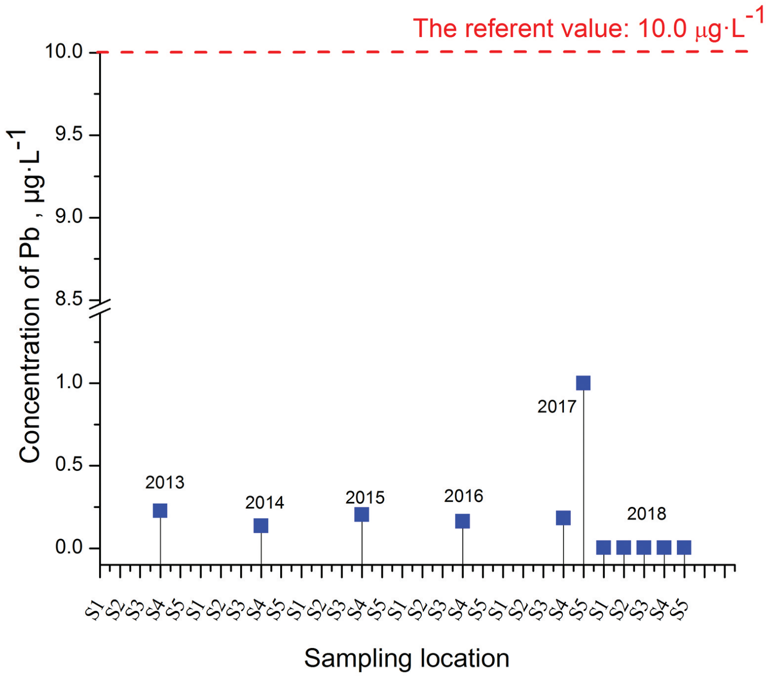

Fig. 3. Concentration of total Pb in studied period.

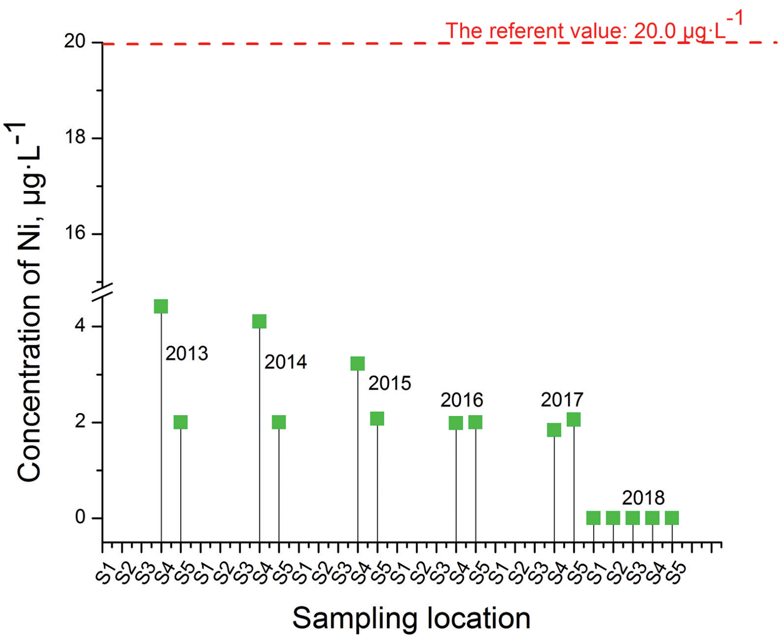

Fig. 4. Concentration of total Ni in studied period.

essential for plants and animals (Poonkothai and Vijayavathi 2012, Zerner 1991). Compared with other transition metals, nickel is a moderately toxic element and its higher concentration can cause a skin disorder known as nickel-eczema (Kristiansen et al. 2000).

In summary, the total content of metals in the samples could be arranged in the following order S1 > S3 > S4 > S2 > S5 (Table 3). The highest portion of metals was observed in S1 showed to be associated with most industrial plants located around the sampling point (Fig. 1).

In order to assess the relationship between individual metals in water, a statistical cluster analysis (CA) was performed using the collected data for six metal ions over the whole study period. A correlation between studied objects and entire data sets was explored using Hierarchical approach, which is the most widely used CA technique (Farmaki et al. 2012). In this study, the cluster analysis was applied for identified Variables' similarities. The dendrogram showed in Figure 5 displaying a significant reduction in dimensionality and complexity of the initial data. Smaller distance between the objects (Euclidean distance) was used because it is preferred for objects similarity interpretation. The data were standardized (mean of 0 and variance of 1) by means of Ward's method, which is the most common way of calculating the distance between two clusters. Thus, one cluster was identified as divided into two subgroups (Fig. 5). The first of them includes Cr, Zn, Mn and

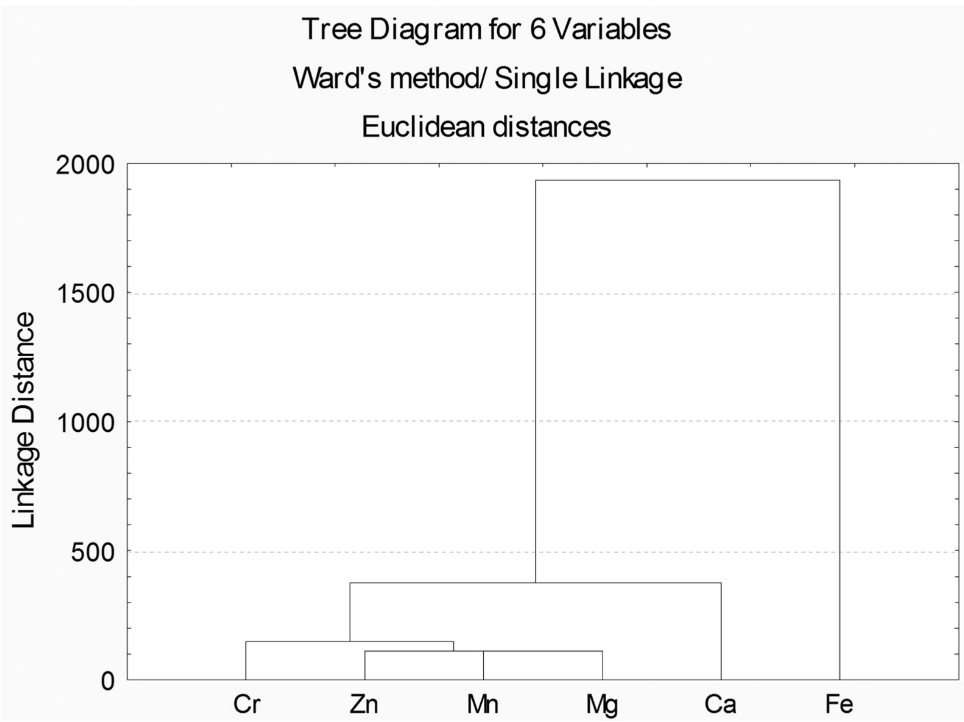

Fig. 5. Dendrograms of the cluster analysis using Ward's method (Euclidean distances) for six variables showing the division of important metals of surface water samples.

Table 3. The mean value for each metal during the period 2013–2018 and total metal content.

Component, μg·L^{-1} / Sampling stations	S1	S2	S3	S4	S5
	***n*/mean ±CI**	***n*/mean ±CI**	***n*/mean ±CI**	***n*/mean ±CI**	***n*/mean ±CI**
Ca, mg·L^{-1}	21/67.12 ±0.03	21/67.29 ±0.01	21/22.60 ±0.02	21/60.345 ±0.002	21/62.6 ±0.1
Mg, mg·L^{-1}	17/15.94 ±0.05	21/14.25 ±0.02	21/17.41 ±0.01	21/15.348 ±0.002	21/15.496 ±0.001
Mn	17/17.72 ±0.08	21/15.675 ±0.007	21/19.97 ±0.03	21/10.354 ±0.003	21/11.678 ±0.003
Fe	17/91.98 ±0.03	21/50.195 ±0.005	21/185.14 ±0.02	21/122.81 ±0.04	21/48.60 ±0.02
Cu	9/0.006 ±0.001	12/2.914 ±0.003	21/2.48 ±0.01	10/2.857 ±0.002	9/2.729 ±0.004
Zn	9/40.01 ±0.01	21/9.13 ±0.02	12/10.13 ±0.02	9/7.24 ±0.07	10/16.41 ±0.04
Pb	5/0.0013 ±0.0002	5/0.003 ±0.001	6/1.20 ±0.02	12/0.182 ±0.002	21/1.00 ±0.001
Hg	17/**68.15 ±0.05**	5/0.07 ±0.02	12/<0.0010	21/0.213 ±0.003	6/0.228 ±0.002
Cd	21/**14.90 ±0.02**	12/0.002 ±0.001	12/0.002 ±0.001	12/0.0224 ±0.0002	12/0.073 ±0.002
As	5/10.0 ±0.2	12/10.1 ±0.6	12/9.62 ±0.04	12/1.016 ±0.001	12/10.40 ±0.08
Cr	10/0.010 ±0.007	21/0.499 ±0.004	21/0.338 ±0.003	21/0.328 ±0.003	17/1.875 ±0.004
Ni	10/4.1 ±0.2	10/4.1 ±0.2	10/3.00 ±0.01	21/3.115 ±0.002	12/2.029 ±0.003
Total heavy metal content, μg·L^{-1}	246.9	92.7	231.88	148.14	95.02

Note: *n* is sampling number; CI is the confidence intervals, calculated by the formula (1); Total heavy metal content is calculated excluding the concentration of calcium and magnesium; the values in bold are above the permissible reference values according the Bulgarian ordinances – No 9 (2001), No 11 (2002), No 12 (2002), No 18 (2009), EQSPSSOP (2015).

$$\Delta\bar{y} = \frac{s \cdot t_{tabl}(P = 95\%; n-1)}{\sqrt{n}}, \qquad (1)$$

where: *s* is standard deviation; t_{tabl}(*P*=95 %; n–1) is table value for statistic criteria; *n* is number of individual results during study period.

Mg, which represent anthropogenic pollutants as discussed above. The second is represented by Ca and Fe. The noted hierarchical cluster analysis of data showed a correlation between some important metal concentrations, which proves that the increase of Fe concentration could be mainly related to the increase of the unregulated industrial pollution in the catchment area.

On the basis of the obtained monitoring data, an ecological and chemical assessment of Yantra River was made (Table 4), (RBMP-DR 2010–2015, 2016–2021; Ordinance No H-4 2012). Despite the variety of the industrial activities along the Yantra river catchment, the river quality is not changed according with previously studied period (1990–2012) (Raynova 2015).

Table. 4. Ecological and chemical assessment of Yantra River.

Water body code	Sampling stations	Ecological	Chemical
BG1YN700R1017	S1 (BG1YN00079MS200) S2 (BG1YN00079MS190)	Moderate	Excellent
BG1YN307R1127	S3 (BG1YN00059MS130)	Excellent	Good
BG1YN130R1029	S4 (BG1YN00319MS030) S5 (BG1YN00001MS010)	Moderate	Excellent

As a whole the river water is assessed as good (Table 4) and may be used for different purposes (Table 2). The set goals for environmental protection of surface water bodies with codes BG1YN700R1017, BG1YN307R1127, and BG1YN130R1029, situated in Danube region, are pollution prevention and keeping good chemical and ecological status.

Conclusions

Heavy metals, even in small doses, possess toxic properties leading to adverse effects on human and ecosystem health. In river water, they are naturally found. The heavy metals are often getting into the environment through dissolution of water-soluble salts, soil erosion, and weathering of rocks but often, their presence in high concentrations is a result of human activities – industrial wastewater discharge and agricultural runoff, as well as illegal landfills. On the basis of this study it could be concluded that the concentrations of heavy metals such as Fe, Mn, Mg, Ca, As, Cu, Cr, Zn, Pb and Ni in the analyzed period are well below the permissible limits of the Bulgarian surface water standard excluding Hg and Cd in some of the sampling points along Yantra river. The data analysis showed that the total metals content was highest in the S1, as the mercury and cadmium content until 2017 were above the permissible limits. It could be associated with the most industrial plants located around the sampling point. In recent years the Bulgarian legislation in the field of environment protection becomes more restrictive, which leads to imposing stricter measures to enterprises, discharging their wastewater into the sewage systems and surface water bodies. This explains the fact that the concentrations of most of the analyzed metals in the waters of the Yantra River over this five-year period are well below the permissible limits, and the concentrations of cadmium and mercury have also decreased over the last two years of the analyzed period. From this point of view, the Yantra River water could be safe for different purposes. The obtained results could be useful for policymakers for water quality management and inclusive monitoring programs should be taken to prevent of further unwanted impact.

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LEGISLATIVE FRAMEWORK FOR ENVIRONMENTAL PROTECTION FROM ELECTRONIC INDUSTRY IN EUROPEAN UNION, THEIR ADAPTATION CHALLENGES AND ASSOCIATED GAPS IN DEVELOPING COUNTRIES: PAKISTAN CASE STUDY

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Abstract

This article targets current situation of Restriction of Hazards Substances (RoHS), Registration, Evaluation and Authorization of Chemicals (REACH), Waste of Electrical and Electronic Equipment's (WEEE) and Responsible Minerals Initiative (RMI) compliance and management in developing countries. The study focus is on adaptation challenges and related environmental protection issues within Pakistan. The emphasis is on how European Union (EU) and other global legislation can mitigate the environmental challenges triggered by EEE (Electrical and Electronic Equipment's) products if EU practices are considered as a role model for developing countries. The aim of this study is threefold: a) to review the challenges in adaptation of environmental legislation already in place in EU and other countries related to electronic industry; b) to review the existing practices related to EEE's related environmental and human health hazards within Pakistan; and c) to discuss the recommendations on how the legislations if adopted in a developing countries like Pakistan can minimize the adverse influence of EEE's on the environment and human health and support national development cause. The study finds that there are huge gaps to reach the optimum level of adequate EEE's base level management (production) and e-waste recycling. To the best of authors' knowledge for Pakistan this is the first time a study on adopting key legislations like EU RoHS, EU REACH, and The Dodd-Frank Wall Street Reform 2010 has been carried out. Similarly, 2019 EEE's Tsunami (Phthalates invasion) are highlighted for first time for government and policy makers in developing countries like Pakistan. This study recommends that switching to these legislations can be useful to tackle the rapidly growing EEE's related environmental, public health and other social issues.

Key words: conflict minerals, environmental policy, e-waste, phthalates invasion, regulated substances, WEEE.

Introduction

The innovation cycles for many EEE products in recent years are quite short, and such products generally have potential to contain materials and components which can be considered hazardous as per EU standards (Press … 2008). To abate the level of hazardous materials, there are regulations in place that control the threshold level of certain hazards substances that are used in EEEs. EU has RoHS, REACH, WEEE legislations in place. RoHS restricts the limit of substances at very fundamental stage, while REACH makes sure that incase of any Substances of Very High Concern (SVHCs) the whole supply chain is notified, and WEEE come into place at the end of life cycle stage, when the EEEs are abandoned. Kandil (2013) estimated that after 2017 the global e-waste will rise to 65.4 million tons annually needing to be disposed safely. Pakistan with a population of over 200 million is prone to various issues and adverse effects of EEE's from both environment and human health perspective.

The EEE's impacts in Pakistan are discussed in various research studies earlier, of these studies (Abbas 2010) is one of the primary researches that provide awareness regarding the important issue of lack of recycling facilities for e-waste management in Pakistan. The country mobile subscribers rose from 1.277 million in 2005 to 100 million by 2011 (Abbas 2011). As per 2018 mobiles subscribers are 151 million (Anonymous 2018). This shows increasing usage trend in recent years. Due to lower income and buying power, people in Pakistan mostly prefer buying used and old phone as compare to buying new. Pakistan already import EEEs which are near their end of life cycle and there is no reliable statistics available on the amount of used electronics arriving in Pakistan (Iqbal et al. 2015). By looking at previous research studies focus was mostly on e-waste management issues. To the best of author's knowledge there is no literature available on RoHS, REACH and Dodd-Frank Consumer Act 2010 possible adaptation challenges and recommendations in Pakistan. The authors believe it is a high time for these legislations to be assessed to be part of Pakistan environmental protection future policies.

Objective of the study

The objective of this study is threefold: a) to review regulated substances environmental legislation already in place in EU and other developed countries; b) to review the existing practices related to EEE's related environmental and human health hazards within Pakistan; and c) to discuss the gaps of environmental regulation and recommend on possible legislation for developing countries, i.e. Pakistan. To meet the objectives of the study it is important to discuss a brief background of regulated substances legislation. Further details are provided in section 4.

Method

This is a narrative assessment that emphasized importance of having legislative framework dealing with electronic industry environmental and social challenges in developing countries. This study considers EU and other developed countries as a benchmark due to their existing strict legislation ensuring environmental protection from electronics during manufacturing, operation and end of life stage (disposal of electronic waste). Furthermore, a survey about environmental legislative framework awareness was conducted with engineers working in electronic industry manufacturing and environmental protection in EU and Pakistan.

Legislation Background – Developed Countries

RoHS Directive

RoHS Directive is an EU directive that administers the restriction on use of certain hazardous substances in electrical and electronic equipment's. RoHS directive has been enforce since 2003 with a recast under the name of RoHS II that was published in Official Journal of European Union and enforced on 1 July 2011 (Directive EU 2015/863 2015). According to Annex II of RoHS Directive (RoHS 2017) as shown in Table 1 it restricts the use of certain substances including: Lead, Mercury, Cadmium, Hexavalent Chromium, PBB (Poly Brominated Biphenyl) and PBDEs (Poly Brominated Diphenyl Ethers) under a threshold level of 1000 ppm except for cadmium (100 ppm) in a homogenous material in EEE. In 2014, EU Commission revealed the intention to add four new phthalates: DBP (Dibutyl phthalate), BBP (Butyl benzyl phthalate), DEHP (Bis (2-ethylhexyl) phthalate) and Diisobutyl Phthalate (DIBP), with maximum concentration value 0.1 % by weight (1000 ppm) in homogenous material. Starting 22 July 2019, the total number of RoHS substances is 10 (Table 1). In addition to these restrictions this directive is known for CE (Conformité Européene) which means European Conformity. The CE marking shows the conformity of EEE/products with all EU legislation applying to the product. This CE mark is a key indicator of EEE compliance with EU legislative requirements. In a nutshell this legislation prohibits and minimize the use of toxic substances at manufacturing level. Any electronic product that is set to be placed on EU market must meet all EU RoHS requirements regardless of where it was manufactured.

REACH Regulation

REACH is an EU Regulation since 2006 with objective to guarantee a high-level safety of public health and the environment, also encouraging development of alternative approaches for assessment of hazards of substances and making sure trouble-free circulation of the substances in the internal EU and Turkish market. It is established on the basis that it is the responsibility of the manufacturers, importers and other subsequent users to make sure that they produce, make available on the market or use the substances that have minimal or no adverse influence on the environment or the human health. Its terms are emphasized by the preventive principle (Regulation (EC) No 1907/2006). REACH deals with Substances of Very High Concerns (SVHCs) as of July 2020, there are 209 SVHCs on European Chemical Agency (ECHA) list (ECHA 2020). According to REACH Regulation manufacturers, importers or distributers has the responsibility to notify the customers of any SVHCs presence.

WEEE Directive

WEEE is an EU Directive 2002/96/EC of the European Parliament and of the Council (January 2003) dealing with the most rapidly growing problem of waste generated by EEE. Some researchers have found that 30–50 million tons of items from electronics industry are abandoned annually (Fogarasi et al. 2014, Menikpura et al. 2014). In 2016, Europe including Russia produced 12.3 Mt of electronic waste with 4.3 Mt only recycled (Baldé et al. 2017). Studies predicted that around 50 million tons of e-waste will be generated around the world in 2019 (WEEE Forum … 2019). All EU member states contribute towards electronic waste collection, recovery and recycling costs.

Table 1. List of substances covered by RoHS and REACH legislation, WEEE Directive covers all equipment using current and Dodd–Frank Wall Street reform and Consumer Protection Act covers 3TGs.

Substance	Concentration	Intrinsic property	Presence in EEE assessment source is (BOMCheck 2019)	Relevant legislation
Cadmium	100 ppm	Carcinogenic	Likely	EU RoHS
Lead	1000 PPM	Carcinogenic, affect nervous system	Very likely and in some cases RoHS exemptions are used.	
Mercury	1000 PPM	Carcinogenic	Likely	
Chromium VI	1000 PPM	Toxic effects in multiple organ systems	Likely	
Polybrominated Biphenyls	1000 PPM	Carcinogenic, endocrine disruptor	Likely, mostly used as flame retardants	
Polybrominated diphenyl ethers	1000 PPM	Carcinogenic, endocrine disruptor	Likely, mostly used as flame retardants	
DEHP	1000 PPM	Reproductive toxic, impair fertility	Likely. It is widely used as a plasticizer in polymer products, mainly PVC	EU RoHS since July 2019
BBP	1000 PPM	May impair fertility, toxic to aquatic organisms	Likely. It is used in polymer products, mainly PVC	
DBP	1000 PPM	Reproductive toxic, very toxic for aquatic organisms	Likely. It is often used, in combination with other phthalates, in flexible PVC	
DIBP	1000 PPM	Risk to impair fertility, may cause harm to unborn child	Likely. It is used, in combination with other phthalate plasticizers, in rubber, chlorinated rubber and PVC	
REACH SVHCs (Substances of very high concern)	1000 PPM	Variable: Toxic for production, very persistent very bio accumulative, carcinogenic, mutagenic, PBT-persistent Bio Accumulative toxic	As of 16 July 2019, there are 201 substances on REACH candidate list many of them are found in electronics. (ECHA 2019)	EU REACH

Dodd–Frank Wall Street reform and consumer protection Act – responsible mineral initiative RMI

The Dodd-Frank Wall Street reform and consumer protection Act section 1502-1504 of the United States of America requires persons and companies to disclose their mineral sourcing origin. The act is in place since 2010 (Dodd-Frank … 2010). Prior to this reform and consumer Act, RMI formerly known as Conflict Free Sourcing Initiative (CFSI) started in 2008 and was adopted by few of the leading companies in electronics business. The idea was to assist them to be updated about their choices on 3TGs (Tin, Tungsten, Tantalum and Gold) conflict-free sourcing in their raw material supply chains. The RMI provided companies with means and resources that can enhance the regulatory compliance and encourage responsible 3TGs sourcing from war-affected and high-risk areas globally but especially in Africa. The aim is that a RMI template is distributed among the supply chain and information on sourcing of minerals (country of origin) used in their products are obtained. Revenues from conflict minerals sourced from the Democratic Republic of the Congo (DRC) have backed conflict, severe human rights abuses, and labour and environmental violations in the county for many years. Corporations that depend on 3TGs for their design and manufacturing products or parts are aware of these social and environmental abuses. Companies are keen on taking necessary action to avoid the benefits from these mineral businesses continue to fund the conflict in any form, these companies are encouraging conflict free sourcing in these areas (RMI 2017). Conflict minerals reporting is a requirement in USA but currently EU companies are also demanding their supply chain to also provide information on 3TGs sourcing using same CFSI reporting template. For example, Germany is making it a requirement for supply chain to do their due diligence for importers of 3TGs and their ores originating from conflict-affected and high-risk areas (Regulation (EU) 2017/821).

Situation in Developing Countries: Case study of Pakistan

In order to assess the legislative framework situation in developing countries a case study of Pakistan was selected. Table 2 provides an overall situation in fast growing economies of China, India, Bangladesh and of the selected case study country.

Table 2. Existing legislation in developing countries.

Legislation	China	India	Bangladesh	Pakistan
RoHS	China RoHS 2	E-waste (Management and Handling) Rules 2011. From May 2014 restricts 6 RoHS substances	Management of WEEE, Draft Regulations, March 2017	PEPA, 1997. National Environment Policy (2005) and Import Policy Order (2016)
REACH	China REACH MEP Order 7	Hazardous Substances (Classification, Packaging and Labelling) Rules, 2011	Bangladesh Environment Conservation Act (BECA) 1995. No list of substances restriction is available.	Same as above
WEEE	Regulation on Recovery Processing of Waste Electrical and Electronic Products	E-waste (Management and Handling) Rules 2011.	Management of WEEE, Draft Regulations, March 2017.	Same as above
Legislation Covering 3TGs		None	None	None

Pakistan is signatory to number of United Nations and other international treaties to safeguard Environment. It formulated and enacted the Pakistan Environmental Protection Act in 1997 (PEPA 1997). This Act has some clauses which in broader sense cover hazardous substances under EU RoHS, EU REACH and EU WEEE legislation but electronic waste is discussed along with other type of waste. Article 31 of PEPA (1997) allowed to make further rules and regulation and Pakistan formulated IEE/EIA Regulation 2000 and Hospital Waste Management Rules 2005 but no rules were developed to handle e-waste when the environment was handled entirely by Federal.

After the 18th Amendment of 2010, Environment was transferred from Federal to Provincial jurisdiction. Subsequently the provinces in haste formulated their own provincial laws, but they are mostly based on same PEPA 1997. For example, Punjab Environmental Protection Act 2012 is issued as an amendment to PEPA 1997 with replacement of term 'National' to 'Punjab'. Other provincials are not much different either. No further rules and regulations were formulated by any province till date related to EEEs. Hence, Pakistan currently have no direct legislation dealing with environmental challenges from electronic industry and specifically electronic waste at both national and provincial level.

On 16th March 2017 Bangladesh Ministry of Environment and Forests published draft rules establishing national requirements for WEEE management, prompting a reasonable concern for EEE producers with manufacturing companies in the country. While not exactly matching the typical EU RoHS, the draft does contain RoHS-based requirements which for sure will impact electronic industry (Compliance… 2017).

From Table 2 it is evident that Pakistan among all these developing countries in the region needs some serious efforts to make legislation like RoHS, REACH, WEEE and conflict minerals reporting for its electronic industry sector.

Discussion

Adaptation challenges – environmental issues due to legislative gaps and recommendations

For a country like Pakistan the adaptation challenges to these legislations along with environmental and social issues caused by these legislative gaps are not very different from those already faced by Vietnam, India, Bangladesh and China which are briefly discussed in this section.

Adaptation challenges

1. Underdeveloped electronic sector and professional awareness

With 7.8 % growth in 2018 still only 3.24 % is contributed to Gross Domestic Product (GDP) by Pakistan electronic industry sector and this is due to the lack of industries in Pakistan (State Bank … 2018). In past one of the reasons was unstable security situation that huge electronic manufacturing companies were reluctant to setup their production facilities. Recently Samsung, Huawei and Nokia have shown interest to setup their mobile phone manufacturing facilities in Pakistan (Business Recorder … 2019). If successfully installed these manufacturing facilities will boost underdeveloped electronic sector of the country.

2. Professional awareness

A survey was conducted as a part of this research and 52 professional in environmental industry from EU and Pakistan participated. Participants were asked to answer some basic questions concerning electronic industry environmental challenges. The survey results in Table 3 clearly indicate, that due to lack of legislation and underdeveloped electronic sector in Pakistan even the professionals working in the environmental protection and electronics sector have very limited knowledge of the threats posed by toxic substances from electronics.

Table 3. Survey results – awareness about environmental challenges from electronic industry among engineers, in %.

Respondent engineers	Q1: Are you aware of harmful substances in electronics?		Q2: Do you know any legislation for environmental protection from electronic industry?		Q3: Do you know cross-wheelie bin sign?		Q4: Your facility have electronic waste segregation?		Q5: Do you know what CE marking is?		Q6: Do you know 3TGs in electronics?	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
EU	90	10	55	45	100	0	100	0	90	10	67	33
Pakistan	65	35	50	50	80	20	40	60	20	80	30	70

3. Communication gap

There is a serious need to improve communication and cooperation between ministries of environment at both federal and provincial level, Pakistan Customs Authorities, ministry of commerce and telecommunication in Pakistan.

4. Customer approach

Lau and Wang (2009) reported that currently many customers are not aware of China RoHS existence, and it is important to add that currently in Pakistan where is no awareness of RoHS, REACH or other legislations related to EEE among the public, therefore they are unable to act as a driving force to make the electronic industry realize its obligations for safer environment. A good example is of public awareness currently practiced in EU where people are aware of the influence of mismanagement of the electronic equipment and try to buy only from reliable companies that are having a take-back system and are trying their best to go green in electronic industry. Customers demand for green and environmentally friendly electronics can be a huge driving force for electronic companies to adopt environmentally friendly products (Doua and Sarkis 2013), and for enforcing RoHS, REACH and other similar legislations.

5. Suppliers responsibility

For many suppliers in Pakistan environment and sustainability is not a priority due to the lack of strict legislation that are practiced in EU countries. Many companies have not done their homework on how they can look for more sustainable alternatives for hazardous substances in their electronic products. There is very limited research on how to go about and adopt more green technologies in the supplier and manufacturing chain.

6. Enforcement of existing regulations

Pakistan being a signatory of Basel convention is still unable to fully comply with its agenda due to lack of awareness and research, and that is why WEEE and other similar legislations (RoHS) are a challenging task especially with the rapid growth of information and communication technology.

7. Lack of electronic industry top management support

Pakistan has mostly small to medium size electronic industry business firms that are reluctant to adopt any new regulations that would affect their profit plans. Testing substances for RoHS and REACH compliance with proper WEEE disposal would add to the financial burden. Doua and Sarkis (2013) reported similar challenges to RoHS regulations implementation in China.

Environmental and social issues caused by legislative gaps

This section provides details on the environmental and social issues caused by legislative gaps.

1. Illegal e-waste arrival in Pakistan

Any EEE's produced and exported into the developing countries both as a new or an e-waste product has different implications. Efthymiou et al. (2016) discussed already known routes for black-illegal e-waste trade that flows from USA, EU and Australia to the developing countries. This flow has been referred to as unfair flow by Wang et al. (2016), they also reported that China ends up receiving and processing 70 % of e-waste by 2012. Pakistan being a close business partner of China due to its geographical position end up receiving a huge portion of such used or near-end of life cycle EEE's as well. Imran et al. (2017) reported that USA exports only 1 % of its e-waste to Pakistan but another study indicated that around 80 % of the US' e-waste is routed to developing states including to Pakistan (Panambunan-Ferse and Breiter 2013). It is worth mentioning that majority of countries (Taiwan, Japan, China, EU and others) indicated as top exports to Pakistan by Imran et al. (2017) are having their own regulations that are somewhat similar to EU-RoHS. These regulations to some extent is believed to be limiting the amount of release of hazardous substances presence in the EEE's/e-waste arriving in Pakistan but it depends on the end of life period of the products.

2. Exposure of human health to toxic substances

Toxic and non-toxic components in the EEE's and e-waste restricted by RoHS and REACH in EU and other countries are not separate that makes the workers more prone to the hazardous substances. A special disturbing scene is that these informal recycling businesses violating the labour laws by employing child labourers as young as 8–18-year-old, they work without any necessary protective equipment's like goggles, face mask or gloves (Abbas 2011). Figures 1–3 show examples of violation of occupational health standards.

Most work is carried about by these children and other adults with bear hands without any Personal Protective Equipment (PPE). Most of them are from poor urban areas with very little or no education and have no awareness what so ever about the threats of hazardous substances presence in e-waste. The children at the development stage are exposed to neurotoxicants, endocrine disrupters and other carcinogenic substances found in electronic waste.

3. Environmental pollution

Environmental pollutants transport phenomena to water, soil and air contin-

Fig. 1. Female worker in informal e-waste recycling areas working without gloves (Photo source: Giovanni Porzio, obtained with permission).

Fig. 2. Female workers residing near informal e-waste recycling areas holding an infant in one of the most contaminated area (Photo source: Giovanni Porzio, obtained with permission).

Fig. 3. Child labourers in scrap areas are exposed to very high level of toxic substances from e-waste informal recycling (Photo source: Giovanni Porzio, obtained with permission).

ues from these informal e-waste recycling practices. Syed et al. (2013) reported that Lyari River is most frequently used as a trashing site if the waste is not dumped into some landfills. Iqbal et al. (2015) also highlighted this environmental issue in their study. The uncontrolled burning of electronic products in Lyari area is causing air, soil and water pollution, this local impact can turn into a regional issue as Lyari flow into Arabian sea and mangroves associated with Indus Delta are found to be heavily contaminated with metal content (Siddique et al. 2009). These toxic metal contents from EEEs burning and trashing can be certainly one of the prime reasons for this pollution. For 2016 Europe including Russia has the highest electronic waste regional collection rate of 35 % (Baldé et al. 2017) most of this collected back through proper take-back program with minimum threat to environmental pollution. Pakistan still has a long way to go in terms of controlling environmental pollution from the electronic waste.

4. Social issues

People who are residing in nearby areas are even if not directly related to the e-waste informal recycling burning are exposed to high level of pollutants including Lead, Mercury, Hexavalent Chromium and other pollutants from plastic burning, etc., their wages are very low in comparison to the working hours they should deal with (Imran et al. 2017).

Recommendations

Government role

Pakistan government should adopt these EU legislations as China did for RoHS.

China has shown its intent and commitment towards tackling restricted substances issues in EEEs. Similar measures have shown by many other countries by introducing their own RoHS these countries include Japan, Republic of Korea, India, Vietnam, UAE and recently Bangladesh have joined the list. Now for developing countries these new legislations would make electronic companies think of establishing their production facilities in countries like Bangladesh and Vietnam as cost of manufacturing is very low, this would give people more jobs and a good social status, at the same time their products can compete in EU market by fully complying to EU-RoHS and REACH type of legislations. Pakistan should allocate more funds, as there are currently not enough available for implementation of requirements of international agreements like Basel Convention (Abbas 2010) and this is the reason e-waste intrude into Pakistan. There should be effective communication between various ministries as the issue with EEE's will further grow an estimate suggests that by 2030, the developing countries (Pakistan is one of them) would have to get rid of double the number of computers in comparison to developed countries (Sthiannopkao and Wong 2013). The government should encourage the electronic industry sector to keep themselves updated regarding EU RoHS, WEEE and ECHA development for REACH (SVHCS) future candidates.

Public awareness

People should be educated through various channels. Iqbal et al. (2015) suggested TV commercials and printed media articles could help in making the public aware of hazards of e-waste. Not many people in Pakistan are aware of green environment and sustainability, making them aware to look for CE mark and China RoHS type logo while buying new products. This should encourage suppliers to provide more environmentally friendly electronic products.

Corporate and producer extended responsibility

Being a developing country that is prone to the toxic substances from EEE's, a fee of 2–5 % of the original cost of the product production should be charged from the country (Wang et al. 2016), which is making the EEE/e-waste available on the Pakistani market, this fee could be used to fund the development of a formal recycling facility in the country. All the manufacturing and supplier of electronic products should be obliged to put their sustainability and environmental regulatory information on WEEE, RoHS REACH and mineral sourcing and what are their plans for take back program. Pakistan should go one more step forward and be a leader in developing countries to encourage the suppliers to disclose their conflict minerals information for the EEE's placed on its market, this will help support conflict free sourcing of minerals.

Regional cooperation with better environmental legislations

Many countries in the region have planned or already adopted RoHS type legislations including China, India, Bangladesh, Vietnam and UAE. There is huge scope of these legislations to be enforced on government level as they have profound impacts on the betterment of environment in EU countries. The RoHS aspect of the managing EEEs related environmental issues is not the only legislation that need to be enforced but nonetheless a positive sign that the country would be committed to addressing its growing e-waste problem both upstream and through end-of-life management. Time has come for Pakistan to catch up with global RoHS and other EU and global environmental legislations developments.

In the past due to political instability and lack of active legislative structure environmental legislations were not progressed coherently. Pakistan provincial legislative institutes missed an opportunity in 2010 when the environment came under the provincial jurisdiction and they instead of upgrading the laws simply scaled down PEPA 1997 to their respective provincial jurisdiction. Since the democratic governments have now being elected regularly and there is smooth continuation of democratic process the lawmakers should develop the rules and regulation to adopt to new challenges. It must be noted that PEPA 1997 was developed when the electronic mobile industry was not of the scale as it is now. Therefore, countries like Pakistan should upgrade their rules and regulation imminently.

Watch out for 2019 EEE's Tsunami

Four new phthalates that have approached their sunset date in July 2019 includes DBP, BBP, DEHP, DIBP as discussed earlier in RoHS introduction part of this paper are now regulated for their concentration in electronic products at value of 0.1 % by weight (1000 ppm) in homogenous material per Directive EU 2015/863 (2015). The electronic companies which are not able to come up with other suitable alternatives for these phthalates in their products will look for alternate market as EEE's with above threshold limits of these phthalates cannot be placed on EU market. The best option for them would be to sell their products in developing countries with no strict regulations on these hazardous substances. Lack of EU RoHS type of legislation in many developing countries including Pakistan had to face 22 July 2019 triggered Tsunami of electronic products containing these hazardous substances. This shift of market preference and phthalates containing electronic products invasion would create more challenges in electronic industry and its subsequent waste management in developing countries.

Conclusions

With rapid increase in severe environmental risks originating from EEE's and other related products, Pakistan and other developing countries should recognize that RoHS, REACH, WEEE and RMI type of legislation are no longer an option but a necessity these days considering their lack/outdated legislative framework. However, their adaptation will face multiple challenges as discussed in this study for Pakistan case, but there is no escape from adopting these similar legislations as EU and many developed countries did. Developing countries like Pakistan should make sure that any EEE manufactured or arriving at the country's market should be required to pass the RoHS, REACH, WEEE and possibly RMI compliance requirements. Compliance with these regulations can help Pakistan to minimize the impacts of EEE's driven challenges to environment and human health at very root level especially after the 2019 EEE's Tsunami when additional regulated substances were added to EU RoHS. Pakistan could lead as an example by adopting RMI and request at least as a starting point suppliers and manufacturers like

Samsung, Nokia, LG Electronics to provide details of their mineral sourcing areas this will help reduce the environmental and social abuses in other areas such as Congo and other African countries. It is recommended that policy makers in developing countries like Pakistan come up with extended producer responsibility requirements, companies should contribute financially to ensure end of life electronic products proper take-back and safe disposal like in European Union.

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RED FOX (*VULPES VULPES*) AS THE MAIN VECTOR OF ANIMAL'S RABIES IN THE FOREST-STEPPE ZONE OF REPUBLIC OF MORDOVIA

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Abstract

Rabic infection currently determines the epidemic and epizootic state in many countries. In this study, we revealed the value of red fox (*Vulpes vulpes* Linnaeus, 1758) as a carrier of rabies in the forest zone. Altogether, the research was conducted in 22 districts and the city of Saransk during the period from 2009 to 2018. The work uses a comprehensive epizootological analysis, including methods of modern diagnostics, veterinary and sanitary statistics, epizootological examination, as well as other methods generally accepted in epizootology. When determining the boundaries of the risk of rabies virus in the Republic, we found that the main clusters of epizootic foci of infection are located on the territory of Bolsheignatovskiy, Ichalkovskiy, Chamzinskiy and Atyashevskiy districts. It was found that the expansion of the infection zone over the years on the territory of the Republic is associated with the dynamics of the main host of the pathogen – red fox. In our Republic, the population density of foxes varies from 1.1 to 1.6 individuals per 1000 ha. In some areas, it is twice the average. The highest population density was observed in 2010 – 1.6 individuals per 1000 ha. The same year, there was a large proportion (66 %) of infected foxes with rabies, compared to other animals. The first rise of diseases coincides with the period of fox rut (early spring) and the second-with the settlement of young animals (autumn-winter).

Key words: forest ecosystem, rabies virus, tank, wild animals.

Introduction

Rabies is a very dangerous deadly infectious disease caused by rabies virus *Rabies virus* (*Lyssavirus*, family Rhabdoviridae). It is specific encephalitis in animals and humans. Rabies virus is a well-adapted pathogen of the mammalian nervous system, where it mostly infects neurons. This virus is typically transmitted by animal bites, although some cases of aerosol contamination have been described. Virus particles from the saliva of infected animals or progeny virus particles produced by muscle infection enter the nervous system via a sensory nerve through nerve spindles or via the neuromuscular junctions where motor axons bifurcate in invaginations of the muscle surface. Rabies virus particles then travel along the spinal cord to the brain, before spreading to the salivary glands. Virions are excreted in the saliva and are transmitted to other hosts by bites (Rupprecht et al. 2002, Belotto et al. 2005, Lafon, 2005).

Rabic infection currently determines the epidemic and epizootic state in many countries. Rabies is registered annually on all continents and in all natural and climatic zones. It occupies an extremely important place in pathology, since the disease affects a large number of species of domestic and wild animals. The disease is a deadly danger to humans (Jackson et al. 2003, Hemachudha et al. 2013, Morters et al. 2013, Fooks et al. 2014, Al-Abaidani et al. 2015, Hampson et al. 2015, Paroshin et al. 2017).

In Russia human deaths when dealing with dogs have constantly been recorded since the 16th century. The share of dogs in human hydrophobia infection reached 85 % from the end of the 19th to the middle of the 20th centuries. The epidemic value of foxes is cyclical. In the late 18th century fox rabies in Russia was not known. People died from hydrophobia after fox bites in the early 19th century, but since 1825 cases of human hydrophobia due to contacts with foxes stopped. Fox again started infecting people only since the 1940's. In some years from 1970 to the 1990's, fox was the source of hydrophobia in 50–52 % cases. In the 21st century, its role in human infection has decreased to 16 %, but the value of that predator in animal infection increased to almost 50 % (Deviatkin et al. 2017, Sidorov et al. 2016, 2019).

The problem of studying rabies in Mordovia was covered by us and our colleagues (Chumakov and Alferina 2009, Gurina 2009, Tolokonnikova et al. 2015, Alferina et al. 2016). However, it requires constant monitoring.

The purpose of this article is to study the role of the red fox as carrier or reservoir of rabies.

Material and Methods

The work uses a comprehensive epizootological analysis, including methods of modern diagnostics, veterinary and sanitary statistics, epizootological examination, as well as other methods generally accepted in epizootology. The work was carried out in all regions of Mordovia. The Republic of Mordovia is located in the centre of the European part of Russia. Its extreme points are defined by geographical coordinates 42º11' – 46º45' E and 53º38' – 55º11' N (Fig. 1). The maximum distance from west to east is 298 km and the distance from north to south is 57 to 140 km. The area of the republic is 26,200 km^2. Features of the geological structure of Mordovia are determined by its location in the central part of the Russian Platform and the north-western slopes of Volga Upland. In the western part, Volga Upland reaches Oka-Don Lowlands.

The climate of the region is moderately continental with pronounced seasons throughout the year. The influx of direct solar radiation in Mordovia varies from 5.0 in December to 58.6 kJ/cm^2 in June. Total radiation throughout the year is 363.8 kJ/cm^2, the radiation balance is 92.1 kJ/cm^2. The average annual air temperature varies from 3.5 to 4.0 °C. The average temperature of the coldest month (January) is in the range of –11.5 to –12.3 °C. Temperature drops down to –47 °C occur. The average temperature of the warmest month, i.e. July, is in the range of +18.9 to +19.8 °C. Extreme temperatures in the summer reach 37 °C. The

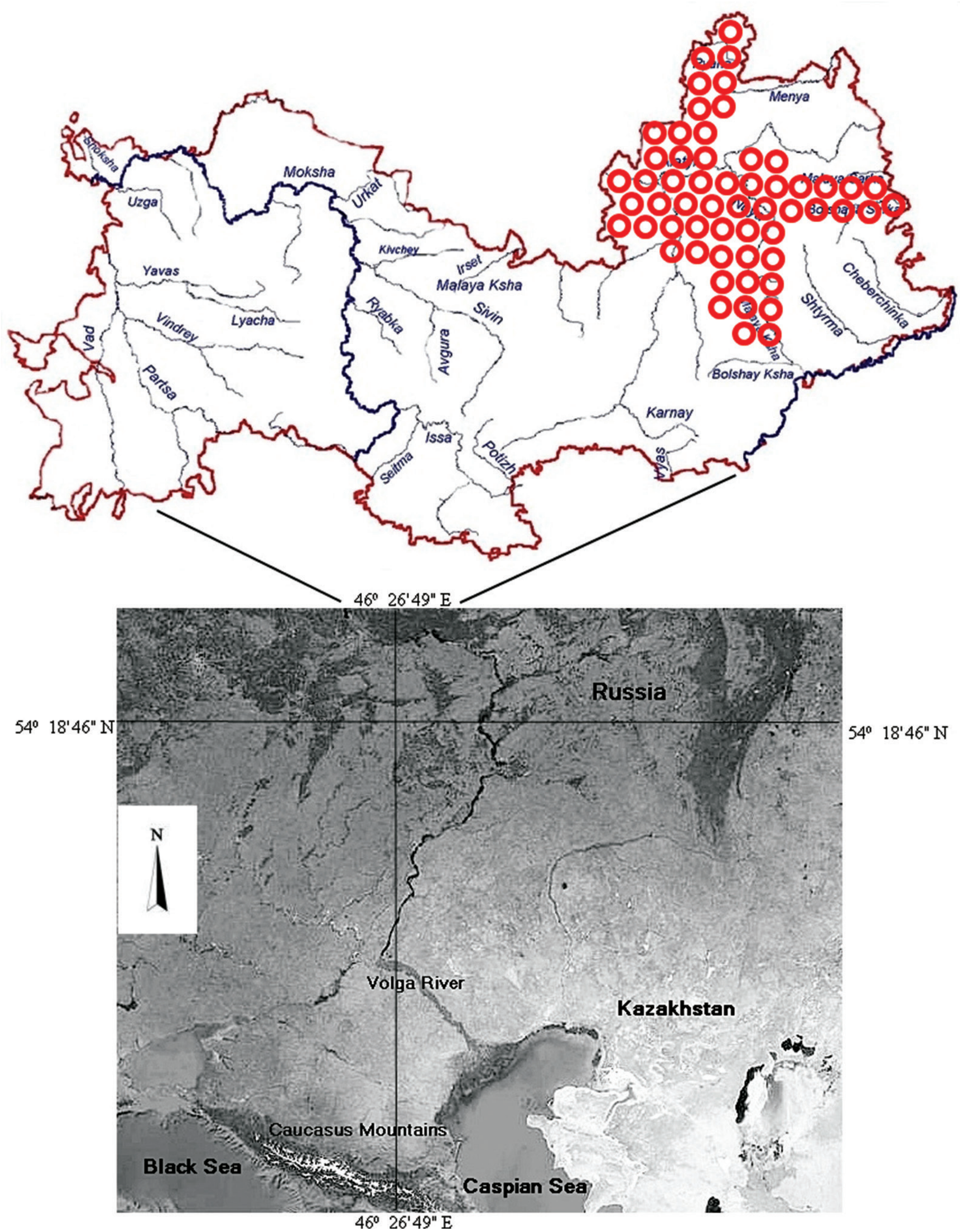

Fig. 1. Location of districts with high infection of foxes with rabies.
Note: red circle – Bolsheignatovskiy, Ichalcovskiy, Chamzinskiy, Atyashevskiy districts.

average annual precipitation in the territory of Mordovia is 480 mm. Over the course of observation lasting many years, periods of more and less humidification were noted, ranging between the minimum and maximum values of 120–180 mm. Distribution of precipitation across the territory is not very diverse. The average long-term value of evaporation is calculated to be in the range of 390–460 mm (Yamashkin 1998). The total number of foxes studied for rabies was 386 individuals, of which 145 were infected.

Results and Discussion

The habitat of the red fox is extremely diverse. The fox, as a species, is a fairly sedentary animal, it does not tend to migrate. The development of new territories is carried out by young animals (Andreychev et al. 2015). With the beginning of autumn, foxes older than six months leave their parents' territory, migrate to a distance of about 20 km and begin an independent life. It is believed that the territory controlled by a single fox family, depending on the food supply and the nature of the habitat, is 2–10 km^2. In our Republic, the population density of foxes varies from 1.1 to 1.6 individuals per 1000 ha (Andreychev et al. 2012). In some areas, it is twice the average. The highest population density was observed in 2010 – 1.6 individuals per 1000 ha. The same year, there was a large proportion (66 %) of infected foxes with rabies, compared to other animals. The dependence of the number of registered sick foxes on the overall population dynamics in Mordovia is observed. In particular, with the total number of foxes in Mordovia in 2017 was 3294 individuals, the share of foxes infected with rabies accounted for 63 % of cases among all animals. A similar situation was observed in 2010. When the number of foxes was 3738 individuals, the share of foxes infected with rabies accounted for 66 % of cases among captured animals. In 2014, with a fox population of 2441 individuals, rabies-infected foxes accounted for 38 % of cases among captured animals.

Our data confirm the results obtained for Russia as a whole. Based on the analysis of the rabies rate of animals for the period of 1960–2006 and on the assessment of the number of wild dogs (Canidae) for 1981–2006 and their infection, a prevailing role of red fox in spreading of rabies among wild mammals (91.7 %) is established. The high correlation between the number of this predator and infected animals was confirmed (Sidorov et al. 2010).

When determining the boundaries of the risk of rabic infection in the Republic, we found that the main clusters of epizootic foci of infection are located on the territory of Bolsheignatovskiy, Ichalkovskiy, Chamzinskiy, and Atyashevskiy districts (Table 1). These districts are unfavourable for other infectious diseases (Andreychev et al. 2019, Andreychev and Boyarova 2020). It was found that the expansion of the zone of infection over the years on the territory of the Republic is associated with the dynamics of the main host of the pathogen – red fox. The number directly depends on the dynamics of the number of small mammals, which are the main food resource of the predator. Earlier, we traced a similar dependence of the population dynamics of other predators on the dynamics of the number of their victims (Andreychev et al. 2014, 2016a, 2016b; Andreychev and Lapshin 2017).

Table 1. Dynamics of the number of infected red foxes with rabies by region.

District	Years										Total by district
	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	
Ardatovskiy	2	2	2	0	0	0	0	1	0	0	7
Aturevskiy	0	3	0	0	1	0	0	0	1	0	5
Atyashevskiy	8	2	1	0	2	3	0	0	0	0	16
Bolshebereznikovskiy	0	2	1	0	0	1	0	0	0	0	4
Bolsheignatovskiy	7	3	0	0	0	1	0	1	1	1	14
Dubenskiy	0	0	0	0	0	0	0	0	0	0	0
Elnikovskiy	0	0	1	0	0	0	0	0	0	0	1
Zubovo-Polyansky	0	0	0	0	1	0	0	0	0	0	1
Insarskiy	2	1	0	0	0	0	0	0	0	0	3
Ichalkovskiy	6	7	2	0	0	2	1	0	0	4	22
Kadoshkinskiy	0	0	0	0	0	0	0	0	0	0	0
Kovylkinskiy	1	2	0	1	1	1	2	0	0	0	8
Kochkurovskiy	1	4	1	0	0	1	0	0	0	1	8
Krasnoslobodskiy	0	1	2	0	0	1	1	0	0	0	5
Lyambirskiy	0	2	0	0	0	1	0	0	0	1	4
Romodanovskiy	1	1	0	0	0	0	0	0	0	1	3
Ruzaevskiy	2	1	0	0	1	1	0	0	1	0	6
Staroshaigovskiy	3	1	0	0	0	1	0	0	0	2	7
Tengushevskiy	1	0	0	0	0	0	0	0	1	1	3
Temnikovskiy	1	1	0	0	0	0	0	0	0	0	2
Torbeyevskiy	0	0	0	0	0	0	0	0	0	0	0
Chamzinskiy	0	6	5	2	0	2	0	0	0	0	15
Saransk	4	2	0	0	0	3	0	0	1	1	11
Total per year	39	41	15	3	6	18	4	2	5	12	145

Note: grey color indicates areas with a high percentage of infected foxes.

In the long-term dynamics of the time boundaries of epizootic manifestations of rabies, we were able to establish a pronounced periodicity with a synchronous alternation of declines and rises in epizootic tension. The results of the analysis of archival documents in the dynamics of rabies over the last ten years, you can select multiple cycles of rise and decline, which suggests a cyclical upswing rabies infection in the country, which manifests itself with an interval of 3–4 years (Fig. 2). The smallest percentage of infected foxes among other animals was noted in 2012 and 2015. The peaks of rabies infection were observed in 2009, 2010, 2013, and 2017.

We were able to establish the annual dynamics (seasonality) for the research period from 2009 to 2018, due to the cyclical biological activity of the main host of the pathogen. The first rise of diseases coincides with the period of fox rut (early spring) and the second – with the settlement of young animals (autumn-winter). There were 17 cases of foxes infected with rabies in February and 22 cases in March (Fig. 3). There were 17 cases of foxes infected with rabies in November and 14 cases in December. The fewest

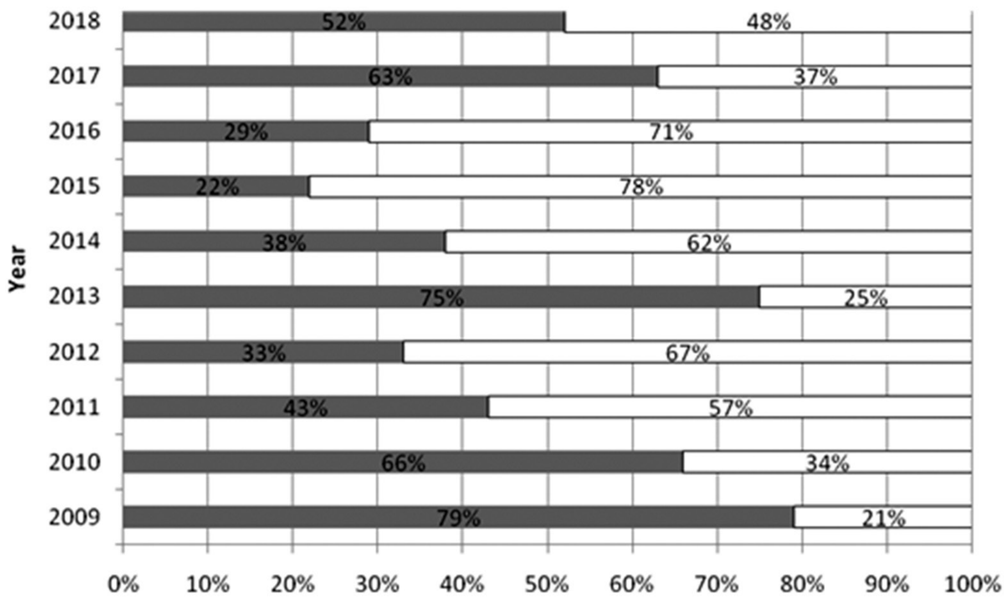

Fig. 2. Percentage of foxes infected with rabies (grey) to other animals (dogs, cats, cattle, small cattle) (white) in Mordovia.

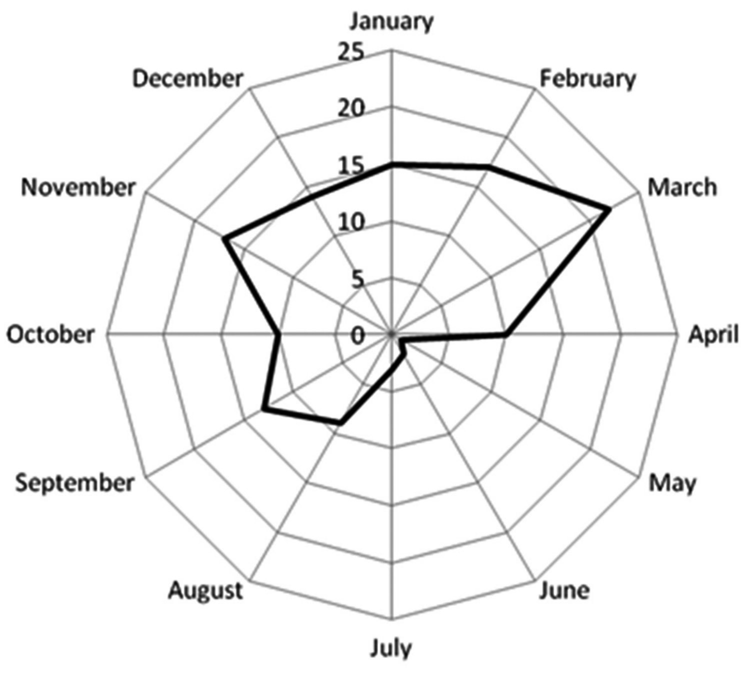

Fig. 3. Seasonal cycles of rabies among foxes in 2009–2018.

cases were reported in May (1), June (2) and July (3). Thus, foxes infected with rabies in Mordovia are registered monthly.

The analysis of the results of epizootological monitoring of rabies indicates that the territory of Mordovia is unfavourable for the incidence of rabies. When studying the pathogenicity spectrum of rabic infection, it was found that rabies was registered in foci (human settlements) among large and small cattle, domestic and unproductive animals (dogs and cats), as well as in autochthonous epizootic foci among wild carnivores (mainly red foxes). The degree of involvement in the epizootic manifestation of rabies infection of animals of various species is directly dependent on the probability of their population contact with the population of the obligate host – the fox. The formation of foci of rabies is associated with the natural and geographical conditions of the region. When determining the boundaries of the risk of rabies infection in the Republic, we found that the main concentrations of epizootic foci of infection are located in the territories of four districts in the North-Eastern part. In the dynamics of the time boundaries, there is a pronounced periodicity with synchronous alternation of declines and rises in epizootic tension, and in the annual dynamics – seasonality due to the cyclical biological activity of the main host of the pathogen.

In 1999, six cases of rabies were detected in animals in Mordovia, including one case in red fox, three cases in domestic animals, and two cases in farm animals. In 2001, Mordovia was classified as a poor region for rabies in the group with a low intensity of epizootic process. This group includes regions with the number of registered disadvantaged localities from one to five per year (Dudnikov 2002). Thus, it can be stated that for the period from 2013–2017, the region could be classified as a group with an average intensity of the epizootic process (the number of registered unfavourable points from seven to nineteen per year). And in 2018, Mordovia was included in the group with a high intensity of the epizootic process (the number of registered disadvantaged points is 20 or more per year).

Since 2000, there have been 4 cases of hydrophobia related deaths in Mordovia: one in Krasnoslobodskiy district (2003), one in Zubovo-Polyanskiy and Temnikovskiy districts (2004), and one in Ardatovskiy district (2009) (Alferina et al. 2016).

The situation with the distribution of rabies among different groups of animals in Mordovia is similar to the Volga region. In this economic region in 1999, a total of 553 rabies cases were identified, of which 244 were wild animals, 160 domestic carnivores, and 149 farm animals. In 2000, only 251 rabies cases were identified, of which 144 were wild animals, 69 domestic carnivores, and 39 farm animals (Dudnikov 2002). Thus, the share of wild animals, which are most often represented by common foxes, in Volga region's rabies accounted for 44.1 % in 1999, and 57.3 % in 2000.

Data for Mordovia is comparable to other regions of Volga region. The situation with the increase in the number of rabies among animals every year is typical for Nizhny Novgorod region. In 2005, the number of registered cases of rabies among animals there increased by 1.8 times compared to 2004. In 2006–2008, the epizootic situation remained tense with the involvement of 50 % of the region's territory (23 districts). At the same time, there was an increase in the degree of involvement of foxes (up to 63 % of the total number of sick animals) against the background of an increase in their mortality by 1.7 times (Pashkina et al. 2016). In Ulyanovsk region, over 20 years of research, it is known that the sources of rabies are distributed evenly – 50 % wild carnivores (fox) and domestic animals (dog) (Nafeev et al. 2014). In Chuvashia, the share of red foxes in rabies is 60.6 %. This is significantly more than in dogs and cats (20.5 %) and farm animals (17.9 %) (Tikhonov et al. 2016). Foxes are the main reservoir of rabies virus in the Republic of Tatarstan. They account for 53.9 % of the total registered cases (Hismatullina et al. 2016). Two-thirds of all rabies cases in Kirov region were detected in red foxes (Savinykh et al. 2019).

The situation with the main role of the fox in rabies is typical for other regions of Russia. In Altai territory during the period from 2001 to 2008, the share of red foxes in the spread of rabies accounted for from 50 to 85 %, on average – 66.5 % (Andreycev and Baryshnikov 2009). Red foxes in Crimea account for 55% of all cases of rabies (Yantsev et al. 2013).

A large role of the fox belongs to the spread of rabies in the countries of the Commonwealth of Independent States. The main reservoir of the rabies pathogen in Ukraine is still the red fox – 38.1 % of all animals and 89.4 % of all wild animals (Pechinka and Gley 2013). The main reservoir of the rabies virus in Belarus is wild carnivores. Foxes account for 91.5 % of all rabies cases in wild animals (Usenya 2011). In the Republic of Moldova, during the research period from 2009 to 2018, foxes account for an average of 19.4 % of all rabies-infected animals (Starchuk et al. 2019).

Our data are consistent with data on rabies in foxes in Europe. In particular, with the deterministic, compartmental model (Anderson et al. 1981). Their research as a simple mathematical model for the overall dynamics of the interaction between fox populations and rabies is presented – the fox population is divided into susceptibles, infected that are not yet infectious, and infectious individuals. The model helps to explain epidemiological patterns observed in Europe, including the 3- to 5-year cycle in fox populations infected with rabies, threshold densities and average levels of prevalence of infection.

The situation is similar in Asia and Africa. However, there the dynamics of rabies disease is associated with the dynamics of the number of dogs (Knobel et al. 2005, Zhang et al. 2005, Hampson et al. 2009).

In Mordovia, we often registered dead fox cubs. They were often eaten by other animals. It remains questionable whether this is related to rabies. However, some facts are known from the literature. Although rabid cubs (3-month old) are sometimes reported, the number is insignificant, and these animals are most likely infected by adult territory members. The limited spatial activity of the cubs reduces the probabilities of encountering a rabid intruder considerably. Their intraspecific contacts are limited to group members. Thus, cubs are not involved in the maintenance of the chain of rabies infection, and can be considered as dead-end hosts (Vos 2003).

One of the main and effective methods of specific prevention of rabies is timely and effective immune prevention. It is based on the use of anti-rabies vaccines (Rosatte et al. 2007). As a result of oral vaccination of foxes against rabies, this virus disease has almost been completely eradicated from West and Central Europe (Vos 2003). Highly effective vaccines for oral vaccination of foxes, wolves and stray dogs in Mordovia are the drugs ‘Oralrabivak’ and ‘Sinrab’. It is optimal to add

1.8 mL of vaccine per 1 bait. The most attractive lures are chicken heads and meat briquettes from the production of canned food and sausages. In the conditions of Mordovia, the optimal layout is 20 baits per 1 km^2 of territory (Tolokonnikova et al. 2015).

Conclusion

We found, that the red fox is the main source of rabies in the region. This is possible due to its high number (up to 4700 individuals). We conclude, that the red fox in the Middle Volga region has favourable conditions for existence. In the dynamics of the time boundaries of epizootic manifestations of rabies in the Republic, there is a pronounced periodicity with synchronous alternation of declines and rises in epizootic tension, and in the annual dynamics – seasonality due to the cyclical biological activity of the main host of the pathogen. We recommend, that the control of rabies spread is carried out by timely and effective immune vaccination of red foxes.

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TEMPORAL AND SPATIAL DIETARY SHIFTS OF A GENERALIST TOP PREDATOR: LONG-TERM STUDY OF AN EAGLE OWL *BUBO BUBO* POPULATION

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Abstract

Changes in land use practices such as livestock rearing and crop farming are crucially important for conserving biodiversity in the modern world. This study characterizes the shifts in the diet of the Eagle Owl *Bubo bubo* population in SE Bulgaria mainly as a result of agro-economic changes and depopulation of settlements. The share of predominant prey species in preferred weight classes from urban and open (mainly gallinaceous birds) habitats decreased significantly after the collapse of traditional livestock husbandry and local abandonment of agricultural land. These species were replaced by wetland ones when available and small rodents especially during population eruptions. The Northern white-breasted hedgehog *Erinaceus roumanicus* and the European hare *Lepus europaeus* contributed more as prey for Eagle Owls. Other significantly increased replacement prey species in the Eagle owl diet consisted of predatory mammals and birds as well as many smaller species typical of open and wood-shrubby habitats. High proportions of the latter prey groups characterized the annual diets of the studied population after resumption of farming operations and indicated at least temporary food shortages for Eagle Owls. The characteristic direction of diet shifts at the subpopulation level in the particular breeding clusters responded to the agro-economic changes and the landscapes of hunting territories. Despite diet changes, the average prey weight and the food niche breadth of successfully breeding Eagle Owl pairs differed insignificantly at a population level over the time series, except during two years with vole outbreaks.

Key words: birds' diet characteristics, feeding ecology, food stress, superpredation.

Introduction

The Eagle Owl *Bubo bubo* (Linnaeus, 1758) is a highly eclectic predator with preferences for mammals and birds. Its diet is driven, however, by local composition of the food supply, temporal fluctuations in prey abundance, and accessibility of potential prey species in diverse hunting habitats (Mebs and Scherzinger 2008, Penteriani and Delgado 2019). The number of preferred prey mostly between 200 and 2000 g in the hunting territory reflect the dominance of a prey species in the Eagle Owl diet (Korpimäki et al. 1990, Marchesi et al. 2002, Schweiger and Lipp 2011, Shin et al. 2013). Reduced supplies of preferred prey lead to increasing Eagle

Owl predation on less frequently used alternative species, with greater prevalence of variety of smaller prey as food niches necessarily expand (Dalbeck 2003, 2005; Obuch and Karaska 2010; Demay et al. 2015; Tobajas et al. 2015). Meanwhile, the Eagle Owl is adapting to urbanized areas by hunting common urban species such as rats (*Rattus* spp.) and feral pigeons (*Columba livia f. dom*., Gmelin, 1789) (Marchesi et al. 2002, Cugnasse 2017). This top predator plays an important role in sustaining prey populations by hunting selectively substandard individuals (Mebs and Scherzinger 2008, Penteriani and Delgado 2019) and affects top-down the community of mesopredators (Lourenço et al. 2011, 2018). The Eagle Owl can serve as an indicator of the high biodiversity and the comparative importance of areas for protection (Sergio et al. 2006, Milchev and Menzel 2017). Therefore, changes in its food spectrum and the fluctuations in diet indicate local environmental changes and the effectiveness of conservation strategies.

Policy changes in Bulgaria since 1990 have restored private land ownership, leading to progressive abandonment locally of agricultural land when agricultural production was no longer economically viable under changed market conditions. Farming was reactivated with agricultural subsidies after the accession of Bulgaria to the European Union in 2007. The livestock sector, especially traditional extensive pasture livestock husbandry, collapsed and never substantively recovered (Milchev et al. 2012). Species from open agricultural lands, pastures, and from urban habitats were expected to be strongly affected by the transformation of economic processes (Plieninger et al. 2014, van der Zanden et al. 2017). The Eagle Owl diet in the Strandzha Mountains had included important prey from the urban species (rats, feral pigeons, 26.5 % by prey number) and open area species (voles, gallinaceous birds, 26 %) before the politico-economic changes in 1988–1989 (Simeonov et al. 1998). Therefore, the traditionally agricultural and livestock grazing region area of south eastern Bulgaria including Strandzha Mountains was chosen for studying the changes in Eagle Owl diet at population and subpopulation levels.

Material and Methods

The study area of approximately 10,000 km² in south eastern Bulgaria has predominantly hilly or flat relief, from which ridges of Strandzha and Sakar mountains and several hills rise averaging 300–400 m a.s.l. (Fig. 1, see also Milchev et al. 2019). The eight clusters with Eagle Owl localities were defined mainly by similar landscapes and existing economic conditions (Table 1). Thirteen characteristics of the main part of Eagle Owl hunting territories within a 2-km radius around occupied rocks (Penteriani and Delgado 2019) indicate the predominant types of habitats in each clusters. Clusters 7 and 8 stand out the most; the first one is the most wooded cluster with small portions of open and urbanized areas, while the second is the richest in wetlands and urbanized areas. Oaks (*Quercus* spp.) dominate the prevalent deciduous forests, and field crops of grain, sunflower, and rape and vineyards comprise the agricultural lands. The towns and villages in cluster 8 along the seacoast suffered the least from the progressive human depopulation of the study area while the most negatively affected area was the border with Turkey.

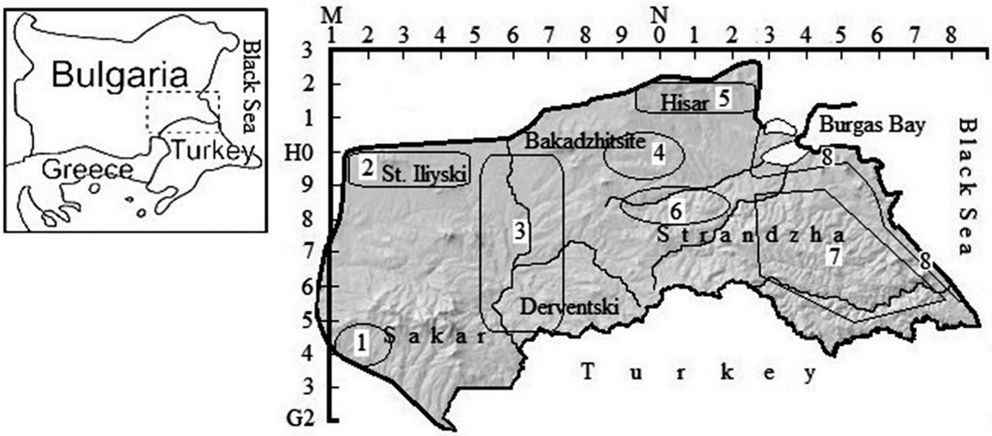

Fig. 1. The eight clusters with Eagle Owl *Bubo bubo* localities in SE Bulgaria with marked 10-km UTM grid.

Table 1. Landscape characteristics (average value) of the main Eagle Owl *Bubo bubo* hunting territories (2 km radius around the 53 occupied localities) in 8 clusters of SE Bulgaria.

Landscape characteristics	**1 (*n* = 2)**	**2 (*n* = 2)**	**3 (*n* = 9)**	**4 (*n* = 7)**	**5 (*n* = 5)**	**6 (*n* = 7)**	**7 (*n* = 11)**	**8 (*n* = 10)**
Open, %	72.3	58.7	61.6	81.3	77.6	51.0	28.5	35.2
Urban, %	2.0	4.5	5.1	4.0	6.9	4.4	1.4	8.1
Wood-shrub, %	22.1	32.7	30.6	13.8	13.9	43.1	67.9	35.0
Wetland, %	1.3	0.6	1.5	0.6	1.1	1.0	1.4	21.4
Rock, %	2.3	3.5	1.2	0.4	0.4	0.5	0.8	0.3
Habitat heterogeneity	39.0	21.0	33.6	34.9	22.2	37.0	31.5	25.9
Topographic relief	19.0	11.0	16.9	12.4	14.6	19.4	32.2	14.6
Altitude, m	177	228	171	96	204	92	123	64
To building, m	1867	1803	1945	1235	1458	1348	2383	1156
To road, m	960	725	1581	1116	788	1167	1524	666
To water body, m	35	871	387	59	380	58	113	575
To open areas, m	67	44	51	33	16	102	116	63
Farmland	1	0	1	1	0	2	3	2

Abbreviations and symbols: *n* – number of Eagle Owl localities in brackets; Landscape characteristics: 1) Open (%) – percentage of open areas (pastures, agricultural, and arable lands); 2) Urban (%) – percentage of urban areas (settlements, detached buildings, and main road networks); 3) Wood-shrub (%) – percentage of woodlands and shrub lands; 4) Wetland (%) – percentage of wetland habitats (open water area and areas covered with aquatic vascular vegetation); 5) Rock (%) – percentage of rocky areas; 6) Index for habitat heterogeneity – number of borderlines between the 5 main groups of habitats described above cut by the 2 diagonals in the cardinal directions N, S, E, and W; 7) Index for variability in topographic relief – number of 25-m contour lines cut by the 2 diagonals in the cardinal directions N, S, E, and W; 8) Altitude (m a.s.l.) of the occupied site; 9) To building (m) – distance (m) to the nearest building; 10) To road (m) – distance (m) to the nearest paved road; 11) To water (m) – distance (m) to the nearest wetland; 12) To open (m) – distance (m) to the nearest open area; 13) Farmland use at a cluster level: 0 – without abandoned farmland, 1 – partially abandoned farmland, but the majority has been re-cultivated since 2007, 2 – completely abandoned farmland and re-cultivated in a minor part since 2007, 3 – completely abandoned farmland.

The study is based on 62,314 prey specimens from 367 taxa collected in 53 Eagle Owl localities studied between 1994 and 2013 (Milchev and Georgiev 2019). Mammals and birds were most frequently hunted, while other vertebrates and arthropods were negligible with total 7.8 % by number and 0.8 % by biomass respectively. Voles (*Microtus* spp.) 18.2 %, typical mice (*Mus* spp.) 8.2 % and Northern white-breasted hedgehog (*Erinaceus roumanicus*, Barrett-Hamilton, 1900) 5.9 % were the most numerous prey species by number. The greatest contribution to food biomass was supplied by Northern white-breasted hedgehogs 22.9 %, European hare (*Lepus europaeus*, Pallas, 1778) 8.9 %, Norway rat (*Rattus norvegicus* (Berkenhout, 1769) 6.5 % and Common Moorhen (*Gallinula chloropus* (Linnaeus, 1758) 5.5 %.

The comparisons of diet samples (a breeding period diet per breeding locality per year) between years were based on the following estimators: *i*) average prey weight: total biomass divided by total prey number; *ii*) food niche breadth as computed: FNB = $1/\Sigma p_i^2$, where p_i is the proportion of prey category *i* by number (FNBn) or by biomass (FNBb) in the actual diet sample (Levins 1968). The larger values indicate a higher dietary diversity.

The large number of prey taxa was categorized for the analyses into 8 main prey species/groups (Fig. 2A-H). The grouping was based on the prey predominance in the annual diets (a breeding period diet of the study population per year) in combination with taxonomic affinity and habitat similarity between species. The generalized linear model and polynomial regression tested the relationship between the proportions of prey species/groups (dependent variable) in the annual diets versus years (independent variable). Differences in the means of average prey weight and of FNB of the successful pairs between years were computed with one-way ANOVA. Tukey *post hoc* test was used to test the differences between years when ANOVA results were significant. Arcsine-transformed values were tested in statistical analyses when the variables were proportions, while logarithmic transformations were applied for the count data. A chi-square test computed the differences between the diets in Strandzha Mountains before (Simeonov et al. 1998) and after the start of the economic transformation. The significance level was $p < 0.05$. All means are reported as an arithmetic mean ± standard deviation. The analyses were implemented with the PAST 3.01 software (Hammer et al. 2001).

Principal component analysis was used for studying the pattern of distribution of the prey groups in annual diets of the population and in diets of the clusters during five-year periods (CANOCO v. 4.5; ter Braak 1995). The samples were the separate annual diets or cluster diets,

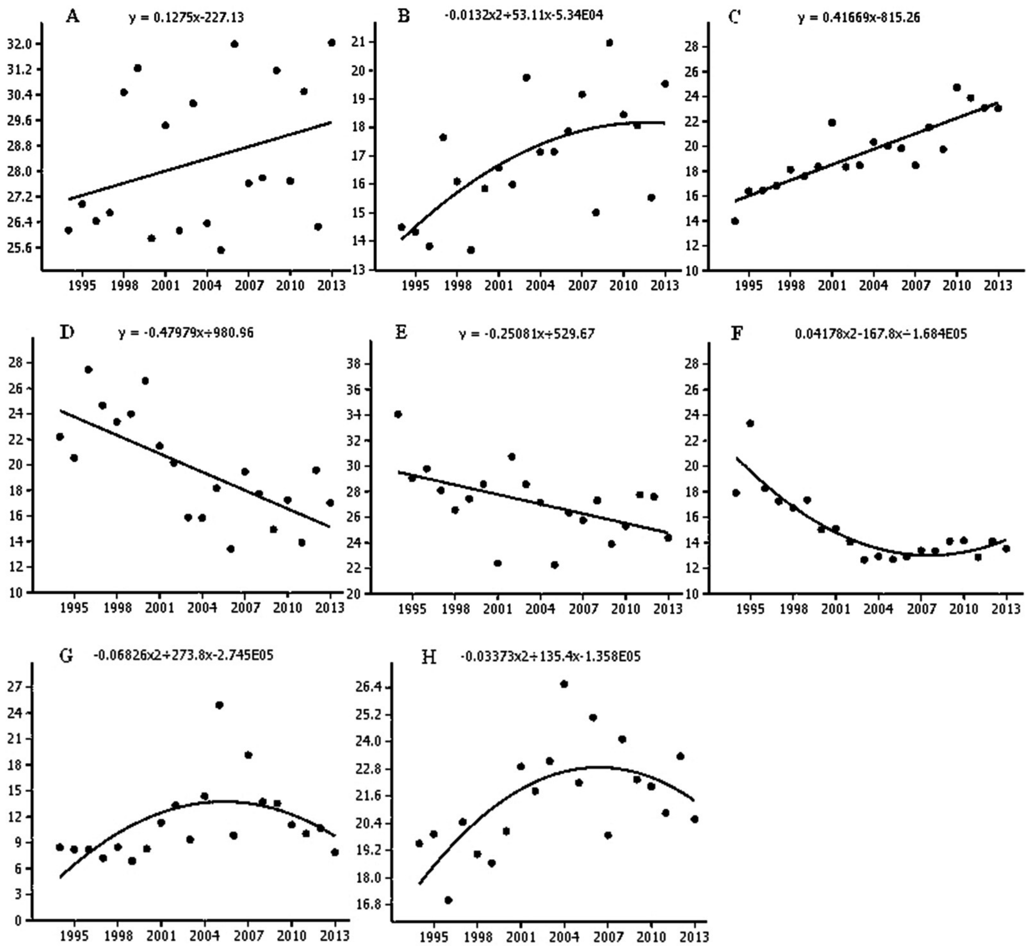

Fig. 2. Relationship between proportion of the main prey species/groups (% by biomass) in annual diets of the Eagle Owl *Bubo bubo* population in SE Bulgaria and study years.

Note: A – *Erinaceus roumanicus* – generalized linear model (GLM), $G = 0.38$, $P = 0.537$; B – *Lepus europaeus* – polynomial regression (PR), $F = 5.943$, $P < 0.05$; C – Superpredation – GLM, $G = 5.89$, $P < 0.05$; D – Urban species – GLM, $G = 8.1$, $P < 0.01$; E – Wetland species – GLM, $G = 7.06$, $P < 0.01$; F – Open area non-passerines – PR, $F = 30.991$, $P < 0.001$; G – *Microtus*, *Apodemus*, *Mus* – PR, $F = 4.224$, $P < 0.05$; H – 'other animals' – PR, $F = 6.574$, $P < 0.01$. Abbreviations and symbols: abscissa, years; ordinate, % by biomass (arcsine-transformed).

while the variables were the proportions of the main prey species/groups (% by biomass) in the respective diets. The variables are represented by arrows and the annual diets by circles on the ordination diagram. The arrows show the weightings of the variables in the first two principal components. The angles between the arrows approximate to the correlations among variables. Most important in the analysis were species with longer arrows and sharper angles with the ordination axes (ter Braak 1995, Lepš and Šmilauer 2003).

Results

The shares of three main prey species/groups increased during the study, Northern white-breasted hedgehog, European hare and superpredation (predatory mammals and birds), but the increase of hedgehogs was not statistically significant (Fig. 2A-C). Urban and wetland species and open areas non-passerines were predated in decreasing proportions (Fig. 2D-F). Predation on voles with mice and 'other animals' after an initial increase has declined during the most recent five-year period studied (Fig. 2G, H). The proportions of some individual species within the main prey groups have changed even in the opposite pattern. Adult European hares were hunted in decreasing proportion, while the proportions of smaller species within 'other animals' of open habitats (larks, buntings, shrikes, grasshoppers, field mice and Günther's vole (*Microtus guentheri* (Danford and Alston, 1880) and forest-shrub land habitats (thrushes, finches and longhorn beetles) increased significantly. The house rat (*Rattus rattus* (Linnaeus, 1758) with an increasing proportion over the last five-year period was an exception in the group of urban species. Important wetland species such as Common Moorhens and Common Coots (*Fulica atra* Linnaeus, 1758) supplied varying proportions for the food biomass over the years, but without statistically significant trend.

Figure 3 displays changes in the importance of main prey species/groups for Eagle Owl diets over the years. The first axis (eigenvalues 0.528) distributes the annual diets and prey in two groups. The positive part of the axis corresponds to the annual diets between 1994 and 2000 and higher level of hunting on reported prey species/groups mostly with significant decreases in proportions during the study period (urban and wetland species, open areas non-passerines). The majority of prey with significant increases in diet proportions over time and the later annual diets fall into the opposite part of the chart. The second axis (eigenvalues 0.224) distributes the annual diets according to the predominance by biomass of voles and mice along its positive part and Northern white-breasted hedgehog in combination with superpredation, European hare and 'other animals' along its negative part. The diets in 2005 and 2007 were exceptions, resulting in peaks in hunting on voles and mice (17.8 % and 10.8 % by biomass, respectively). Most annual diets at the end of the study and prey with significant increases in diet proportions correlated with the negative part of the second axis.

Average prey weight of the successful breeding pairs was 224.7 ±79.0 g (range 70.1 – 562.3 g) with significant difference in the means between years ($F_{19, 206} = 7.63$, $p < 0.001$). The smallest values for average prey weight corresponded with the two years of hunting on abundant voles (132.3 ±70.2 g in 2005, 138.8 ±55.9 g in 2007). Tukey tests calculate significant differences exclusively between the average prey weights in these two years with almost all other years.

Food niche breadth varied with significant differences in the mean FNBn (13.34 ±6.70, range 1.98 – 34.04) between years ($F_{19, 206} = 3.76$, $p < 0.001$) but insignificant ones in the mean FNBb (8.79 ±3.61, range 1.90–20.38, $F_{19, 206} = 0.85$, $p = 0.65$). Tukey tests indicate 10 significant differences only between FNBn in the two years with vole predation, respectively with the narrowest niches (6.66 ±5.10 in 2005, 6.90

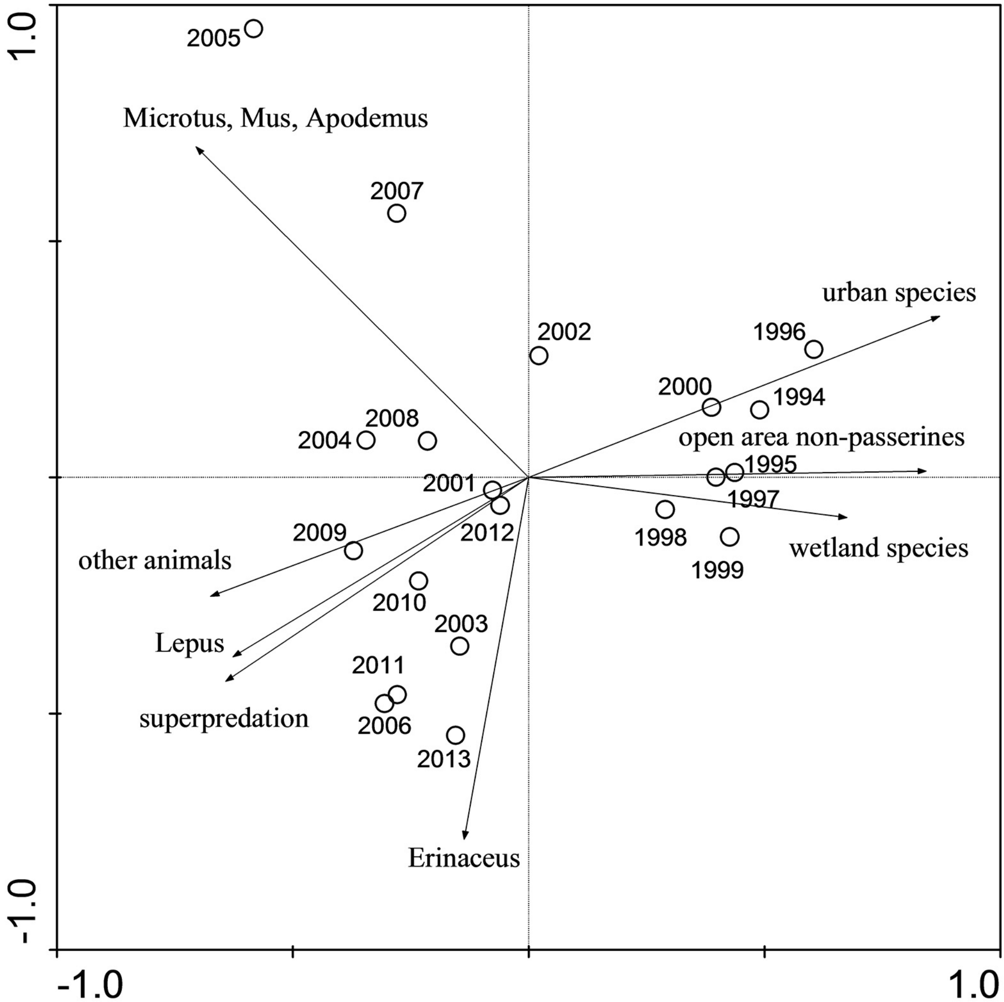

Fig. 3. PCA ordination of main prey species/groups (% by biomass) and annual diets of the Eagle Owl *Bubo bubo* population in SE Bulgaria, 1994–2013.
Note: arrows – prey species/groups; open points – annual diets.

±3.38 in 2007) and FNBn in the other years.

The Eagle Owl diet in the Strandzha Mountains (clusters 6, 7 and a locality from cluster 8) changed significantly after 1990 (χ^2 = 678.8, N = 8, p < 0.001, Table 2). The frequency of urban species and voles with mice decreased significantly and their proportions collapsed by 20.9 % and 12.7 % by number, respectively. Wetland species and the diverse group 'other animals' were hunted significantly more frequently (increase by 11.9 % and 18.4 % by number, respectively). Thrushes (*Turdus* spp.) increased most among the 'other animals' by 7.6 % by number. The level of superpredation doubled to 5.4 % by number of prey individuals.

Table 2. Eagle Owl *Bubo bubo* diet in the Strandzha Mountains, SE Bulgaria in 1988–1989 (Simeonov et al. 1998) and 1994–1998 (present study).

Prey species/groups	1988–1989		1994–1998	
	N	% *N*	*N*	% *N*
Erinaceus roumanicus	140	8.1	247	8.5
Lepus europaeus	21	1.2	46	1.6
Superpredation *	42	2.4	158	5.4
Urban species *	475	27.5	191	6.6
Wetland species *	324	18.8	894	30.7
Open area non-passerines	106	6.1	157	5.4
Microtus, Apodemus, Mus *	414	24.0	329	11.3
Other animals *	207	12.0	885	30.4
Total	1729	100	2907	100

Note: Abbreviations and symbols: *N* – number of specimens; % *N* – percent by number; * – prey with a significantly different frequency between the two periods.

Figure 4 presents the ordination of the eight clusters according to the proportions of the main prey species/groups in diets during the four five-year study periods. The first axis (eigenvalue 0.340) distributes clusters according to the diet proportions of mainly urban species to the positive part of the axis. Higher proportions of Northern white-breasted hedgehog in combination with more ‘other animals’ and superpredation correlated with the negative part of the second axis (eigenvalue 0.315). The higher proportions of wetland species and more urban species were associated with the positive part of the axis. Urban species were the most important prey in clusters 3, 4 and 5 during the initial two five-year periods. Eagle Owls in cluster 8 along the Black Sea coast depended greatly on wetland species in all four five-year periods. Wetland species were an essential part of the diet also in cluster 1, but in combination with more superpredation and the smallest proportion of urban species. Wetland species dominated the diet in the richest of open habitats cluster 4 during the third five-year period and in the most wooded cluster 7 during the first five-year period. Hedgehogs, ‘other animals’ and predators became the primary prey in cluster 7 in subsequent periods when the cluster correlated with the negative parts of both axes. Clusters 1, 3 and 4 changed their diet composition drastically in the final five-year period and correlated with the negative parts of both axes like cluster 7. The prey from predators and ‘other animals’ groups was in line with the share of their main habitat in Eagle Owl hunting territories in these clusters during the last five-year period. Most typical forest species were predated with the highest proportions in the wooded cluster 7, while the diets in clusters 1, 3 and 4 included a larger share of typical inhabitants of open and urbanized landscapes (Table 3).

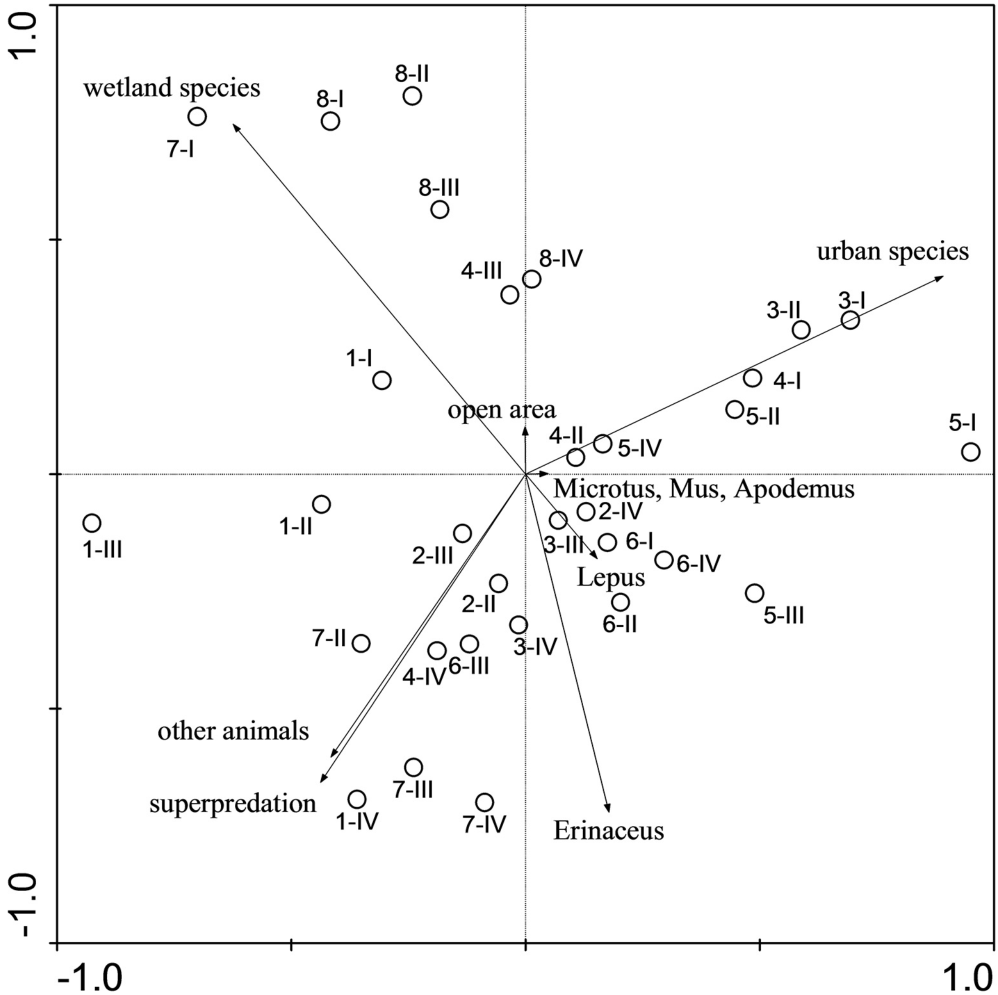

Fig. 4. PCA ordination of the clusters with Eagle Owl *Bubo bubo* localities in SE Bulgaria during four five-year periods between 1994 and 2013 according to the average proportions (% by biomass) of main prey species/groups.

Note: arrows – prey species/groups; open points – cluster diets where an Arabic numeral is a cluster number while a Roman numeral is a consecutive five-year period.

Table 3. Inhabitants (% by biomass) of forest, open and urbanized habitats from the prey groups of predators and ‘other animals’ in Eagle Owl *Bubo bubo* diets in clusters 1, 3, 4 and 7 of SE Bulgaria during the fourth five-year study period, 2009–2013.

Prey	Clusters			
	1	3	4	7
Mostly woodland-scrubland inhabitants, subtotal	6.5	7.5	9.8	24.8
Glis glis (Linnaeus, 1766), *Sciurus vulgaris*, Linnaeus, 1758	0.1	0.3	0.5	5.6
Columba palumbus, Linnaeus, 1758, *C. oenas*, Linnaeus, 1758, *Scolopax rusticola*, Linnaeus, 1758	4.3	3.0	2.8	9.8
Strix aluco, Linnaeus, 1758	0.1	0.04		3.5
Accipiter spp.	0.9	0.4	1.2	0.3
Turdus spp.	0.5	3.0	3.9	3.7
Garrulus glandarius (Linnaeus, 1758)	0.7	0.8	1.4	1.9
Mostly open and urban species, subtotal	19.1	7.3	8.7	1.7
Tyto alba (Scopoli, 1769), *Athene noctua* (Scopoli, 1769), *Asio* spp.	6.2	5.0	5.9	1.6
Circus spp.	0.3	0.2	0.6	0.1
Falco spp.	1.0	0.1	0.2	
Pica pica (Linnaeus, 1758), *Corvus* spp.	11.6	2.0	2.0	
Total	25.6	14.8	18.5	26.5

Discussion

The Eagle Owl is an opportunistic generalist and the variable proportions of prey in its diets are highly dependent on fluctuations in the population of potential prey species (Mebs and Scherzinger 2008, Penteriani and Delgado 2019). The decline in traditional livestock husbandry, along with the progressive depopulation of settlements, was expected to adversely affect populations of urban species and their proportions in its diet. The present study confirms this assumption.

The abandonment of arable land and pastures, and the subsequent restart in agriculture do not correlate with similar changes in the proportions of their main resident species in the Eagle Owl’s diet. Open area non-passerines including mainly gallinaceous birds decreased their proportion in the diet while the proportions of European hare increased over the same period. Predation data on Chukar (*Alectoris chukar* (J. E. Gray, 1830) conformed well to data about the extinction of this species as a breeding bird in the study area (Gruychev 2016). The European hare inhabited Bulgarian lowland habitats with an average density of 1.8 hares/100 ha, but clusters 2 and 3 had 2–4.9 hares/100 ha at the end of the study (Zhelev et al. 2013). Despite the low population number of European hares, the Eagle Owls increased significantly the proportions of hares in its food biomass. The European hare was dominant or subdominant prey in 32.3 % of diet samples of successful breeding pairs of Eagle Owls (*n* = 226 diets; Milchev and Georgiev 2019), but mainly in clusters 3 and 4 (63 %, *n* = 73) with partially abandoned farmland. Lagomorphs have been exceptionally desirable prey for the Eagle Owl across its entire range (Penteriani and Delgado 2019). However, the local status of the European hare population appears to have increased, benefitting from the abandonment of pastures and cropland.

Exceptions with peaks in proportions of voles and mice were recorded twice when the Eagle Owls switched to hunt the numerous voles. These instances of vole proliferation were the first ones since 1988 (Miltschev and Georgiev 2009). Peaks of the vole population are rare and not cyclical in SE Bulgaria because they are limited by summer droughts (Straka and Gerasimov 1977). Eagle Owls shifted to hunt temporarily numerous voles, as recorded in other parts of its range at higher latitudes (Korpimäki et al. 1990, Schweiger and Lipp 2011, Andreychev et al. 2014). The proportions of voles correlated positively with hunting on typical mice and field mice. Bezzel et al. (1976) and Dalbeck (2003) also reported that field mice were predated as incidental prey in the course of hunting on voles.

The declining share of wetland species such as some ducks could not be attributed solely to the progressive drying up of small ponds previously used mostly as livestock water sources (Milchev and Menzel 2017). Some waterbirds, predominantly Common Moorhens and Common Coot, have remained important prey species in SE Bulgaria.

It turned out that the Eagle Owls did not compensate the reduced hunting on preferred urban and wetland species or gallinaceous birds, principally by predating hedgehogs much more intensively. Northern white-breasted hedgehogs supplied the principal share of food biomass in fluctuating proportions with a non-significant upward trend. The steadily growing share of superpredation, as well as small insect prey and small passerines in SE Bulgaria was considered as indicator of food shortages (Mebs and Scherzinger 2008, Lourenço et al. 2018). The gradual changes in studied population diet led to a stable alteration in the diet structure at the end of the second five-year period. Prey with increased shares in the Eagle Owl diet over time, including the above-mentioned predators and small animals, characterized the population diet over the next two five-year periods.

The Eagle Owl optimizes its diet according to the available food supply in its permanent breeding territory, balancing between the energetic input of the prey and the costs of prey capture, transport to the nest and eating according to the optimal foraging theory (Dalbeck 2003, Mebs and Scherzinger 2008). The large number of prey species in varying proportions in the diets of successful owl pairs explained the variability established in average prey weight and FNBn over the years. Significant changes in these diet characteristics occurred only in both years with peaks in vole populations: the narrowest food niches and low average prey weight. No other prey was so profitable for most breeding pairs simultaneously in one year even though significant changes in the share of a large number of prey species were in evidence over the years.

The differences among hunting territories and among available food supplies in habitats definitely influence Eagle Owl diets of specifics (Dalbeck 2003, Schweiger and Lipp 2011, Shin et al. 2013, Penteriani and Delgado 2019). The localities of Eagle Owl pairs in cluster 8 included the largest urbanized area within the hunting territories and were closest to buildings. The localities were mainly in a coastal resort area with small crop farming and livestock grazing but with the largest wetland of all clusters. Wetland species dominated Eagle Owl food in this cluster. The diets in cluster 1 were also dominated by wetland species, harvested mainly from ponds used for livestock. Draining these abandoned small ponds as a measure to avoid local flooding most likely led to the distinct change in diet from waterbirds toward hedgehogs, superpredation and 'other animals' over the last five-year period. Higher shares of the last three prey groups and European hares compensated for the reduced proportion of urban species in clusters 3, 4 and 5. The diet in clusters 1, 3 and 4 approximated that in the wooded clusters 7, where the decline in the share of urban species took place before the beginning of this study. The increased diet share of Northern white-breasted hedgehog, a habitat generalist and the non-homogeneous groups of predators and 'other animals' appeared to eliminate the significance of habitat characteristics on Eagle Owl diets in these several clusters. Eagle Owls hunted prey species in groups of predators and 'other animals' living in forested or open and urbanized habitats in proportions corresponding with the presence of these habitats in each cluster also over the last five years.

The Eagle Owl is an endangered species according to the national Red Data Book (Golemanski 2015). Abandonment of arable land, collapse of traditional livestock farming and depopulation of human settlements in SE Bulgaria did not positively affect the dietary structure of the Eagle Owl breeding population. The highest values of superpredation and hunting otherwise neglected small prey as indicators of a high level of food stress coincided with recovery of intensive farming during the last five-year study period. Many predatory mammals and birds were found in the Eagle Owl diet in a neighbouring geographical area in Central Bulgaria during the same period (Milchev and Gruychev 2015) as an exception in the Balkan Peninsula (Lourenço et al. 2011). Economic changes have taken place with varying intensity and in diverse natural conditions across Bulgaria. Therefore, our results may not be replicable in other parts of the country. However, the negative tendencies in the diet structure of this top predator exist in one eleventh (9 %) of the Bulgarian territory. Combined with high breeding losses from direct and indirect anthropogenic factors (Milchev et al. 2019), the stability of its population is uncertain.

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UKRAINIAN FOREST GOVERNANCE SYSTEM IN THE CONTEXT OF INSTITUTIONAL REFORMS: DIAGNOSIS OF PERFORMANCE

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Abstract

Every forest governance model development has unique features and lessons to be learned. The institutional reforms in Ukrainian forestry are still in progress but there is lack of assessment and diagnosis of its impacts on Ukrainian forest governance. This paper provides such an assessment for the current status of the Ukrainian forest governance system, using FAO and PROFOR approach. It is based on the assessment of three fundamental pillars of forest governance (I) policy, legal, institutional and regulatory frameworks, (II) planning and decision-making processes, and (III) implementation enforcement and compliance. A set of indicators, developed within these pillars, was assessed by 53 experts. The results show that Ukrainian forest governance is far from achieving the status of 'good forest governance', and can be described rather as 'poor forest governance'. However, some positive tendencies such as performing well the functions of planning, organization, and control in the Ukrainian forestry are identified.

Key words: decision-making process, forest policy, institutional reforms, sustainable forest management, Ukraine.

Introduction

The concept of forest governance usually is considered through the shift from 'old' governance to 'good' governance (Arts and Visseren-Hamakers 2012). The 'old' governance refers to traditional state-centered and top-down management approach, in which the functions of planning, organization, motivation, and control were concentrated in state authorities (Agrawal et al. 2008, Rametsteiner 2009, Arts and Visseren-Hamakers 2012). The emergence of the 'good forest governance' concept could be explained by new governance trends: decentralized and participatory forest governance, market initiatives (forest certification, payment for forest ecosystem services), public-private partnerships (Agrawal et al. 2008, Arts and Visseren-Hamakers 2012). Cashore (2009) defines that the practice of 'good (forest) governance' requires attention to five key components: a) overarching principles and criteria, b) institutional fit, c) policy substance (ends), d) policy instruments (means), and e) policy evaluation. Although new governance trends, especially decentralized governance and forest certification, are worldwide represented, the process of transition to good forest governance varies from country to country (Lindahl et al. 2017). Ukraine is a case of a country with a long path to democratic institutionalization (Aliev 2017), so 'good governance practices' are not yet sufficiently studied, especially for forest sector.

The forests cover only 15.9 % of Ukraine's territory. That is why according to the Forest Code the priority is given to performing water protection, sanitary, hygienic, health, recreational, aesthetic, educational, and other ecological and social functions of forests, and corresponding limitations of their commercial use. The forests are distributed unevenly over geographical zones. The forest areas are concentrated mainly in the north (Polissya) and west (Ukrainian Carpathians) (State Forest Resource Agency of Ukraine 2019).

According to Land and Forest Codes of Ukraine, there are three forms of forest ownership: state, communal and private (Land Code of Ukraine 2002, Forest Code 1994). The vast majority of forests are state-owned. In the process of land delimitation, about 1.3 million ha (13 %) of forest land plots were assigned to communal forest lands. The share of private forests is less than 0.2 % (State Forest Resource Agency of Ukraine 2019). In terms of forest management, forests are provided to permanent use of enterprises, institutions and organizations of several dozens of ministries and departments. By institutional subordination, the largest area of state forest lands is managed by the State Forest Resource Agency of Ukraine – 73 %, 7 % – state-owned lands, not provided for use and allocated to reserved lands, 2 % – Ministry of Environment and Natural Resources, 5 % – other ministries and departments.

The institutional transformation in the forest sector that took place after the collapse of the Soviet Union was not as radical in Ukraine as in the other post-socialist Central European countries. This first of all due to the keeping the same system of administration and absence of forest ownership restitution. At the same time revision of forest legislation modified forest authorities' responsibilities reflecting the changing power of different political actors. These changes reflect the challenges facing Ukraine namely the administrative decentralization, social dissatisfaction, weak institutions, and their capacities (Yakymenko et al. 2019). Reforms in the forest sector have stagnated during a long time due to the lack of broader policy and economic reforms (Lazdinis et al. 2009). At the same time, the Ukrainian forest governance system and forestry legislation have been constantly changing during the last two decades (Dubovich et al. 2018). The changes were not transforming principally the rules of the game as they were caused mainly because of changes of governments, political appointments, and their vision of institutional architecture of the most bodies responsible for forests and forestry. The most recent institutional change is shifting the function of formation and implementation of state policy in the field of forestry and hunting to the established Ministry of Energy and Environmental Protection (instead of separate ministries for environment and energy in all previous Ukrainian governments) (Cabinet of Ministers of Ukraine 2019). Generally, such consolidation creates risks of environmental issues being relegated to the background compared to energy issues, but the State Forest Resource Agency has a sufficient degree of autonomy and this will not adversely affect its functioning.

The challenges of reforming Ukrainian forest governance, especially institutional, economic, and political/legal challenges are widely discussed in the scientific literature. It is emphasized that in Ukrainian forest sector reforms were not as radical as, for example, in agricultural sector. Also forest governance relies mainly on state authority, without properly functioning market incentives (Nijnik and Oskam 2004, Soloviy et al. 2017).

The study of Dubovich et al. (2018) showed that a holistic approach to reforming is not taken. Some studies provide the Ukrainian forest governance system assessment, but describe key objectives and problems of state-owned forests or focus only on communal forest governance (Storozhuk 2016, Michel 2016). Therefore, at present, a comprehensive approach to the assessment and diagnostics of the whole forest governance system is not applied.

This study aims to assess forest governance system in Ukraine based on indicators that are most heavily discussed in political and academic environments, and reflect the national context. Such an assessment is the basis for identifying governance pillars and components that need improvement and allows the identification of priority measures for the forest governance system development in Ukraine.

Methods

Many international initiatives on sustainable forest management (SFM) criteria and indicators were developed. Forest Stewardship Council (FSC) Principles and Criteria, and Criteria and Indicators Toolbox were among the first (FSC 1996, Prabhu et al. 1999). 'The Pyramid: A diagnostic and planning tool for good forest governance' has extensively described the elements of such kind of governance (Mayers et al. 2002). Several international institutions have also developed guidelines focused on specific regions to assess SFM. However, within these approaches, governance-related indicators are very poorly included (World Bank 2009).

The most popular methodological approaches applied to forest governance assessment are Framework for Assessing and Monitoring Forest Governance and Governance of Forests Initiative Indicator Framework (FAO 2011, Davis et al. 2013). The FAO approach has already been adapted for assessment and diagnosis of forest governance systems in Bosnia and Herzegovina, Georgia, Azerbaijan, Vietnam, Thailand, and other countries (Avdibegović et al. 2014, Michel 2016, Gritten et al. 2019). According to this approach, the fundamental pillars are divided into 13 main components. In particular, the components of the first pillar 'Policy, legal institutional and regulatory frameworks' examine the quality of forest policy, laws and regulations, compliance with sectoral policies, financial incentives, and tools to address fairness in the allocation of forest resources and benefits. The components of the second pillar reflect stakeholder participation in forest governance, transparency of decision making, and accountability of management processes. The issues of forest resources administration, law enforcement, combating forest corruption, cooperation, and coordination between executive forestry entities are the components under the third pillar 'Implementation, enforcement and compliance' (FAO 2011, Kishor and Rosenbaum 2012).

In this paper, we used the FAO approach as it is flexible and allows to select indicators that reflect the current state of forest governance and to reach the research purpose.

The FAO approach is based on the

application of the expert survey method in order to investigate the implementation of fundamental pillars, as well as the compliance of forest governance system with the above-mentioned principles (FAO 2011). Therefore, the methodological design of the questionnaire was developed taking into account the need to include questions that would cover all pillars and principles of the forest governance system (Table 1).

Table 1. Methodological design of the questionnaire.

Pillar	Principals	Component	Indicators
I	2, 3, 6	Forest-related policies and laws	2. How are activities of the Ukrainian forestry authorities on protection, use and reproduction of forests coordinated with each other?
I	2, 3	Forest-related policies and laws	8. How do current Ukrainian programs for protection, use and reproduction of forests contribute to the achievement of sustainable forest management?
I	4, 6	Institutional frameworks	10. Does internal conflict of interest take place in the system of Ukrainian central executive authorities that responsible for the formation and implementation of forest policy?
II	1, 6	Transparency and accountability	4. To what extent political environment affects forestry authorities?
II	4, 6	Transparency and accountability	5. Does the lobbying of economic interests of forest industry companies affect on institutional reforms in the Ukrainian forestry?
II	1, 5, 6	Stakeholder participation	6. What forms of stakeholder participation are implemented?
II	1, 5, 6	Stakeholder capacity and action	7. Please, assess participation of stakeholders in forest governance reformation on 6-point scale (0 – do not take part, 5 – active participation).
III	2, 3, 6	Forest law Enforcement	1. To what extent programs for protection, use and reproduction of forests have been implemented?
III	2, 3	Forest law Enforcement	3. How are measures for adaptation of forests to climate change and mitigation of climate change implemented?
III	2, 3, 5	Forest law Enforcement	9. How efficiently do international agreements and conventions on the protection, use and reproduction of forests execute?
III	2, 4	Administration of forest resources	11. To what extent the qualifications of forest workers correspond to the needs of development of Ukrainian forest sector?
III	1, 2, 4, 5	Administration of forest resources	12. To what extent are management functions performed by central executive authorities responsible for the formation and implementation of forest policy?
III	2, 4, 6	Administration of forest resources	13. Assess the effectiveness of the implementation of following measures in the Ukrainian forestry: forest certification, auction wood trade, moratorium on exports of round wood, timber tracking system.

Note: Principals: 1 – accountability, 2 – effectiveness, 3 – efficiency, 4 – fairness/equity, 5 – participation, 6 – transparency.

The group of 53 experts from 6 regions of Ukraine (Lviv, Kyiv, Kharkiv, Volyn, Zakarpattia, Rivne oblast) participated in the survey. The experts are chosen from these regions because in Lviv, Kyiv, Kharkiv historically are concentrated the oldest forestry research centers in Ukraine, and Volyn, Zakarpattia, Rivne oblast are among the most forested areas. The experts represent the following professional categories: 12 – scientists who study the issues of the reforming of Ukrainian forest governance system and forest policy, 8 – representatives of environmental NGOs, 27 – directors and employees of State Forestry Enterprises (practitioners), 6 – representatives of forestry-related sectors (administration of Nature Protected Areas, touristic companies, agricultural enterprises and water supply companies). The survey was conducted online through the SurveyFace tool and by submitting and collecting completed questionnaires by employees of forestry enterprises.

Each expert scored the proposed indicators on the four-point scale: 0 – worst situation within the indicator/nonperformance of indicator, 1 – partial performance, 2 – significant performance, 3 – best situation within the indicator/full performance of indicator, based on their own experience, knowledge and perception.

Stakeholder participation in Ukrainian forest governance, including such forms as information and consultation is regulated by the Forest Code of Ukraine, the Laws of Ukraine ‘On Information’, ‘On Environmental Impact Assessment’, ‘On Environmental Protection’ (Verkhovna Rada of Ukraine 1991, 1992, 2017). Also national FSC standard has detailed requirements for stakeholders’ involvement. Previous studies show that stakeholders, including local communities, are involved in forest governance, but their participation is quite passive (Kravets et al. 2012, Pavlishchuk 2014), so indicators 6–7 are not identified as evaluative, but included to diagnose the degree of involvement of separate stakeholders’ groups.

Results

Pillar I ‘Policy, legal, institutional and regulatory frameworks’

Survey results show that the coordination of activities on forest protection, use, and reproduction by forestry authorities is estimated as ‘partial’ (Fig. 1). Although practices aimed at achieving sustainable forest management have been implemented (for example, forest certification as a voluntary instrument of forest policy), experts assess the contribution of forest programs to SFM as a ‘partial’.

The issue that should be stressed on the assessment of forest policy and laws component is that at present the system of forest legislation is well developed, although there is no separate law that highlights the long-term vision and goals of Ukrainian forest policy (Soloviy et al. 2018). Therefore, considering the national context, while developing and assessing the indicators of the first pillar, we are more likely to assess the quality of forest legislation than the quality of forest policy.

Pillar II ‘Planning and Decision Making Processes’

The components include indicators to assess the transparency of the forest governance system and its independence from external influences and stakeholder participation in the forest governance process. According to the results, the political envi-

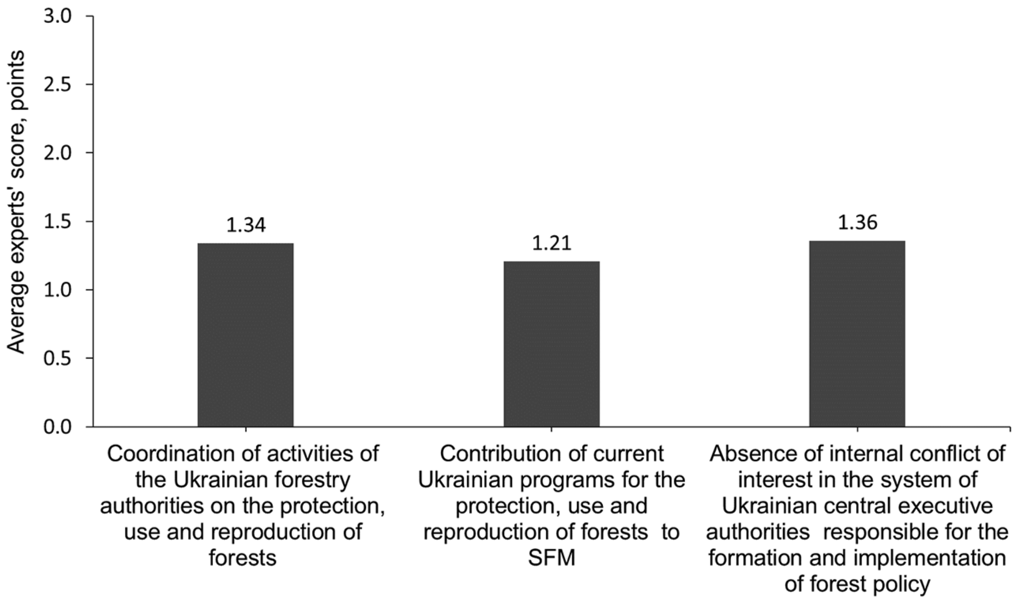

Fig. 1. Indicators of the first pillar.

ronment has a greater influence on the institutional reforms in forestry than the policy of lobbying of economic interests of forest industry companies (Fig. 2). The average expert score of the influence of the political environment on forestry authorities is 1.15 points, while the average expert score of the impact of lobbying on institutional reforms in forestry is 1.6 points.

The results confirm that information and consultation are the main forms of stakeholder participation (Fig. 3). The most active stakeholder groups are the local community, industry (wood-processing, furniture manufacturing, paper production) enterprises, environmental

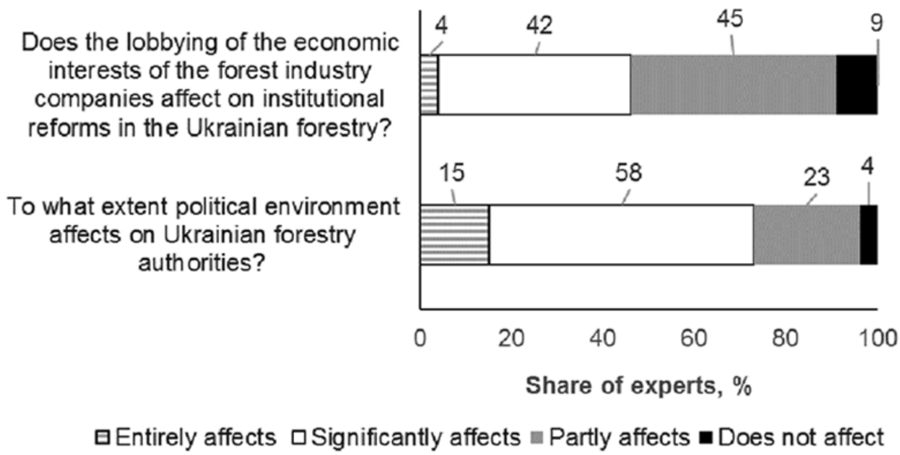

Fig. 2. Indicators of transparency and accountability.

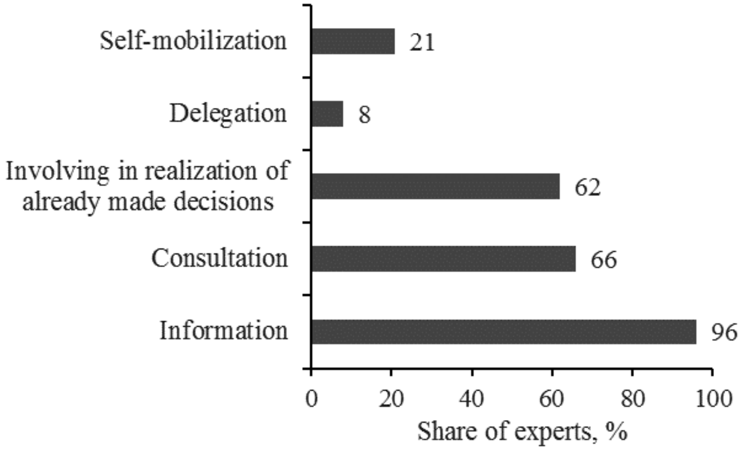

Fig. 3. Forms of stakeholders' participation.

NGOs, and administration of nature protected areas.

In Table 2 it can be seen that participation of touristic companies, agricultural enterprises, and water supply companies is quite passive, although they are users of the regulatory and cultural forest ecosystems services.

Table 2. Assessment of participation of stakeholders in forest governance system.

Stakeholders	Average experts' score, points				
	Information	Consultation	Involving in realization of already made decisions	Delegation	Self-mobilization
Local community	3.41	2.71	2.12	1.38	2.23
Environmental NGO	2.98	2.67	1.78	0.65	2.19
Industry (wood-processing enterprises)	2.95	2.90	2.77	1.30	2.08
Agricultural companies	0.64	0.70	0.50	0.30	0.40
Tourism companies	1.26	1.19	0.64	0.25	0.50
Water suppliers	0.39	0.54	0.37	0.15	0.25
Administration of nature protected areas	3.76	3.71	3.35	1.69	2.69

Note: Average experts' score on 6-point scale, where 0 – do not take part, to 5 – active participation.

Pillar III 'Implementation, Enforcement, and Compliance'

Since 1993, the State Forest Resource Agency of Ukraine has been participating in the Ministerial Conference on the Protection of Forests of Europe. And Ukraine has signed relevant international agreements to ensure sustainable forest management (e.g. Protocol on Sustainable Forest Management to the Framework Convention on the Protection and Sustainable Development of the Carpathians) (State Forest Resource Agency 2014). However, as it is illustrated in Figure 4, the effectiveness of the implementation of international agreements and conventions is assessed by the experts as 'partial'.

The level of implementation of national programs for protection, use, and reproduction of forests receives a higher average expert score than the level of implementation measures towards forestry adaptation and mitigation to climate change, although both indicators are close to 'partial implementation'.

According to the experts, the performance of the functions of planning, organization, and control in forestry is assessed as 'significant', while motivation and regulation – 'partly' (Fig. 5). The average expert score of the compliance of qualifications of forestry workers with the actual needs of the development of the forest industry is 1.8 points (approximate to 'correspond significantly'.

Over the last decade, several initiatives have been implemented in the forest governance system to combat forest corruption, maintain forestry in accordance with the SFM concept, and improve the timber pricing policy. The forest certification and timber tracking system are considered as the most effective instruments, and the ban on the round wood export as the least effective one (Fig. 6).

The total expert score of forest governance system based on the analyzed indicators is 1.4 points. It means it tends to the score 'poor forest governance'. Therefore, most forest government elements need to be improved. However, there are

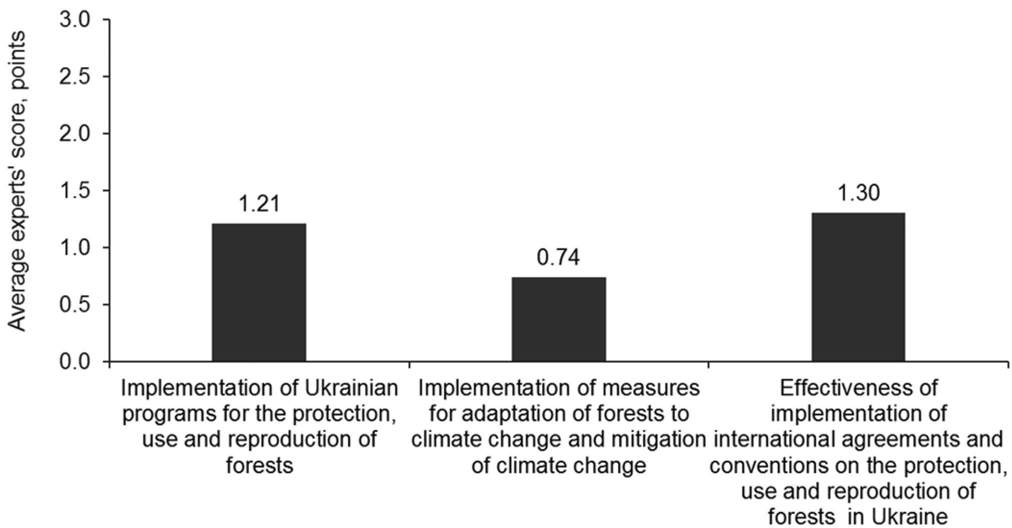

Fig. 4. Indicators of forest law enforcement in forest governance system.

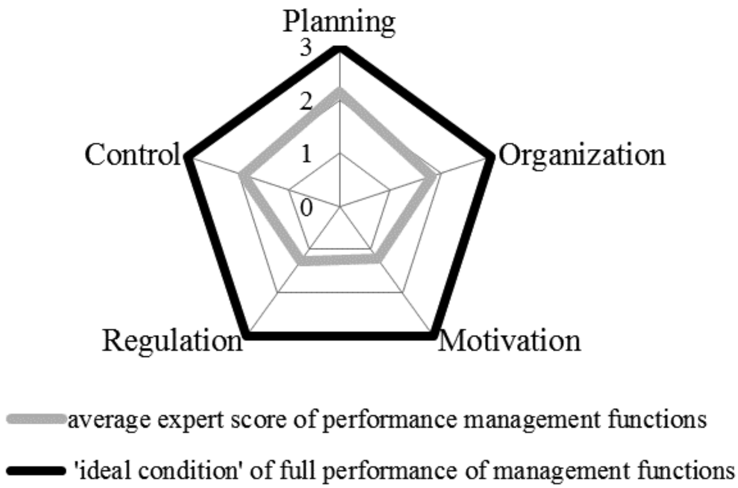

Fig. 5. Performance of management functions in forest governance system.

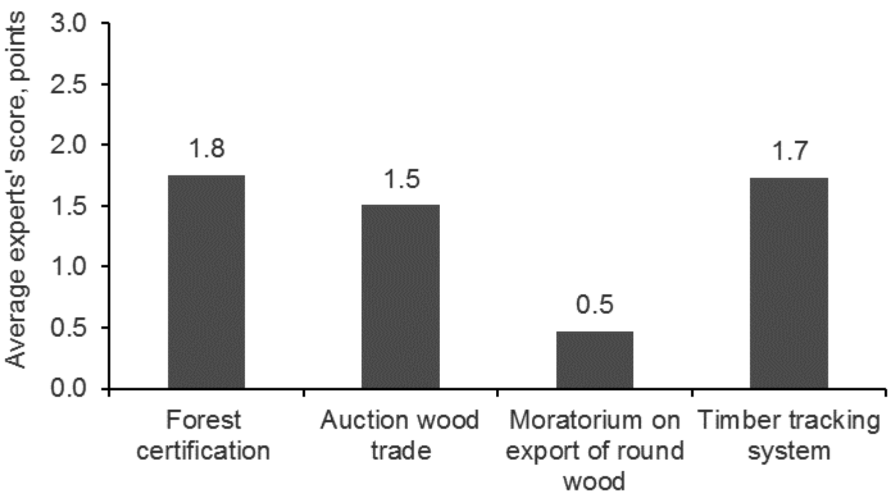

Fig. 6. Expert evaluations of measures in forestry.

some positive findings such as performing the functions of planning, organization, and control and the compliance of forestry workers' qualifications with the actual needs of forestry development.

Taking into account the current status of the forest governance system, we also asked experts to assess the importance of measures to reform it (Table 3).

According to the expert opinions, the most important measures that can contribute to the institutional reform are the adoption of the Law of Ukraine 'On National Forest Policy', developing and implementing actions to stimulate forest planting on private lands, the institutionalization of the payment for forest ecosystem services.

Table 3. Evaluation of measures.

Measures for reforming the forest governance	Average experts' score
Adoption of the Law of Ukraine 'On National Forest Policy' with vision and goals of the national forest policy	4.17
Development and implementation of measures to stimulate forest planting on private lands	3.21
Institutionalization of payment for forest ecosystem services	3.21
Changing personnel policy in forestry sector	2.96
Inspection on function performance by central executive authorities responsible for the formation and implementation of forest policy	2.88
Enhancing stakeholder participation in forest governance	2.72

Note: Average experts' score on 6-point scale, where 0 – not important, to 5 – very important.

Discussion

Forest policy and forest governance in the coming decade will be driven by demographic, economic, environmental, technological, and governance trends (Wolfslehner et al. 2020). These trends will lead to changes in perceptions of forests and nature, and their value, increase demand for bio-based products and use of wood tracking software applications, strengthen synergies between forest and sectoral policies, including climate and environmental policies, and determine more active stakeholder participation in governance.

The survey results show that such trends are partially represented in the Ukrainian forest governance system. Digitalization in forestry is assessed by experts as effective due to the presence of an electronic timber tracking system, which is administered by a separate state enterprise 'Forestry Innovation and Analytical Center'. Implementation of the system provides marking of all harvested wood with unified tags and barcodes that register the origins of wood, its qualitative and quantitative indicators, and ensure automation of preparation of primary documentation, wood accounting and control (Forestry Innovation and Analytical Center 2020).

The implementation of measures to adapt Ukrainian forestry to climate change is assessed as 'partially effective'. The impact of climate change on forestry is especially significant in the Ukrainian Carpathians, where spruce forests are drying up on a big scale (Buksha 2019), but relevant to other regions as well. The Concept of implementing state policy in the field of climate change until 2030 (Cabinet of Ministers of Ukraine 2016) defines the development of a medium-term action plan for the forestry adaptation to climate change. However, up to date, neither the action plan nor the financial sources for the adaptation measures have been identified.

Participatory approach is represented in the forest governance system, but it is a kind of passive participation – when individuals are informed of decisions *ex-post facto*, with shift to active participation – when individuals influence on decision. The active participation is facilitated by the Law of Ukraine 'On Environmental Impact Assessment' (2017), according to which communities are notified in advance about main felling (both clear cutting and gradual felling). Also they should be informed about related report on Environmental Impact Assessment and have possibility to propose measures towards its implementations.

Decentralization in forestry looks promising in the long run, when the results of the Ukrainian decentralization reform of local self-government, launched in 2014, become more visible. According to Tkach and Torosov (2015), the newly formed united territorial communities should be more active in decision-making process both in communal and state forests, as the share of forest revenues that remain in local budgets will increase.

For Ukraine and for its forest sector, progressive changes in formal and informal rules are crucial. Institutional transformations in Ukraine's forestry have started, but the rules of the game and the arrangements have not changed substantially so far (Soloviy et al. 2017). The rigid sector-based forest governance, focusing on national level economic gains from forestry as opposed to considering local benefits of forests, and reliance on technical expertise instead of local knowledge seem to be emphasized (Sarkki et al. 2009). As stressed in recent studies (Khvesyk et al. 2019) transformation of relations in forest sector is possible through broad range of measures which includes, but not limited to following: achieving decentralization of governance system; equality of market access to forest raw materials and forest products; financial sustainability of forestry; transparency of the timber market functioning, openness for investment and innovation; promoting the development of territorial communities; transition to green economy based on environmentally friendly and energy efficient processes in-depth timber processing; integrated use of multiple forest resources and services.

The approaches to forest governance assessment and their application criticized first of all due to the difficulty of integrating all its elements and even a different understanding of forest governance which as a very broad term (Arts and Visseren-Hamakers 2012, Giessen and Buttoud 2014). Therefore, why its application can be useful? Several studies have shown that forest governance assessment is useful as a learning and practice-oriented tool that will highlight the potential benefits and disadvantages of the current governance system and ways to improve it (Campese et al. 2016). This is also true for the Ukrainian case study.

The study presented in this paper gives an assessment of forest governance in Ukraine based on the set of indicators. In this relation two methodological challenges can be outlined. Firstly, in comparison to other similar studies, the set of indicators was not developed by a group of experts but proposed by the authors on the basis of FAO approach and analysis of the studies on improving forest governance in Ukraine. On the other hand, the inclusion of a large number of indicators resulted in experts' reluctance to participate in the survey. The experts involved in this survey represented 6 regions of Ukraine, and inclusion of experts from all regions can be reasonable to approach comparative assessment. The using of SurveyFace tool is convenient for all groups of experts, with the exception of forestry workers (practitioners) for whom the paper version of the questionnaire is usually preferred.

Conclusions

The application of the FAO approach as an analytical framework for assessing the forest governance system of Ukraine allows determining the degree of transparency, accountability, participation, efficiency, effectiveness and fairness in the allocation of forest resources at the national level. The assessment of indicators within three elements shows progress towards good forest governance.

The survey results show that the forest governance system needs to be improved within all elements: political, legal, institutional and regulatory frameworks; planning and decision making process; implementation, enforcement, and compliance. The biggest gaps were found in the independence level of the forest governance system from the influence of the political environment and the effectiveness of the implementation of ban on raw wood exports. The participation of some stakeholder groups is also critically low.

It is recommended to carry out further research on the forest governance system assessment and diagnosis by deepening the approach and including new indicators, in particular under the components: financial initiatives, economic instruments and distribution of forest income, forest enforcement and anti-corruption measures. In the context of new administrative decentralization reform in Ukraine, which envisages the formation of united territorial communities, it is important to move from passive forms of participation to active ones. In addition, also it should be mentioned that setting a mechanism for periodic assessment of forest governance system can be helpful in monitoring the real progress towards good forest governance.

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RADIAL INCREMENT OF BEECH (*FAGUS SYLVATICA* L.) IN THE UKRAINIAN CARPATHIANS

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Abstract

The objects of the study were pure and mixed middle-aged beech stands that grow on humid fertile hornbeam-beech forest type conditions on the southern and northern slopes at the altitude of 550–600 m a.s.l. We found a correlative relationship between beech radial increment and climate indices. Average radial increment of beech varies within the limits of 1.71 to 2.41 mm depending on tree composition with the maximum in the mixed stands. The share of early wood and late wood of the annual ring of beech fluctuates within the limits of 83–88 % and 12–17 %, respectively. In the mixed stands that grow on the northern slopes, annual radial increment of beech is maximal. In the mixed and pure forest stands average radial increment of beech on the northern side of the slope compared to the southern side is higher. It has been found that radial increment of the south-facing side of beech trees growing on the southern mountain slope and the north-facing side of them growing on the northern mountain slope is higher in comparison to the opposite side of the trees. Radial increment extremes in many cases are identical with climate indices extremes. In the years of the most intensive solar activity beech radial increment is minimal. Relationship between beech increment and climate indices is stronger in the pure beech stands than it is in the mixed stands. Relationship between beech increment and air temperature as well as precipitation becomes weak in the mixed stands and it is moderate in the pure stands. Beech increment correlates greatly with humidity deficiency, air temperature and precipitation, which is especially marked during the period from 2003 to 2013. Beech wood radial increment correlates closely with air humidity and humidity deficiency both in the pure and in the mixed stands.

Key words: climate indices, correlations, early wood, forest ecology, late wood, slope aspect.

Introduction

The beech (*Fagus sylvatica* L.) is a valuable and typical species of the Carpathian region and in other mountain ranges in Europe. This species demands air humidity, soil moisture, as well as the richness of the soil. In the Ukrainian Carpathians it grows within 250–1450 m a.s.l. Beech is the forest-forming species in the fertile and fairly fertile site type. It is one of the most shade-tolerant, soil-improving and wind-resistant tree species (Hensiruk 2002).

The beech forests of the Ukrainian Carpathians perform important protective, environmental and other ecological functions. The most of the beech forest types are characterized by the highest classes of water regulation (Polyakov 2003).

Beech is characterized by high productivity, and its wood is widely used in various industries. It regenerates in a natural way and that is why the majority of beech forests in the Carpathian conditions are natural and very often uneven-aged.

For the assessment of the forest ecosystems state, most researchers use dendrochronological and dendroclimatic methods based on the study of the radial increment of individual trees and forest stands (Schweingruber 1996, Hughes 2002, Matveev 2003, Speer 2009, Sheppard 2010). Radial increment is the most universal and comprehensive measure of forest growth throughout its life span, and therefore provides an opportunity to identify their reactions to the impact of external factors (Mölder and Leuschner 2014, Augustaitis et al. 2015, Remeš et al. 2015, Bosela et al. 2018, Šimůnek et al. 2019). The studying of radial increment dynamics of the mountain beech forests which grow in climate change conditions (Mazepa and Shyshkanynets 2013) is important to assess condition and productivity of stands and develop their predictive models.

Annual growth of beech is most significantly influenced by the climate and atmospheric air pollution. Correspondent to a significant warming trend from 1990–2010, average beech growth declined in the mixed beech-fir forest sites across Continental Europe, including Bosnia and Herzegovina, Germany, Italy, Romania and Slovakia (Bosela et al. 2018). The studies of Augustaitis et al. (2015) showed that frost in winter months and heat in June, along with drought in the vegetation period, limited beech tree growth outside its natural distribution range in northeast Europe (Poland, Lithuania and Latvia). Higher air concentration of surface ozone and sulphur deposition level reinforced the negative effect of the detected key meteorological variables on beech growth, while higher air concentrations and deposition of nitrate had a positive effect. These factors explained about 50 % of the total variation in increment indices of beech trees at sites on the northeastern edge of their range. In the eastern Krkonoše Mountains, the Czech Republic the growth development of beech stands was significantly affected by air pollution load in 1977–1989, and increasingly frequent climate extremes in recent years (since 2010) (Šimůnek et al. 2019). The positive influence of temperatures on beech increments was recorded in winter, early spring, and especially in July and August of the current year. Conversely, precipitation in the previous year had higher impact on radial increment, with prevailing negative correlation. The plots were negatively affected by the decrease in sum of precipitation in February and March, but it was the temperature that influenced the beech increment most significantly.

The aim of the research is studying the characteristics of the radial wood increment of beech stands depending on the main climate indices on the slopes of different aspects in the mountain forests of the Transcarpathian region of the Ukrainian Carpathians.

Material and Methods

We selected beech stands of the Nyzhni Vorota forest district in the Volovets State Forestry Enterprise in order to study the characteristics of beech stands radial increment and the influence of external environmental factors. On each sample area in the biogroups of 10 trees at the height of 1.3 m by means of Pressler drill core samples of wood were selected and 20 cores in the North-South directions were taken for each biogroup (Mazepa 2009). The width of early and late wood was measured by means of the microscope MBC-1 with an accuracy of 0.1 mm.

We compared absolute and relative growth values and carried out dendrochronological series analysis over the past 24 years depending on climate factors in order to evaluate radial increment of the forest stands.

Along with the amounts of precipitation and average temperature for the calendar year and the vegetation period a number of complex meteorological (climatic) indices (climate humidity, hydrothermal coefficient (0_1), complex hydrothermal index (0_3), complex hydrothermal coefficient (0_2), the coefficient characterizing the supply of moisture (k) were tested to establish correlations. Climate humidity was determined by the formula (1) of Vorobiov (1967).

$$W = \frac{R}{T} - 0.0286 \times T, \qquad (1)$$

where: R is the amount of precipitation for the months with positive monthly average temperatures, T is a sum of positive monthly temperatures.

Hydrothermal coefficient O_1, O_2, O_3 were determined by means of formula of Bitvinskas (1974). Hydrothermal coefficient O_1 was determined by the formula (2).

$$O_1 = \frac{t_0 \times 100}{V_0}, \qquad (2)$$

where: t_0 – average air temperature for the hydrological year (the period from the previous October to the current September); V_0 – precipitation (mm) for the hydrological year.

Complex hydrothermal coefficient of O_2 was determined by the formula (3).

$$O_2 = \frac{t_0}{V_0 \times k}, \qquad (3)$$

where: t_0 and V_0 have the same meaning as in the formula (2), and k is a coefficient characterizing the supply of moisture and is determined by the ratio of the amount of precipitation during four hydrological years to average perennial rainfall for the same period – by formula (4).

$$k = \frac{V_3 + V_2 + V_1 + V_0}{4V_{avr}}, \qquad (4)$$

where: V_0 – precipitation for the hydrological year, V_1 – precipitation in the previous hydrological year, etc.; V_{avr} – rainfall of the hydrological year (long run average).

Integrated hydrological indicator O_3 reflects the influence of climatic factors on the growth of stands in four years. It is determined by the formula (5).

$$O_3 = (V_3+2V_2+3V_1+4V_0)\cdot(t_3+2t_2+ +3t_1+4t_0)\cdot t_0 /10\times10\times100, \qquad (5)$$

where: V is precipitation for a hydrological year, t – average annual temperature over the same period, indexes 0, 1, 2, 3 – years (current, previous, etc.).

Statistical processing of research results was conducted by methods of mathematical statistics (Dospekhov 1985) using MS Excell and Statistica 6.0 software.

For the analysis of meteorological parameters in the region of the mountain beech forests Nyzhni Vorota weather station data for 1992–2012 years were used. The meteorological station is located in the district of the upper reaches of the Latorytsya river, at altitude of 500 m a.s.l. and at a distance of 4.5 km from our sample plots.

The results of the analysis of climate indices (Table 1) show that in the study region magnitude of the major climate indices tends to increase.

Table 1. Dynamics of the main climate indices.

Climate indices	Period, years 1992–2001	2002–2012	1992–2012	Long-term average 1949–2000
Precipitation (annual average), mm	1128	1146	1138	1046
Precipitation during the vegetation period, mm	725	742	734	693
Average annual temperature, °C	7.1	7.6	7.3	6.6
Average temperature during the growing season, °C	12.8	13.3	13.0	12.2
Average humidity, %	78.5	79.6	79.1	-
Average humidity during the vegetation period, %	76.1	77.3	76.7	-
Average annual humidity deficit, mb	3.3	3.2	3.2	-
Average humidity deficit during the growing season, mb	4.7	4.6	4.6	-

Note: '-' means data is absent.

Climate indices changed most intensively in the period 2002–2012. Compared to the standard climate norm, average monthly air temperature during the growing season increased from 12.2 °C to 13.3 °C, and average annual temperature – from 6.6 °C to 7.6 °C. The annual rainfall increased from 1046 mm to 1146 mm. The dry years with the maximum annual average air temperature and the minimum rainfall during the growing season were: 2000, 2007, 2012.

To study the characteristics of radial increment of the beech stands and the impact of external environmental factors we selected forest stands of the Nyzhni Vorota forest district in the Volovets State Forestry Enterprise. Silvicultural description of the forest stands, where sample plots (SP) were laid is shown in Table 2. As can be seen from the stated in the table data, the middle-aged pure and mixed beech stands grow under conditions of humid hornbeam fertile beech forest type on the slopes of southern and northern aspect at the altitude of 550–600 m a.s.l.

Table 2. Silvicultural inventory indices of test sites.

No sample plot	Tree composition	Age, years	Site class	Aspect slope	Altitude, m
1	60B30Hb10Sp	44	II	SW-20°	600
2	50B48Hb1Br1As	49	II	NE-20°	600
3	98B2Hb	57	Ia	S-25°	550
4	98B2Hb	54	I	NE-20°	550

Note: B – beech, Hb – hornbeam, Sp – spruce, Br – birch, As – aspen.

Results and Discussion

The comparison of statistical indices of the dendrochronological series of radial increment in the fertile beech forest type on their composition and direction of trees according to the four cardinal points of the world made it possible to note similarity in the value of average annual increment for the same composition of the forest trees (Table 3). Depending on tree composition average increment changes in the range of 1.71–2.41 mm and reaches the highest value in the mixed stands (SP 1 and 2). Radial increment of the studied beech stands, depending on slope aspect also differs. Thus, average radial increment of the southern side of a beech tree which grows on the southern mountain slope and the northern side of a tree on the northern mountain slope is higher in comparison with the opposite side of it.

It should be noted that in the mixed and pure forest stands average increment on the northern side is higher compared to the southern side of the slope, but in the mixed stands, this difference is minimal. In the mixed stands the variability of the annual increment is higher, as it is evidenced by the standard deviation and variation coefficient (Table 3). Negative excess for series of the forest stands increment on the southern mountain slopes testifies to their smoothness in comparison with the ranks on the northern slopes.

Table 3. Statistical characteristics of radial increment series of the middle-aged beech forest stands (1989–2012).

No SP	Tree composition	Aspect slope	Tree side	Increment, mm			Standard deviation of selection	Excess	The coefficient of variation
				average	maximum	minimum			
1	60B30Hb10Sp	SW-20°	N	2.29	2.91	1.63	0.37	-0.89	16.4
			S	2.53	3.26	2.08	0.36	-0.61	14.2
			average	2.41	3.08	1.89	0.35	-0.87	14.7
2	50B48Hb1Br1As	NE-20°	N	2.50	3.62	1.59	0.46	0.19	18.4
			S	2.34	3.70	1.54	0.48	1.16	20.7
			average	2.42	3.66	1.56	0.47	0.65	19.3
3	98B2Hb	S-25°	N	1.69	2.29	1.28	0.26	0.33	15.1
			S	1.73	2.2	1.25	0.29	-1.06	16.7
			average	1.71	2.16	1.27	0.24	-0.53	14.3
4	98B2Hb	NE-20°	N	1.92	2.58	1.29	0.30	-0.26	15.6
			S	1.82	2.41	1.25	0.30	-0.15	16.5
			average	1.87	2.34	1.27	0.26	0.14	13.9

Growth curves in the pure and mixed stands vary synchronously (Fig. 1). In the pure stands natural reduction of radial increment of beech can be traced (SP 3 and 4). We observed reduction of radial increment in the mixed stands prior to 2000 year (SP 1 and 2), and then a sharp increase of it, in our opinion, due to the intensive thinning. Years with peak growth of beech in most cases, coincide with the years of climatic extremes. Thus, the minimum increment of beech was observed in 2000 and 2007, and the maximum in 1998, 2004, 2005 and 2010 years. Accordingly, in 2000 and 2007 it was recorded maximum solar activity (119 units in Wolf number) and the maximum average air temperature (8.4 °C). In 1998 the maximum amount of precipitation (1521 mm) and minimal humidity deficit (2.6 mb) were registered. The humidity deficit describes the conditions of heat transfer through the evaporation of moisture from any surface.

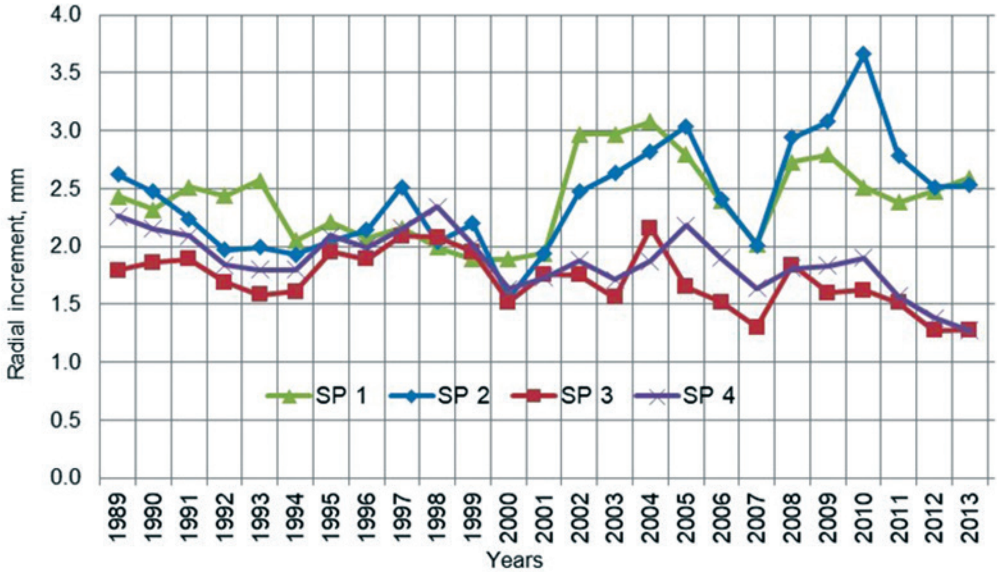

Fig. 1. Dynamics of radial increment of beech on the slopes of different aspect.

As it was shown by our studies (Shyshkanynets and Mazepa 2014), the percentage of early and late wood in the beech annual rings varies in the range of 83–88 % and 12–17 %, respectively.

The formation of early and late wood of beech is affected by climate, the influence of which on the northern slopes is more effective than on the southern slopes (Table 4).

Table 4. The correlation coefficients of early and late wood of beech, climate indices and Wolf numbers.

Indices	Aspect			
	SW-20°		NE-20°	
	Early wood	Late wood	Early wood	Late wood
The amount of precipitation (mm) for: - calendar year	-0.09	-0.05	0.28	0.15
- the vegetation period	0.05	-0.02	**0.34**	0.16
- hydrological year	0.005	0.12	**0.41**	0.27
- May	-0.09	0.1	**0.51**	**0.39**
-June	-0.05	0.16	0.11	0.16
- July	-0.01	-0.26	0.24	0.02
Temperature (°C): - calendar year	0.02	0.12	-0.13	0.32
- the vegetation period	0.03	0.36	-0.19	0.32
- hydrological year	-0.16	0.08	-0.16	0.07
- May	0.25	0.15	0.05	0.07
- June	-0.02	0.04	-0.02	0.24
- July	0.09	**0.48**	0.0	0.4
Air humidity (%) for: - calendar year	-0.11	0.23	0.23	**0.44**

Indices	Aspect			
	SW-20°		NE-20°	
	Early wood	Late wood	Early wood	Late wood
- the vegetation period	-0.05	0.26	**0.41**	**0.43**
- hydrological year	-0.12	0.23	0.22	**0.47**
- May	0.1	0.31	**0.47**	**0.44**
- June	-0.22	0.15	0.2	0.29
- July	0.07	0.04	**0.39**	0.24
Humidity deficit (mb): - calendar year	0.09	-0.07	**-0.40**	-0.27
- the vegetation period	0.06	-0.06	**-0.43**	-0.26
- hydrological year	-0.01	-0.11	**-0.46**	**-0.36**
- May	0.09	-0.1	-0.30	-0.27
- June	0.1	-0.19	-0.24	-0.17
- July	-0.07	0.11	**-0.35**	-0.07
Integrated climate indices: - *W*	-0.08	-0.22	0.12	-0.09
-*k*	0.09	0.28	0.09	0.31
-O_1	-0.08	-0.02	**-0.42**	-0.15
-O_2	-0.06	-0.06	**-0.40**	-0.30
Wolf numbers	-0.04	0.06	-0.13	-0.30

Note: the numbers in bold indicate a reliable correlation at a 95% level of significance.

Correlation analysis of the main climate indices and beech increment showed that the largest number of moderate and significant correlation is formed in the pure beech stands on the southern and north-eastern slopes (Table 5). Relationship between beech increment and climate indices in the mixed beech stands on the north-eastern slope is somewhat lower compared to the pure beech stands, but on the south-western slope such dependence is not visible.

Table 5. The correlation coefficients of the average radial increment of beech, climate indices and the Wolf numbers (1992–2013 years).

Indices	Aspect (No SP)			
	SW (1)	S (3)	NE (2)	NE (4)
The amount of precipitation (mm) for: - calendar year	-0.09	**0.45**	0.27	**0.39**
- the vegetation period	0.0004	**0.53**	0.28	**0.50**
- hydrological year	0.009	**0.40**	**0.38**	**0.37**
- May	-0.04	0.28	**0.50**	0.29
- June	-0.002	0.16	0.19	0.19
- July	-0.06	**0.44**	0.12	0.18
Temperature (°C): - calendar year	0.007	**-0.58**	0.09	**-0.63**
- the vegetation period	-0.06	-0.33	-0.008	-0.17
- hydrological year	-0.18	**-0.49**	-0.01	**-0.44**
- May	0.2	**-0.50**	0.015	**-0.41**

Indices	Aspect (No SP)			
	SW (1)	S (3)	NE (2)	NE (4)
- June	-0.03	**-0.34**	0.11	**-0.39**
- July	0.13	-0.29	0.22	-0.19
Air humidity (%) for: - calendar year	-0.04	0.17	**0.43**	**0.33**
- the vegetation period	-0.04	**0.50**	0.28	**0.64**
- hydrological year	-0.02	0.06	**0.44**	0.24
- May	0.18	0.24	**0.54**	0.29
- June	-0.12	0.11	0.33	0.21
-July	0.07	0.25	0.32	0.14
Humidity deficit (mb): - calendar year	0.01	**-0.61**	**-0.38**	**-0.64**
- the vegetation period	-0.06	**-0.52**	**-0.41**	**-0.46**
- hydrological year	-0.11	**-0.64**	**-0.46**	**-0.67**
- May	-0.0002	**-0.45**	**-0.36**	**-0.44**
- June	-0.002	-0.28	-0.29	**-0.35**
- July	-0.08	**-0.37**	-0.23	-0.23
Integrated climate indices: - *W*	-0.15	**0.50**	0.07	**0.43**
- *k*	0.04	**-0.40**	0.18	**-0.50**
- O_1	-0.11	**-0.62**	-0.28	**-0.57**
- O_2	0.06	**-0.44**	-0.34	**-0.35**
Wolf number	-0.11	0.13	-0.32	0.18

Note: the numbers in bold indicate a reliable correlation at a 95% level of significance.

Together with humidity deficit, air humidity, air temperature and precipitation radial increment of beech wood forms the greatest number of significant correlations. Integrated climate indices and beech increment form a significant and moderate correlation in the pure beech stands (SP 3 and 4). In the mixed stands (SP 1 and 2) relation closeness is absent or weak. The absence of correlation between radial increment and complex climate indices of the mixed beech stands testifies to their higher resistance to climate change compared to the pure beech stands.

Analyzing the correlation of the main climate indices and beech increment for the ten-year period, we found that in the last period (2003–2013) the closeness of relation was higher compared to the previous period (1992–2002) (Table 6). Radial

Table 6. The correlation coefficients of average radial increment of beech and climate indices for ten-year periods.

Indices	Aspect (No SP)			
	SW (1)	S (3)	NE (2)	NE (4)
1992–2002				
The amount of precipitation (mm) for: - calendar year	-0.30	**0.47**	-0.003	**0.39**
- the vegetation period	-0.12	**0.60**	0.27	**0.62**
Temperature (°C): - calendar year	-0.002	**-0.55**	-0.33	**-0.55**
- the vegetation period	-0.08	**-0.52**	**-0.45**	**-0.48**

Indices	Aspect (No SP)			
	SW (1)	S (3)	NE (2)	NE (4)
Air humidity (%) for: - calendar year	**-0.52**	**0.60**	0.06	**0.56**
- the vegetation period	**-0.36**	**0.70**	0.28	**0.65**
Humidity deficit (mb): - calendar year	**0.39**	**-0.73**	-0.32	**-0.66**
- the vegetation period	0.29	**-0.74**	**-0.41**	**-0.66**
Humidity of climate (*W*)	-0.16	**0.43**	0.09	**0.38**
2003–2013				
The amount of precipitation (mm) for: - calendar year	0.07	**0.59**	**0.63**	**0.49**
- the vegetation period	0.18	**0.58**	**0.56**	**0.46**
Temperature (°C): - calendar year	**-0.58**	**-0.47**	-0.27	**-0.60**
- the vegetation period	-0.32	-0.12	-0.08	0.15
Air humidity (%) for: - calendar year	-0.1	0.32	**0.40**	**0.69**
- the vegetation period	0.13	**0.51**	**0.41**	**0.75**
Humidity deficit (mb): - calendar year	**-0.39**	**-0.77**	**-0.63**	**-0.87**
- the vegetation period	**-0.43**	**-0.66**	**-0.59**	**-0.53**
Humidity of climate (*W*)	-0.04	**0.55**	0.33	**0.44**

Note: the numbers in bold indicate a reliable correlation at a 95% level of significance.

increment of beech wood together with humidity deficit forms particularly high inverse correlation. In our opinion this is due to more intensive increase of average air temperature in comparison to average annual humidity, especially during the last decade (Mazepa and Shyshkanynets 2013).

Conclusions

In the Ukrainian Carpathians the average radial increment of beech, depending on the composition of trees varies in the range of 1.71–2.41 mm and is the largest in the mixed stands. The proportion of early and late beech wood in the annual ring is 12–17 % and 83–88 %, respectively.

Average increment and characteristics variability of the mixed stands is higher compared to the pure stands. Radial increment of beech on the southern slopes of the south side of a tree and on the northern slopes on the north side of a tree is larger in comparison with the opposite side of a tree. On the northern slopes the annual increment is larger compared to the southern slopes. Extreme values of radial increment, in most cases, coincide with the critical values of climate indices. In the years of the most intensive solar activity radial increment of beech is minimal.

In the pure beech stands relationship between beech increment and climate indices is more substantial in comparison with the mixed forest stands. The beech increment correlates greatly with humidity deficit, air humidity, temperature and rainfall, which is especially observed in the last decade (2003–2013). Significant correlation between annual beech wood increment and air humidity as well as humidity deficit was found in the pure and mixed stands. The correlation of radial increment, temperature and precipitation in the mixed stands is weak and in the pure stands it is moderate. No significant correlation is obtained between Wolf numbers and beech radial increment neither for the annual growth, nor for the early and late wood.

The mixed beech stands compared to the pure beech stands are more resistant to climate change.

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AVAILABILITY AND UPTAKE OF PHOSPHORUS IN SOILS OF FOREST ECOSYSTEMS

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Abstract

The aim of this work was to assess the availability and uptake of phosphorus in the soils of four forest ecosystems in Greece, i.e. a maquis, a deciduous oak, a beech and a fir forest. The highest concentrations of total P were observed in FH (mixture of F and H) horizons (914 to 1269 mg·kg^{-1}). In the mineral layers (up to 80 cm depth), the range was 439 to 850 mg·kg^{-1}. The percentages of the available P with regard to the total P were very low. In the FH horizons, they ranged from 2.4 % in the fir plot to 6.5 % in the beech plot. In the mineral layers, all percentages were less than 1.0 % in all forest types. The concentrations of available P were significantly and positively affected by the contents of soil organic C, total nitrogen and the clay percentage. Negative and significant relations were observed for the ratios P/C, P/N and the percentage of sand, whereas the content of total P did not affect the availability of P significantly. The P uptake by trees was based on the calculation of the above ground litterfall. It was found that, at least for the fir plot, the amounts of available P in soils up to the depth of 20 cm were not enough to cover the tree needs. It is hypothesized that the extra P needed is acquired by the dissolution of secondary minerals by root exudates.

Key words: extraction, litterfall, phosphorus, soil, tree uptake.

Introduction

For many forests of the world, growth and nutrient cycling are limited by phosphorus. There is increasing evidence that it is immobilized in the first stages of organic matter decomposition to a greater extent than nitrogen (Attiwill and Adams 1993). Lang et al. (2016) hypothesized that at sites rich in mineral-bound P, plant and microbial communities use it from primary minerals and hence there is not a tight P cycle. In contrast, a tight cycle is crucial at sites poor in mineral bound P. A tight nutrient cycle exploits even the minute concentrations. The concentration of P in soil solution is very low. It ranges from 0.001 mg·L^{-1} in very infertile soils to 1 mg·L^{-1} in heavily fertilized soils (Belyazid and Belyazid 2012). The bulk of soil P is immobilized in the organic and inorganic soil fractions. The distribution sinks and their competiveness are topics of fundamental importance for the understanding the cycling of P in a forest environment (Attiwill and Adams 1993). Currently, there is evidence that anthropogenic environmental changes affect P nutrition by disrupting its cycling (Jonard et al. 2014). The authors suggested that excessive N deposition brought about reduced root and mycorrhizae growth, which resulted in reduced P uptake. In addition, higher tree growth due to increased CO_2 in the atmosphere require higher P uptake.

The total P content in forest soils is not a good predictor of tree nutritional status. Very often, a significant correlation with the foliar P content, in different tree species cannot be found (Ilg et al. 2009). It is inferred that most soil P is unavailable or not directly plant available. Other approaches employ the quantification of a number of different P fractions in mineral soils (Hedley et al. 1982). It is very often that researchers use only one extractant to assess available P. Different extractants correlate with distinct pools of P in soils and the extractability of a given pool is affected by soil properties (Wuenscheret al. 2015).

It was decided to use the Olsen phosphorus (OP) to assess the available P in soils. This method uses sodium bicarbonate ($NaHCO_3$) to extract plant available P. Originally it was developed for calcareous soils where P complexes with Ca. In the OP extraction, calcium carbonate ($CaCO_3$) is formed and in this way, the activity of Ca in the solution decreases as $CaCO_3$ precipitates. At the same time, the dissolution of Ca phosphate is promoted. Furthermore, the high pH (8.5) of the extractant enhances phosphate desorption from Al and Fe oxides (Wuenscher et al. 2015). Later the OP was found to apply with success to acid soils as well (Tchuenteu 1994, Bair and Davenport 2012). Do et al. (2007) argued that when this method is applied to acid soils the threshold for P deficiency should increase.

The aim of this work was to assess the total and available P in soils of four forest ecosystems in Greece and identify the dependence of the available P on soil physical and chemical parameters. In addition, the uptake of P was assessed taking into account parameters of the net primary production such as the fluxes of above ground litterfall of the forests. The calculated uptake needs for P was compared with the amounts of available P in soils to check the sufficiency of the soil P pools for the tree nutrition with this element. As mentioned above, the tree nutrition with regard to P has deteriorated and in this respect, this work will contribute to the understanding of P uptake.

Materials and Methods

Sites description

All sites from which the material was collected belong to the Intensive Monitoring Survey of the ICP Forests network (UN-ICP-Forests 2020). They represent important forest ecosystems in Greece and this was the reason they were selected. Information about the plots is given in Table 1.

Table 1. Characteristics of the areas.

Area	Altitude, m	Soil parent material	Soil type	Vegetation
Amfilohia	360	Flysch	Haplic Luvisol	Maquis species: *Quercus ilex L., Arbutus unedo L., Phillyrea latifolia L.*
Ossa Mountain	740	Mica schist	Dystric Cambisol	*Quercus frainetto* Ten.
Ossa Mountain	890	Mica schist	Haplic Alisol	*Fagus sylvatica L.*
Karpenisi	1170	Flysch	Humic Alisol	*Abies borisii-regis* Mafft.

Note: Soil types are presented in accordance with FAO-UNESCO (1988); the term 'maquis' is defined as a scrubland vegetation of the Mediterranean region composed primarily of leathery, broad-leaved evergreen shrubs or small trees (Encyclopedia Britannica 2020).

Soil samples collection and pretreatment

It was carried out by means of systematic sampling in the summer of 2007. Inside the plots, along three lines having a distance of 25 m from each other, six soil pits (5 m away from each other) were excavated. From each pit, the organic horizons litter (L) and the FH layers were collected with a metallic frame having an area of 15×15 cm. Actually the L and the FH horizons together form the forest floor. In other forests, especially in northern countries, the F and H horizons are distinguishable but not in the Mediterranean forests where decomposition is fast. Mineral soil layers were collected with a shovel from the depths 0–10 cm, 10–20 cm, 20–40 cm and 40–80 cm. Six samples (approximately 2 kg each) per horizon and soil depth were mixed to have three pooled samples per horizon and depth. The bulk density of the mineral soils was measured with a cylinder of known volume (129 cm^3). The value was converted to bulk density of fine earth (<2 mm) after subtracting the volume of coarse material. The percentage of large stones was estimated visually in the field. All soil samples were transferred to the laboratory and were air-dried. Apart from the L layer, the other samples passed through a 2 mm sieve. For the total analysis for P subsamples of all soil layers were pulverized in a ball mill and stored for analysis. All results were expressed in 105 ^{o}C dry weight of soil.

Litterfall collection and pretreatment

Ten littertraps (cylindrical plastic buckets), each having a collecting area of 0.242 m^2, were placed systematically along a straight line in all plots, approximately 0.50 m above ground at a distance of 10 m from each other. The litterfall sampling for the fir and beech plots covers the period 2009 to 2017, for the maquis plot the years 2013 to 2017 and for the oak plot the 2010 to 2019 period. Collection of litterfall was done monthly or longer periods, depending on the accessibility of the area due to snow and the sufficiency of the material for chemical analysis. At each collection, a composite sample was formed by pooling the content of the ten littertraps. Three fractions were formed: foliar, woody (twigs, bark debris) and the rest consisted of flowers, fruits, lichens, mosses, insect frass, pollen and whatever did not belong in the first two categories. The fractions were dried at 80 ^{o}C for 48 h and weighed. Each fraction was ground in a blade mill, passed through a 1.0-mm screen (20 mesh) and stored for analysis. All results were expressed in 105 ^{o}C dry weight of litterfall material.

Physical and chemical analysis

Soil texture analysis

The texture analysis (in sieved soil) was carried out with the pipette method (Miller and Miller 1987).

Chemical analysis

The pH was measured electrometrically (pH meter ORION 410-A) by a glass electrode in water (1:5 soil weight per volume of water). The concentrations of total N and organic C in soils were measured with a CN analyzer (Farina et al. 1991) (model Vario MAX, Elementar Company). For the determination of total P in ground litterfall and the L horizon subsamples were digested in a 2:1 mixture of nitric and perchloric acids (HNO_3/$HClO_4$) (Zasoski and Burau 1977). Available P was extracted with a $NaHCO_3$ solution (Olsen and Sommers 1982). Total P in litterfall, soil L horizon and available P were determined with the ammonium molybdate method in a UV spectrophotometer (Varian-Cary model). Total P in soils (apart from the L horizon) was measured by means of the Energy Dispersive X-Ray Fluorescence (ED-XRF) method (Vanhoof et al. 2004) (XEROS model of the TURBOQUANT Company).

Data handling and statistical analysis

The amounts of P (available) in soils were calculated taking into account the P concentrations, bulk density and the masses of soil per layer after subtracting the estimated volume of rocks. The litterfall fluxes were calculated by multiplying the P concentrations of each fraction by its dry weight. The annual total litterfall fluxes of P were found by summing up the annual litterfall fluxes of all fractions.

For the soil properties the average values of the three replicates were calculated and the coefficients of variations as the percentages of the standard deviations over the means. The same procedure was followed for the annual fluxes of P in total litterfall but the replicates here were the yearly fluxes. Table 4 contains the amounts (kg·ha^{-1}) of available P in the various soil layers. This data was used to assess the available P for the take up.

The statistical dependence of available P was examined for a variety of soil parameters in the mineral soil layers by means of the Principal Component Analysis (PCA). The predictors chosen were pH, clay and sand, total P, N, C and the ratios C/N, P/C and P/N. The dependence of P on pH and soil texture is well known (Brady 1984). The other variables represent elements and ratios, which in the authors' opinion may affect available P. Nutrient ratios, have often been used to assess tree nutrition (Michopoulos 2013; Tessier and Raynal 2003). The replicates in space of the soils collected were also included in the PCA calculations. PCA technique is very useful when the number of the initial correlated variables is large (Zitko 1994).

Results

The percentages of available P, with regard to total P, calculated from Table 2 ranged from 2.4 % in the fir plot to 6.48 % in the beech plot with regard to the FH layer. In the mineral layers, all percentages were below one. It is worth noting

Table 2. Soil properties in the four forest sites.

Layer	pH	Clay, %	Sand, %	C, %	Total N, $g \cdot kg^{-1}$	Total P, $mg \cdot kg^{-1}$	Available P, $mg \cdot kg^{-1}$
Maquis							
L				49.4 (2.8)	11.2 (8.5)	351 (17)	
FH	6.6 (3.7)			26.0 (7.1)	13.6 (8.2)	914 (4.3)	35.6 (14)
0–10 cm	6.26 (2.0)	23.6 (6.2)	20.3 (8.9)	5.0 (14)	3.03 (13)	565 (2.6)	3.17 (40)
10–20 cm	6.18 (6.6)	24.2 (14)	21.6 (15)	2.73 (17)	1.89 (14)	481 (0.8)	1.04 (44)
20–40 cm	6.12 (2.3)	26.0 (19)	21.2 (22)	1.44 (17)	1.15 (14)	441 (5.4)	0.59 (65)
40–80 cm	6.53 (5.7)	29.3 (15)	20.1 (22)	0.86 (14)	0.83 (11)	439 (9.4)	0.73 (7.0)
Oak							
L				45.9 (3.1)	12.6 (5.8)	634 (4.3)	
FH	6.19 (1.6)			20.1 (9.5)	9.62 (10)	924 (6.4)	45.8 (7.7)
0–10 cm	5.35 (0.7)	16.2 (3.8)	38.9 (3.6)	3.46 (14)	1.98 (15)	680 (2.1)	4.43 (0.39)
10–20 cm	5.45 (1.5)	16.6 (2.3)	36.4 (5.2)	20.9 (16)	1.23 (19)	624 (6.6)	1.21 (1.1)
20–40 cm	5.43 (1.2)	16.1 (2.5)	34.5 (8.2)	1.18 (3.7)	0.725 (3.9)	643 (3.6)	0.082 (98)
40–80 cm	5.42 (0.7)	13.6 (4.2)	46.6 (3.2)	0.57 (17)	0.378 (13)	632 (2.8)	0.043 (53)
Beech							
L				47.3 (0.6)	14.3 (4.2)	685 (7.0)	
FH	5.75 (4.9)			26.9 (3.5)	13.6 (1.1)	956 (2.5)	62.0 (0.03)
0–10 cm	4.83 (2.4)	20.0 (5.5)	26.4 (10)	4.32 (4.5)	2.09 (8.2)	633 (2.7)	4.39 (25)
10–20 cm	5.08 (2.1)	21.8 (2.1)	22.8 (0.8)	2.95 (8.7)	1.54 (11)	630 (8.9)	1.32 (17)
20–40 cm	5.24 (0.6)	21.5 (0.3)	21.3 (4.2)	2.09 (12)	1.20 (8.6)	661 (4.8)	1.27 (33)
40–80 cm	5.27 (1.1)	15 (4.1)	30.4 (6.6)	0.908 (3.2)	0.582 (2.2)	585 (11)	3.35 (118)
Fir							
L				50.8 (0.7)	12.3 (9.3)	881 (12)	
FH	6.48 (0.2)			23.0 (14)	12.2 (13)	1269 (5.6)	30.5 (6.0)
0–10 cm	6.07 (2.0)	27.2 (2.0)	29.5 (1.2)	5.12 (20)	3.31 (16)	850 (3.1)	5.26 (28)
10–20 cm	5.77 (3.1)	31.2 (2.1)	24.7 (8.1)	3.36 (10)	2.45 (9.4)	825 (3.0)	2.68 (14)
20–40 cm	5.54 (1.2)	33.0 (6.7)	21.0 (6.0)	2.75 (3.5)	2.12 (4.4)	818 (1.5)	2.90 (38)
40–80 cm	5.32 (1.5)	34.9 (14)	23.2 (11)	1.53 (19)	1.35 (15)	731 (4.9)	6.60 (37)

Note: in parentheses are the coefficients of variation.

that the fir plot had the lowest percentage of available P in the FH horizon and the highest percentages in the mineral soils in all plots.

The variability, expressed by the coefficients of variation, of soil properties are showed in Table 2. The available P showed the highest one especially in deeper soil horizons.

The results of Figure 1 depicting the PCA axes and the vectors of the parameters are combined with Table 3. The component 1 represents the horizontal axis of Figure 1, whereas the component 2 the vertical one. The first principal component is the linear combination of x-variables that has maximum variance (among all linear combinations). It accounts for as much variation in the data as possible. The second principal component is the linear combination of x-variables that accounts for as much of the remaining variation as possible, with the constraint that the correlation between the first and second component is 0 (Zar 1999).

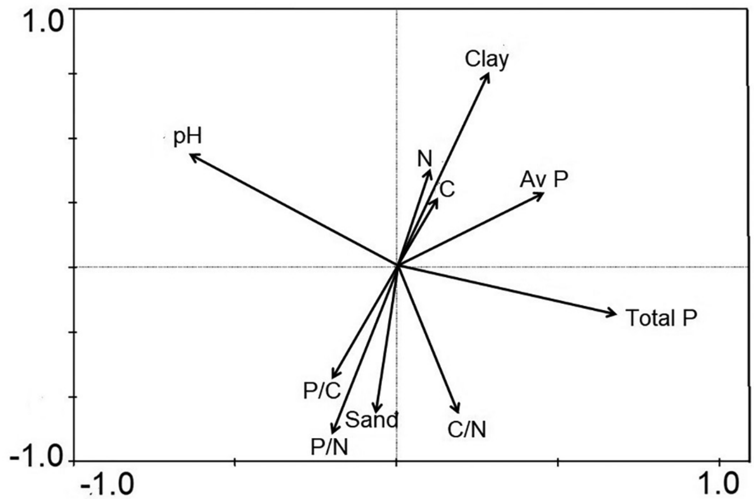

Fig. 1. PCA ordination diagram for the relation of available P with the soil properties.

Table 3. Component matrix with the relations of the available P and soil parameters derived from the PCA.

Indicator	Component	
	1	2
Available P, $mg \cdot kg^{-1}$	0.632	0.295
Total P, $mg \cdot kg^{-1}$	0.325	0.452
pH	0.245	-0.742
C, %	0.846	0.403
N, $g \cdot kg^{-1}$	0.909	0.184
C/N	0.065	0.866
Clay, %	0.607	-0.589
Sand, %	-0.617	0.550
P/C	-0.889	-0.110
P/N	-0.920	0.096

As the available P had the highest value there (0.632 in the component 1), the component 1 was chosen to check the results of the PCA. Values around 0.600 or higher are considered satisfactory as they explain at least 40 % of the variability of the parameters (Comrey 1962). Therefore, from Table 3 the parameters having a satisfactory and positive relationship with the available P are the organic C, total N and the clay percentage. Negative relationships were observed for the sand percentage, the ratios P/C and P/N.

In comparison with the three other plots, the maquis plot had the lowest amounts of available P (Table 4) in the deepest mineral layers due to the high percentage of rocks. This fact shows the importance of calculating the rock percentages even only visually when there is need to express the nutrients in amounts per area in soils. It must be also pointed out that the amounts of available P are based on the concentrations of the OP. Other extractants (water, 0.01 M $CaCl_2$, Mechlich, etc.) would have given different amounts of available P. In any case, it is a reliable indication for P concentrations and the same conclusion should be drawn for the calculated amounts of P in soils.

Table 4. Amounts of available P in the soils.

Layer	Maquis	Oak	Beech	Fir
	$kg \cdot ha^{-1}$			
FH	1.05	0.08	0.27	2.43
0–10 cm	1.74	3.21	2.54	3.26
10–20 cm	1.12	1.15	1.17	2.28
20–40 cm	1.08	1.52	1.58	4.69
40–80 cm	0.54	1.50	3.49	10.8

In terms of absolute numbers, the concentrations of available P are considered satisfactory for the FH layers but not for the mineral when the thresholds for agricultural soils (11 mg·kg^{-1}) (Bai et al. 2013) are taken into account.

Table 5 shows the means and ranges of the aboveground total litterfall measured in the field, whereas Table 6 contains the means and ranges of the belowground litterfall calculated from literature.

Table 5. Annual fluxes of P in the total aboveground litterfall and coefficients of variation (CV).

Indicator	Maquis	Oak	Beech	Fir
Average, kg·ha^{-1}·yr^{-1}	2.82	3.55	2.67	5.74
Range, kg·ha^{-1}·yr^{-1}	1.88–4.25	2.36–4.81	1.60–4.04	3.67–8.71
CV, %	29	20	29	33

Table 6. Annual fluxes of P in the belowground litterfall (fine roots production) calculated from Table 5 and literature.

Indicator	Maquis	Oak	Beech	Fir
	kg·ha^{-1}·yr^{-1}			
Average	2.07	2.61	1.96	4.22
Range	1.38–3.12	1.73–3.54	1.18–2.97	2.70–6.40

Discussion

Magnitudes of total and available P in soils

The concentrations of total P in the FH horizons were found consistently higher than those in L layer and mineral soils. In comparison, Yanai (1992) found somewhat higher values for total P in the forest floor of the Hubbard Brook experimental forests (with the exception of fir, which had similar values), 70 years of age in USA. Schoenau et al. (1989) examined the forms of P in prairie and boreal forest in Canada. In native Chermozen they found similar concentrations of total P in the FH horizons of Luvisol and Gleysol but much lower concentrations in the FH of Chermozens. In the mineral horizons, the results were similar. In any case, the concentrations of total P in our work were within the range of 200–800 mg·kg^{-1} found in mineral soils (Cross and Schlesinger 1995) with the exception of the fir plot, which slightly exceeded the upper limit. This high concentration of total P in the soils of the fir plot should be ascribed to the relatively high contents of organic C and clay in comparison with the other three plots (Table 2). Other authors found considerably higher concentrations of total P in forest soils (37–1773 mg·kg^{-1}) in the top soil (0–15 cm) (Achat et al. 2009).

As mentioned in the results section, the percentages of available P with regard to total P calculated from Table 2 ranged from 2.4 % in the fir plot to 6.48 % in the beech plot with regard to the FH layer. In the mineral layers, all percentages were below one. Schoenau et al. (1989) found higher percentages of available P (extracted also with sodium bicarbonate) approximately 12 and 13 % in the FH horizons, whereas in the mineral soils the percentages values were above one. These high percentages in those cold climates should be attributed to slower decomposition rates in comparison with the forest types in the Mediterranean zone.

The question that arises is about the high difference between the magnitudes of available, total P and the origin of the latter in mineral soils, which is appreciably high even in soil depth. According to Bowman and Cole (1978), $NaHCO_3$ solution can extract highly labile organics of low molecular weight containing P but not resistant organic P that is strongly associated with the mineral fraction. The resistant P also comprises inorganic P bonded to Al and Fe sesquioxides. As time passes, some labile P forms (including OP) can be included in soil minerals and thus increase the residual P fraction. Therefore, the high amounts of total P in the 20–40 cm and 40–80 cm of the plots can be either resistant organic or inorganic P. In future times, these fractions can replenish the labile P pools when the latter are exhausted by plant uptake (Smeck 1985).

The available P presents higher variability in deeper soil horizons than those of the total P. This might be due to the different mineralization rates of organic P in the soil. McGill and Cole (1981) argued that there is an independent mineralization process of organic P (apart from the one following the C mineralization) caused by enzymes released by plant roots. One might expect that this increased variability should have occurred in the rhizosphere zone and not so much in deeper soil layers. Maybe the hydrological path (which becomes more complicated in depth) of the root enzymes can affect the concentrations of available P in deeper horizons as well.

Factors affecting available P in soils

The PCA analysis showed that both organic C and total N (Table 3) affect the available P in soils (positively). This means that the release of P from organic matter depends on the C necessary for energy and N to cover the N needs of microorganisms. The negative effects of P/N and P/C emphasize the dependence of C and N. The positive dependence of available P on the clay percentage has to do with the mineralization of organic P but also with dissolution of secondary minerals containing P through root exudates. The PCA did not disclose a satisfactory relationship with the total P. Similarly, Liu et al. (2014) found a significant correlation of available P with organic C and total N but not with total P in a subtropical forest situated in southern China. It is also logical that the sand percentage had a significant negative effect. This soil fraction cannot withhold any P compound.

P uptake by forests

An indirect way to assess the P uptake by plants is by estimating the litterfall input to the forest floor. Trees have to take up all the P they lose in litterfall (Cole and Rapp 1981). The averages and the ranges of the above ground total litterfall fluxes for P are showed in Table 5. The values are close to those mentioned in literature. Yanai (1992) found 4 $kg \cdot ha^{-1} \cdot yr^{-1}$ in a hardwood forest (Hubbard Brook), whereas Hansen et al. (2009) measured 2.1 to 3.3 $kg \cdot ha^{-1} \cdot yr^{-1}$ of P in litterfall fluxes in five even-aged stands of Norway spruce, Sitka spruce, Douglas-fir, European beech and common oak at three different locations in Denmark.

The below ground litterfall has not been calculated in this work but it must be taken into account that the mycorrhizae fungi can account for 50 % of the annual throughput of biomass (Fogel 1980). In this work, we have not calculated the fine root production and mortality. A rough estimate to apply could be that from An et al. (2017) who worked in variety of deciduous and conifer forests. They assessed the carbon flux of fine roots to be 25 % of the Net Primary Production, whereas that of the above ground litterfall was 34 %. Furthermore assuming that the P fluxes in fine roots tissues are approximately the same with the aboveground litterfall, then the numbers in Table 5, both means and ranges, should be multiplied by 25/34 to find the fluxes of fine roots. So a new Table 6 can be made from Table 5 containing the fine root production and mortality. Admittedly, that is an approximation, which helps nevertheless to make a rough picture of the P uptake needs. If we add the calculated P flux of the fine roots (Table 6) to the respective values in Table 5, the total litterfall above and below ground in the four forest types can be found. For example, for the fir forest one has to add the averages fluxes 5.74 and 4.22 $kg \cdot ha^{-1} \cdot yr^{-1}$ of tables 5 and 6, respectively to find the average total flux of P 9.96 $kg \cdot ha^{-1} \cdot yr^{-1}$. The same calculations have to be made for the ranges. Of particular interest are the highest ranges of the new litterfall. They become 7.4, 8.4, 7.0, and 15.1 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the maquis, oak, beech and fir forests, respectively. We calculated the highest range because we want to know the extreme situation needs. The values represent the highest needs for uptake. From these numbers the P inputs to the ecosystems should be subtracted. When considering them to a forest, one has to mention the deposition input which is low (0.05–1.7 $kg \cdot ha^{-1} \cdot yr^{-1}$) and the weathering input from apatite, also low (0.05 to 1 $kg \cdot ha^{-1} \cdot yr^{-1}$) (Belyazid and Belyazid 2012). If from the above-mentioned numbers the total input (let us say 3 $kg \cdot ha^{-1} \cdot yr^{-1}$) is subtracted, we have 4.4, 5.4, 4.0, and 12.1 $kg \cdot ha^{-1} \cdot yr^{-1}$ needs for P uptake for the respective plots (for the highest range of litterfall). To check that the available P in soils suffices for these needs, we have to see the amounts of available P in soils in Table 4. Up to the depth of 20 cm the sums of amounts are 3.9, 4.4, 4.0 and 8.0 $kg \cdot ha^{-1}$. This depth was chosen because P uptake usually takes place in the surface soil layers. This was the case for the sugar maple stands in the Quebec Appalachians (Pare and Bernier 1989) and in stands for *Banksia* spp. in Australia (Grierson and Attiwill 1989). It appears that the fir stand (more than the others) cannot cover its needs for P uptake only from the available P in soil as determined by $NaHCO_3$. These needs are probably met by P dissolved from soil minerals through the exudation of low molecular organic acids such as oxalic acids (Plassard and Dell 2010). This happens when the litterfall, both above and below ground, or in other words, when the net primary production is high and there is need to replenish the nutrients invested in it.

Conclusion

The release of P from the soil organic matter depends on the C necessary for energy and N to cover the N needs of microorganisms. It was also found that the clay percentage also affected positively the availability of P. The FH horizon is the most important soil layer to supply P to the forest trees as it contains the highest percentage of available P with regard to the total P amount. For the fir forest, the dissolution of minerals in the soils through roots exudates is also essential as the needs for uptake can exceed the amounts of available P in the surface soil layers from which the P uptake usually takes place.

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REACTION OF POPLAR SEEDLINGS AFTER SELECTIVE HERBICIDES TREATMENT

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Abstract

The reaction after treatment with selective herbicides was carried out in a nursery plot for production of one-year-old poplar saplings from the type 0/1 from the cultivars ′I-214′ and ′Vernirubens′. Water solutions of the 4 herbicides (H1–H4) with a concentration 5 ml.l^{-1} and a control variant were tested. Treatments were carried out in five terms: May, June, July, August and September. Final measurements were carried out after the growing season. The estimation of the influence of each herbicide was done after the following indices: survival, height and diameter of the stem; thickness and number of first grade roots; relative moisture of wood of poplar saplings. The results showed that tested herbicides do not influence on the survival of saplings with the exception of a negative influence on cultivar ′Vernirubens′, caused by the herbicide H2. Saplings of cultivar ′I-214′ have faster growth in height than cultivar ′Vernirubens′. Tested cultivars show specific reaction to applied herbicides and best tolerance to H3 (350 g.l^{-1} Butachlor + 350 g.l^{-1} Propanil). Herbicides H1 (150 g.l^{-1} Fluazifop-P-butyl) and H4 (240 g.l^{-1} Oxyfluorfen) oppress less the growth of saplings at both cultivars, compared to the outlined depressive effect of the herbicide H2 (240 g.l^{-1} Clethodim). As most suitable was determined the herbicide H3. In the course of production, weed control could be carried out with the help of this herbicide during all phoenophases of the development of saplings.

Key words: cv. I-214, cv. Vernirubens, poplar seedlings, *P.* × *canadensis*, reaction, selective herbicides.

Introduction

Traditionally in the forestry practice in the moderate climate zone countries, including Bulgaria, poplar plantations have a significant share in afforestations. The interest to poplar growing is determined by the fast growth of poplars, their high productivity in short rotation and the constant big demand of timber by consumers. The sites in forest territories of Bulgaria, suitable for poplar-growing, are about 26,000 ha. Predominantly, approved cultivars of Canadian poplar hybrids (*Populus* × *canadensis* Moench) are used. The biggest share is of cv. I-214 – about 44 %, and the share of the cultivars ′Bachelieri′, ′CB-7′, ′I-45/51′, ′Vernirubens′, ′Agate F′, ′MC′, etc. is 5–8 % each. During the last 8 years (2012–2019), 5000–8000 dka of poplar plantations were established in Bulgaria and their share is average 40 % of the totally afforested areas (operational information from the Executive Forest Agency for the period 2012–

2019). The data show a trend of increasing, which determines constant necessity of poplar saplings and optimisation of their production.

Basic problem in the production of poplar saplings is the phytosanitary status of production areas and especially of weeds. Weeds are determined as one of the most limiting factors for the success of nursery production (Vasic and Konstantinovic 2008, Vasic et al. 2015). Bigger distance between planting rows and bigger access of light in the initial stage of development of saplings predispose to large-scale development of weeds (Vasic et al. 2007). Poplars are particularly demanding of both moisture and rich soils. Weeds do not overgrow saplings but compete them predominantly for water and nutrients (Kauter et al. 2003, Wagner et al. 2006, Kabba et al. 2007, Tobisch 2007). Some authors draw the attention to the most significant period of maximal negative influence of weeds and determine it as critical point. They also determine the critical period for weed control (Knezevic et al. 2002, Bukun 2004). The weed control is also carried out according to economic indices of the obtained income and expenses for weed control. The point of maximal loss of yield during the critical period is determined as critical point (Otto et al. 2009). The knowledge about losses caused by weeds in the production is important for the timely control (Dorado et al. 2009). These losses are estimated as bigger towards losses caused by insect pests and diseases. Hybrid poplars are very sensitive to the competition of other vegetation, especially during the first growing period (Buhler et al. 1998, Coll et al. 2007). It is determined that weeds show most unfavourable influence in the initial phase after rooting of poplar cuttings (Anselmi and Giorcelli 1983, Kauter et al. 2003, Dhiman and Gandhi 2011). In the presence of strong weeding during this period, the growth is depressed and dyeing of saplings is also observed. Thus towards the end of the growing period the quality and quantity of production are unsatisfactory. Broeckx et al. (2012) point out that only when proper weed control is carried out it could be expected that losses should be fewer than 20 %.

During an experiment with weed control through various methods, including combinations with paddy straw mulch and plastic mulch strips, Kaur et al. (2016, 2018) determine that the effect of elimination of weeds with preparations (Pendimethalin with 1 $kg \cdot ha^{-1}$ and Alachlor with 2.5 $kg \cdot ha^{-1}$) for improving the *Populus deltoides* saplings growth is expressed through bigger height (11–19 %) and diameter (20–28 %) growth. The results from the best variants are similar to the control one through mechanical manual weeding.

Thomas et al. (2001) compare the use of opaque polyethylene mats and glyphosate treatment (applied at a rate of 2.1 kg active ingredient per ha in midsummer of the second and third growing seasons). The experiment was carried out during four growing seasons. The authors report that the opaque polyethylene mats significantly improved poplar height growth only for the first growing season. After the four growing seasons, hybrid poplars treated with glyphosate were significantly taller than those in either the mat or control treated plots. Diameter at breast height was also significantly greater in the herbicide treated plots than in either the mat or control treated plots. Total volume was also significantly greater in herbicide plots than in either the mat or control plots.

Hansen and Netzer (1985) report that weeds do not cause saplings loss but influence negatively on their growth proportionally to the competition level of weeds. Depressive influence of weeds is determined, compared to variants with manual weeding, and the height of poplar saplings is less with 8–15 %, diameter – with 6–18 % and above-ground biomass – with 21–58 %. Similar results are presented by Marino and Gross (1998) and Sixto et al. (2001).

Recommendations for herbicides using in nursery production are given by James et al. (2003). Oxyfluorfen, Simazine and Izoxaben are suitable before the occurrence of the weeds, efficient against broad-leaved weeds. Oryzalin, Pendimethalin and Prodiamin are efficient in the control with grasses and some small-seed broadleaved weeds. Fluazifop-butyl, Sethoksidym and Clethodim are selective herbicides to be used after germination, used for grasses control. Glyphosate, Paraquat and Glufosinate are non-selective herbicides for after germination, used for wide-various weed control. Bentazon, Halosulfuron-methyl and Imazaquin are efficient after germination. Metolachlor is pointed out as especially designed for nurseries. Chloroacetanilides are used for preliminary treatment against annual grasses and some perennial broadleaved weeds (Soltani et al. 2011). Metribuzin is Triasin herbicide, which inhibits photosynthesis (Flores-Maya et al. 2005). This herbicide could be applied in control with annual grasses and broadleaved weeds (Majumdar and Singh 2007). Especially for poplar nurseries, as efficient and with good selectivity towards non-rooted cuttings, herbicides Tiazopir (Sixto et al. 1999) and Oxyfluorfen (Sixto et al. 2001) are recommended. For the systematic herbicide Glyphosate it is mentioned that it kills plants of many species through its translocation in plants and therefore its application should be very careful to avoid losses on saplings (Dhiman and Gandhi 2011).

The selection and application of herbicides is complicated task because it is related to many factors – the herbicide chemical composition and structure, quantity, method and time of application, climatic and meteorological conditions, soil type (Prasse 1985). The humus content in soil is of particular importance. It has direct effect on the herbicide activity due to its adsorption ability. The content of colloid particles decreases the herbicide activity due to the big tendency of herbicides to joining (Spark and Swift 2002).

The mechanical weed control is expensive, slow and low-efficient. The manual trenching between the rows is the mostly applied method for weed control, and elimination of weeds around the plants in the rows is difficult. There are muddy periods caused by rains or watering and the conditions do not allow in-time mechanical weeding. These problems demand application of chemical tools for in-time and efficient weed control (Kaur 2016).

Berthelot and Bonduelle (1993) point out that the expenses for treatment with herbicides are considerably less than those for the traditional weeding. Experiments of Dhiman and Gandhi (2011) for integrated control through herbicides, including straw mulch and polyethylene, have shown that the expenses for chemical weed control are 6–7 times less compared to the mechanical way.

The mechanical weed control in forest nurseries in Bulgaria is also basic production expense (Vatov and Zahov 1980), and meanwhile the capabilities of herbicides for production optimisation are not revealed (Iliev 2007, Iliev and Iliev 2010). Investigations are directed mainly to their capabilities for application in agriculture (Keremidchiev et al. 1980, Abrahamson

1987). Important reason to rely more on herbicides is also the still unsolved problem with the manpower.

Experiments with application of herbicides in forestry in Bulgaria have been carried out for the first time in 1957 (Vatov and Zahov 1981). Their more organised practical application is carried out since 1978, after the development of a Programme for extension of the application of herbicides in forestry (Vatov and Zahov 1980). Results from application of herbicides in forest nurseries and plantations have been also published by Garelkov et al. (1962), Vatov and Zahov (1980, 1981) and Bochev (1985, 1988). More recent studies on the application of selective herbicides in forest nurseries are carried out by Iliev (2007), Iliev and Iliev (2010) and Iliev et al. (2013). For the time being, there is no published information about application of herbicides in the production of poplar saplings.

It is considered that between 50 and 400 man-hours per 1 dka are necessary for manual weeding in forest nurseries (Abrahamson 1987, South 1994). The use of selective herbicides can reduce the necessary time for manual weeding with 80–87 % (Abrahamson 1987).

The aim of the investigation is to determine the reaction of poplar saplings to the application of some selective herbicides for production improvement.

Object and method of work

The study was carried out in Dragor nursery, State Forest Enterprise Pazardzhik, in the central part of South Bulgaria (42°41′52" N 23°19′18" E) at an altitude 204 m a.s.l. The average annual temperature is $T_{av.}$ = 11.7 °C. The average annual amount of precipitations is W_{ann} = 568 mm. The soil in the nursery is Mollic Fluvisols, very deep, with clayey-sandy mechanical composition in the surface layer 0–35 cm. The overall moisture capacity is 29.75 %. It is very poorly stocked with humus and poor of nitrogen. The stock of assimilate phosphorus is poor, and the stock with assimilate potassium is very good. The soil reaction on the surface soil layer (0–35 cm) is neutral.

Experiments were carried out in a plot for production of one-year-old poplar saplings of the type 0/1 from *P.* × *canadensis* cv. I-214 and cv. Vernirubens.

In October the sample plot was ploughed in a depth 30–35 cm, and in the beginning of March next spring the soil was tillaged in a depth 10–12 cm. The shoots used for cuttings were obtained in the beginning of February. The cuttings were cut into 22 cm long pieces with diameter of the thin edge not less than 0.8 cm, and of the thick edge not more than 1.8 cm. The cuttings were stored in a fridge camera at a temperature 2 °C and relative air humidity 90 %. The planting was carried out in the middle of March. The planting scheme is two-row with 1.85 m between couples of rows and 0.50 m between rows. The distance between cuttings in the row is 0.30 m.

The experimental variants include 3 repetitions, 30 saplings each. The repetitions were developed consequently along the length of the double rows

Water solutions of the herbicides H1 (150 $g.l^{-1}$ Fluazifop-P-butyl), H2 (240 $g.l^{-1}$ Clethodim), H3 (350 $g.l^{-1}$ Butachlor + 350 $g.l^{-1}$ Propanil) and H4 (240 $g.l^{-1}$ Oxyfluorfen) with concentration 5 $ml.l^{-1}$ and control variant were tested. The concentration is selected according to the directions of the producers and the maximal high value among the recommended ones was applied.

Treatments were carried out every month in 5 terms: from May to September. The moment of spraying is in the beginning of each month. Within the same terms, the heights of saplings from control variants were measured, and final measurements were carried out in the beginning of November after the end of the growing season.

The estimation of the effect of the herbicides was carried out according to the indices: survival; rate of growth of saplings; height and diameter of the stem; thickness and number of first grade roots and relative moisture of wood of poplar saplings.

For the determination of the relative moisture of the saplings, 3 saplings with average dimensions from each variant were taken out with their root system after the end of the growing season, cut into pieces and dried at a temperature 105 ±2 °C until they reach constant weight at two consequent accounts.

The statistical analysis of results was carried out with the help of SPSS 11. The mean and standard error were estimated by One-Way ANOVA followed by a post hoc LSD test at $p \leq 0.05$.

Results

All applied herbicides brought to sustainable suppression of the grass vegetation and to experimental variants clean of weeds.

The survival of saplings in both investigated cultivars was synonymous from statistical point of view, within the range from 84 to 100 % (Table 1). Proved lower percentage of survival (62 %) was obtained only for cv. Vernirubens, variant H2.

Table 1. Influence of selective herbicides on the survival of the saplings.

Variant/ herbicide	Survival, %	
	'I-214'	'Vernirubens'
Control	86.67 ±3.84 b	94.45 ±2.22 b
H1	100.00 ±0.00 b	87.78 ±6.19 b
H2	84.44 ±7.28 b	62.22 ±14.19 a
H3	100.00 ±0.00 b	97.78 ±1.11 b
H4	94.43 ±5.56 b	92.22 ±4.01 b

Note: The mean and standard error within a column followed by the same letter were not significantly different.

As early as the initial treatment, the two cultivars reacted with various growth in height depending on the applied herbicides. For cv. I-214 (Table 2) with biggest height growth, statistically different from all other variants, were the saplings from the control – average 229 cm. Best effect on the growth in the treated variants was obtained applying H3, when the average height of the saplings reached 205 cm.

Table 2. Influence of selective herbicides on the growth of cv. I-214.

Variant / herbicide	Stem height, cm	Thickness of saplings in the basis of the stem, mm	Thickness of roots, mm	Av. number of roots 1-st grade
Control	229.16 ±8.12 g	21.45 ±0.67 f	4.30 ±0.17 e	29.11 ±2.52 d
H1	161.01 ±5.60 d	18.18 ±0.63 e	2.98 ±0.19 b	19.50 ±2.41 bc
H2	99.04 ±3.88 b	12.80 ±0.38 b	2.53 ±0.13 ab	16.22 ±1.99 b
H3	205.43 ±6.51 f	20.12 ±0.66 f	3.68 ±0.18 cd	24.22 ±2.49 cd
H4	173.78 ±5.07 de	15.71 ±0.45 d	2.21 ±0.14 a	21.33 ±1.98 bc

Note: The mean and standard error within a column followed by the same letter were not significantly different.

At cv. Vernirubens, however, saplings treated with H3 reached bigger average height – 179 cm compared to the control – 146 cm (Table 3).

Herbicides H1 and H4 showed identical effect on the height growth for both cultivars. Slowing in height growth for both cultivars was caused by the herbicide H2, and saplings remained with heights 99 cm ('I-214') and 55 cm ('Vernirubens').

Table 3. Influence of selective herbicides on the growth of cv. Vernirubens.

Variant / herbicide	Stem height, cm	Thickness of saplings in the basis of the stem, mm	Thickness of roots, mm	Av. number of roots 1-st grade
Control	146.02 ±5.47 c	18.25 ±0.51 e	4.08 ±0.19 de	23.78 ±0.72cd
H1	113.64 ±4.92 b	13.99 ±0.46 b	2.68 ±0.14 ab	16.89 ±2.37 b
H2	54.90 ±2.77 a	8.41 ±0.31 a	2.10 ±0.14 a	7.00 ±1.08 a
H3	178.67 ±5.59 e	20.00 ±0.49 e	3.57 ±0.16 c	63.67 ±0.78 e
H4	107.86 ±3.93 b	11.73 ±0.31 b	2.28 ±0.11 a	22.56 ±1.59 c

Note: The means and standard error within a column followed by the same letter were not significantly different.

View of representative saplings of the variants at cv. I-214 and cv. Vernirubens is presented in Fig. 1.

The criterion thickness in the stem basis showed that for both tested cultivars the herbicide H3 does not exhibit oppressing effect. The cultivar 'I-214' forms thicker stems – from 20 mm (H3) to 22 mm (control). At cv. Vernirubens the stem diameter is within the frame from

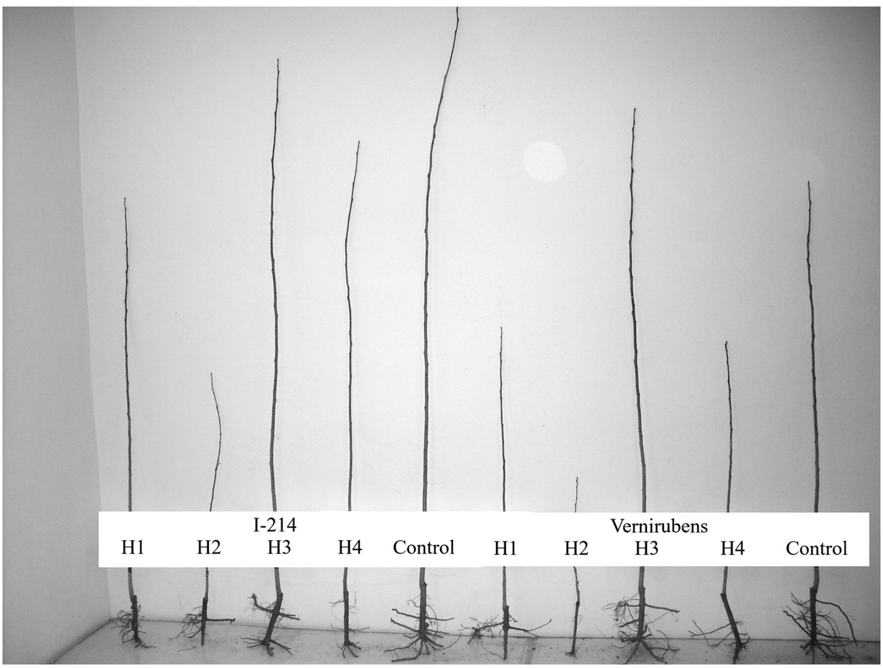

Fig. 1. Representative saplings of the variants at cv. I-214 and cv. Vernirubens.

18 mm (control) to 20 mm (H3).

The herbicide H2 oppressed most strongly the growth in thickness of the saplings in both cultivars, and the herbicide H3 oppresses least the growing of roots in thickness and showed identical effect on both cultivars.

The development of the roots system is related not only to the thickness but to the number of formed roots, as well. With highest number of roots at cv. I-214 were the saplings from variants H3 and control – average number of roots from 24 to 29.

At cv. Vernirubens most powerful root system was developed at variant H3 – average 64 roots, followed by variants H4 and control (23 and 24, respectively). Poorest root system was observed at the saplings treated with Select – average 7.

The results from the water content of tissues showed that from all tested variants increased value is available for variant H2, and the relative moisture is from 65 % to 68 %. For all other variants it is within the frame from 48 % to 54 % (Table 4).

Table 4. Influence of selective herbicides on the relative moisture of saplings.

Variant / herbicide	Relative moisture, %	
	'I-214'	'Vernirubens'
Control	50.50 ± .04 b	48.59 ±3.73 b
H1	48.20 ±0.88 b	53.11 ±0.98 b
H2	64.87 ±1.07 a	67.56 ±4.68 a
H3	53.33 ±1.42 b	53.58 ±1.56 b
H4	48.25 ±3.74 b	53.14 ±3.41 b

Note: The means and standard error within a column followed by the same letter were not significantly different.

The statistical analysis of results (Table 5) proved the influence of the two tested factors – cultivar and herbicide – according to growth indices. Only moisture of saplings was influenced by applied herbicide only.

Table 5. Statistical significance of factors influencing on the development of saplings.

Factors	Statistical significance of factors influencing on:											
	Thickness of saplings in the root cervix		Growth in stem height		Number of roots 1-st grade		Survival of saplings		Thickness of roots of 1-st grade		Moisture of saplings	
	F	Sg	F	Sg	F	Sg	F	Sg	F	Sg	F	Sg
Cultivar	85.23	0.000	229.20	0.000	15.13	0.000	6.16	0.013	6.16	0.013	1.47	0.228
Herbicide	108.50	0.000	130.20	0.000	82.12	0.000	50.86	0.000	50.86	0.000	4.42	0.003

Note: F – Fisher's exact test; Sg – significant level.

Conclusion

The obtained results outlined the following conclusions and recommendations:

Tested herbicides do not influence on the survival of saplings, except for negative effect on cv. Vernirubens, caused by the herbicide H2 (240 g.l^{-1} Clethodim).

The cv. I-214 saplings show faster height growth compared to cv. Vernirubens for all variants.

Tested cultivars show specific reaction to used herbicides and best tolerance to the herbicide H3 (350 g.l^{-1} Butachlor + 350 g.l^{-1} Propanil). The specific reaction is expressed in the fact that cv. I-214 shows less tolerance to influence of herbicides than cv. Vernirubens.

Herbicides H1 (150 g.l^{-1} Fluazifop-P-butyl) and H4 (240 g.l^{-1} Oxyfluorfen) oppress less the saplings growth for both cultivars compared to the outlined depression effect of the herbicide H2 (240 g.l^{-1} Clethodim).

The effect of the tested herbicides showed that it cannot be counted only on the instructions of the producer for selectivity. The herbicide H4 (240 g.l^{-1} Oxyfluorfen), oppressing dicotyledonous plants, brought to comparatively good growth indices of the saplings. The herbicide H2 (240 g.l^{-1} Clethodim), oppressing monocotyledonous plants, caused slowing in the growth and phytotoxic reaction of the saplings.

In the course of production, the best for weed control is to be carried out through the herbicide H3 (350 g.l^{-1} Butachlor + 350 g.l^{-1} Propanil), which can be applied during all phoenophases in the development of saplings.

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FEATURES OF BREEDING NEW WILLOW VARIETIES FOR CREATION OF ENERGY PLANTATIONS

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Abstract

An effective way to solve the problem of becoming energy more expensive can be found in the use of organic mass of growing woody plants, which are obtained from special energy plantations. Certain positive changes in energy policy in Ukraine in recent years have contributed to a marked increase in the percentage of alternative renewable energy in the energy balance of the country – from 0.5 % of the amount of consumed fuel and energy resources in 1998 to 5.8 % at present. In this paper we evaluated the possibility of obtaining new high-yield willow hybrids based on domestic and foreign cultivars for the creation of energy plantations in the conditions of Polesia and the Forest-Steppe of Ukraine. Breeding works were carried out as interspecific hybridization. Hybridization was conducted in spring 2018 on the cut branches in the laboratory and on branches inseparable from the bush, on which before the flowering female inflorescences was isolated to prevent uncontrolled pollination. The best of the two-year-old hybrid willow forms we have obtained in the course of our research reach a height of 3.0–3.2 m, which indicates their high biological potential and the potential for use in creating energy plantations.

Key words: cultivar, hybrids, interspecific crosses, plant biometric indicators, renewable resource, *Salix*, selection.

Introduction

Energy is becoming more and more expensive every year, and its quantity is constantly decreasing, so there is a need to ensure energy security of Ukraine, which leads to the search for alternative sources of raw materials for energy needs (Afonin and Fuchylo 2012a, 2012b; Geletukha 2015; Fuchylo and Sbytna 2017).

Representatives of willow species (*Salix* L.) are gaining popularity in Europe (Liberg 1994, El Bassam 2010, Fuchylo 2011, Caslin et al. 2012), the north of the US, and Canada as one of the promising biomass crops for use in bioenergy, biofuels and high value products in the chemical industry (Heller et al. 2003).

The main advantages of this culture are: genetic diversity for breeding and reproduction; short rotational growing period; high biomass capacity – significant potential for bioenergy generation; positive balance of greenhouse gases; carbon deposition in the aboveground and underground parts of the energy willow (Liberg 1994, Taylor at al. 2019).

Certain positive changes in Ukraine's energy policy in recent years have contributed to a significant increase in the percentage of alternative renewable energy in the country's energy balance – from 0.5 % of fuel consumed in 1998 (Geletukha and Martsenyuk 1998) up to 5.8 % nowadays (Chmeruk 2018).

About 5000 ha of energy plantations have been created in Ukraine now (Fuchylo et al. 2018). These are quite significant indicators, but they are not sufficient for a more complete solution of the problem of replacement of fossil energy by renewable energy raw materials, so it is very important for our country to expand the area of energy plantations and increase their productivity (Roik et al. 2015).

One of the effective ways to ensure high productivity of energy plantations of willow is the development of gene pools of this broad genetic base using Ukrainian and foreign material. The theoretical potential of Ukraine for the cultivation of energy willow and other energy plants is about 5 million ha of marginal land. Technical capacity is about 1.5 million ha. Economic potential is 2 billion USD. But a significant part of these lands belongs either to oligotrophic or oligomesotrophic hydrophytes or *vice versa* to xerophytes and mesoxerophytes, which in turn significantly complicates the creation of energy willow plantations on them. Existing cultivars are not very suitable for such a composition of lands. Expanding the genetic diversity of willows will solve these problems. Therefore, our goal is the selection of new high-yielding and resistant to negative factors varieties for specific soil and climatic conditions, and land categories (Smart et al. 2007, Afonin and Fuchylo 2012b, Fuchylo et al. 2016, Fuchylo et al. 2018).

The purpose of the study is to evaluate the possibility of obtaining new high-performance willow hybrids on the basis of domestic and foreign cultivars for the creation of energy plantations in the conditions of Polissia and Forest-Steppe of Ukraine.

Materials and Methods

Pollen for pollination of female flowers was obtained by germinating branches prepared to open anthers and placed at room temperature (20–22 °C) at a relative humidity of 40–50 %. Under these conditions, the pollen mass matures within 2–3 days. The pollen was shaken from branches onto paper, placed in test tubes, and stored in a refrigerator until crossbreeding. Thus, pollen of male plants of Basket willow (*S. viminalis* L.) varieties 'Ternopilska' and 'Sven' and Fragile willow (*S. fragilis* L.) of the variety 'Adam' (Ukrainian Institute … 2020) were obtained (Melezhyk and Maurer 2016a, 2016b). Table 1 shows list of cultivars selected for research.

Table 1. List of species and cultivars selected.

Order number	Name	Sex	Origin
1	*S. alba* L.	female	Ukraine
2	*S. fragilis* L.	female	Ukraine
3	*S. viminalis* 'Tora'	female	Sweden
4	*S. alba* 'Lisova pisnia'	female	Ukraine
5	*S. viminalis* 'Tordis'	female	Sweden
6	*S. dasyclados* Wimm.	female	Ukraine
7	*S. viminalis* 'Inger'	female	Sweden
8	*S. fragilis* 'Adam'	male	Ukraine
9	*S. viminalis* 'Ternopilska'	male	Ukraine
10	*S. viminalis* 'Sven'	male	Sweden

Hybridizations were conducted in spring 2018 on the cut branches in the laboratory and on branches not separated from the bush, which before the flowering was isolated female inflorescences to prevent uncontrolled pollination.

White willow (*S. alba* L.), Wool-twig willow (*S. dasyclados* Wimm.), Fragile willow (*S. fragilis* L.), three varieties of Swedish breeding on the basis of the basket willow – 'Tora', 'Tordis' and 'Inger' and a hybrid of White and Fragile willow 'Lisova pisnia' were used as female plants (Melezhyk and Maurer 2018).

The time of pollination of female flowers was determined by the condition of the pestles. Pollination of pre-isolated inflorescences was carried out immediately before the beginning of their bloom, when the blades of the uterus receptacles have acquired their inherent size, shape and colour. The pollination was carried out with a soft brush, which was pollen collected and evenly distributed over the inflorescence and immediately isolated with paper bags. When the seeds ripen, the branches with insulators were cut. The seeds were separated by grinding in a sieve with a hole diameter of 2 mm and immediately sown in plastic containers on a well-moistened soil-sand mixture. Within 1–2 h after sowing, the seeds have turned bright green – through the thin shell, chlorophyll-bearing seedlings can be seen. The seedlings appeared in 24–36 h. When the plants reached stages 3–4 of true leaves (mid-June), they were transplanted into larger-capacity plastic containers where they were gradually adapted to grow outdoors. By the end of the first growing season, 70 % of the planted seedlings died, despite the fact that the automatic irrigation system was constantly working and shading of the area was created. Thus, we have obtained individuals of ten hybrid combinations, among which selected plants with intensive growth, resistant to disease and pests and adapted to local climatic conditions. According to the results of wintering, plants that were well tolerated in the unfavourable winter period were selected (Gorielov et al. 2014).

The selected plants were planted in open soil according to the scheme 0.5×0.7 m in November 2018. During the growing season, 4 manual soil inspections were carried out, which contributed to the complete destruction of weeds and intensive growth of seedlings.

Periodic determinations of the height and diameter of hybrid plants and the processing of the obtained results were carried out according to conventional methods (Dospekhov 1973, Fuchylo et al. 2018).

Results

Studies have shown that after the end of the first growing season of almost 200 obtained hybrids (100 %) remained 69 (34.5 %). Their height ranged from 2.4 to 48.5 cm, and the diameter near the root neck – from 1.0 to 4.0 mm. Among the offspring of hybrids where three or more plants were preserved, the highest average height had *S. viminalis* hybrids. 'Tordis' × *S. viminalis* 'Ternopilska' – 39.0 cm and *S. viminalis* 'Tordis' × free pollination – 28.0 cm. Their average diameters were 3.1 and 2.2 mm, respectively. Table 2 shows morphometric characteristics of hybrid willow plants before planting in open ground and at the end of the second growing season.

After two years of research, it was found that the resulting two-year hybrids have a height of 120.0 to 320.0 cm.

Table 2. Morphometric characteristics of hybrids before planting in open ground.

Order number	Hybrid combination (female × male)	2018				2019			
		Height, cm		Root neck diameter, mm		Height, cm		Root neck diameter, mm	
		M	m	M	m	M	m	M	m
1	*S. alba* L. × *S. fragilis* 'Adam'	16.0	0.24	3.0	0.10	320.0	12.00	16.8	0.96
2	*S. dasyclados* Wimm.× *S. fragilis* 'Adam'	16.5	-	3.1	-	320.0	-	16.0	-
3	*S. viminalis* 'Inger' × *S. viminalis* 'Ternopilska'	48.5	4.35	3.9	0.36	280.0	21.00	13.2	1.24
4	*S. fragilis* L. × *S. viminalis* 'Ternopilska'	18.0	0.80	3.6	0.14	240.0	14.00	12.4	1.00
5	*S. viminalis* 'Tora' × *S. viminalis* 'Ternopilska'	22.0	1.20	3.3	0.22	310.0	24.00	14.2	1.15
6	*S. viminalis* 'Tora' × *S. viminalis* 'Sven'	24.0	0.65	2.4	0.10	275.0	13.00	13.8	0.94
7	*S. viminalis* 'Tordis' × *S. viminalis* 'Ternopilska'	39.0	2.20	3.1	0.12	215.0	15.00	12.0	1.06
8	*S. alba* 'Lisova pisnia' × free pollination	22.0	1.25	3.8	0.24	320.0	21.00	16.0	1.12
9	*S. alba* 'Lisova pisnia' × *S. fragilis* 'Adam'	22.0	1.30	4.0	0.26	240.0	18.50	13.2	1.14
10	*S. viminalis* 'Tordis' × free pollination	28.0	0.60	2.2	0.10	315.0	19.00	16.0	1.10
	Average value	25.6	1.38	3.2	0.18	283.5	17.50	14.4	1.08

Note: M – mean value, m – mean value error.

The best among the studied hybrids had a height of 320.0 cm *S. alba* × *S. fragilis* 'Adam' (hybrid 1/16), *S. dasyclados* × *S. fragilis* 'Adam' (hybrid 2/2) and *S. alba* 'Lisova pisnia' × free pollination hybrid 4/9), 315.0 cm – *S. viminalis* 'Tordis' × free pollination (hybrid 5/3), height 310.0 cm small *S. alba* × *S. fragilis* 'Adam' (hybrid 1/13) and *S. viminalis* 'Tora' × *S. viminalis* 'Ternopilska' (hybrid 3.7).

These promising plants will be cloned and their vegetative offspring will be tested in test cultures with the prospect of obtaining clone variety status.

The study of the growth characteristics of the obtained hybrids during the second growing season showed that the intensity of increasing their height reaches maximum values in the period from mid-June to mid-August (Fig. 1).

The diameter near the root collar during the second year of vegetation increased almost similar to the height, but the intensity of this process was higher in most hybrids in the first half of summer (Fig. 2). This applies in particular to almost all hybrids that are 300 cm or more in height.

The special interest is presented by landings of fast-growing willow, that require the selections of sorts which countries adapted to the ground-climatic terms. This is considered on the basis of the presence of large areas of unoccupied marginal land (more than 5 million ha), as well as the high level of Ukraine's poten-

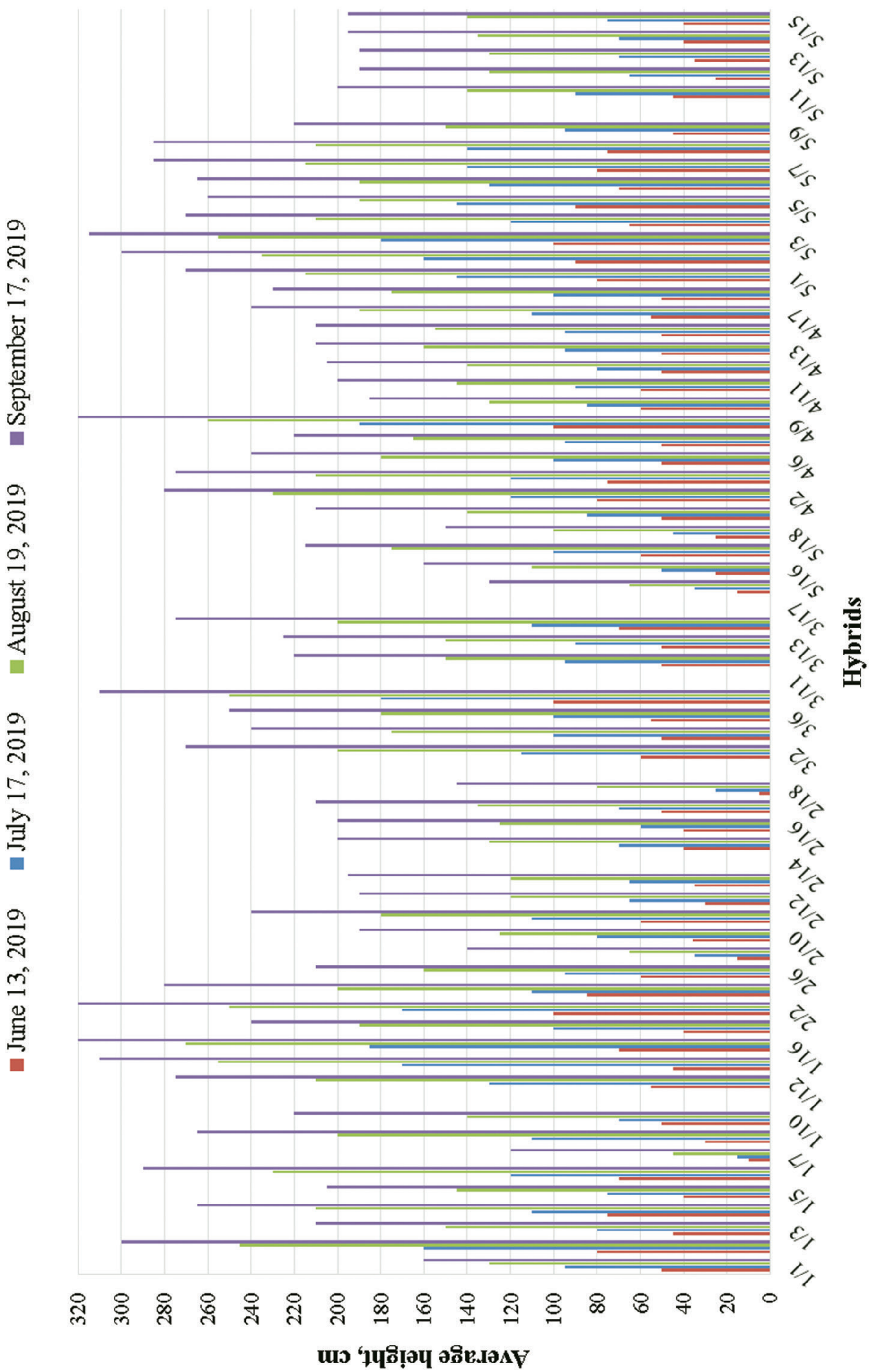

Fig. 1. Growth in height during the second growing season.

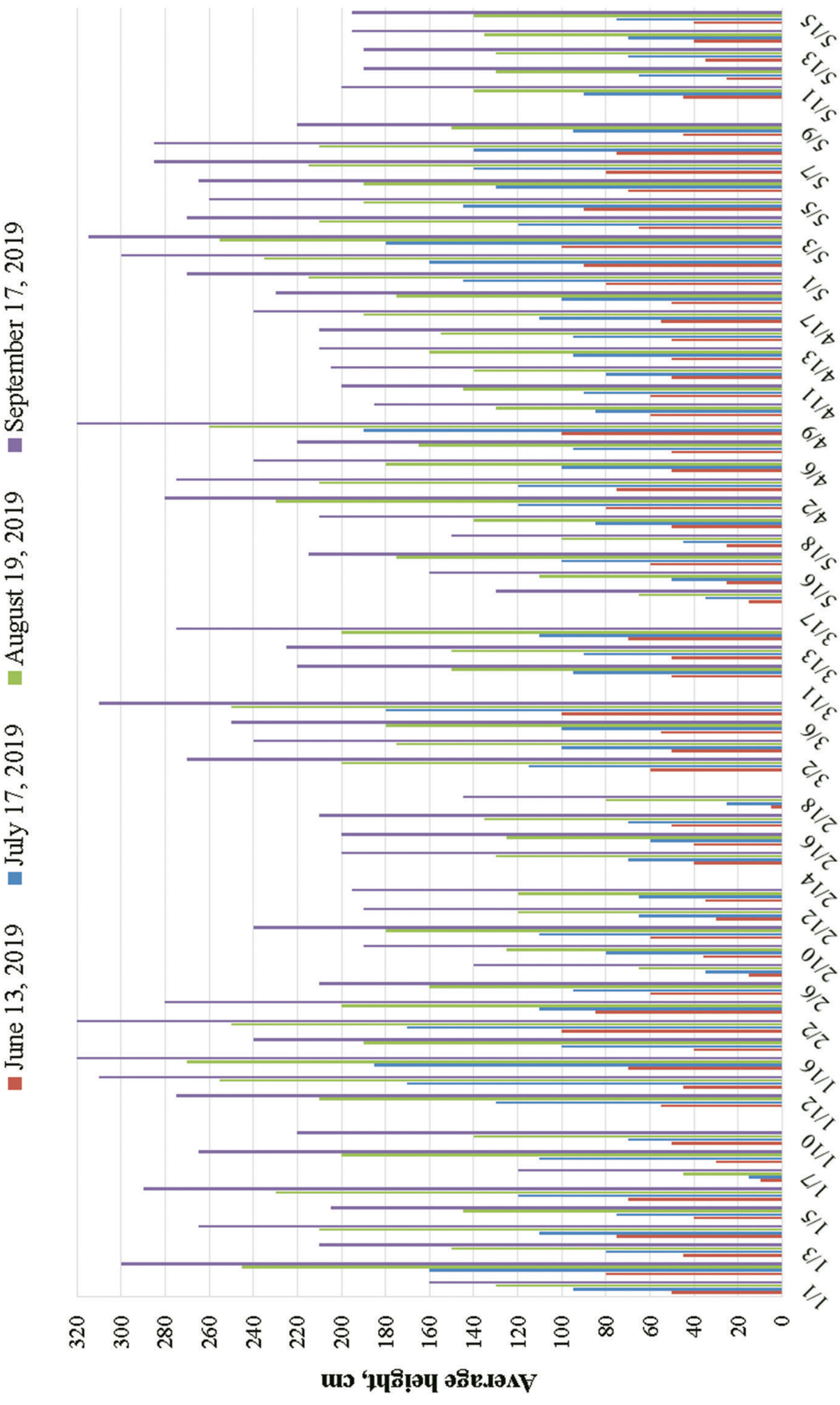

Fig. 2. Growth in stem diameter near the root collar during the second growing season.

tial for growing energy crops.

So, hybridization of the most productive willow varieties with native species will allow to receive cultivars that will yield high biomass yields and have a high growth rate while maintaining resistance to specific growing conditions. By bringing willow varieties to different regions of Ukraine, we will be able to significantly accelerate the growth of energy plantations throughout the territory, not just in some areas.

Discussion

Cross-species willow hybridization to bring new emerging cultivars is widely used in leading countries in the world. With state support, in 1990, a breeding program for the fast-growing willow at the Long Ashton Research station in the UK, based on a collection created by Kenneth Stott, replaced by Kevin Lindegard, started. In 1997 Svalof Weibull Willow Cultivation Industrial Breeding Program began in Sweden, under the direction of Stig Larson (Karp et al. 2011, Sharma et al. 2017).

The breeding of new varieties of willow in North America was initiated at the University of Toronto which research focused on local willow species. Beginning in 1983, it was the first attempt at extensive crossing with willow species from US collections, which provided important information and the ability to obtain interspecific hybrids. At present, SUNY College of Environmental Science and Forestry is developing new willow hybrids.

According to the book ‘Genetic Improvement of Bioenergy Crops (Vermerris 2008), the most productive and commercially successful willow cultivars can be distinguished. Table 3 shows list of willow hybrids as commercial bioenergy crops.

Table 3. Willow hybrids bred and selected as commercial bioenergy crops.

Breeding country	Cultivars	Parental species and hybrids
Sweden Svalof Weibull	′Orm′, ′Jorr′, ′Jorunn′	*S. viminalis*
	′Tora′, ′Bjorn′	*S. schwerinni* × S. *viminalis*
	′Torchild′, ′Tordis′	(*S. schwerinni* × *S. viminalis*) × *S. viminalis*
	′Sven′, ′Olof′, ′Asgerd′	*S. viminalis* × (*S. schwerinni* × *S. viminalis*)
	′Loden′, ′Gubrun′	*S. dasyclados*
	′Inger′	*S. trianda* × *S. viminalis*
Great Britain	′Endeavour′	*S. viminalis* × *S. schwerinnii*
	′Discovery′	*S. viminalis* × (*S. schwerinni* × *S. viminalis*)
	′Resolution′	(*S. viminalis* × (*S. schwerinni* × *S. viminalis*)) × (*S. viminalis* × (*S. schwerinni* × *S. viminalis*))
	′Tera Nova′	(*S. trianda* × *S. viminalis*) × *S. linderstipularis*
	′Ashton Stott′	*S. viminalis* × *S. burjatica*
	′Nimrod′	(*S. schwerinni* × *S. viminalis*) × *S. linderstipularis*
	′Quest′	*S. viminalis* × (*S. schwerinni* × *S. viminalis*)
USA SUNY-ESF	′Fish Creek′, ′Onondaga′, ′Alle-gany′	*S. purpurea*
	′Millbrook′, ′Oneida′	*S. purpurea* × *S. miyabeana*
	′Sherburne′, ′Canastota′	*S. sachalinensis* × *S. miyabeana*
	′Otisco′, ′Tully Champion′, ′Owasco′	*S. viminalis* × *S. miyabeana*

Having analyzed the experience of international and domestic experts with our results, we have concluded that during breeding of new fast-growing willow cultivars, hybrids from crossing native species such as white and three-willow with the most productive varieties of foreign and domestic breeding are the most productive.

Conclusion

The use of hybridization in the process of obtaining highly productive and sustainable forms of willow has a great potential, in particular – to improve the productivity of energy plantations. The best of the two-year-old hybrid willow forms we have obtained in the course of our research reach a height of 3.0–3.2 m, which indicates their high biological potential and potential for use in the creation of energy plantations. After further research, the best of them will be submitted for registration in the National Register of Plants. According to the results obtained, it is advisable to use white, woolly, willow, 'Lisova pisnia' hybrid, Swedish breed 'Tora' and 'Tordis' to obtain growing hybrids of willow during crosses as female plants. As pollinators, the varieties of *S. viminalis* 'Ternopilska' and the cultivar of our breeding *S. fragilis* 'Adam' have positively proved themselves.

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THE GROUND BEETLES (COLEOPTERA: CARABIDAE) FROM A SIGNIFICANT, BUT POORLY STUDIED REGION IN NW BULGARIA. PART 2: ECOLOGY

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Abstract

This is the second part of the study representing the first overall assessment of the ground beetle fauna in the region of the Zlatiya plateau. The study aimed at analyzing the ecological structure of the carabid fauna represented by 6598 adult carabid specimens, belonging to 138 species, 49 genera and 20 tribes. The dominant structure was characteristic with the presence of 2 eudominants numbering 26 % of all specimens (*Harpalus rufipes* and *Harpalus tardus*), 4 dominants (26 %), 1 subdominant (3 %), 17 recedents (27 %) and 114 subrecedents (18 %). The analysis of the life forms showed only a slight predominance of the zoophages (71 species; 52 %) over the mixophytophages (67 species; 48 %). Similar ratio (50: 50 %) is mostly approaching to the typical for the steppe zones, for the orchards from the forest-steppe zones, and for the vast deforested territories across Europe. Humidity preferences analysis showed the larger share of the mesoxerophilous carabids. The prevalence of the macropterous carabids reflected their higher mobility and adaptiveness.

Key words: carabid communities, dominance structure, life forms, wing morphology, Zlatiya.

Introduction

Ground beetles (Coleoptera: Carabidae) represent one of the largest beetle families with cosmopolitan distribution and with decisive importance for the functioning of ecosystems. The high taxonomic richness, the large numbers and the diverse life specializations are the reasons they cover the entire environmental spectrum of fundamental natural gradients. These substantive arguments lie at the base of the possibility ground beetles and their communities to be widely used as bioindicators of terrestrial environment in the system of biological monitoring (Desender and Baert 1995, Luff 1996, Cranston and Trueman 1997, Rainio and Niemelä 2003, Pearsall 2007, Rainio 2009). Their agronomic significance together with the high diversity and well-defined ecological niches, explain their common use in ecological analyses, habitat quality evaluation and studies of ecosystem succession (Desender et al. 1994, Luff 1996, Rainio and Niemela 2003, Pearce and Venier 2006, Pearsall 2007, Ludwiczak et al. 2020) or anthropogenic impact (Paoletti and Bressan 1996, Avgin and Luff 2010, Langraf et al. 2016).

Carabids and their communities are excellent model for ecological and conservation research (Langraf et al. 2016). Many studies have been conducting with regards of different ecological and morphological characteristics of carabids. Ecological parameters of their communities can be used in assessing the state of the environment and predicting the trends in the future development of the ecological systems. This way, synecological studies are often used to characterize the influence of the biota on the environment, and the impact of human activity on the functioning, productivity and changes in the ecosystems.

This study aimed at analyzing the ecological structure of the carabid coenoses in relation to main ecological parameters, e.g. dominance structure, species richness, similarity between carabid communities, life form categorization, wing development, and humidity preferences, with a subsequent assessment of the environmental trends and anthropogenic impact in the studied area.

Materials and Methods

The design of the field work and the details about the material, including the full species list with all ecological data compiled, were presented in the first part of the research (Teofilova and Kodzhabashev 2020b). We used 97 pitfall traps, set in 8 different by vision and structure territories. In each sampling site, 12 traps were set, only in site VI the traps were 13 (Table 1). The entire period of sampling was 236 days. Sampling sites II, III, IV, and V were studied during the whole period. Sampling sites VI, VII and VIII were studied for 188

Table 1. Sampling effort and activity density of carabids in the studied habitats, and share of the species found in only one habitat (further called 'specific' or 'unique').

	Sampling site	Number of traps		Trap-days		Specimens		No ex./ 100 trap-days	Species	
		Set	Collected [sampling period]	Total number	% realised	Number	Share, %		Number	Unique sp./site
I	Harvested, not plowed wheat field	12	9 [1]; 2 [2]	606	37.0	182	3.0	30.0	24	4
II	Micro dam, shore	12	8 [1]; 9 [2]; 7 [3]	1874	69.0	637	10.0	34.0	70	22
III	Micro dam, pseudo-steppe-like habitat	12	10 [1]; 9 [2]; 9 [3]	2172	77.0	944	14.0	43.5	51	5
IV	Ogosta River shore	12	12 [1]; 9 [2]; 6 [3]	1965	69.0	1641	25.0	83.5	58	5
V	Mesophilic oak forest	12	7 [1]; 7 [2]; 11 [3]	2056	73.0	1413	21.0	68.7	38	3
VI	Loess steppe-like habitat near Shishmanov Val Dam	13	13 [2]; 12 [3]	2343	96.0	1186	18.0	50.6	53	13
VII	Loess steppe-like habitat near the Danube River	12	3 [2]; 9 [3]	1170	52.0	313	5.0	26.7	37	1
VIII	Grazed pasture with bushes	12	10 [2]; 11 [3]	1981	88.0	282	4.0	14.2	33	0
	Total	97		14167	71	6598	100	46.6	138	53

days (two collecting periods), and sampling site I was studied only for 135 days (two collecting periods). The material was collected thrice, the first sampling period (spring) [1] being of 48 days, the second (summer) [2] of 87 days, and the third (autumn) [3] – 101 days. Four of the sites were sampled twice, which was considered in calculating the activity density of carabids. 'Activity density' is the number of specimens per 100 trap-days during the trapping period.

This part of the research encompassed some ecological characteristics of the carabid communities.

In order to determine the dominance structure, the relative abundance (or degree of dominance) was used: $D = (n_i/N)\cdot 100$, where n_i is the number of individual representatives of each species, and N – their total number. The classical four-level classification of Tischler (1949) for invertebrates, modified by Sharova (1981) with the initiation of a 5th category 'eudominant', was adopted: eudominants (> 10 % of all individuals); dominants (5 to 10 %); subdominants (3 to 5 %); recedents (1 to 3 %); subrecedents (< 1 %). For the assessment of the species with a significance < 1 %, we used the following 5-ball scale, according to calculations based on Pesenko (1982): single species (represented by 1 ex., D < 0.02 %); random (2–7 ex., D = 0.02–0.1 %); sporadic (8–20 ex., D = 0.1–0.3 %); very rare (21–33 ex., D = 0.3–0.5 %); rare species (34–66 ex., D = 0.5–1 %).

Species richness in both studied habitats was calculated using the Margalef's species richness index (Margalef 1958) [$D_{Mg} = (S–1)/\ln N$] and Menhinick's species richness index (Menhinick 1964) [$D_{Mn} = S/\sqrt{N}$], where S is the number of species, and N is the number of specimens.

Categorization of the species in respect of their life forms followed the classification of Sharova (1981). Species were also classified into three groups according to their hind wing development: winged or macropterous (always possessing wings), wing dimorphic/polymorphic (only part of the population being fully winged), and brachypterous (wingless), according to the commonly accepted classification of Den Boer et al. (1980).

According to their ecological requirements in terms of humidity, the established carabid species were divided into six categories (Teofilova 2018a): hygrophilous, mesohygrophilous, mesophilous, mesoxerophilous, xerophilous, and eurybionts.

The data were processed with MS Excel and PRIMER 6 (Clarke and Gorley 2005).

Results

During the study, a total of 6598 adult carabid specimens were captured. Beetles belonged to 138 species, classified into 49 genera and 20 tribes (see Teofilova and Kodzhabashev 2020b). In total, over the entire study period, 14167 trap-days were realised with the exposure of the 97 pitfall traps. This accounted for 71 % of the potentially possible trap-days (Table 1). These sampling results could be considered successful and used for ecological analyses. The only exception was the sampling site I (an arable agricultural area), where collection periods were limited by the agricultural programmes. For this site we considered that the life cycles of its inhabitants were ephemeral, characteristic of similar natural communities in the steppes of Eurasia, as well as for agrocoenoses with a one-year crop cycle and subsequent ploughing for the following year. The sampling success in all other sampling sites was more than 50 % (even > 90 % in site VI), making the biological information suitable for analysis.

Dominance structure and rare species

The dominance structure of the entire carabid complex was characterised by the presence of 2 eudominants (with a total number of 26 % of all caught specimens), 4 dominants (26 %), 1 subdominant (3 %), 17 recedents (27 %) and 114 subrecedents (18 %). The eudominant species were *Harpalus tardus* (Panzer) and *H. rufipes* (De Geer), dominants were *Pterostichus melas* (Creutzer), *Abax carinatus* (Duftschmid), *Calathus fuscipes* (Goeze), and *Brachinus crepitans* (Linnaeus), and the subdominant species was *Ophonus laticollis* Mannerheim (Table 2). The dominance structure showed a presence of seven dominant species (*D* > 3 %) (5 % of all species). Subrecedents (*D* < 1 %) were 83 % of the species and 18 % of the specimens. Together with the recedent species (*D* = 1–3 %) (17 species, 12 %), the total share of the recedent community component made up 95 % of the species. Thus, the ratio of the quantitative significance of the dominant community component to the recedent component was 55 % : 45 %, and the qualitative ratio was 5 % : 95 %.

Twenty-five of the 114 subrecedent species were presented with one individual (18 % of the species and only 0.4 % of specimens) (Table 3). These single species were most numerous in the coastal territory, due to the many extra- and intrazonal phytophilic species. In other habitats, this category was represented by 1–3 species. Most of these species are usually less abundant due to their stenotopic nature.

Table 2. Dominance structure.

Category	Species	No
eudominant	*Harpalus rufipes*, *Harpalus tardus*	2
dominant	*Abax carinatus*, *Brachinus crepitans*, *Calathus fuscipes*, *Pterostichus melas*	4
subdominant	*Ophonus laticollis*	1
recedent	*Amara anthobia*, *A. convexior*, *Anchomenus dorsalis*, *Calathus ambiguus*, *Carabus coriaceus*, *C. ullrichi*, *Harpalus atratus*, *H. caspius*, *H. flavicornis*, *H. rubripes*, *H. subcylindricus*, *Ophonus azureus*, *O. cordatus*, *O. sabulicola*, *Platyderus rufus*, *Stenolophus mixtus*, *Trechus quadristriatus*	17
subrecedent	all the rest	114

Table 3. Subrecedent species (*D* < 1 %) in the different sampling sites.

Category	I		II		III		IV		V		VI		VII		VIII		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Single	2	1.5	12	8.7	3	2.2	2	1.5	3	2.2	2	1.5	1	0.7	-	-	25	18.1
Random	8	5.8	18	13.0	10	7.2	14	10.1	3	2.2	13	9.4	6	4.3	5	3.6	46	33.3
Sporadic	4	2.9	12	8.7	10	7.2	10	7.2	5	3.6	10	7.2	5	3.6	4	2.9	25	18.1
Very rare	2	1.5	2	1.5	3	2.2	4	2.9	2	1.5	4	2.9	5	3.6	4	2.9	10	7.2
Rare	1	0.7	4	2.9	5	3.6	6	4.3	3	2.2	6	4.3	5	3.6	5	3.6	8	5.8
Total	17	12.4	48	34.8	31	22.4	36	26.0	16	11.7	35	25.3	22	15.8	18	13.0	114	82.5

Random species (D < 0.1 %) were 46 (33 % of the carabid fauna and 3 % (183 ex.) of the total catch). They showed a strong presence in the coastal habitats. Sporadic (D = 0.1–0.3 %) were 25 species (18 %), accounting for 5 % (328 ex.) of all specimens. Very rare (D = 0.3–0.5 %) were 10 species (7 %) with 4 % (266 ex.) of all specimens. Eight species were rare, accounting for 6 % of the area's carabid fauna and 27 % (1788 ex) of the total catches.

The communities in sampling sites I, VII, VIII and VI were clearly distinguishing by the number of rare species in them, respectively by their spatial, structural and functional capacity, reflecting the degree of successional development: e.g. arable land – 17 rare species, pasture – 18 species, abandoned pasture after fire – 22 species, and loess steppe formed secondary at the place of an abandoned pasture – 35 species.

Diversity indices and similarity

The α-diversity of carabid communities calculated with Menhinick's and Margalef's indices showed that humid habitat near the lake shore (site II) had the highest species richness. The lowest values were in the climax oak forest (V) and intensive wheat field (I) (Table 4).

Table 4. Species richness of carabid communities in Zlatiya Plateau.

Indicators	Sampling sites							
	I	II	III	IV	V	VI	VII	VIII
Number of specimens, N = 6598	182	637	944	1641	1413	1186	313	282
N, %	3	10	14	25	21	18	5	4
Number of species, S = 138	24	70	51	58	38	53	37	33
S, %	17	51	37	42	27	38	27	24
$D_{Mg} = (S–1)/\ln N$ = 15.6	4.4	10.7	7.3	7.7	5.1	7.4	6.3	5.7
$D_{Mn} = S/\sqrt{N}$ = 1.7	1.8	2.8	1.7	1.4	1.0	1.5	2.1	2.0

According to the taxonomic structure and species abundance, the similarity dendrogram showed that the quantitative significance of the species (based on their abundance) strongly depended on the way of modern land management and the succession processes that took place in the recent past (Fig. 1). Sampling sites with similar microclimatic, edaphic and physiographic conditions (II and III, IV and V) were grouped at a relatively high degree of similarity. These natural habitats were separated, allowing the hypothesis that the quantitative significance of the carabids is indicative of the degree of anthropogenic influence. In this case, the heavily influenced habitats I and VII (eroded slopes and abandoned pastures overgrown with weeds and synbovine ruderals) were grouped as a single couple with a very low degree of similarity. They were clearly separated from those that have become 'loess pseudo-steppes' after the cessation of the intensive grazing (VI, VIII), and from the natural riparian (II, III) and forest (IV, V) habitats with relatively poorly modified biotic parameters of the environment.

The grouping of the habitats by qualitative similarity (species composition of carabids) was analogous to the quantitative, but even more definite (Fig. 2). Clearly distinguishable were the three habitat types according to their anthropogenic impact: arable land (I), loess pseudo-steppes and pastures (VI, VII, VIII), and natural habi-

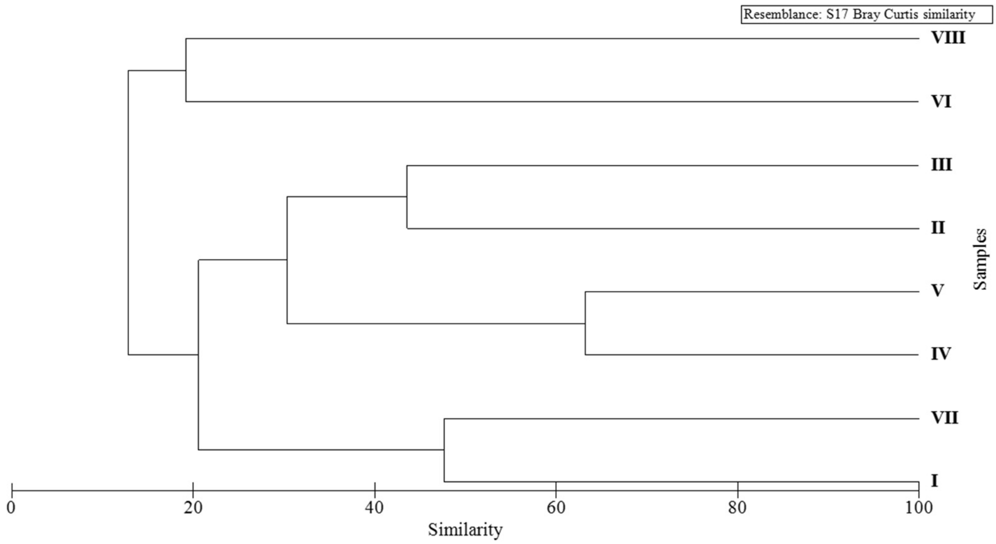

Fig. 1. Dendrogram of the similarity based on quantitative significance.

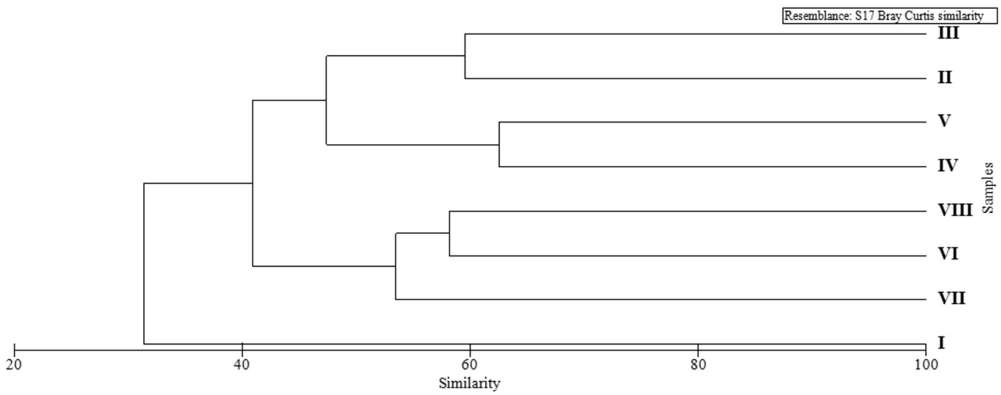

Fig. 2. Dendrogram of the similarity based on qualitative significance.

tats (II and III, IV and V).

Life forms

The 138 ground beetle species belonged to two classes and 18 life form groups proposed by Sharova (1981) – 12 zoophagous and 6 mixophytophagous. The life forms of each species were given in the first part of the study (Teofilova and Kodzhabashev 2020b). The analysis of the life forms occurring in the whole complex of ground beetles showed only a slight predominance of the zoophages (71 species; 52 %) over mixophytophages (67 species; 48 %). In quantitative aspect the ratio was quite different: zoophagous were 3011 ex. (46 %), while mixophytophagous were 3587 ex. (54 %). It was noticeable the significant percentage of the mixophytophagous harpaloid geohortobionts, mainly due to the increased presence of species from the genus *Harpalus* (Table 5).

Table 5. Life forms of the ground beetles from the Zlatiya Plateau.

Life forms		**No sp.**	%	**No ex.**	%
	Class: Zoophagous				
	Life form subclass: 1.1 – Phytobios				
1.1.2	Stem-dwelling hortobionts	1	0.7	1	0.01
1.1.3	Leaf-dwelling dendrohortobionts	2	1.4	3	0.04
	Life form subclass: 1.2 – Epigeobios				
1.2.2	Large walking epigeobionts	5	3.6	316	4.8
1.2.3	Running epigeobionts	2	1.4	13	0.2
1.2.4	Flying epigeobionts	2	1.4	32	0.5
	Life form subclass: 1.3 – Stratobios				
	Series: 1.3(1) – crevice-dwelling stratobionts				
1.3(1).1	Surface & litter-dwelling	16	11.6	327	5.0
1.3(1).2	Litter-dwelling	18	13.0	1038	15.7
1.3(1).3	Litter & crevice-dwelling	11	8.0	498	7.5
1.3(1).4	Endogeobionts	1	0.7	1	0.01
1.3(1).6	Bothrobionts	1	0.7	24	0.4
	Series: 1.3(2) – digging stratobionts				
1.3(2).1	Litter & soil-dwelling	11	8.0	755	11.4
	Life form subclass: 1.4 – Geobionts				
1.4.2(1)	Small digging geobionts	1	0.7	3	0.04
	Zoophagous total	**71**	**52.0**	**3011**	**45.6**
	Class Mixophytophagous				
	Life form subclass: 2.1 – Stratobios				
2.1.1	Crevice-dwelling stratobionts	7	5.1	202	3.1
	Life form subclass: 2.2 – Stratohortobios				
2.2.1	Stratohortobionts	18	13.0	1471	22.3
	Life form subclass: 2.3 – Geohortobios				
2.3.1	Harpaloid geohortobionts	33	23.9	1812	27.5
2.3.1(1)	Crevice-dwelling harpaloid geohortobionts	1	0.7	1	0.01
2.3.2	Zabroid geohortobionts	5	3.6	67	1.0
2.3.3	Dytomeoid geohortobionts	3	2.1	34	0.5
	Mixophytophagous total	**67**	**48.0**	**3587**	**54.4**

Note: the first figure in the index shows the class of life form, the second shows the subclass, and the third indicates the life form group; the figure in brackets after the subclass shows the series, if any.

When examining in detail the structure of the life forms, the significance of three subclasses was emphasised. Of the class Zoophagous, the most significant was the subclass Startobios comprising 58 species (42 % of all species) and 1888 ex. (29 % of all specimens), and of the class Mixophytophagous – the subclass Geohortobios with 42 species (30 %) and 1914 ex. (29 %), as well as the subclass Stratohortobios with 18 species (13 %) and 1471 ex. (22 %).

The most numerous life form groups were the harpaloid geohortobionts (33 species; 24 %) and the stratohortobionts (18 species; 13 %) from class Mixophytophaga, and the litter-dwelling stratobionts (also 18 species; 13 %) and the surface & litter-dwelling stratobionts (16 species; 12 %) from class Zoophaga (Table 5).

Wing morphology

The degree of hind wing development allowed distinguishing of three groups of carabids: brachypterous (hind wings shorter than elytra or missing), macropterous (winged), and dimorphic (some individuals have fully developed wings, others only vestigial ones). For one species in our study (1 %) there were no data about its wing morphology. Macropterous beetles represented 69 % (95 species) of all collected carabid species. Pteridimorphic species were 22 % of all (31 species), and brachypterous were only 8 % (11 species) (Fig. 3).

Humidity preferences

Analysis of the humidity preferences (Fig. 4) of the ground beetles showed the prevalence of the mesoxerophilous carabids (50 species, 36 % of all established species). Mesophilous were 27 species (20 %). Less represented were mesohygrophilous (17 species), xerophilous (15 species), and hygrophilous (14 species) carabids.

Discussion

The established average activity density for the entire study period, with 6598 specimens collected over a period of 14167 trap-days, was 46.57 specimens per 100 days, meaning a relatively high activity density averaged for Bulgaria (Teofilova 2013, Kodzhabashev 2016). The large range of variation of the average activity density of the areas studied was remarkable. The minimum value of 14.24 ex./100 trap-days was recorded in the intensively grazed pasture, where the regular removal of plant biomass was probably an extremely important factor in the energy capacity of the habitat, leading to a limitation of the density of carabids in general. Such low values of activity density have the arable lands, recently abandoned pastures or

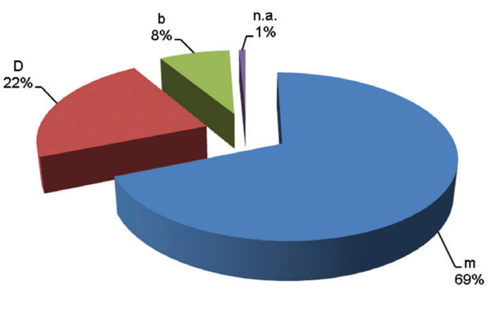

Fig. 3. Wing morphology of carabid species in Zlatiya plateau.

Note: m – macropterous, D – wing di(poly) morphic, b – brachypterous, n.a. – no data.

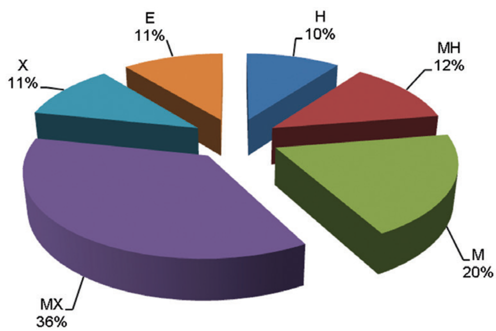

Fig. 4. Humidity preferences (number of species) of the carabids.

Note: H – hygrophilous, MH – mesohygrophilous, M – mesophilous, MX – mesoxerophilous, X – xerophilous, E – eurybiont.

synanthropic habitats, where, depending on the strength and duration of human impacts, a trend in biocoenoses degradation is observed (Aidamirova 2011, Sukhodolskaya et al. 2020).

Especially high were the values of activity density (respectively, of ecosystem productivity) in the forest and riparian habitats (83.51 and 68.73, respectively), which can be explained with their climax state. The activity density values usually correspond proportionally with the numbers of the specimens caught, when the total percentage of trap-days realised was more than 50 % and the samplings were carried out throughout the study period.

According to the results obtained about the activity density, the number of specimens and species collected, and the species unique to each territory, we may divide the habitats into two categories: 1) natural habitats with a natural course of ecological development, in or near a climax state, and 2) synanthropic habitats with varying degrees of influence depending on the purpose and manner of the land use. Forest and riparian habitats, in addition to their high activity density, had a rich and stable species composition and a relatively large number of specific species. In the synanthropic habitats, we found the significant differences in their ecological characteristics to be typical. Relatively low activity density, poor fauna, and lack or limited number of specific species were found in the wheat field (I), followed by the intensively grazed pasture (VIII), and the regularly burnt grassland on the Danube coast (VII). The exception was the abandoned pasture on the shore of the Shishmanov Val Dam (VI), where processes of advanced secondary succession were observed, consisting in relatively high activity density, rich species composition and presence of a large number of specific species. This was probably due to the relatively long period of lack of anthropogenic intervention and subsequent successional processes under the specific conditions of the changed environment. The absence of conditions for the development of woody vegetation due to erosion and degradation processes, given the strong slope and subsequent pasture exploitation after the deforestation, were the reason for the establishment of perennial xerophilic grass formations, creating an environment and conditions for the development of a specific carabid coenose representing a complex of heterogeneous in origin, distribution, taxonomic and ecological affiliation species.

The analysis of the dominance structure showed the presence of eudominant species, which seems typical for anthropogenically influenced and unsustainable ecosystems, and was also established by Kodzhabashev and Mollov (2000), Kostova (2004), and Teofilova (2015). There is a proportionate imbalance and a distorted ecological structure lacking evenness between the species. The relative share of species with significance less than 1 % of total catches was 114 species (83 %), and that of dominant species (*D* > 3 %) was only 7 species (5 %). Similar ratio was established in the region of Cape Emine, where dominant species (*D* > 3 %) were 7 %, and the sudrecedends (*D* < 1 %) were 82 % of the species (Teofilova 2015).

Rare species (*D* < 1 %) were represented by 1177 specimens (20 %), and the dominant species (*D* > 3 %) had 3615 specimens (55 %). The ratio in the region of Cape Emine was similar, i.e. 15 % and 60 %, respectively (Teofilova 2015). Such results seem characteristic of plain territories with developed agriculture, where arable land covers all suitable terrains, and natural and semi-natural habitats are severely limited to small patches preserved mainly along the steep gullies and the banks of rivers and standing water bodies. For the carabid fauna, these habitats act as local refugia of extremely high conservation importance for the survival of most species.

The quantitative significance was 26 % for the two eudominants (*Harpalus tardus* and *H. rufipes*), 26 % for the four dominants (*Pt. melas*, *A. carinatus*, *C. fuscipes*, and *Br. crepitans*), and 3 % for the subdominant species *O. laticollis*. The two eudominants in the region of Cape Emine (*Carabus coriaceus* Linnaeus and *Chlaenius nitidulus* (Schrank) had similar abundance – 35 % of all specimens (Teofilova 2015). *Harpalus rufipes* is a widespread Palaearctic, ecologically plastic eurybiont. It is dominant in most researches with pitfall traps (Popov and Krusteva 1988, Shishiniova et al. 2001, Aidamirova 2011, Kodzhabashev 2016, Trushitsyna et al. 2016, Putchkov et al. 2020, Sukhodolskaya et al. 2020). In agrocoenoses it can reach very high densities, especially if it lacks competition due to intensive chemical agriculture. This species is registered in all sampling sites, having relatively low density and numbers only in pastures and dry pseudo-steppes. The second eudominant, *H. tardus*, is eurytopic with preferences for shady habitats. In studies of the carabid coenoses of Dobrudzha this species was dominant in forest habitats (Penev 1993, Kodzhabashev 2016). Its absence in the arable territories is inexplicable, given its great ecological plasticity and its occurrence in other anthropogenised areas of the Danube Plain (Kodzhabahsev 2016). Two of the other dominants (*C. fuscipes* and *Br. crepitans*) are eurytopic in open habitats, and have usually high numbers and density in both natural and synanthropic coenoses, including agrocoenoses (Shishiniova et al. 2001, Aleksandrowicz et al. 2009, Aidamirova 2011, Teofilova 2013, Kodzhabashev 2016, Trushitsyna et al. 2016, Putchkov et al. 2020). The other three species, *A. carinatus*, *P. melas* and *O. laticollis*, have high densities in forest habitats. We may consider them as indicators of that type of habitat for the area of the Zlatiya plateau, along with *Nebria brevicollis* (Fabricius), *Leistus rufomarginatus* (Duftschmid), *Notiophilus rufipes* Curtis, *Carabus ullrichi* Germar, *Myas chalybaeus* (Palliardi), *Platyderus rufus* (Duftschmid), *Amara saphyrea* Dejean. As quantitative indicators for the secondary steppe and agricultural habitats in the Western Danube Plain, we could classify *Calathus ambiguus* (Paykull), *C. erratus* (Sahlberg), *Amara aenea* (De Geer), *Ophonus azureus* (Fabricius), *O. cribricollis* (Dejean), *Harpalus rubripes* (Duftschmid), and *H. zabroides* Dejean.

Recurring ecological model in biocoenotic researches is the presence of a few abundant species and the predominance of the variety of rare species (Preston 1962), which is confirmed by the results of this study, showing the greatest number of species from the category of the subrecedents. The presence of many species with very low numbers may also be due to a methodical deficiency, i.e. many phytophilous species rarely climb down and move on the ground substrate, which is the reason for their low density in pitfall traps. The large share of the subrecedents ($D < 1$ %), i.e. 114 species (83 %), requires their detailed examination. The share of these species was found to be between 40 and 45 % in highly urbanized territories in Ukraine (Putchkov et al. 2020). The group of subrecedent species was divided according to the scale developed on the basis of the faunal abundance used by

Pesenko (1982). The species represented with only one specimen, called single (see Table 3), have always been of research interest. Species-rich genera and whole tribes do not fall into the traps or fall accidentally, with single specimens, due to low activity, excessively small size, and endogeic or horto-dendrobiont way of life. Such are the whole tribes Clivinini, Dyschiriini, Trechini, some Bembidiini, and some genera of the Lebiini and Harpalini.

Highly impressive was the small number of subrecedents in the forest habitat, possibly due to the climax state of the old mesophilic deciduous forest, with expressed evenness of the species and stable dominant structure of typical forest species. The most affected habitats, such as the agricultural lands, also have very few rare species (17 in total), the possible cause being in the severely disturbed dominant structure due to intensive land use and the lack of microhabitat diversity. High number of recedent species had the coastal habitats, where extra- and intrazonal (mostly phytophilic) species were recorded in low numbers, as well as the open secondary habitats with a low degree of recent anthropogenic intervention – the two abandoned pastures. Their modern appearance is of loess pseudo-steppes, overgrown with xerophilic and xerothermic herbaceous vegetation. In these habitats, rare species represent a sum of typical steppe and thermophilic xerobionts, and plastic species in a process of initial expansion. In general, the dominant structure of these communities is imbalanced, with a preponderance of the dominant community component and a great number of rare species, which is characteristic of communities in an initial phase of succession. Stenotopic species in these habitats are relatively few (5–6 species), but their presence can be considered an evidence of refugium for this type of fauna in the given area.

Cluster analysis of the communities' similarity showed higher rates of taxonomic similarity between associations, in comparison with the communities from the region of Cape Emine, where the habitats were much more diverse (Teofilova et al. 2015). More homogenous were the agrocoenoses near the city of Sofia (Shishiniova et al. 2001, Kostova 2004). The dendrograms showed that geographical location is essential for the spatial distribution of the habitats. However, the grouping also showed the succession path and the ecological transformation of degraded pastures, after the cessation of grazing and the change in edaphic and hydrothermal conditions. The change of the pasture management regime was a prerequisite for the establishment of complexes of plastic species of ground beetles, characteristic of the arid territories in the steppes and forest-steppes of Eurasia and open habitats of the Mediterranean. The deforestation of the Danubian plain in NW Bulgaria has radically changed the natural vegetation appearance and the natural faunistic complexes along with it. The vast arable and synanthropic territories have fundamentally changed the landscape and microclimate in the area. The persistent tendency towards steppification, as a consequence of mass aridization and soil erosion lead to the establishment of specific faunistic complexes.

In relation to the life forms, the same ratio between the two classes (Zoophages: Mixophytophages) as in our study was found by Kodzhabashev and Mollov (2000) when exploring open habitats in synanthropic habitats around Sofia, as well as by Kodzhabashev (2016), in open semi-natural areas around the Srebarna Reserve. Ratio 54: 46 % was found in xerophytic pseudo-maquis communities from the low mountain belt of southern Pirin Mts. (Teofilova 2020). Similar ratio (50: 50 %) between the classes of life forms is mostly approaching to the typical for the steppe zones and for the orchards from the forest-steppe zones, as well as the vast deforested territories across Europe (Sharova 1981). According to Sharova (1981), the normal ratio between the two classes is 60: 40 % for the forest-steppe areas, and 70: 30 % for the nemoral forest zone. Close to the normal ratios between the classes were established in the Srebarna Reserve – 60: 40 % (Kodzhabashev 2016), the region of Cape Emine – 57: 43 % (Teofilova 2013, 2015), and the Lower Tundzha Valley – 62.5: 37.5 % (Teofilova 2017). The average ratio in Bulgarian grasslands was calculated as 55: 45 % (Teofilova 2018b). Close to the normal for the nemoral zone was the ratio in the Western Rhodopes Mts. – 67: 33 % (Teofilova 2018a). Quantitative data about the life forms further demonstrate the anthropogenic impact in the study area, where the ratio increases to 54 % in favour of the mixophytophages. We found a predominance of the stratobiont zoophages, which has also been established amongst Dagestan carabids (Mammaevna et al. 2011), and harpaloid geohortobionts from class Mixophytophaga, which were also the most species rich category in pseudomaquises in SW Bulgaria (Teofilova 2020), as well as in the Eastern Rhodope Mts. (Teofilova and Kodzhabashev 2020a).

The structure we established most likely results from the changes in landscape and land use that have led to habitat xerophytisation due to deforestation and ploughing for agricultural purposes, the subsequent monocultural intensive farming, and soil erosion with ‘stripping‘ of the base sediment (loess), which is heavily drained and easily evaporates soil moisture. Vast areas sown with cereals are close in microclimate factors to the natural steppes, but are monocultural. All these changes lead to strong structural changes of the spectra of life forms and, accordingly, to a change in the ratio of the two classes due to the replacement of the indigenous carabid fauna with a new thermo-xerophilic one.

Carabidae are mainly meso-, meso-hygro- and hygrophilous, and xerobionts have a relatively limited distribution in arid landscapes (Kryzhanovskij 1983). In contrast to the small share of the eurybionts (4 %) found in the Eastern Rhodope Mts. (Teofilova and Kodzhabashev 2020a), they accounted for 11 % here. Eurybionts were 8–10 % in sub-Mediterranean Balkan pseudomaquis habitats in SW Bulgaria (Teofilova 2020), and 9 % in the region of Cape Emine (Teofilova et al. 2015). The same share of mesoxerophiles (36 %) as in Zlatiya, was found in the Eastern Rhodope Mts. too (Teofilova and Kodzhabashev 2020a). According to Kryzhanovskij (1965, 1983), carabid eurybionts are mostly species with extensive Palaearctic or Eurasian ranges, and they are also ecologically plastic which provides them conditions for life in synanthropic and semi-natural environments, such as arable lands, pastures and forest plantations. The large number of eurybionts in the Zlatiya Plateau probably stems from the presence of extensive agricultural lands, where the synanthropic environment favours species with high ecological plasticity. In the studied region, forest habitats have undergone a strong reduction and after their transformation into agricultural lands, the mesophilous species composition has been proportionally changed and replaced by mesoxerophilous, i.e. there was a process of aridization of the habitats and, accordingly, of carabid coenoses, due to the replacement of mesophilous forest species with eurybionts. That explains the lower presence of the mesophiles (20 %). Similar changes have been found in other plain areas where the forest massifs have been destroyed and the land has been turned into agricultural, such as the Upper Thracian Lowland (Teofilova 2020a) and Dobrudzha (Kozhabashev 2016), as well as in xerothermic pseudomaquis habitats from the southern Pirin Mts. and Struma Valley, where deforestation took place (Teofilova 2020). As a comparison, in the Western Rhodope Mts., the share of the mesophilous carabids was 37 % of the species and 64 % of the specimens, due to the mountain and forest species (Teofilova 2018a).

The proportions and ratios between the different carabids' wing morphs, and their flight capabilities, respectively, can be used as indicators of the specific environmental conditions and tendencies in the development of carabid faunas and carabid coenoses in a given geographical area, since the migratory component comprises mainly macropterous species, whereas the stable component comprises mainly brachypterous species and predominantly brachypterous morphs of dimorphic species (Chernov and Makarova 2008). Such targeted and specialized studies on the ratio, proportions and structure of the wing forms of ground beetles in Bulgaria have not been conducted, with the exception for the analysis of the wing polymorphism of carabids in oilseed rape fields (*Brassica napus* L.) (Teofilova 2020b).

Specifically, for the Zlatiya Plateau, all mass eurytopic species were winged forms, explaining their wide range of distribution (Kryzhanovskij 1965). Wingless forms were only 8 %, of which six (*Carabus ullrichi*, *C. convexus*, *Myas chalybaeus*, *Pterostichus melas*, *Abax carinatus*, *Platyderus rufus*) are forest species, and another four (*Zabrus spinipes* (Fabricius), *Acinopus ammophilus* Dejean, *Licinus cassideus* (Fabricius), *Microlestes fulvibasis* (Reitter) can be classified as thermophilic stenobionts. It is probable that the requirements for specific environmental conditions are causing the lack of winged forms in these species, and the availability of the extensive spatial resource does not require the ability to disperse and move by flight. The reduction of the forests as a living resource in the Zlatiya can be taken as a synanthropic change that occurred relatively late, as evidenced by the presence of wingless forest forms, although they were not many. The value of di(poly)morphic species is difficult to be assessed in comparative terms, given the lack of information. Among these species we found both intrazonal epigeic hygrophiles (*Nebria brevicollis, Dyschirius globosus* (Herbst)*, Bembidion lampros* (Herbst), *Stomis pumicatus* (Panzer), *Pterostichus anthracinus* Illiger, *Pt. nigrita* (Paykull), *Pt. strenuus* (Panzer), *Pt. ovoideus* (Sturm), and forest species (*Leistus rufomarginatus*, *L. ferrugineus* (Linnaeus), *Ophonus laticollis*, *Harpalus atratus* Latreille). Within this group we also found some of the open habitats' species characteristic of the vast synanthropic habitats – agricultural land and pastures. They probably develop winged or wingless form, according to the need to fly, depending on the phase of distribution and expansion. This group included the polymorphic forms of the genera *Calathus*, *Ophonus*, *Harpalus*, *Microlestes*, and *Brachinus*.

Since macropterous wings are mainly used for dispersal flights, winged species seem normally especially abundant in scattered or disturbed habitats, e.g. cultural land. On the other hand, brachypterous species often are stenotopic (e.g. forest) inhabitants with a low dispersal ability (Kryzhanovskij 1965, Kromp 1999, Chernov and Makarova 2008). Our results are in accordance with Gray's hypothesis, that the proportion of flight capable pioneer species should increase with increasing disturbance, and the proportion of flightless species should decrease (Gray 1989). Very similar results about the wing morphology of carabids were obtained about oilseed rape fields in four European countries (Bulgaria, Germany, Romania and Switzerland) (Teofilova 2020b).

Conclusions

Activity density, combined with the number of specimens caught and the number of species identified, can be considered as a function of the spatial resource, productivity of the habitat and its energy capacity, allowing the values to be included in the interpretation of the habitats' biodiversity in the analysis of their nature, origin and condition.

The established dominant structure is characteristic of highly anthropogenically affected areas, where the share of ecologically plastic species is severely increased, and that of sporadic and random species is low. The imbalance in species distribution is strongly pronounced. The dominant component of the community (*D* > 3 %) includes seven species, representing 55 % of the specimens caught, and the remaining 131 species (*D* < 3 %) consisted of only 45 % of the total numbers.

Rare carabid species, qualitatively and quantitatively, have a relatively low presence both in the heavily influenced, and in climax communities, and gradation in their number can be used as a criterion for the degree of successional development, as there is a visible increase in their number as the succession progresses.

The two types of analysis of the similarity of the carabid coenoses – in quality and quantity, complement each other and reinforce the argumentation about the primacy of the natural landscape and microclimate for the area, as well as the changes occurred in the carabid communities after the mass deforestation, agricultural cultivation (ploughing), soil erosion and subsequent steppification as a result of strong microclimatic and microhabitat aridization.

There is a direct link between life forms and their taxonomic and zoogeographical affiliations (discussed in the Part 1 of the paper, see Teofilova and Kodzhabashev 2020b). Neareastern, Euro-Asiatic (steppe) and Mediterranean elements include mainly xero- and mesoxerophilous species of genera and tribes adapted for living in open habitats, regardless of the origin of grass communities and monocultures. The increased presence of eurybionts and the considerable share of the mesoxerophilous component are probably resulting from the drastic anthropogenic changes that have led to a significant reduction of forest habitats and their replacement by a 'cultural steppe'.

By the changes in the ratios between the wing morphs of carabid coenoses and the specific forms of the wing polymorphic species, the speed of environmental changes could be analysed, and the trend in regional microclimatic changes could be predicted. The prevalence of the macropterous carabids reflects their higher mobility and adaptiveness, and evidences the initial stage of formation of coenoses, as well as the unstable state of carabid populations.

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INVENTORY OF ALLERGENIC POLLEN URBAN DENDROFLORA AS A BASIS FOR DESIGNING HEALTHIER GREEN INFRASTRUCTURE

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Abstract

The paper considers the potential impact of allergenic pollen urban dendroflora on public health. The aim of the study was to make quantitative and qualitative analysis of urban park woody taxa emitting allergenic pollen in order to increase the awareness of landscape architects and medical practitioners. The case study was conducted in the second largest park in Bulgaria – Sveti Vrach, Sandanski. The town is famous as climatotherapy and spa resort of international importance. The total number of the investigated trees was 2673, and the number of shrub specimens – approximately 2640. The results from the detailed inventory of park dendroflora estimated that the total number of taxa was 199 in terms of species and the infraspecific taxa, belonging to 49 families and 109 genera. Analysis of the systematic structure of the examined specimens was performed. Geographic distribution, reproductive characteristics, foliage permanence, and allergenicity level of taxa were also analysed. Considering the allergenic potential value of tree species, 34.9 % of tree specimens had a high allergenic potential, 10.6 % of tree individuals had a very high allergenicity level, while 4.8 % – a moderate one. As a result, half of all the researched trees (50.2 %) had a moderate to very high allergic potential. The most frequent shrub species had moderate to low/very low alergenicity potential. Only 4.9 % of shrub specimens had a high to very high allergenicity level. Tree density and Diversity Index were also calculated, as factors that most affect the final allergenicity value of the park. Adopted strategies for risk mitigation has been suggested and addressed to future urban greening initiatives and landscape design for achieving biodiversity-friendly green infrastructure and sustainable urban environment.

Key words: allergenic plants, biodiversity, ecosystem services, green spaces, tree pollen, urban planning.

Introduction

The subject of health-related ecosystem services of urban green spaces is a global issue in urban planning (Roy et al. 2012, Salmond et al. 2016, Vasiljevic et al. 2018). In the last decades, a great number of studies have suggested positive effects of green spaces on the mental and physical health status of population (Pretty et al. 2005, Gidlöf-Gunnarsson and Öhrström 2007, Lee and Maheswaran 2011, Ward Thompson et al. 2012, Dzhambov and Dimitrova 2014, Grazuleviciene et al. 2014, Jonker et al. 2014, Markevych et al. 2014, Staneva et al. 2016).

Urban greenery may have a positive impact on the reduction of psychophysiological stress, associated with time spent in outdoor physical activities in a natural environment, such as urban forest, parks or other residential green spaces (Sugiyama et al. 2008, McCurdy et al. 2010, Balseviciene et al. 2014, Harting et al. 2014, Nieuwenhuijsen et al. 2014). However, the topic of green infrastructure and human health has been approached very differently by natural science and epidemiology (Kumar et al. 2019). Along with the multiple environmental, economic, social and cultural benefits that contribute to human well-being, urban trees pose some health hazards determined as 'ecosystem disservices' (Lyytimäki and Sipilä 2009, Tomalak et al. 2011, Cariñanos et al. 2017, Ćwik et al. 2018, Battisti et al. 2019). Some epidemiological studies directly link urban green spaces with allergic sensitization to pollen and asthma (D'amato et al. 2007, DellaValle et al. 2012, Scharte and Bolte 2012, Lovasi et al. 2013, Andrusaityte et al. 2016).

Allergic respiratory response to pollen in sensitized individuals, pollinosis, allergic rhinitis, hay fever, allergic rhinoconjunctivitis or asthma exacerbations, is an extremely common disease worldwide (Wuthrich et al. 1995, Strachan et al. 1997, Bousquet et al. 2001, Dales et al. 2004, Ridolo et al. 2007, Greiner et al. 2012). The prevalence of pollinosis at some European locations is presently estimated to be up to 40 % and has a remarkable clinical impact since pollination period covers about half the year (D'amato et al. 2007). There is a lack of sufficient data to build up a clear picture of prevalence of asthma and pollinosis in Bulgaria. The values obtained in Hristov (2013) study for the prevalence of asthma and allergic rhinitis (95 % CI): 14.9 % (13.5–16.2 %) and 39.3 % (37.5–41.1 %), respectively, differ significantly from those accepted for the country so far.

Tree pollen emissions can be considered main source of emission of coarse particulate matter in urban environments (Cariñanos et al. 2017). Nikolov el al. (2015) palynological survey shows that the tree pollens are a significant part (78.2–83.8 %) of total pollen grains in the air. According to Nikolov el al. (2015), tree pollen allergens play a key role in the sensitization and in the occurrence of allergic symptoms in hypersensitive individuals in Bulgaria.

In relation to higher ambient air pollutant concentrations and the interaction between pollen and air pollutants, urban citizens have shown to be more prone to pollen allergies (asthma and allergic rhinitis) than those who live in rural areas (Von Ehrenstein et al. 2000, Riedler et al. 2001, D'Amato et al. 2007).

Tree pollen grains are released higher above the ground and can be transported and deposited in large distances from its source (Skjøth et al. 2007, Galan et al. 2008). The production and dispersion of pollen depends on the plant taxon and meteorological factors, such as temperature, precipitation, humidity, wind speed and direction, as well as diurnal rhythm and thunderstorm events (Latalowa et al. 2005, Emberlin et al. 2007, Sofiev et al. 2012, Elliot et al. 2013, Ščevková et al. 2015). There is some evidence that climatic warming over the past decades resulted in earlier and lengthened pollen seasons for many tree species and earlier seasonal appearance of respiratory symptoms and their prolonged duration, respectively (Emberlin et al. 1997, Frei 1998, Emberlin et al. 2002, Fitter and Fitter 2002, Beggs 2004, Ziska et al. 2011, Vardoulakis and Heaviside 2012).

Among the main sources of allergenic pollen emissions in urban areas are species from some of the most commonly used ornamental tree genera in European cities: *Acer* L., *Cupressus* L., *Pinus* L.*,* *Platanus* L., *Quercus* L. and *Tilia* L. (Cariñanos et al. 2017). Depending on geographical location, a wide variety of trees are common producers of pollen aeroallergens in Europe (e.g. genera of birch (*Betula*), alder (*Alnus*), hazel (*Corylus*), oak (*Quercus*), olive (*Olea*), and cypress (*Cupressus*)). The most allergenic tree pollen is produced by birch in North, Central and Eastern Europe, and by olive and cypress in the Mediterranen regions (D'amato et al. 2007).

The value of overall allergenic risk that species pose in the area they grow was epitomized in the Index of Urban Green Zone Allergenicity (IUGZA), developed by Cariñanos et al. (2019). Cariñanos et al. (2019) postulate that a forest-type landscape design, with high density of tree per 1 ha, and monospecific tree plantations, contribute to the remarkable increase in the IUGZA. High biodiversity environment is well appreciated not only in the landscape design along with the other recreational values and health and well-being benefits of urban green spaces (Fuller et al. 2007, Hoyle et al. 2017, Gao et al. 2019). Many authors suggest that contact with biodiversity-rich environments can boost immunological tolerance and reduce sensitivity to allergens (Hanski et al. 2012, Rook 2013).

Asthma and allergic rhinitis adversely alters the quality of life of suffers, their performance and productivity (Bousquet et al. 1994, Meltzer 2016). Furthermore, the costs for treating rhinitis and asthma are a considerable annual financial burden on patients and healthcare systems (Nathan 2007, Pawankar 2014).

Since the associations of urban green spaces with allergic conditions are diverse and the effect of greenery on health is complex, Andrusaityt et al. (2016) suggests that further investigation on species composition of green spaces, air quality and population groups should be conducted.

Sveti Vrach Park dendroflora was selected for object of study, as Sveti Vrach is one of the largest parks in Bulgaria. Moreover, the town of Sandanski is well-recognized natural sanatorium for the treatment of respiratory diseases, famous as climatotherapy and spa resort of international importance (Kostadinov et al. 1983). It attracts a large number of visitors annually as one of the most popular recreational areas in the region, offering a wide range of active and passive leisure activities.

The purpose of this study is to perform quantitative and qualitative analysis of urban park woody ornamentals producing allergenic pollen in order to increase the awareness of landscape architects, medical practitioners and sensitized park visitors to this health issue with large-scale socio-economic impact.

Object and Methods

Study area

The study was conducted in 2019 in the Sveti Vrach Park, Sandanski (41°34′15″ N; 23°17′00″ E; 200 m a.s.l). This centennial park is one of the largest urban parks in Bulgaria and according to its master plan covers an area of 335 dka. The first plantations from *Pinus sylvestris* and *Pinus nigra* established in 1916 (Penzov 2016) nowadays stand out in the historical core of the park, along with the centuries-old riparian *Platanus orientalis* trees. The scope of the inventory included the historical part, which is declared a monument of garden and park art, and the east, north and south extentions.

In terms of climate, Sandanski falls whithin the South Bulgarian climate province, Sandanski-Petrich climate region (Sabev and Stanev 1963). The climate is characterized by a mild winter with a positive January mean temperature – +2.5 °C, and a long and hot summer with July and August average temperature 24.6 °C. The mean annual temperature for Sandanski is 13.9 °C. Northwest and west winds prevail with an average speed of 2 m/s. The mean annual precipitation is 534 mm, most of it being concentrated in the winter months. Moisture deficit during the major part of the growing season – lasting from last decade of June to the first decade of October is common. Sandanski is the driest region in the country, with the lowest relative humidity in all of Bulgaria (53–54 % for July and August).

Urban park woody taxa composition was identified by applying the route method, inventorying the specimens along the whole alley network and in the depth of the groups and massifs. Due to the different dendrometric characteristics, species were divided by their biological type into two groups – trees and shrubs (incl. lianas and subshrubs). Their absolute and relative specimen quantities were presented, apart from those applied as hedges and groundcovers.

The names of the species and cultivated varieties are consistent with the International Plant Names Index (IPNI 2020) and the RHS Horticultural databases (RHS 2020). In addition, the reproductive traits of dioecious specimens were determined in case they were evident. The permanence of species foliage – evergreen or deciduous was considered. Floristic elements of indigenous species were established after Assyov et al. (2012).

The allergenicity rating of the surveyed woody species was determined after Ogren Plant-Allergy Scale (OPALS) (Ogren 2015) and PollenLibrary (2020). According to these scales species with a ranking of 1 on the scale are least likely to cause allergenic reactions in most people, whereas plants assigned a 10 should be regarded as highly allergenic. In general, plants that rank from 1 to 5 should be considered low-risk plants. Two allergenic ratings are used for dioecious species in which male and female reproductive attributes occur on separate individuals.

Tree density (trees per hectare) and Shannon-Wiener Diversity Index (*H*) (Shannon and Weaver 1949) were also calculated, as factors that most affect the final allergenicity value of green spaces (Cariñanos et al. 2019) by formula (1).

$$H = -\sum_{i=1}^{k} \cdot p_i \cdot \log(p_i)\,, \qquad (1)$$

where: $p_i = \dfrac{n_i}{N}$, n_i is number of individuals of species *i*, *N* is the total number of samples.

Shannon-Wiener Diversity Index (*H*) accounts for both abundance and evenness of the species present. The equitability (evenness) (E_H) can be calculated by the formula (2).

$$E_H = \frac{H}{H_{\max}}\,, \qquad (2)$$

where $H_{max} = \log(N)$.

Equitability assumes a value between 0 and 1 with 1 being complete evenness.

The climate analysis of the site is based on the data of Hydrological and Meteorological Service of Sandanski (meteorological station 190 m a.s.l.) (BCRB 1983, CRB 1990).

Results and Discussion

Sandanski is located in Southwestern Bulgaria, in the Sandanski-Petrich valley, between the Kresna and Rupel gorges. The town is situated amphitheatrically on the farthest southwestern slopes of Pirin Mountain along the lower flow of the Sandanska Bistritsa River. Another waterbody in the Sveti Vrach Park is a pond.

Climate. The environmental variables, which most affect the floristic composition of the parks, as well as production and dispersion of pollen, are: temperature, precipitation, humidity, wind speed and direction. The southern Bulgarian geographical location and low altitude of the city of Sandanski in combination with the specific relief of the region is a prerequisite for the formation of specific favourable microclimate, influenced by the Mediterranean. The whole park territory is situated along a river, which contributes to air circulation and relative humidity levels. On the other hand, steep slopes frame the park territory in its central part. The relationship between wind direction and pollen dispersion is positive since these winds would transport pollen to the city located mainly southwardly.

The varied native flora of this climatic region is influenced by the Mediterranean (Gateva and Delkov 2001). The environmental conditions and the irrigation of Sveti Vrach park theritory allow the establishment of many exotic species. However, the warm and arid climate would result in earlier and lengthened pollen season.

Woody species composition

A detailed inventory of Sveti Vrach Park woody dendroflora was carried out in 2019. Urban park dendroflora consists of totally 174 species identified. The total number of taxa was 199 in terms of species and the infraspecific taxa, belonging to 49 families and 109 genera. Vakarelov et al. (1982) established 176 taxa in the park. The results of the two inventories cannot be compared, because the scope of our inventory includes additionally the contemporary extentions of the park theritory northwards and southwards.

The analysis of the systematic structure of Sandanski park dendroflora revealed the predominant families, genera and taxa among Gymnosperms (23.1 %) and Angiosperms (76.9 %). The Gymnosperms include 46 taxa belonging to 4 families, 16 genera, 35 species, 4 varietas and 12 cultivars, while the Angiosperms include 153 taxa belonging to 45 families, 93 genera, 139 species, 2 subspecies, 3 varieties and 20 cultivars. Amongst the Gymnosperms families, the Cupressaceae (11.6 %) is represented by 9 genera, 16 species, 2 varieties, 9 cultivars, followed by Pinaceae (10.1 %) – 5 genera, 17 species, 2 varieties, 2 cultivars, Ginkgoaceae (0.5 %) – 1 species and Taxaceae (1.0 %) – 1 species, 1 cultivar. Amongst the Angiosperms families, the Rosaceae (10.05 %) is represented by 12 genera, 19 species, 3 cultivars, followed by Oleaceae (6.5 %) – 7 genera, 13 species, Leguminosae (5.0 %) – 9 genera, 9 species, 1 cultivar, Caprifoliaceae (5.0 %) – 3 genera, 9 species, 1 variety, 1 cultivar, Sapindaceae (4.5 %) – 3 genera, 8 species, 1 cultivar, Salicaceae (4.0 %) – 2 genera, 7 species, 2 varieties, 1 cultivar, Berberidaceae (3.0 %) – 3 genera, 6 species, Hydrangeaceae (3.0 %) – 3 genera, 4 species, 3 cultivars, Malvaceae (3.0 %) – 2 genera, 4 species, 3 cultivars, Moraceae (2.5 %) – 4 genera, 4 species, 1 cultivar, Bignoniaceae (2.0 %) – 2 genera, 4 species, Apocynaceae (1.5 %) – 2 genera, 3 species, Betulaceae (1.5 %) – 3 genera, 3 species, Buxaceae (1.5 %) – 1 genera, 1 species, 2 cultivars, Cornaceae (1.5 %) – 1 genera, 3 species, 1 cultivar, Ebenaceae (1.5 %) – 1 genera, 3 species, Fagaceae (1.5 %) – 2 genera, 3 species, Lythraceae (1.5 %) – 2 genera, 2 species, 1 cultivar, Adoxaceae (1.0 %) – 1 genera, 2 species, 1 cultivar, Asparagaceae (1.0 %) – 2 genera, 2 species, Celastraceae (1.0 %) – 2 genera, 2 species, Elaeagnaceae (1.0 %) – 1 genera, 1 species, 1 cultivar, Juglandaceae (1.0 %) – 2 genera, 2 species, Lamiaceae (1.0 %) – 2 genera, 2 species, Magnoliaceae (1.0 %) – 1 genera, 2 species, Platanaceae (1.0 %) – 1 genera, 2 species. The other 19 families (Anacardiaceae, Aquifoliaceae, Araliaceae, Asclepiadaceae, Cannabaceae, Hamamelidaceae, Hypericaceae, Lauraceae, Meliaceae, Myrtaceae, Paulowniaceae, Pittosporaceae, Polygonaceae, Rutaceae, Simaroubaceae, Solanaceae, Tamaricaceae, Ulmaceae, Vitaceae) – are represented by one species each. The genera represented with the largest number of species were *Lonicera* – 7 spp.; *Acer* – 6 spp.; *Fraxinus* – 5 spp.; *Pinus* – 5 spp.

The total number of the surveyed trees was 2673, and the number of shrub specimens – approximately 2640 (Table 1). The number of shrub individuals is approximative, because some shrub species were planted in large groups or hedges which made counting difficult.

The number of angiosperm tree specimens (56.8 %) slightly exceeded Gymnosperm tree specimens (43.2 %). The biological type analysis showed that trees amount 56.9 %, while shrubs – 37.4 %, lianas – 4.0 %, subshrubs – 1.7 % of the total number of species.

In the dendrological list, 36.6 % of species belong to Bulgarian dendroflora, including the adventive species from the Mediterranean, Asia and North America, while the majority (63.4 %) were introduced or hybrids, respectively.

With reference to sexual attributes, 81.0 % of the species were hermaphrodite or monoecious (had both sexes on the same specimen), while 11.5 % were dioecious (separated sexes in different specimens). In 7.5 % of the listed species male and female reproductive organs occur dioeciously or monoeciously. Our study faced some limitations in sex determination of individuals, because some specimens were planted or grew in dense large communities or hedges, others were sheared or their crowns were inaccessible, third were not yet reproductively mature.

The foliage permanence was also considered, because it affects the pollen dispersion. Deciduous species (78.5 %) in the dendrological list dominated over the evergreen species (21.5%), while deciduous tree specimens were 56.1 % of all the examined trees.

Species allergenicity level

Considering the allergenic potential value of tree species, 34.9 % of tree specimens had a high allergenic potential, 10.6 % of tree individuals had a very high allergenicity level, while 4.8 % – a moderate one (Table 1). As a result, half (50.2 %) of all the examined trees had a moderate to very high allergic potential.

The most frequent allergenic species of Gymnosperms were members of Cupressaceae family (Table 2). The most common allergenic pollen tree species of Angiosperms were: *Platanus orientalis*, *Ulmus minor*, *Alnus glutinosa*, which are autochthonous and are part of natural riparian forest.

The indigenous dominant species *Platanus orientalis* has a notable presence in

Table 1. Species allergenicity level.

Allergenicity level	Species and infraspecific taxa	Specimens number	Specimens %
9–10 Very high	**Trees**	**282**	**10.55**
	Cryptomeria japonica (Thunb. ex L.f.) D.Don	6	0.22
	Cupressus arizonica Greene /’Pyramidalis’/	33	1.23
	Cupressus sempervirens /var. *horizontalis*, var. *pyramidalis*/	67	2.51
	Juniperus communis ‘Hibernica’ ♂	1	0.04
	Juniperus excelsa M.Bieb.	1	0.04
	Juniperus virginiana L.	3	0.11
	Taxus baccata L. ♂	3	0.11
	Acer negundo L. ♂	35	1.31
	Acer saccharinum L.	4	0.15
	Alnus glutinosa (L.) Gaertn.	56	2.10
	Betula pendula Roth	23	0.86
	Broussonetia papyrifera (L.) L’Hér. ex Vent. ♂	2	0.07
	Maclura pomifera (Raf.) C.K.Schneid. ♂	3	0.11
	Morus alba L.	35	1.31
	Olea europaea L.	1	0.04
	Quercus suber L.	3	0.11
	Salix alba L.	6	0.22
	Shrubs	**92**	**3.48**
	**Juniperus × pfitzeriana* (Späth) P.A.Schmidt	25	0.95
	**Juniperus sabina* ‘Tamariscifolia’	32	1.21
	Laurus nobilis L. ♂	7	0.26
	Ligustrum japonicum Thunb.	10	0.38
	Ligustrum ovalifolium Hassk.	8	0.30
	Ligustrum vulgare L.	10	0.38
7–8 High	**Trees**	**933**	**34.90**
	Chamaecyparis lawsoniana (A.Murray bis) Parl.	85	3.18
	Chamaecyparis pisifera /’Ellwoodii’, ‘Plumosa’, ‘Squarrosa’/	9	0.34
	× *Cupressocyparis leylandii* ‘Silver Dust’	1	0.04
	Ginkgo biloba L. ♂	2	0.07
	Platycladus orientalis (L.) Franco /’Aurea Nana’/	101	3.78
	Taxodium distichum (L.) Rich.	1	0.04
	Thuja occidentalis L. /’Smaragd’, ‘Columna’/	75	2.81
	Thuja plicata Donn ex D.Don	3	0.11
	Ailanthus altissima (Mill.) Swingle	40	1.50
	Celtis australis L.	52	1.95
	Fraxinus americana L.	43	1.61
	Fraxinus pennsylvanica Marshall	20	0.75
	Fraxinus excelsior L.	4	0.15
	Gymnocladus dioica (L.) K.Koch ♂	8	0.30
	Ilex aquifolium L. ♂	8	0.30
	Juglans regia L.	57	2.13
	*Liquidambar styraciflua*L.	6	0.22

Allergenicity level	Species and infraspecific taxa	Specimens	
		number	%
	Platanus orientalis L.	246	9.20
	Platanus occidentalis L.	2	0.07
	Populus alba L.	11	0.41
	Populus x canescens (Aiton) Sm.	1	0.04
	Populus nigra var. *italica* Münchh. ♂	3	0.11
	Quercus rubra L.	9	0.34
	Ulmus minor Mill.	146	5.46
	Shrubs	**37**	**1.40**
	Buxus sempervirens L. /'Suffruticosa'; 'Arborea'/	17	0.64
	Corylus avellana L.	5	0.19
	Fontanesia phillyreoides Labill.	15	0.57
	Hedera helix L.	*	*
5–6 Moderate	**Trees**	**128**	**4.79**
	Sequoiadendron giganteum (Lindl.) J.Buchholz	4	0.15
	Acer tataricum subsp. *ginnala* (Maxim.) Wesm.	3	0.11
	Acer palmatum Thunb.	1	0.04
	Acer platanoides L. /'Globosum'/	27	1.01
	Acer pseudoplatanus L.	25	0.94
	Aesculus hippocastanum L.	20	0.75
	Castanea sativa Mill.	1	0.04
	Catalpa ovata G.Don	3	0.11
	Catalpa speciosa (Warder ex Barney) Warder ex Engelm.	2	0.07
	Diospyros sp.	10	0.37
	Diospyros lotus L.	7	0.26
	Diospyros kaki L.f.	6	0.22
	Fraxinus angustifolia subsp. *oxycarpa* (Willd.) Franco & Rocha Afonso	5	0.19
	Fraxinus ornus L.	3	0.11
	Pterocarya fraxinifolia (Poir.) Spach	10	0.37
	Salix cinerea L.	1	0.04
	Shrubs	**749**	**28.35**
	**Celastrus orbiculatus* Thunb.	8	0.30
	Cornus sericea 'Flaviramea'	2	0.08
	Cotinus coggygria Scop.	1	0.04
	Elaeagnus × submacrophylla 'Limelight'	12	0.45
	Forsythia × intermedia Zabel	511	19.34
	Laburnum anagyroides Medik.	38	1.44
	**Lonicera japonica* Thunb. /'Halliana'; var. chinensis (P. Watson) Baker/	30	1.14
	Lycium barbarum L.	10	0.38
	Salix eleagnos Scop.	1	0.04
	Syringa vulgaris L.	125	4.73
	Tamarix tetrandra Pall. ex M.Bieb.	1	0.04
	Viburnum tinus L.	10	0.38

Allergenicity level	Species and infraspecific taxa	Specimens	
		number	%
	Trees	**1127**	**42.16**
	Cedrus deodara (Roxb. ex D.Don) G.Don	76	2.84
	Picea abies (L.) H.Karst.	33	1.23
	Picea omorica (Pancic) Purk.	1	0.04
	Picea orientalis (L.) Peterm.	1	0.04
	Picea pungens Engelm. /'Glauca'/	55	2.06
	Pinus halepensis Mill.	18	0.67
	Pinus nigra J.F.Arnold	366	13.69
	Pinus pinea L.	6	0.22
	Pinus strobus L.	1	0.04
	Pinus sylvestris L.	32	1.20
	Pseudotsuga menziesii /var. *Viridis* (Schwer.) Franco; var. *glauca* (Beissn.) Franco/	23	0.86
	Albizia julibrissin Durazz.	63	2.36
	Catalpa bignonioides Walter	15	0.56
	Cercis siliquastrum L.	36	1.35
	Eriobotrya japonica (Thunb.) Lindl.	3	0.11
	Gleditsia triacanthos L. /'Inermis'/	21	0.79
	Koelreuteria paniculata Laxm.	31	1.16
	Magnolia grandiflora L.	18	0.67
	Magnolia kobus DC.	12	0.45
3–4 Low	*Malus nidzwetzkyana* Dieck ex Koehne	4	0.15
	Melia azedarach L.	22	0.82
	Paulownia tomentosa Steud.	2	0.07
	Prunus dulcis (Mill.) D.A.Webb	2	0.07
	Prunus mahaleb L.	1	0.04
	Prunus serrulata 'Kanzan'	2	0.07
	Prunus cerasifera Ehrl. /'Pissardii'/	11	0.41
	Pyrus communis L.	9	0.34
	Robinia pseudoacacia L.	186	6.96
	Sorbus intermedia (Ehrh.) Pers.	3	0.11
	Styphnolobium japonicum (L.) Schott	7	0.26
	Tilia platyphyllos Scop.	25	0.94
	Tilia tomentosa Moench	25	0.94
	Tilia cordata Mill.	17	0.64
	Shrubs and subshrubs	**1599**	**60.52**
	Acca sellowiana (O.Berg) Burret	1	0.04
	Amorpha fruticosa L.	57	2.16
	Berberis julianae C.K.Schneid.	5	0.19
	Berberis thunbergii DC.	8	0.30
	Berberis vulgaris L.	28	1.06
	**Campsis radicans* (L.) Seem.	31	1.17
	Citrus trifoliata L.	1	0.04
	**Cotoneaster dammeri* C.K. Schneid.	3	0.11

Allergenicity level	Species and infraspecific taxa	Specimens number	Specimens %
	**Cotoneaster horizontalis* Decne.	3	0.11
	Cornus alba L.	20	0.76
	Cornus sanguinea L.	3	0.11
	Deutzia scabra Thunb. /'Candidissima', 'Plena'/	113	4.28
	Deutzia gracilis Siebold & Zucc.	80	3.03
	Euonymus japonicus Thunb.	16	0.61
	**Fallopia aubertii* (L.Henry) Holub	3	0.11
	Hibiscus syriacus L. /'Red Heart', 'Woodbridge', 'Monstrosus'/	210	7.95
	Hydrangea arborescens 'Annabelle'	1	0.04
	**Hypericum calycinum* L.	*	*
	Lagerstroemia indica L. /'Durant Red'/	67	2.54
	Lonicera fragrantissima Lindl. & J. Paxton	35	1.32
	Lonicera maackii (Rupr.) Maxim.	1	0.04
	Lonicera nitida E.H. Wilson	*	*
	Lonicera pileata Oliv.	*	*
	Lonicera tatarica L.	3	0.11
	Nerium oleander L.	15	0.57
	Osmanthus fragrans Lour.	8	0.30
	**Parthenocissus quinquefolia* (L.) Planch.	25	0.95
	Philadelphus coronarius L.	85	3.22
	Photinia x fraseri 'Red Robin'	5	0.19
	Physocarpus opulifolius Maxim.	15	0.57
	Pittosporum tobira (Thunb.) W.T.Aiton	1	0.04
	Prunus laurocerasus L.	80	3.03
	Punica granatum L.	45	1.70
	Pyracantha coccinea M.Roem.	35	1.32
	Rosmarinus officinalis L.	6	0.23
	**Ruscus aculeatus* L.	3	0.11
	**Spiraea cantoniensis* Lour.	101	3.82
	Spiraea japonica L.f.	68	2.57
	**Spiraea x vanhouttei* (Briot) Zabel	160	6.06
	**Symphoricarpos orbiculatus* Moench	128	4.84
	**Vinca major* L.	*	*
	**Vinca minor* L.	*	*
	Vitex agnus-castus L.	1	0.04
	Weigela florida (Bunge) A. DC.	23	0.87
	**Wisteria sinensis* (Sims) Sweet	15	0.57
	Yucca gloriosa L.	86	3.26
	**Periploca graeca* L.	5	0.19
1–2 Very low	**Trees**	**203**	**7.59**
	Abies alba Mill.	21	0.79
	Abies cephalonica Loudon	2	0.07
	Abies concolor (Gordon) Lindl. ex Hildebr.	3	0.11
	Abies pinsapo Boiss.	15	0.56

Allergenicity level	Species and infraspecific taxa	Specimens	
		number	%
	Cedrus atlantica (Endl.) Manetti ex Carrière /'Glauca'/	84	3.14
	Cedrus libani A.Rich	2	0.07
	Ginkgo biloba L. ♀	2	0.07
	Taxus baccata 'Fastigiata' ♀	5	0.19
	Acer negundo L. ♀	16	0.60
	Broussonetia papyrifera (L.) L'Hér. ex Vent. ♀	1	0.04
	Ficus carica L.	26	0.97
	Ilex aquifolium L. ♀	4	0.15
	Cydonia oblonga Mill.	1	0.04
	Maclura pomifera (Raf.) C.K.Schneid. ♀	3	0.11
	Morus alba 'Pendula' ♀	1	0.04
	Salix babylonica L./var. *pekinensis* 'Tortuosa'/ ♀	17	0.64
	Shrubs	**165**	**6.25**
	Chaenomeles × superba (Frahm) Rehder	10	0.38
	Mahonia aquifolium (Pursh) Nutt.	145	5.49
	Mahonia japonica (Thunb.) DC.	2	0.08
	Nandina domestica Thunb.	5	0.19
	Viburnum opulus 'Roseum'	3	0.11
	Total trees	**2673**	**100**
	Total shrubs	**2642**	**100**

Note: ♂ – males, ♀ – females, * – groundcover, liana, hedges.

Table 2. Characteristics of the most-frequent allergenic tree and shrub species in Park Sveti Vratch.

Species	Origin	Foliage permanence		Sexual/ reproductive characteristics		Allergenicity level
		Ever green	Deciduous	Monoecious or hermaphrodite	Dioecious	
Trees						
Platanus orientalis	Med		✓	✓		High
Ulmus minor	Eur-Med		✓	✓		High
Chamaecyparis lawsoniana	West. N-Am	✓		✓		High
Thuja occidentalis	N-Am	✓		✓		High
Cupressus sempervirens	E-Med-Anat-OT	✓		✓		Very high
Juglans regia	Eur-As/Paleo		✓	✓		High
Alnus glutinosa	Med-CAs		✓	✓		Very high
Celtis australis	Med		✓	✓		High
Acer negundo	Adv (N-Am)		✓		✓	♂ Very high ♀ Very low
Fraxinus americana	Adv (N-Am)		✓	✓	✓	♂ High ♀ Very low

Species	Origin	Foliage permanence		Sexual/ reproductive characteristics		Allergenicity level
		Ever green	Deciduous	Monoecious or hermaphrodite	Dioecious	
Ailanthus altissima	Adv (N-Ch)		✓	✓	✓	♂ High ♀ Very low
Cupressus arizonica	N-Am	✓		✓		Very high
Shrubs						
Forsythia × intermedia	Hybr		✓	✓		Moderate
Syringa vulgaris	Carp-Bal		✓	✓		Moderate

Note: the abbreviations for 'Origin' are in accordance with Conspectus of the Bulgarian vascular flora: Distribution maps and floristic elements (Assyov et al. 2012).

the park landscape, with its centennial specimens, reaching more than 2.5 m DHB and huge crown volumes. Many authors (Varela et al. 1997, Alcázar et al. 2004, Gioulekas et al. 2004, Biçakçi and Tosunoğlu 2019, Cariñanos et al. 2020) suggest that *Platanus* genus is one of the main causative agents of tree pollen allergy in the Mediterranean area because of its abundant presence in urban green infrastructure.

Alnus glutinosa, *Betula pendula* and *Juglans regia* are highly allergenic species. *Alnus glutinosa* and *Juglans regia* were growing along the river banks, where the air circulation is good and probably pollen can be dispersed by air currents.

Another species with high degree of allergenicity is *Olea europaea* (Gioulekas et al. 2004), which was presented by a single specimens in the park theritory, but is quite common as an orchard tree in the private gardens in Sandanski. Its frequent use should be reconsidered**.**

Fraxinus is another Oleaceae genus emitting highly allergenic pollen (Guerra et al. 1995). The reproductive attributes of ash trees show a continuum from pure male to pure female trees with a variety of intermediate hermaphrodite stages that can vary between years (FRAXIGEN 2005). Moreover, the cross-reactivity that can be established between Oleaceae species (e.g. Fraxinus spp., *Forsythia* spp., *Ligustrum spp., Syringa* spp. etc.) due to the presence of shared allergens must be taken into consideredation (Pajarón et al. 1997, Rodríguez et al. 2001). The large scale use of non-native species from genus *Fraxinus* (63 specimens) in comparison to the use of native ones (12 specimens) is not appropriate. Moreover, the native species *Fraxinus angustifolia* subsp. *oxycarpa* and *Fraxinus ornus* have lower degree of allergenicity and higher ornamental qualities.

Cypress trees are also prominent ornamentals in the park Sveti Vratch scenery. However, they have very high value of allergenic potential (Gioulekas et al. 2004, Charpin et al. 2013, Biçakçi and Tosunoğlu 2019). Additionally, another Cupressaceae taxa – *Cryptomeria japonica* (Japanese cedars), is one of the most severe allergenic tree species (Okuda 2003, Yamada et al. 2014). Cross-reactivity among some Cupressaceae species from genera *Juniperus, Thuja, Cupressus* and *Chamaecyparis* has been suggested (Schwietz et al. 2000).

Acer negundo, *Ailanthus altissima* and *Robinia pseudoacacia* generate pollen sensitization (Ballero et al. 2003, Compés et al. 2006, Ribeiro et al. 2009). Disurbingly, these species are wide spread and are included in the list of invasive alien species in Bulgaria and EU (Petrova et al. 2012). Ailanthus could be monoecious, andromonoecious, hermaphrodite or dioecious tree (Kowarik and Säumel 2007, Holec et al. 2014).

Male individuals from Moraceae family (e.g. *Maclura* spp., *Broussonetia* spp., *Morus* spp.) dispersing their allergenic pollen can cause severe respiratory symptoms (Navarro et al. 1997, Qazi et al. 2019).

The most frequent shrub species had moderate to low/very low alergenicity potential (tables 1 and 2). Only 4.9 % of shrub specimens had a high to very high allergenicity level. It is not recommendable to establish hedges or large plantations of allergenic shrubs, even sheared, as they are a rich local source of pollen emitted at human height (Alcázar et al. 1999).

Urban green zone allergenicity

The value of overall allergenic risk that species pose in the area they grow Urban Green Zone Allergenicity (IUGZA) (Cariñanos et al. (2019) considers a variety of plant characteristics and factors to evaluate the allergenic potential of urban green spaces. The lack of phoenological and aerobiological monitoring records and clinical statistics on patient sensitization in this region so far prevent estimation of IUGZA for Park Sveti Vrach and assessment of the real threat of allergenic pollen taxa to park visitors.

The density of existing trees, species richness and the Shannon Index are the characteristics and factors that most affect the final allergenicity value (Cariñanos et al. 2019). Results from the inventory of arboreal ornamentals in Sveti Vrach Park show a very good value of species richness – 199 taxa. Taking into account the abundance of species, Shannon's Index = 4.17 indicate a great diversity due to the high number of taxa and the evenness of park woody ornamentals – 0.79. A very low average density of 79.8 trees/ha was established.

Therefore, the recommendations given by Cariñanos and Casares-Porcel (2011) for designing and maintenance of green spaces with low allergy impact should be followed in Sveti Vrach Park management. They include: increased urban biodiversity, controlled introduction of exotic species, invasive species management, botanical sexism avoidance, avoidance of formation of large focal pollen sources and screens, selection of low pollen producing species, adoption of appropriate management and maintenance strategies, involvement of experts and consultants at the planning process, establishment of local by-laws guidelines for low allergy impact design of urban green spaces. Additionally, McInnes et al. (2017) suggest developing detailed maps at national level of allergenic pollen producing taxa in order to improve understanding of relationships between allergenic pollen exposure and human health outcomes.

Conclusions

Sveti Vrach Park in Sandanski is a real 'dendrological hotspot' in a town – natural sanatorium – for the treatment of respiratory diseases, climatotherapy and spa resort of international importance. Considering the allergenic potential value of species, which could represent a risk to the citizens and visitors, almost half of the trees and 4.9 % of shrub specimens had high or very high allergenic potential. The highly allergenic members of Cupressaceae, Betulaceae, Moraceae, Oleaceae and Salicaceae families should be avoided in the design of new green areas and revitalization of existing ones in the urban park territory, unless female specimens, female cultivars or species with lower degree of allergenicity are selected. *Acer negundo, Ailanthus altissima* and *Robinia pseudoacacia*, as an invasive species should be controlled and even removed and destroyed in some of the park sectors.

Phoenological and aerobiological monitoring records of allergenic pollen producing plants and clinical statistics on patient sensitization in Sandanski region should be done by specialists in order to identify the taxa that triggers pollenosis or asthma on a local level and to assess the real threat of allergenic pollen taxa to park visitors.

The research on allergenic woody plant taxa in Sveti Vrach Park provides useful risk assessment information to medical practitioners and local authorities responsible for city landscaping. Urban green infrastructure planning and managment should consider the allergenicity criterion. Interdisciplinary expertise in these processes is crutial for establishment of sustainable low allergy-risk green spaces.

Despite the fact that alergennic pollen can be transported in long distances by the wind, steps can be taken to minimize the pollen load and potential hazard in surrounding urban area. Providing public information on that subject could also help affected citizens with pollenosis and pollen-related asthma to take self-imposed preventative measures.

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CROWN BIOMASS OF SCOTS PINE AND BLACK LOCUST IN NORTHERN STEPPE OF UKRAINE

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Abstract

The formation of live biomass of forests is premised on many factors, among which the dominant ones are stands growth conditions and biometric characteristics. The purpose of this research is to develop reference materials for the evaluation of crown live biomass structural components for stands of black locust (*Robinia pseudoacacia* L.) and Scots pine (*Pinus sylvestris* L.) for the conditions of the Northern Steppe of Ukraine. The field research has been carried out in Scots pine and black locust stands of artificial origin subordinated to the State Forest Resources Agency of Ukraine. The research employed the classical forest mensuration techniques for collecting and processing experimental material on 40 test plots. The modelling was preceded by the stage of statistical analysis of the input dataset, which characterizes the live biomass of forest stands' crown. Mean age, diameter, and height of stands, as well as their relative stocking, have been identified as informative and statistically significant predictors. We have developed the two- and three-factor allometric regression equations for assessment of crown live biomass components – branches over bark and foliage based on the biometric indices of stands. The adequacy of the models has been estimated by the determination coefficient (0.47–0.71) and 'Fisher's F-test. In black locust and Scots pine stands with the same average diameter and relative stocking, live biomass of branches and foliage will be larger in those, where the mean height of the main species is smaller. The obtained allometric equations for assessing the biophysical indices of live biomass subject to this research can be used for practical forestry purposes in the course of forest inventory, determination of harvest volumes, and assessment of ecosystems services.

Keywords: allometric models, biometric parameters, branch biomass, foliage biomass, *Pinus sylvestris*, *Robinia pseudoacacia*.

Introduction

Assessment of the forests' ecosystem services is based on the understanding of peculiarities of accumulation of aboveground biomass of stands, including growth features, biological productivity patterns, and disturbance regimes (Lakyda et al. 2019). This is especially valid for the assimilative fraction, which secures the process of photosynthesis (Uri et al. 2012, Nord-Larsen and Nielsen 2015, Schepaschenko et al. 2018**).** The approach in forest management currently widely applied for assessing actual biomass of forest-forming tree species both at the level of an individual tree and of a tree stand is based on mathematical models. Allometric regression equations, highlighted in the scientific literature, have been developed taking into account the corresponding edaphic, climatic, and forest growth conditions, and, therefore, they cannot be used for performing this kind of assessments in Ukrainian Steppe, since the factors mentioned above are crucially important from the point of view of the formation of live biomass in forest stands.

Investigation of peculiarities of functioning of tree crowns is an important question from the point of view of automation and facilitation of interpretation of 'stands' parameters, as well as silvicultural, bioecological, and physiologic research linked with advanced research of 'forests' structure and functions.

The study of the forest crown biomass is mainly based on the three most common methods – the method of an average tree; the method of ratios of basal areas of sample trees and stands, and mathematical regression modelling (Satto and Madgwick 1982, Lakyda 2002, Romanovsky et al. 2009).

The most universal and reliable, from the standpoint of statistical significance, is the method by which sample trees are selected in proportion to their diversity in trunk diameter. After that, the obtained initial data are aligned using regression analysis (Lakyda 2002; Vasylyshyn 2016). To evaluate the live biomass components of tree crowns, pipe models developed on the basis of the theory of balance of the xylem transport system of substances in plants have been proposed. In the formulated theory of pipe models, or 'tube models', a tree trunk and a branch are considered as a set of elementary xylem vessels, each of which ends with an elementary mass of the assimilating apparatus (Vasylyshyn 2016).

Usoltsev (1997) states that searching for a rational determination of the number of TSPs and the maximum number of sample trees that should ensure the accuracy of assessing the components of stand live biomass should be based on the variability of the main biometric indices – age, site index, forest type, and cost (time, funds) of field stage of research. Studies by Usoltsev (1999) show that an increase in the number of average sample trees over three units has a very limited on the accuracy of the experiment.

Black locust (*Robinia pseudoacacia* L.) is an introduced species natively present in Northern America. In Ukrainian Steppe, it is used for planting forests and shelterbelts. The multidirectional vectors of management potential of this species are addressed in the scientific publications **(**Rédei 2002, Sitzia et al. 2016, Yuan et al. 2018, Zverkovskyy et al. 2018). Mainly in the introduction ranges, black locust stands of artificial origin are created to stabilize the disturbed and eco-hazardous environment – Loess Plateau (Yuan et al. 2018, An et al. 2019), Western Donbas (Zverkovsky et al. 2018), to obtain the main forest resource – high-quality wood (Rédei et al. 2014, Carl et al. 2018) as a fast-growing 'energy species' for biomass production (Dünisch et al. 2010, Böhm et al. 2011, Mantovani et al. 2014) and as sources of biologically active secondary metabolites (Tian and McLaughlin 2000). Aboveground live biomass of black locust has a substantial business potential due to its compositional structure of biologically active metabolites, mainly polyphenols – robinetin and dihydrobinetin (Tian and McLaughlin 2000, Sanz et al. 2011, Sergent 2014). Bostyn et al. (2018) present the establishment of industrial production of extracts from aboveground live biomass enriched in flavonoids for use in pharmacology. The effectiveness of the use of black locust leaves as biomonitors for assessing air pollutants, the presence of which in the air was determined according to the protocol EPA 3550 C 2007 has been established (Capozzi et al. 2017). Live biomass of Robinia leaves is proposed for use as a raw material for animal fodder by ensiling using lactic acid bacteria (Ni et al. 2016).

Scots pine is a native tree species in Ukrainian Steppe. However, the vast majority of the areas occupied by this species are of artificial origin.

The total area of Scots pine stands within the Dnipropetrovsk region is 21,472.9 ha, black locust stands occupy 17,683.7 ha, which accounts for 24.6 % and 20.3 % of the total forest-covered area, respectively. In conditions of the Steppe zone, black locust is used as a tree species for remediation of industrially damaged areas, with 35.2 % of the total area of *Robinia*'s stands in the study region (Lokhmatov and Gladun 2004, Gulchak et al. 2011). For Scots pine stands, the main functional categories are recreational and therapeutic forests (31.1 % from the total area in the region).

The crown components – branches and foliage – are, in fact, a border zone between the biotic sphere and abiotic medium (Lawlor 1995). They form the inner surface of the forest canopy, act as a filter of atmospheric precipitation, numerous emissions, light and heat fluxes, wind, and noise. The assimilative part is a zone where the main physiological processes are realized in a vegetal organism – photosynthesis, respiration, and transpiration determine links of material transformation and energy fluxes. Currently, the questions related to the research of forest crowns' parameters have been highlighted in publications by scientists from many countries (Parresol 1999, Pretzsch et al. 2002, Sytnyk et al. 2017). Usoltsev (2013) notes that the parameters of absorption of solar radiation by a tree canopy mainly depend on the fractional biomass structure.

In Scots pine trees, the upper and middle sections of a crown play a leading role in the energy transformation, since they have the highest needle packing. Nearly 40 % of active photosynthetic radiation is absorbed by upper crown layers (Usoltsev 2013). The upper crown layer 2–3 m thick is characterized by the highest proportion of foliage (Dilis 1969).

Lokhmatov and Gladun (2004) noted a decrease in the number of shoots and leaves per unit crown volume in black locust, resulting in a progressive increase in its openness and transparency of crowns. Along with the age-driven increase of openness and transparency of crowns of this tree species, intensification of sodding is associated, which leads to a decrease in tree growth.

Due to the absence of similar scientific research for Scots pine and black locust stands in the natural zone of Ukrainian Steppe (Schepaschenko et al. 2019), a study of dependencies of crown live biomass components on biometric indices of stands and development of appropriate reference materials for evaluation are recognized as the main purpose of this research.

Material and Methods

Collection of the experimental material has been carried out on 40 temporary sample plots (TSPs) on the territory of the Northern Steppe of Ukraine, 20 TSPs for each species under research (Fig. 1). The results obtained after the establishing 40 TSPs were statistically significant, as TSPs are homogeneous by their origin and types of forest growth conditions, microclimatic and edaphic conditions. To develop models for assessing the biomass of stands' crowns, we also used biometric data of the actual stands in the steppe zone with the corresponding number of stands of 4739 for black locust and 5158 for Scots pine (Anonymous 2020).

The TSPs were established and processed in accordance with the methodology proposed by Lakyda (2002). Establishing TSPs started with selecting representative forest sites in Scots pine, and black locust stands. Afterward, the compliance of the pre-selected sites with the requirements for forest mensuration sample plots

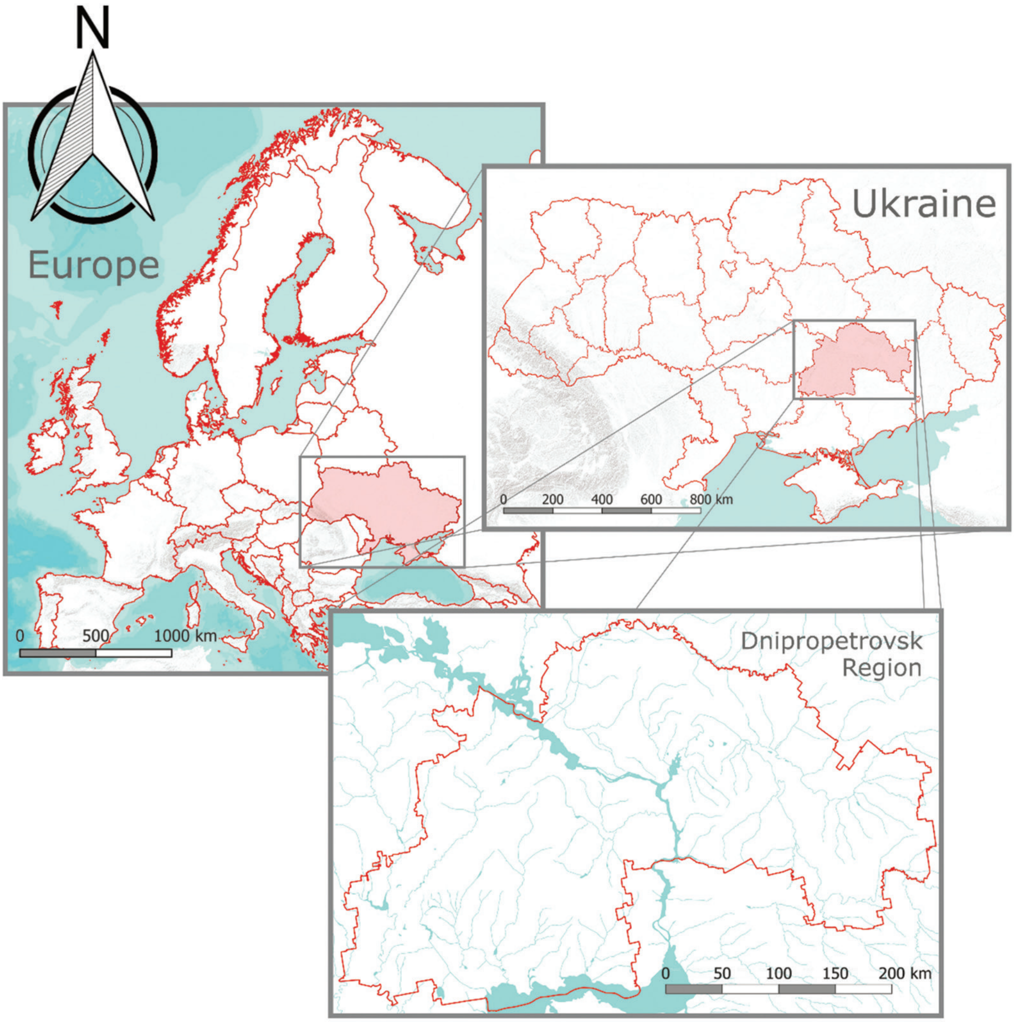

Fig. 1. Study region.

was evaluated.

The predominant shape of TSPs was rectangular or square, with a minimum width and length of sides 50×50 m. After demarcation, a forest mensuration description of TSPs is performed, which begins with determining the terrain characteristics. The TSPs are laid out in single-layer stands located within flat terrain. Tree enumeration in the form of a list of trees at the TSP with identification of their species and diameter at breast height was further carried out.

In order to determine the crown components biomass for the stands under research, at least 3 sample trees (STs) were selected and felled at the TSPs. MTs were selected by the method of tree diameter classes (Zou et al. 2015). The size and shape of the selected sample trees were close to the average for the entire set of trees at a TSP. For each growing sample tree, we have measured the diameter at breast height, and height of a tree. After cutting down, ST's total height and crown length were measured. Further, the crown of each ST was separated from its stem, and the weight of each separate crown component (tree greenery, live and dead branches) was determined by weighing.

To evaluate the density of wood and bark of branches, 2–3 cm thick samples were cut out from the middle parts of live branches of different lengths. The samples were annotated, put into a hermetic bag, and sent to the laboratory. Wood and bark density of the samples was further determined using their measured weight and volume. Subsequently, the samples were dried in an oven at 105 ºC until oven-dry. Re-weighing determined the weight of the samples in an oven-dry state. The volume of samples was determined using the original method proposed by Lakyda (2002).

To determine the percentage of foliage in tree greenery and the dry matter content in foliage, from the lower, middle, and upper parts of an ST's crown, three sample branches were randomly selected. The freshly cut sample branches were weighed, then the foliage fraction was separated, and the defoliated shoots were re-weighed. To determine the dry matter content, three 10-grams samples of fresh foliage were formed, each of which was dried in a drying oven until oven-dry and then weighed again. The total live biomass of ST's foliage in the oven-dry state was calculated employing the content of dry matter in foliage.

To identify the patterns of distribution of biometric indices of stands, determine homogeneity of the experimental data, carry out statistical analysis, we have formed an experimental dataset containing the following parameters: A – mean age of the main tree species, years; D –mean diameter of the main tree species, cm; H – mean height of a stand, m; P – relative stocking of a stand; Ph_{br} – live biomass of crown branches; Ph_f – live biomass of foliage.

Based on the initial data, a regression analysis was performed, which resulted in two- and three-factor allometric equations (1–3) for assessment of the structural crown components of stand live biomass for the two studied tree species:

$$Y = a \times D^b \times H^c \quad (1)$$

$$Y = a \times A^b \times D^c \times H^d \quad (2)$$

$$Y = a \times D^b \times H^c \times P^d \quad (3)$$

Impact factors in the mathematical models are represented by mean age, height, diameter of the main tree species, and relative stocking of a stand.

Biometric parameters of the distribution of absolute values of the main biometric indices of the studied stands are presented in Table 1.

Table 1. Descriptive statistics for the investigated species.

Stand biometric characteristics	Value		Statistics			
	min	max	$\dot{X}$	SD	Skewness	Kurtosis
			Scots pine			
A, years	9	87	54.5	22.9	-0.808	-0.116
D, cm	4.6	40.2	21.8	8.0	-0.416	1.604
H, m	2.8	30.5	19.4	7.2	-1.005	1.225
P	0.13	1.04	0.56	0.17	0.349	3.803
			Black locust			
A, years	3	82	44.8	21.4	-0.175	0.139
D, cm	3.9	26.0	17.7	6.8	-0.704	-0.096
H, m	5.2	21.5	15.7	5.0	-1.065	0.661
P	0.21	1.08	0.74	0.24	-0.039	0.676

Note: SD – standard deviation.

When analyzing the descriptive statistics, the considerable variance of biometric indices of stands becomes obvious. This fact testifies to the significant variability of the experimental data for all the parameters under scrutiny. The statistical sample of experimental data with a degree of freedom of 20 units has a critical value of skewness 0.711 ($p \leq 0.01$), and kurtosis 0.877 ($p \leq 0.01$) (Yancev 2002).

The obtained (actual) skewness values for most of the investigated biometric parameters of stands (except for the mean height for the two species and the mean age for Scots pine) do not exceed the critical values. The predominance of negative values of skewness (except for the relative stocking of Scots pine stands) indicates right-handed asymmetry, testifying a predominance of actual values greater than the arithmetic mean. The excess of the critical value of kurtosis is actually recorded for all the biometric indices of Scots pine stands, whereas such facts have not been registered for black locust stands.

Results and Discussion

The regression coefficients for estimation of Scots pine and black locust crown biomass are presented in Table 2, graphical interpretation of the regression equations residuals – in figures 2 and 3.

The calculated equations have statistically significant coefficients of determination. Their actual values do exceed the critical value (R^2 = 0.25), which justifies their application as the reference materials for assessing live biomass of structural components of tree crowns of Scots pine and black locust in forest stands of the Northern Steppe of Ukraine.

Determination of adequacy of the obtained models by the Fisher criterion has allowed us to establish statistically significant equations: for fractions of branches and needles of Scots pine – model (3) where the driving factors are mean height and diameter of the main species and relative stocking of a stand. For the two studied live biomass fractions (branches and leaves) of crowns of black locust stands,

Table 2. Crown biomass equations for investigated species.

Crown component	a	SE[a]	b	SE[b]	c	SE[c]	d	SE[d]	R^2	F-test F_{real}	F-test F_{table}
Pinus sylvestris L.											
$Y = a \times D^b \times H^c$ (1)											
Needle	0.596	1.020	-0.665	0.695	**1.244**	**0.263**	-	-	0.58	0.55	3.29
Branches	0.465	0.817	-0.273	0.672	**1.128**	**0.245**	-	-	0.59	0.91	3.29
$Y = a \times A^b \times D^c \times H^d$ (2)											
Needle	1.043	1.443	-0.463	0.417	0.292	1.046	0.683	0.540	0.61	0.44	3.29
Branches	0.578	1.136	-0.087	0.452	-0.132	1.043	1.027	0.587	0.59	0.73	3.29
$Y = a \times D^b \times H^c \times P^d$ (3)											
Needle	0.335	0.406	1.220	1.028	-0.233	1.029	**1.842**	**0.576**	0.53	2.38	3.24
Branches	0.477	0.555	1.785	0.968	-0.778	0.968	**1.503**	**0.515**	0.54	**6.66**	3.24
Robinia pseudoacacia L.											
$Y = a \times D^b \times H^c$(1)											
Foliage	1.585	1.073	**-1.800**	**0.823**	2.122	1.010	-	-	0.47	2.85	3.29
Branches	3.234	0.378	-1.341	1.016	**2.936**	**1.294**	-	-	0.69	**8.68**	3.29
$Y = a \times A^b \times D^c \times H^d$ (2)											
Foliage	1.485	0.989	-0.470	0.305	-0.919	1.018	1.869	1.013	0.56	**5.33**	3.24
Branches	0.244	0.418	-0.088	0.378	-1.255	1.133	**2.951**	**1.339**	0.69	**5.55**	3.24
$Y = a \times D^b \times H^c \times P^d$(3)											
Foliage	1.930	1.729	**-1.792**	**0.842**	2.052	1.031	0.072	0.225	0.49	1.79	3.24
Branches	0.465	0.818	-1.228	1.046	2.610	1.336	0.464	0.420	0.71	0.50	3.24

Note: differences at the significance level α = 0.05 are in bold; a–d – regression coefficients; SE – standard error; R^2 – correlation coefficient; F-test – Fisher's test.

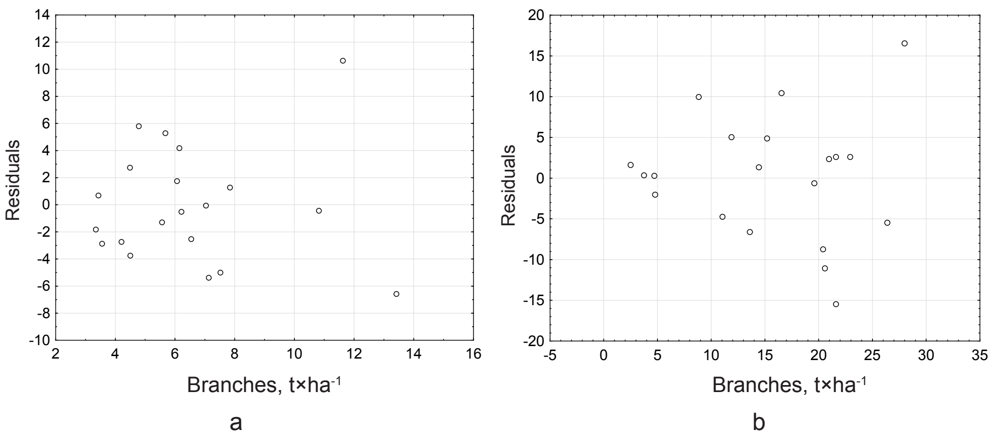

Fig. 2. Residuals of regression equation $Y = a \times D^b \times H^c$ for estimation of branch biomass: a – Scots pine, b – black locust.

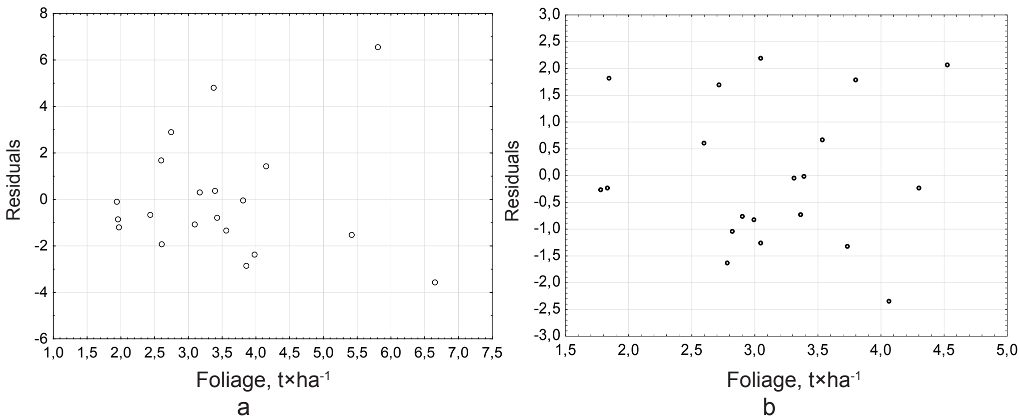

Fig. 3. Residuals of regression equation $Y = a{\cdot}D^b{\cdot}H^c$ for estimation of foliage biomass: a – Scots pine, b – black locust.

the equation (2) accounting for age, mean diameter, and height of a stand was assessed as the adequate one. In other models, the calculated value of Fisher's criterion did not exceed its critical value, which, given the number of degrees of freedom (n) = 20 and the number of driving factors (m) = 2, is equal to 3.29, m = 3–3.24.

The two-factor models developed for assessing live biomass of the studied crown components of both Scots pine and black locust are characterized by a negative coefficient for the mean diameter.

Three-factor models for assessing live biomass of Scots pine branches and needles had negative coefficients when inputting the parameters of age (2) and height (3) of a tree. It should be noted that a negative coefficient for the mean diameter is available to assess the parameter of the branch component parameter.

In the three-factor model (2) for assessing live biomass of Robinia branches and leaves, given the introduction of driving factors – age, mean diameter and mean height, the first two arguments have negative values of regression coefficients.

The basis of changes in the morphological structure of black locust stands' crowns is the course and rate of formation of shoots, live biomass production and increase in leaf area, the nature of their onset to maximum levels.

With the development of black locust stands, there is a change in the energy of trees' growth: the absolute levels of shoot formation and leaf area in trees to a certain age increase and decrease later on. The size of trees increases all the time, and the corresponding growth of shoots and leaves does not occur. This gap increases with age: the number of shoots and leaves per unit volume of a crown decrease, which causes a progressive increase in the finesse and transparency of a crown. Intensification of sod formation in stands of this tree species is associated with the increase in the finesse and transparency of tree crowns, which leads to a weakening of tree growth (Lokhmatov and Gladun 2004).

Figures 4 and 5 visualize the dependency of live biomass of crown components on the main biometric parameters – diameter, height, and age of a stand. Table 3 presents mathematical models of the mentioned dependencies in an analytical form.

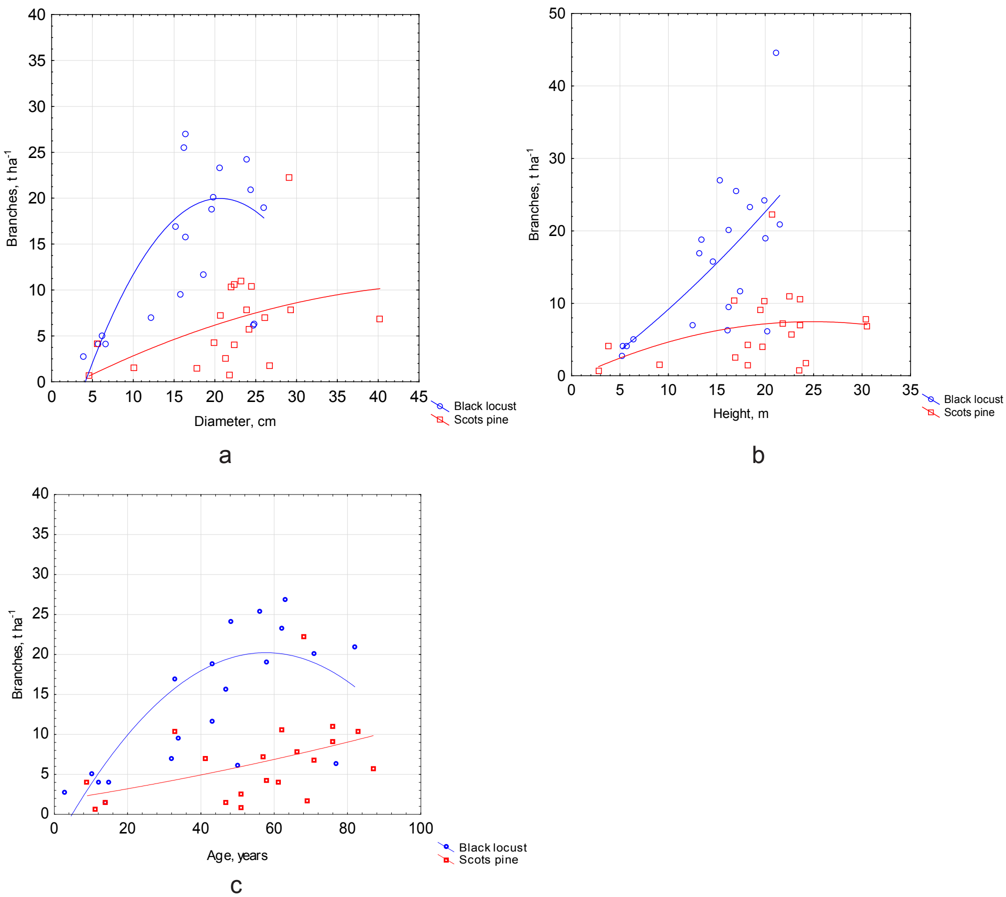

Fig. 4. Non-linear models for Scots pine and black locust describing relationships between branch biomass and mean stand diameter (a), height (b), and age (c).

The weight of the photosynthetic surface fraction is more variable than the stem or total aboveground biomass, as this component responds to age and changes in stand structure (Forrester et al. 2017). A more significant variance of the obtained values of crown live biomass components, as compared with trunk components, is also registered in our research. Within the European continent, a sufficient number of aggregated data on the assessment of crown live biomass for Scots pine stands have been reported to date (Solymos 1973, Albrektson 1980, Vyskot 1983, Bugaev et al. 1988, Santa Regina et al. 1997, Nord-Larsen and Nielsen 2015). A considerable number of equations for Scots pine 'stands' live biomass dependency on biometric parameters is presented in the scientific literature. However, many types of models are still insufficiently described (Forrester et al. 2017). To compare the results for pine stands in the natural zone of Ukraine's Northern Steppe obtained in this research, it was interesting to compare them with the data for the regions, which would have some similarity of natural and climatic conditions. When com-

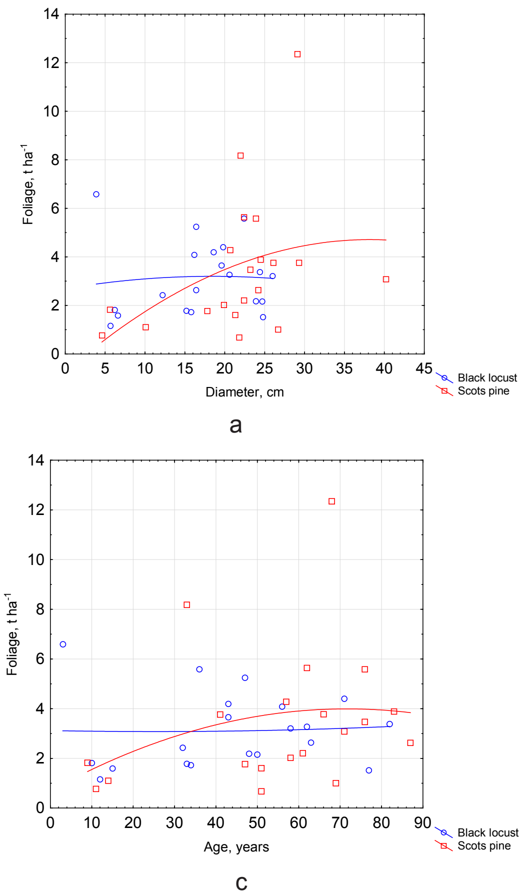

Fig. 5. Non-linear models for Scots pine and black locust describing relationships between foliage biomass and mean stand diameter (a), height (b) and age (c).

Table 3. One-variable regression models for estimation of biomass crown components.

Component of crown	Scots pine		Black locust	
	Equation	R^2	Equation	R^2
Branches	$Ph_{br} = 0.256 \cdot D^{0.964}$	0.49	$Ph_{br} = 0.758 \cdot D^{1.023}$	0.54
	$Ph_{br} = 0.616 \cdot H^{0.703}$	0.41	$Ph_{br} = 0.442 \cdot H^{1.272}$	0.60
	$Ph_{br} = 0.304 \cdot A^{0.702}$	0.45	$Ph_{br} = 1.074 \cdot A^{0.681}$	0.52
Foliage	$Ph_f = 0.741 \cdot D^{0.820}$	0.28	$Ph_f = 1.906 \cdot D^{0.142}$	0.28
	$Ph_f = 0.516 \cdot H^{0.608}$	0.22	$Ph_f = 1.331 \cdot H^{0.286}$	0.38
	$Ph_f = 0.878 \cdot A^{0.591}$	0.23	$Ph_f = 2.516 \cdot A^{0.030}$	0.26

Note: R^2 – determination coefficient.

paring our results with those reported by Dimitrov et al. (1987) for the conditions of Plovdiv (Bulgaria) for coniferous stands in the same age group (30 years), this research shows 35 % higher values for foliage, but one third lower for branches.

When compared with the results by other researchers, the data for 30-years old pine stands did not have a clear trend and showed both similarities (Vyskot 1983, Usoltsev 2010) and significant differences (Bugaev et al. 1988). 11-years old pine stands on sandy soils in Hungary (Solymos 1973), Kazakhstan (Usoltsev 1998), steppe part of Russia (Bugaev et al. 1988) were characterized by significantly higher amounts of crown live biomass (over six times) compared to the results obtained for Northern Steppe of Ukraine.

The aboveground live biomass of black locust stands is assessed to a much smaller extent as compared to Scots pine (Converse and Betters 1995, Burner et al. 2006, Unruh Snyderet al. 2007). Kostov et al. (1992) present the results for Bulgaria (Byala Slatina), noting the following live biomass amounts of crown components (*D* – 14 cm, *H* – 16 m): branches – 11.4 t·ha^{-1}, leaves – 1.9 t·ha^{-1}. In forests of Slovakia, black locust stands with *D* – 18 cm, *H* – 20 m were characterized by the following amounts of live biomass of crown components: branches – 22.7 t·ha^{-1}, leaves – 2.55 t·ha^{-1} (Bencat 1989). Under edaphic and climatic conditions of the Northern Steppe of Ukraine, with a modal relative stocking of 0.9 and equivalent biometric parameters (*D* = 14 cm, *H* = 16 m) and (*D* = 18 cm, *H* = 20 m), the fraction of crown branches amounts to 21.2 t·ha^{-1} and 27.7 t·ha^{-1}, leaves – 4.2 t·ha^{-1} and 4.4 t·ha^{-1}, respectively. This means that for black locust stands in the study region, the amounts of live biomass of crowns' structural components are higher.

Conclusions

The research results on the characteristics of live biomass of structural components of crowns of tree stands can be used for monitoring the state of forests and assessing ecosystem services in the Northern Steppe of Ukraine, including by remote methods, as well as in the course of interpretation of the annual production; in the modelling of the transformation of the active photosynthetic radiation by forest canopy in the analyzed species.

In black locust stands in an even-aged stand under the condition of constant height, an increase in mean diameter leads to a decrease in live biomass of three crown components. At the same time, in Scots pine stands with the same average diameter and height, live biomass of branches and foliage will be larger at a younger age. In other cases, for three-variable models for Scots pine, where the stand's diameter, height, and relative stocking were selected as input parameters, live biomass of crown's components decreased when the stand's mean height decreased at a constant diameter and relative stocking.

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GROWTH AND DEVELOPMENT OF SPECIES AND HYBRIDS OF THE GENUS *POPULUS* L. IN THE RIGHT-BANK FOREST-STEPPE OF UKRAINE

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Abstract

The growth and development of vegetative and generative organs of *Populus alba* L., *P. balsamifera* L., *P. bolleana* Lauche, *P.* × *canescens* (Ait.) Smith., *P. deltoides* Bartr. ex Marsh., *P. nigra* L., *P. nigra* cv. Italica (Du Roi) Moench, P. *simonii* Corr., *P. tremula* L., and European-American *P. deltoides* × *P. nigra* hybrids 'I-45/51', 'Blanc du Poitou', 'Gelrica', 'Marilandica', 'Robusta', 'Serotina', 'Tardif de Champagne', and *P.* × *hybrid*a clones 'Weresina' and 'Witschtejna' were studied outdoor for seven years under current climate change. The development of most *Populus* species and hybrids started in the second half of March – beginning of April when the phase of bud swelling was reached in correspondence of a significant temperature sum from 68 to 112 °C, depending on the species/hybrid considered. In most species, flowering began on April 20th and lasted 9 to 16 days, temperature sum of 93–139 °C, depending on the species. The duration of linear shoot growth was shorter in native species of the genus *Populus* (118–130 days) than in introduced species (135–140 days). Fruiting began when the temperature sum reached 217–234 °C in *P. tremula*, while in *P. balsamifera* it required 413 °C. The other species required an intermediate temperature sum (299–329 °C) to reach the same phase, which was accumulated in the first ten-day period of June. Discolouration of leaves depended on the date of first autumn frosts and during the study period was shifted by two to three weeks later. After analyzing the timing of phenological phases of growth and development, species and hybrids were assigned to phenological groups characterized by early, mid or late beginning and end of the vegetation period. The knowledge of thermal requirements for key phenological stages is expected to be useful for modelling and predicting growth, flowering and fruiting of poplars, which is crucial to create efficient and productive bioenergy plantations and to take anti-allergic measures during fruiting in the Right-Bank Forest-Steppe of Ukraine.

Key words: development, growth, phenology, poplar, thermal time.

Introduction

The seasonal development cycle of plants reflects the interaction of their genotype with the environment. Seasonal cycles play an important role in many sectors of the economy, in particular, decorative gardening, landscaping, phytomelioration, and forestry. Studies of growth and development cycle of introduced plants in connection with climate change are particularly relevant. In the annual cycle of plant development, the periods of entry and exit from deep dormancy and the end of vegetation are essential. The characteristics of seasonal development determine the indicators of ecological plant resistance, i.e. winter hardiness and drought resistance, which define the ability of the species to survive and reproduce in various growth conditions (Lapin 1967, Sergeeva 1971, Sabirova et al. 2018). The results of long-term phenological observations demonstrate successful plant adaptation to soil and climatic conditions.

Phenological observation makes it possible to have an approximate idea of the degree of morpho-physiological periodicity compliance in the annual cycle of development of a particular tree species (Lapin 1967, Sergeeva 1971). Even a short-term (2–3 years) phenological observation provides sufficient material for preliminary assessment of the adaptation of trees and shrubs of the same genus but of different geographical origins (Lapin 1967). Information on the phenological rhythms of growth and development of a particular species is essential for planning and managing new tree plantations.

In Ukraine, the rhythms of development of *Populus* were investigated by Redko (1975), Sluchyk (2000), Litvin et al. (2009), and Danylchuk (2013). In Russia, the seasonal growth rhythm of poplars was examined by Demidova and Durkina (2013), and Medveva and Srodnykh (2014). In other areas with a cold temperate climate, phenological studies of species, hybrids and clones of the genus *Populus* were conducted in Scandinavia by Stener and Westin (2017), in Belgium by Broeck et al. (2003), in Estonia by Lutter et al. (2016), in Iran by Lashkarbolouki et al. (2011) and in Canada by Huybregts et al. (2007).

In Donetsk, seasonal rhythms of development of *Populus* species have been studied by Moskalevskyj and Dem'janenko (2014). The time course of flushing of vegetative and generative buds of six poplar clones ('Strilopodibna', 'Kanadska × Balzamichna', 'Slava Ukrayini', 'Guliver', 'Volosystoplidna' and 'Novoberlinska-3') in Kyiv was analyzed by Khoma and Kutsokon (2019). In Ivano-Frankivsk, the influence of technogenic pollution of urban ecosystems on growth, development and morphometric parameters of shoots of *P. × berolinensis* Dippel. and *P. simonii* Corr. was studied by Sluchyk (2000). The influence of heavy metals on the growth of *P. nigra* cv. Italica (Du Roi) Moench, *P. deltoides* Bartr. ex Marsh., *P. simonii*, *P. candicans* Ait., *P. bolleana* Lauche and Euro-American hybrids in Kryvyi Rih region was investigated by Danylchuk (2013). The phenology of 10 species and clones of poplar in the town of Ovruch in Zhytomyr region was studied by Redko (1975). The phenology of the development of black and balsam poplars hybrids in the forests of the green zone of Kyiv was analyzed by Litvin et al. (2009). Zalesny et al. (2009) studied hybrids of poplar *Aigeiros × Tacamahaca* in the USA (*P. deltoides × P. suaveolens* ssp. *maximowiczii*; *P. nigra × P. suaveolens* ssp. *maximowiczii*) and Euro-American hybrids.

In Scandinavia, field studies on shoot growth of hybrid aspen (*P. tremula* L. × *P. tremuloides* Michx.) and poplar clones (*P. trichocarpa* Torr. & A. Grey, *P. balsamifera* L., *P. maximowiczii* A. Henry and their hybrids) showed considerably higher growth for hybrid aspen than for poplars (Stener and Westin 2017). The researchers link the data obtained with the genetic features of aspen.

Broeck et al. (2003) found out that the flowering time of *P. nigra* and the male clone of *P. nigra* cv. Italica were not synchronized, but *P. nigra* and *P. × canadensis* freely interbred during flowering, which is a potential threat to native plantings of *P. nigra* in Belgium.

In Estonia, Lutter et al. (2016) investigated the phenology of hybrid genotypes of *P. tremula* of northern and southern origin and found out that the genotypes of southern origin were more adapted to climate change as they had a 27-day longer growing season and a tremendous increase in annual shoots.

Lashkarbolouki et al. (2011), in a 22-year-long phenological study, established the phases of the beginning of vegetation, flowering, fruiting and leaf fall of seven *P. deltoides* clones in Iran, and identified the genotypes with different duration of the growing season and productivity.

In the province of Alberta in Canada, the phenology of native and introduced poplars (*P. balsamifera*, *P. davidiana, P. tremuloides*) was assessed to study the occurrence of spontaneous hybridization between them (Huybregts et al. 2007).

The characteristics of seasonal growth of native *P. tremula* L. and introduced *P. alba* L., *P. laurifolia* Ldb., *P. tristis* Fish., *P. deltoides*, *P. nigrosuaveolens* Bogd. in order to obtain poplar in the conditions of the Far North of Russia were studied by Demidova and Durkina (2013). Medvedeva and Srodnykh (2014) reported about the phenological development of *P. alba*, *P. berolinensis*, and *P. alba × P. bolleana* in the city of Yekaterinburg (Russian Federation).

At the biological research station of Bila Tserkva National Agrarian University (Bila Tserkva NAU), the formation of a collection of plants of the genus *Populus* began in 2007. The first cuttings of Euro-American hybrid poplars were obtained from the National University of Bioresources and Nature Management and planted by the Associate Professor I. D. Vasylenko (Ishchuk 2017). In the spring of 2013, the collection was supplemented with introduced and hybrid poplars from the Kryvyi Rih Botanical Garden, and in 2017, with hybrid poplars from the Ukrainian Order ‘Badge of Honor’ Research Institute of Forestry and Agroforestry named after G. M. Vysotskyi of the State Agency of Forest Resources of Ukraine and the National Academy of Sciences of Ukraine (Kharkiv).

Currently, the collection includes 25 species and hybrids, most of which are represented by male individuals aged 1 to12.

Due to global climate change, the timing of phenological events is shifting (Parmesan and Yohe 2003). In Europe, especially during the early spring, global warming changes the phenology of trees (Cleland 2007, Shevchenko 2014), which can lead to different reactions both in individual plants and at the population level. Temperature and photoperiod drive the beginning of the shoot development and growth as well as the acclimatization process (Polgar and Primack 2011). As climate-induced changes in phenology become more apparent over time, the need to quantify these changes is becoming increasingly urgent.

The research aims to find out the difference between the rhythms of development and growth, including flowering and fruiting, of species and hybrids of the genus *Populus* and to establish their dependence on the sum of significant temperatures.

The study is based on collection and

analysis of multi-year phenological observations and meteorological data and is intended to continue a long time into the future. This knowledge is expected to be useful for establishing bioenergy plantations and designing a functional plan of anti-allergic measures in the Right-Bank Forest-Steppe of Ukraine. The data obtained will also help to reduce the risk of spontaneous hybridization of native and introduced poplar species and clones.

Plant Material and Study Sites

We name species and hybrids of the genus *Populus* according to the consensus document on poplar biology (OECD 2000). The International Poplar Commission (IPC) has recognized male specimens of *P. nigra* cv. Italica as the standard for phenological observations and all species and hybrids of the genus are compared with it.

The objects of the research were native juvenile individuals that have not entered the period of flowering and fruiting, pregenerative and generative individuals aged 50 to 80 of the species *P. alba*, *P. nigra*, and *P. tremula*, grown in the urban tree plantations of Bila Tserkva. Among the introduced species, the objects of the research were those introduced since centuries, such as *P. balsamifera*, *P. × canescens*, *P. bolleana, P. deltoides, P. simonii,* and *P. nigra* cv. Italica, aged 60 to 120. Their adult fruiting individuals are widely represented in the plantings of the State Arboretum 'Oleksandriya' of the National Academy of Sciences of Ukraine and Bila Tserkva area. Besides, species and hybrids that were more recently introduced (*P. suaveolens* Fisch., *P. trichocarpa* Torr. et Gray, *P. × euramericana* cv. I-45/51, *P. × euramericana* cv. Blanc du Poitou, *P. × euramericana* cv. Gelrica, P. × *euramericana* cv. Marilandica, *P. × euramericana* cv. Robusta, P. × *euramericana* cv. Serotina, *P. × euramericana* cv. Tardif de Champagne, and *P. × hybrida* clones 'Weresina' and 'Witschtejna') and were mainly represented by young plants up to 12 years old at the biological research station of Bila Tserkva NAU, were also the objects of the research.

Various genetic types, such as greyzem forest, chernozem, sod-meadow, chernozem and meadow, silt-gley and swampy soils, represent the soil cover of the territory. Grayzem forest soils prevail and are characterized by a low degree of saturation, as well as CA+ and MD+. As a result, the process of humification and nitrification is slow. The amount of organic soil (humus) in the surface layer with a thickness of 10–20 cm is 2.7 %. In the horizon of 50–60 cm, it is reduced to 1 %. The boiling line of carbonates is located at a depth of 120–150 cm. In terms of mechanical components, these are medium-loamy soils.

Methodology

We carried out phenological observations during 2012–2018 according to the methodology approved by the Council of Botanical Gardens of the USSR (Lapin 1975) and some modifications according to Weih (2009) and Ghelardini et al. (2014).

The average dates of all phenological phases studied for 7 years (2012–2018) and calculated as the arithmetic mean of the numerical values are represented in the research.

The phase of generative buds swelling was assumed for the beginning of vegetation since generative buds are the first to start vegetation in species of the genus *Populus*. The date of generative buds swelling was recorded after their size was increased by 3–5 mm. The scales covering the buds begin to disperse, and green stripes become visible between them. Therefore, we considered this phase to be the beginning of vegetation. We evidenced the opening phase of the vegetative buds when the integumentary scales parted, and the green tips of the leaves looked noticeably from the top of the bud. In most species, it occurred after or simultaneously with flowering.

We documented the phase of flowering during the period of pollination of male catkins and full opening of female catkins. The fruiting date was considered to be the beginning of splitting of the capsules and appearance of fluff. The end of fruiting was marked by complete fluff release and beginning of abscission of catkins. The features of the seed maturation were yellowing, splitting of the capsules and appearance of fluff.

We evidenced the phase of the linear growth of shoots at the beginning of May. The dynamics of the seasonal growth of shoots was determined by the method of Molchanov and Smirnov (1967). The length of the shoots of a sample of mature trees and 1–8-year-old seedlings was estimated with a measuring ruler every ten days from May 1st till September 1st. Every time ten shoots in the lower, middle and upper zones of the crown of a sample plant were measured. The lignification of shoots was documented by the presence of a protective cork tissue under the bark, which, unlike the epidermis, was darker and had the same density along the entire length.

We evidenced the phase of changing leaf colour from green to yellow after the first autumn frosts in September-October. The phase of leaf fall was determined in October-November. The beginning of any phenological phase was defined as the date when its diagnostic signs were visible in at least 1/3 of a plant's organs.

Having analyzed the periods of vegetation during the years 2012–2018, we defined three phenological groups of species and clones of the genus *Populus*, namely with early (March 1–20), mid (March 21–April 10) and late (April 11–May 1) beginning of vegetation. Similarly, three groups of species with early (August 28–September 30), mid (October 1–25) and late (October 26–November 15) end of vegetation were identified. Based on the timing of the beginning and end of vegetation periods, five phenological groups were distinguished in the studied taxa: (EM) species with early beginning and mid-end, (EL) early beginning and late end, (MM) mid start and mid-end, (ML) mid beginning and late end, and (LE) late beginning and early end.

The sum of significant temperatures (starting with +5 °C) was calculated based on average daily temperatures (data from Bila Tserkva meteorological station) to detect the temperature threshold for the phenological phase of development and growth of the studied species. Average sums of temperatures for each phenological phase during 2012–2018 were identified using the software Statistica and are presented in the research findings.

Results

Climate conditions in the study period

The climate of the Right-Bank Forest-Steppe of Ukraine is moderately continental and relatively mild, with an average annual temperature of 8.4 °C and fluctuations in some years from 5.8 °C to 8.5 °C (Shevchenko 2014). However, over the years of research (2012–2018), the average annual temperature fluctuated between 8.8–9.9 °C, which is higher than the long-term average by 0.4–1.5 °C (Table 1, Fig. 1). During the research period, the winter seasons of 2012, 2013 and 2018 with the minimum air temperature of –25.4 °C were the coldest ones, whereas the overall maximum temperature was +32.9 °C. During the study period, the registered number of frost days was in the range of 110 and 163 (on the average 137). There were 63 days on average when the temperature remained below zero throughout the day. Soil temperature always equal to or below 0 °C was recorded from December to March to a depth of 0.4 m. Late spring frosts occurred in 2013 (May 2–4, to –2.5 °C) and 2017 (May 10–11 on the soil surface to –7.9 °C). The average daily air temperature was always above +5 °C from April 6–8 and dropped below 5 °C from October 26–28. The average length of the growing season was 210 days.

Table 1. Indicators of air temperature, precipitation and relative humidity recorded.

Year	Month												Year average
	1	2	3	4	5	6	7	8	9	10	11	12	
Average monthly air temperature, ºC													
2012	-4.3	-9.9	2.2	11.8	18.2	20.3	22.4	19.7	16.4	10.2	4.5	-5.2	8.9
2013	-4.0	0.3	0.2	9.9	18.5	20.7	19.6	18.9	12.4	9.5	6.5	-0.9	9.3
2014	1.8	-2.0	-3.9	8.5	16.2	17.2	21.1	20.5	14.2	6.7	1.8	-2.0	8.8
2015	-1.3	-1.1	4.1	9.4	16.3	19.6	20.9	20.6	17.6	6.8	4.6	1.7	9.9
2016	2.5	-1.4	2.8	12.4	14.7	19.8	21.6	20.4	15.5	6.4	3.7	-0.3	9.8
2017	-1.5	-0.5	3.1	10.3	14.9	19.7	20.3	21.7	16.3	8.4	3.3	-1.1	9.6
2018	-2.3	-4.2	-2.1	13.3	18.4	20.1	20.5	21.5	16.2	9.9	-2.1	-3.1	8.8
2012–2018 average	-1.3	-2.6	0.9	10.8	16.7	19.6	20.9	20.5	15.5	8.3	3.2	-1.6	9.3
Long term average	-4.6	-2.1	1.6	8.4	14.8	17.8	19.0	18.4	14.9	7.8	2.4	-2.0	8.4
Average monthly precipitation, mm													
2012	32.0	27.0	23.0	38.0	45.0	25.0	69.0	29.0	91.0	37.0	29.0	138.0	583.0
2013	40.0	39.5	92.3	29.6	87.1	20.7	29.6	68.0	155.5	5.7	83.6	64.2	550.7
2014	40.3	33.2	49.1	50.1	118.1	76.5	93.2	48.0	37.6	14.5	63.5	17.7	678.2
2015	16.6	34.1	81.4	15.3	37.6	32.8	82.4	2.4	36.5	31.3	75.0	25.7	662.6
2016	21.5	25.1	48.1	65.8	107.5	50.1	29.9	23.0	3.1	50.6	72.4	35.6	532.7
2017	22.6	18.1	63.1	22.7	42.9	18.8	83.0	16.0	33.2	79.8	71.3	28.4	499.9
2018	32.6	34.6	74.0	8.1	22.8	58.7	128.4	23.9	47.9	22.0	43.2	54.0	550.2
2012–2018 average	29.4	30.2	61.6	32.8	65.6	40.4	73.5	30.0	57.8	34.4	62.4	51.9	579.6
Long term average	35.0	33.0	30.0	47.0	46.0	73.0	85.0	60.0	35.0	33.0	41.0	44.0	562.0
Average monthly humidity, %													
2012	95.0	75.0	73.0	73.0	60.0	68.0	67.0	72.0	67.0	82.0	76.0	77.0	75.0
2013	86.0	77.0	71.0	66.0	62.0	72.0	72.0	70.0	84.0	79.0	80.0	83.0	72.0
2014	82.0	77.0	74.0	84.0	72.0	72.0	71.0	66.0	67.0	69.0	90.0	81.0	65.0

Year	Month												Year aver-age
	1	2	3	4	5	6	7	8	9	10	11	12	
2015	81.0	78.0	71.0	58.0	60.0	60.0	65.0	59.0	68.0	69.0	81.0	80.0	73.5
2016	80.0	75.0	72.0	62.0	75.0	72.0	66.0	66.0	63.0	78.0	83.0	79.0	72.5
2017	82.0	76.0	71.0	56.0	59.0	61.0	63.0	64.0	66.0	82.0	85.0	80.0	70.4
2018	81.0	87.0	86.0	58.0	56.0	64.0	77.0	66.0	71.0	78.0	82.0	87.0	74.4
2012–2018 average	83.4	77.6	74.0	65.3	63.4	67.0	68.7	66.1	69.4	76.7	82.4	81.0	71.8
Long term average	86.0	85.0	82.0	68.0	64.0	66.0	67.0	68.0	73.0	80.0	87.0	88.0	76.0

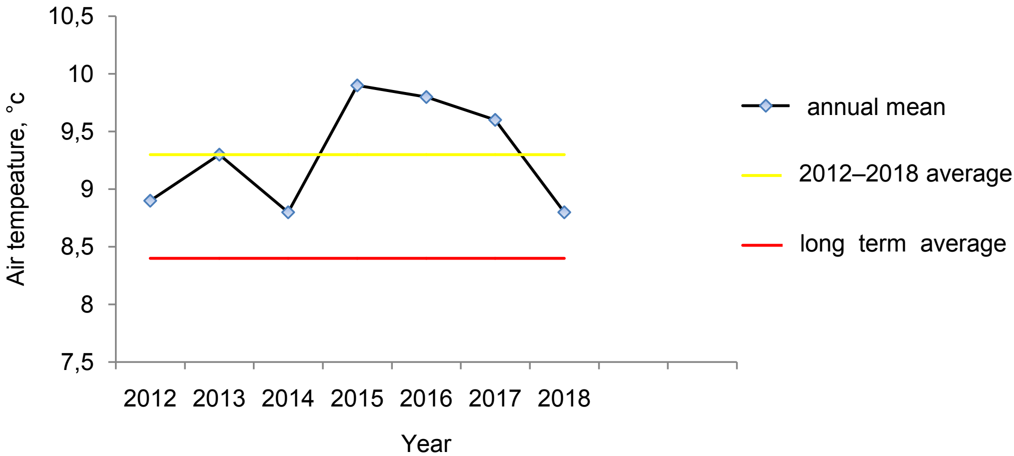

Fig. 1. Fluctuation of average annual temperature in the study years (2012–2018) and its relation to the average one in Bila Tserkva.

The average annual precipitation in the study region is 580 mm, about 80 % of which falls as rain. Precipitation that was lower than average annual amount insufficient precipitation was observed in 2016 and 2017, with annual precipitation being 532.7 mm and 499.9 mm accordingly. We evidenced the best highest amount of precipitation supply in 2014 (678 mm) and 2015 (662 mm). However, even in years with higher amount of precipitation, the distribution of precipitation by month during the year was uneven. We also registered the driest summer periods in 2015–2017 with the minimum amount of precipitation in May-September (191.7 mm, 213.6 mm and 193.9 mm). The snow cover lasted for an average of 60 days, with a maximum thickness of 20 cm, and was not stable. Winters of 2017–2018 were characterized by a thin snow cover and a decrease in frosts days (Ishchuk and Ishchuk 2018).

Growth and development of *Populus* species and hybrids

During 2012–2018, we followed the seasonal development progress of species and hybrids. We defined the following phases of development: swelling and budding of vegetative and generative buds, flowering, linear shoot growth, fruiting, woodiness of shoots, leaf colour change and leaf abscission. The timing of phenological phases of development and growth of vegetative organs of trees is shown in Figure 2.

According to the methodology of phenological observations (Lapin 1975), the starting point of woody plant vegetation is considered to be the phase of swelling of vegetative buds.

In *P. tremula* and *P.* × *canescens*, the appearance of inflorescences and the discharge of the integumentary scales occurred much earlier than the vegetative buds' swelling and bud bursting. In most species of the genus *Populus*, the beginning of the swelling phase of vegetative buds occurred from March 12 to March 25 on average, depending on species and weather conditions. In native species, the vegetation started first in *P. tremula* and *P.* × *canescens* (on average on March 3rd), while *P. nigra* was the last species to start flushing on March 15th. Among the introduced *Populus,* the earliest bud swelling was in *P. deltoides* (on March 25th), while the latest was in *P.* × *hybrida* 'Witschtejna' (on April 5th). In most species of the genus, *Populus* leaf budding was recorded from April 12 to April 28. The first leaves appeared in *P. balsamifera* and *P. alba* (April 17–20 on average). On April 22–26, leafing occurred in *P. nigra* cv. Italica, and then in the other species until the end of April.

After emergence from the bud, the leaf blade of poplars grows by dividing and stretching of cells in the basipetal direction. However, these processes take place with different intensity depending on the influence of external factors, species, leaf age, and tissue considered. Crit-

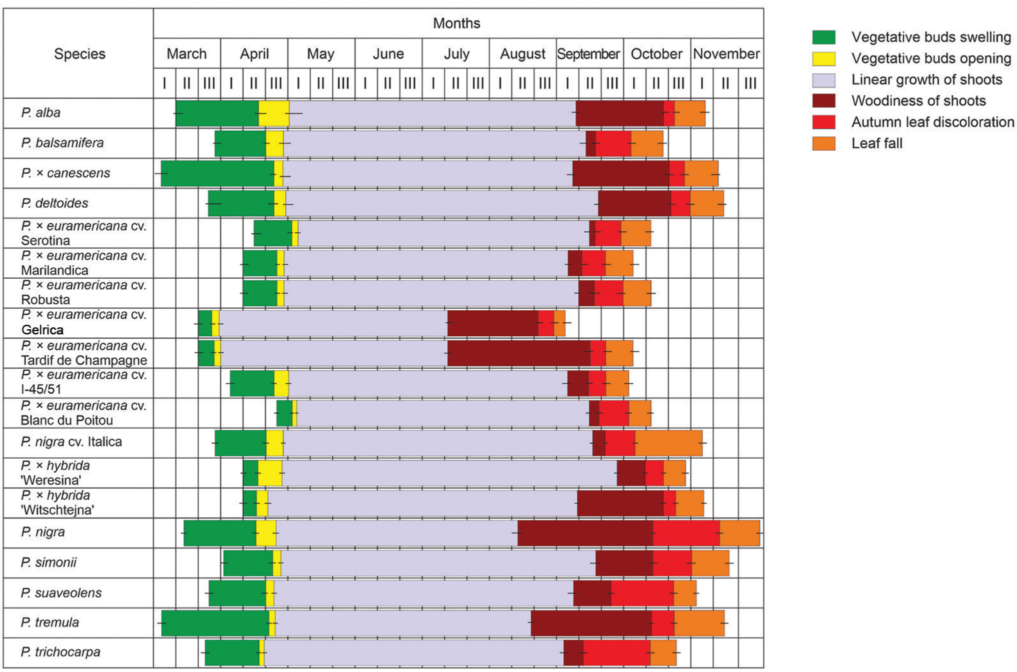

Fig. 2. Seasonal development of vegetative organs.

ical physiological processes occur most actively during leaf growth, which is due to the predominance of cell division over their extension. According to our observations, leaf growth is very intensive, especially during 10–15 days in late April-early May. The end of leaf growth occurred on average on May 10–15, in 20–25 days after leaf flushing depending on the species and weather conditions.

Most introduced species and hybrids enter this phase before mid-May. When the leaves are entirely separated, the stipules fall off. With the cessation of growth, the period of leaf ageing begins. In species with early vegetation period, it occurred on average on May 12–15, while in the rest of the species on May 20–25.

Predicting the linear growth phase is very important for identifying species that are promising for creating short-rotation poplar plantations for bioenergy. According to the results obtained, it was concluded that in the study area the linear growth of shoots in species of the genus *Populus* with early vegetation period begins on average on April 22–26, while in hybrids with late vegetation period, linear shoot growth starts on May 1–5.

The study of annual growth in seedlings of species and hybrids of the genus *Populus* showed that intensive growth of shoots occurred in May-June. In the second half of July and in August, the growth energy of all species and hybrids decreases, and at the end of August, it stops.

Euro-American hybrid 'Gelrica' finished its growth in 100 days, which was faster if compared to a maximum of 140 days in the *P. × hybrida* 'Weresina'. In most indigenous species of the genus *Populus*, the growth of shoots lasted for 118–130 days, while it was longer in introduced species (135–140 days) (Fig. 1). In native species, shoot growth rate peaks at the end of May-June, while in introduced species, and especially Euro-American hybrids, bud growth peaks later in June-July, and slows down in August.

Linear shoot growth was completed in July-September, however not simultaneously in all species and hybrids. Partial lignification of the shoots in the native species was observed at the end of June-July. Complete lignification of the shoots occurred in September and some species before the beginning of November.

As seen in Table 2, *P. trichocarpa* and clones of Euro-American hybrids 'Serotina', 'Marilandica', 'Tardif de Champagne', 'Robusta', 'Gelrica', 'Blanc du Poitou', *P. × hybrida* 'Weresina' and *P.* × hybrida 'Witschtejna' were characterized by the most massive increase at the young age. For other species, the average growth which ranges from 50 to 80 cm per year.

Variations in air temperatures determine the changes in rhythms of the plant life cycle, i.e. bud swelling and burst, beginning of flowering, and fruiting. The calendar dates of certain phenological phases are closely related to temperature conditions, which are the environmental cues for adjustment of biological rhythms of development and which can shift their timing by several days. However, it is possible to trace some trends in the flowering time of poplar species (Ishchuk 2019).

Based on the analysis of average daily temperatures and the timing of phenological phases in species and clones during 2012–2018, the average sum of significant temperatures above a threshold for the beginning of each phase of generative development was identified. Thus, depending on the species, the swelling of generative buds occurred when a temperature sum ranging from 68 to 112 °C was accumulate, bud opening occurred when

Table 2. Growth dynamics of the aboveground part of juvenile (1–3 years old) plants (Biological Research Station of Bila Tserkva, NAU).

Name of the species and hybrids	H_{2013}, cm	H_{2014}, cm	H_{2015}, cm	ΔH_{2014}, cm	ΔH_{2015}, cm	Height, cm			
						M	*m*	σ	*V*
P. alba	23.8	58.2	132.1	34.4	73.9	71.3	7.1	5.8	8.7
P. balsamifera	28.0	63.4	131.9	35.2	68.5	74.4	7.4	6.0	4.2
P. × canescens	26.3	62.9	135.4	36.6	72.5	74.8	7.4	6.8	7.3
P. deltoides	25.6	58.3	115.6	32.7	57.3	66.5	6.7	5.4	6.6
P. × *euramericana* cv. Serotina	28.5	92.0	189.9	63.5	97.9	103.5	10.4	11,2	5.4
P. × *euramericana* cv. Marilandica	32.5	91.8	195.1	59.3	103.3	106.5	10.7	8.6	7.1
P. × euramericana cv. Robusta	33.6	90.0	200.3	56.4	110.3	108.0	10.8	8.7	9.3
P. × euramericana cv. Gelrica	32.9	87.5	190.3	54.6	102.8	103.6	10.3	8.4	4.7
P. × euramericana cv. Tardif de Champagne	30.8	84.5	188.4	53.7	103.9	101.2	10.1	8.2	9.1
P. × euramericana cv. I-45/51	31.9	95.4	199.6	63.5	104.2	109.0	10.9	8.8	8.3
P. × euramericana cv. Blanc du Poitou	28.5	88.4	196.2	59.9	103.8	104.4	10.4	8.4	6.2
P. × hybrida 'Weresina'	36.7	102.8	198.3	66.1	95.5	112.6	11.3	9.1	11.4
P. × hybrida 'Witschtejna'	37.7	104.3	186.4	66.6	82.1	109.5	10.0	8.3	10.7
P. nigra cv. Italica	32.3	75.7	139.5	43.4	63.8	82.5	8.3	6.7	7.6
P. nigra	25.7	54.3	125.9	28.6	71.6	68.6	6.9	5.5	4.8
P. simonii	26.7	57.2	122.1	30.5	64.9	68.7	6.9	5,8	6.2
P. suaveolens	37.1	81.5	173.0	44.4	91.5	97.2	9.7	7.9	4.7
P. tremula	22.4	52.4	108.3	30.0	55.9	61.0	6.1	4.9	5.9
P. trichocarpa	38.9	109.5	194.2	70.6	84.7	114.2	11.4	9.2	7.7

Note: *M* is the arithmetic mean value, *m* is the error of the average value, σ is the mean-square deviation, *V* is the coefficient of variation.

a temperature sum from 84 to 128 °C was reached, while flowering began when a temperature sum of 93 to 140 °C was accumulated. The average sums significant temperatures for fruit set and fruit ripening ranged from 128 to 151 °C and from 299 to 394 °C respectively, while for fruit fall the sum needed was in the range 342–413 °C (Fig. 3). The only exception was *P. tremula*, which requires the lowest sum of threshold effective temperatures for bud swelling (22 °C), opening of generative buds (45 °C), flowering (54 °C), fruit ripening (226 °C) and fruit fall (245 °C).

The knowledge of thermal requirements for flowering and fruiting may enable the development of predictive models for forecasting the periods of poplar pollen and fluff spread, which is especially important for reducing the risk of allergic reactions in the human population. In this study, *P. tremula* was the first species to begin flowering in a few days between late March and the first ten days of April. *P. × canescens, P. alba,* and *P. nigra* followed in the last ten days of April. At the beginning of the last ten days of April,

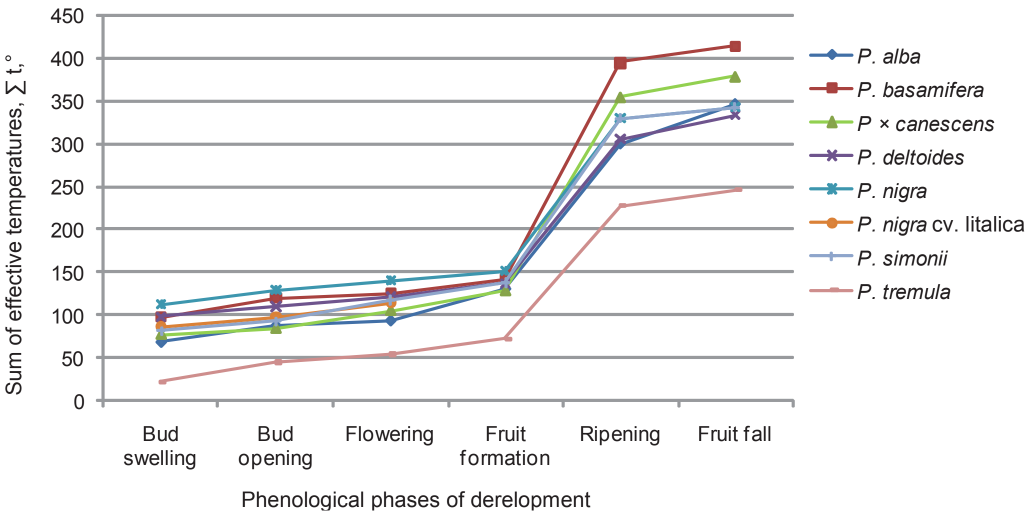

Fig. 3. Average indicators necessary for the beginning of phases of generative organs development.

P. simonii, P. deltoides, and *P. nigra* cv. Italica began to bloom almost simultaneously, while *P. balsamifera* started to bloom from April 25th. However, the leafing of the shoots began first in *P. balsamifera* (Fig. 4).

According to the studies of Starova (1980) and Barna (2002), the period from pollination to fertilization in plants of the genus *Populus* is not visually noticeable and lasts for 1 or 2 days. In the present study, flowering lasted on average from 6.9 days in *P. simonii* to 10 days in *P. tremula* (Ishchuk 2019). Juvenile plants only represent hybrid poplars in our collection, so we have not studied the biology of flowering and fruiting in those hybrids.

The issues of introgressive hybridization between introduced and native poplars, raised by some researchers from Belgium (Broeck et al. 2003) and Canada (Huybregts et al. 2007), seem not to be much relevant for Ukraine at present, because the native species, *P. alba,* and *P.* × *canescens* complete the flowering before introduced species start flowering, while in the case of *P. tremula* there is only a short period overlap.

In the genus *Populus*, the beginning of fruiting is considered to coincide with the beginning of capsules splitting and fluff's appearance. The end of fruiting is defined as the moment of complete fluff release and the beginning of abscission of fruit catkins. The signs of seed maturation are yellowing, splitting of the capsules and appearance of fluff. The timing of boxes' dehiscence is also extended, but much less than the timing of flowering. The fruit matures at the end of May, almost simultaneously in *P. nigra, P. alba, P.* × *canescens.* On June 15–20, they mature in *P. simonii* and *P. balsamifera* and are the latest.

In May, during 2012–2016 and 2018, the ten days average daily temperature was 2–4 °C higher than the long-term average of the month, so the timing of fruit maturation and fruiting lasted less. Among the species, the lowest sum of significant temperatures was required by *P. tremula* (226 °*C*), the highest sum was required by *P. balsamifera* (413 °*C*). To start fruit-

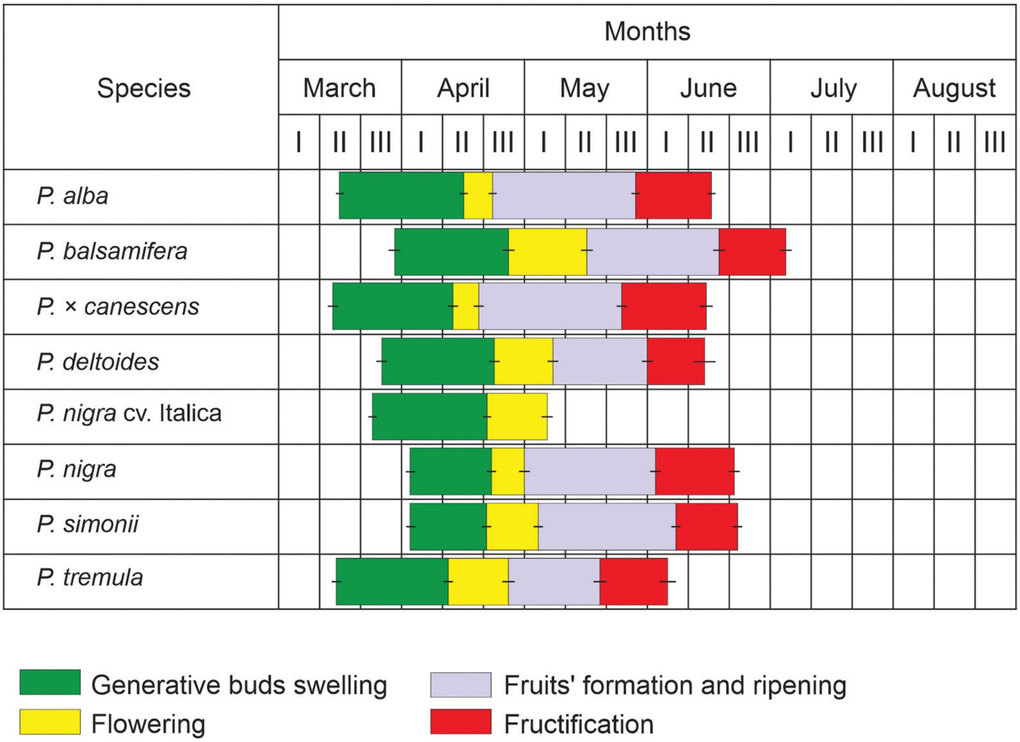

Fig. 4. Seasonal development of reproductive organs of introduced species and hybrids.

ing *P. alba* required a temperature of 299 °C, for *P. deltoides* – 305 °C, for *P. nigra, P. simonii* – 329 °C, and 354 °C for *P. × canescens.* Fruiting occurred between late May and the first ten days of June. It should be noted that in the study of Sluchyk (2000) and Danylchuk (2013), these indicators were higher by 30–50 °C in polluted urban systems.

According to our observations, the leaves change colour with the first frosts. In the past few years, there has been almost no frost in the research area of Bila Tserkva in September. Therefore, the change in leaves colour occurred in October and by the end of the month they fell off.

Based on the analysis of the timing of the start and end dates of the growing season of poplar species and hybrids in the period of 2012–2018, five phenological groups have been defined (Table 3, last column).

Euro-American hybrids 'Gelrica', 'Marilandica', 'Tardif de Champagne', and 'I-45/51' completed vegetation by the end of September. At the beginning of October, the vegetation period for hybrids 'Blanc du Poitou', 'Robusta' was finished. In mid-October, leaf fall was observed in *P. balsamifera, P. trichocarpa*, *P.* × *hybrida* 'Witschtejna', and *P.* × *hybrida* 'Weresina'. Other species of *P. suaveolens, P. trichocarpa, P. nigra* cv. Italica, completed vegetation by the end of October. Only at the beginning of November, defoliation was observed in *P. alba, P. tremula, P.* × *canescens,* P*. deltoides, P. simonii,* and *P. nigra*.

Table 3. Average start and end dates of vegetation of species and hybrids.

Species and hybrids	Vegetation			Phenological group
	Start, date	End, date	Duration, days	
P. tremula	3.03	20.10	231	EM
P. × canescens	3.03	25.10	236	EM
P. alba	10.03	20.10	224	EM
P. nigra	15.03	10.11	240	EL
P. balsamifera	28.03	1.10	209	MM
P. deltoides	25.03	25.10	214	MM
P. × hybrida 'Weresina'	10.04	15.10	181	MM
P. × hybrida 'Witschtejna'	10.04	12.10	185	MM
P. trichocarpa	28.03	15.10	202	MM
P. nigra cv. Italica	23.03	01.11	223	ML
P. simonii	1.04	28.10	20	ML
P. suaveolens	26.03	27.10	216	ML
P. × euramericana cv. Serotina	1.05	25.09	148	LE
P. × euramericana cv. Marilandica	15.04	20.09	158	LE
P. × euramericana cv. Robusta	16.04	28.09	165	LE
P. ×euramericana cv. Gelrica	25.04	28.08	125	LE
P. × euramericana cv. Tardif de Champagne	26.04	20.09	147	LE
P. × euramericana cv. I-45/51	22.04	20.09	151	LE
P. × euramericana cv. Blanc du Poitou	1.05	30.09	153	LE

Note: EM – with the early start and mid-end, EL – an early start and late end, MM – mid start and mid-end, ML – mid start and late end, LE – late start and early end.

Discussion

The research results on the timing of the phenological phases of growth and development of species and hybrids of the genus *Populus* in Bila Tserkva area are consistent with the data obtained by Litvin et al. (2009), who studied the phenology of poplars in Kyiv. When adapting to climatic and environmental conditions, the phenological phases of hybrids can be reduced or prolonged.

In general, the duration of the growing season of *P. balsamifera, P. simonii, P. suaveolens, P. trichocarpa, P. nigra* and *P. nigra* cv. Italica in our research was similar to the data of Litvin et al. (2009). Due to climate change resulting in the warming that was observed during 2012–2018 (Table 1, Fig. 1), the duration of the growing season in poplar increased by 7–10 days if compared to Redko (1975) studies, which were conducted in the mid-1960s in Kyiv area.

Khoma and Kutsokon (2019) studied the phase of budding indoors on cut branches. According to them, the delay of this phase is explained by insufficient light in the room. The photoperiod's effect on the duration of the growing season is confirmed in the studies of Gill et al. (2015) and Way and Montgomery (2014).

According to Medvedev and Srodnykh (2014), the most stable phenological development in Yekaterinburg was shown by *P. alba*, which vegetation period lasted only for 148 days. Hybrid species *P. berolinensis* are characterized by a more extended vegetation period, namely 153 days. Species of the genus *Populus* are winter-hardy, but leaves and young non-woody shoots can often be damaged by spring and autumn frosts (Ishchuk and Ishchuk 2018). In the course of the research, we noted an increase in air temperature. In September of 2012, 2015–2018, the air temperature was even higher than the annual mean temperature, and autumn frosts, that usually occur in this season (Chirkov 1986), were absent. During these years, the phase of leaf discolouration was shifted by 2–3 weeks later.

A more accurate indicator for analyzing phenological phases is the sum of significant temperatures accumulated by each species of poplar at the beginning of each phase. The results of the calculations of the sum of significant temperatures in the research conducted in Bila Tserkva area almost coincided with similar experiments carried out by Redko (1976) in the city of Kyiv for the phases of swelling and opening of generative buds, flowering and fruiting in *P. alba, P. nigra,* and *P. nigra* cv. Italica.

The data obtained on the growth of shoots of Euro-American hybrids 'Serotina', 'Marilandica', 'Robusta', 'Tardif de Champagne', 'I-45/51', 'Blanc du Poitou' coincide with the data recorded by Litvin et al. (2009), who studied the development of clones of these plants in the city of Kyiv. At the same time, Zalesny et al. (2009), who explored the behaviour of poplar hybrids *Aigeiros* × *Tacamahaca* from the northern regions of the United States, transplanted to more southern latitudes, recorded a delay in bud growth in the spring and an early stop in the fall, which led to a decrease in productivity. In our research, shoot growth on 1- to 3-year-old seedlings was most intense in May-June. In Euro-American hybrids, the increase was much higher than in pure species. Among the species, the introduced *P. suaveolens* and *P. trichocarpa* were identified as the most promising for cultivating plantations. Long-term observations of *P. trichocarpa* have confirmed that this species is promising for its stability, growth energy and productivity in the conditions of the Far North of Russia (Demidova and Durkina 2013)

The duration of the growing season depends on biological characteristics of the plant, climate zone and weather conditions of the growing year. This vegetation period for poplars is much more extended than that for other species of woody plants in temperate climates. The daylight factor also plays an essential role here. In this study, the vegetation end in certain species and hybrids of the genus *Populus* was observed at the end of September. However, the majority of species completed the vegetation in the second half of October or in November.

Based on the meta-analysis Gill et al. (2015) concluded that the autumn leaf ageing phase had been significantly delayed at 25–49° north latitude. Moreover, the city of Bila Tserkva, where we conducted the study, is located at 49°47′44″ north latitude. At this latitude, plants are more sensitive to temperature. In more northern latitudes, photoperiod affected the ageing of leaves. The absence of constant links between air temperature and leaf ageing time indicates that various factors can affect autumn ageing. The date of the first autumn frosts is variable in temperate and boreal ecosystems and does not always correspond to a linear cooling trend (Tuskan et al. 2006, Way and Montgomery 2014). Our observations also confirm this fact.

Conclusions

We found out that despite significant differences in the temperature regime in individual years, each species and hybrid of the genus *Populus* is characterized each year by approximately the same amount of sufficient (above +5 °C) temperature sums at which it is possible to predict the start and end of the flowering, seed maturation and fruiting phases. For species it is possible to calculate the sum of significant (above +5 °C) temperatures needed to reach the studied phenological phases. During the study years, the sum of significant temperatures for each phase ranged from 5–20 °C, depending on the study type and year. However, the development phases timing varied from 5 to 10 days, and the duration of the flowering and fruiting phases was 7–12 days, depending on the study type and year.

We defined that most species development starts in the second half of March-beginning of April with the phase of bud swelling when a sum of significant temperatures of 68–112 °C, depending on the species, has been accumulated. Budburst and leaf emergence was recorded in the second half of April in correspondence with an accumulation of a temperature sum of 45–118 °C, depending on the species.

Fruiting began when the temperature sum reached 217–234 °C in *P. tremula*, while in *P. balsamifera* the start of fruiting required 413 °C. The other species (*P. nigra, P. simonii, P. deltoides, P*. × *canescens*) required an intermediate temperature sum (299–329 °C) to reach the same phase, which was accumulated at the beginning of June.

The analyses showed that in native species linear growth of shoots lasts for 118–130 days, while it is longer in introduced species (135–140 days). The Euro-American hybrid 'Gelrica' finished its growth in 100 days, which was faster if compared to a maximum of 140 days in the *P.* × *hybrida* 'Weresina'.

Discolouration of leaves depended on the date of first autumn frosts and during the research was shifted by two to three weeks later. We defined the phenological groups of species with early, mid and late beginning and end of vegetation period after analyzing the timing of phenological phases of growth and development of each taxa.

Thus, taking into account the sum of significant temperatures accumulated over a certain period, it may be possible to model and predict the timing of growth, development, flowering, and fruiting phases of plants of the genus *Populus*. These indicators could be considered when creating bioenergy plantations of poplars and in order to develop anti-allergic measures.

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DIVERSITY, GENETIC RESOURCES AND CONSERVATION OF *TILIA* SPECIES IN BULGARIA

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Abstract

Species of genus *Tilia* (lime) still did not gain the attention they deserve by researchers and forest managers. Their importance as a significant part of forest biodiversity is expected to increase, especially in the light of changing environment, due to climate change and other factors. We present a mini-review about the genetic resources of *Tilia* species in Bulgaria. The survey starts with species diversity, including the standing taxonomic problems, and continues with genetic variation. A thorough evaluation of lime species' genetic resources and their conservation in Bulgaria is performed, facilitated by a specially developed database. Knowledge gaps are identified and some domains for prospective studies are outlined.

Key words: *ex situ*, genetic diversity, *in situ,* Lime species, taxonomy.

Introduction

Limes (genus *Tilia* L.), known also as Linden and Basswoods, are valuable noble hardwood species in Europe due to their economic importance and significance as an integral part of forest biodiversity. They are especially valued for the fragrant and nectar-producing flowers, valuable timber and excellent ornamental properties. The flowers are used in phytotherapy, production of herbal teas and tinctures, but are also exceptionally important for bee-keeping and honey production. The timber is soft, resistant to splitting and used for various purposes, ranging from wood carving to producing artists' charcoal and fuel wood (Delkov 1992, Svejgaard Jensen 2003, Yurukov and Panayotov 2015). Even though currently the *Tilia* species do not enjoy great attention by researchers and forest managers, their significance as a part of the boreal and temperate broad-leaved forests is expected to increase, especially in the light of the changes in environmental conditions due to climate change (Radoglou et al. 2009, De Jaegere et al. 2016). Therefore, studies of their ecology, regeneration (natural and artificial), habitats and response to silvicultural treatments are essential for their successful management and conservation (Petkova et al. 2005, Sultanova et al. 2020). Central to these studies is the assessment of their genetic resources (Maurer and Tabel 1995a, Alexandrov and Dobrev 2011, Black-Samuelsson 2012, Gil and Zajaczkowski 2017). The objective of the present study was to evaluate the state of the knowledge of *Tilia* species diversity and genetic resources in Bulgaria and to identify important knowledge gaps, in relation to their future sustainable use and conservation.

Species diversity was studied by an extensive literature survey and by our own field inventories. The main references were different floras (Browicz 1968, Jordanov and Peev 1979, Raab-Straube 2017+) and the latest comprehensive monograph of the genus (Pigott 2012). A special database was established containing detailed information about the stands with composition of *Tilia* species using information from the current forest management plans (FMPs). The database was organized to allow fast and complete retrieval of information about the available *Tilia* genetic resources.

Species diversity

Traditionally, genus *Tilia* L. has been classified to family Tiliaceae Juss. However, recent phylogenetic studies placed it within Malvaceae Juss. *sensu lato* (Angiosperm Phylogeny Group 2009), which was accepted also in Raab-Straube (2017+). Discussing the phylogenetic position of the genus is beyond the scope of the present review.

Despite the relatively clear-cut and stable morphological characteristics of *Tilia* species, their taxonomy was unnecessarily confusing, controversial and overcomplicated. The comprehensive study of Pigott (2012) resulted in a revised taxonomic structure, consisting of 23 species and 14 subspecies. A total of six species are recognized in Europe, but in spite of this modest diversity, some taxonomical problems still exist. This is at least partly due to the interspecific hybridization occurring in some parts of the species' natural range. The problem was first reviewed by Browicz (1968) in the volume 2 of Flora Europaea. He listed five 'good' species: *T. cordata* Mill., *T. dasystyla* Steven, *T. platyphyllos* Scop., *T. rubra* DC., and *T. tomentosa* Moench., and two species of hybrid origin: *T.* × *vulgaris* Hayne (*T. europaea* L. *pro parte*) and *T.* × *euchlora* C. Koch. Currently, the number of species in Europe was reduced to five and a new species, *T. begoniifolia* Steven was added, replacing *T. rubra* DC., whose taxonomic position and status was re-evaluated (Pigott 2012, Raab-Straube 2017+).

Traditionally, three to four species have been recognized in Bulgaria (Stefanoff 1934, Černjavski et al. 1959, Jordanov and Peev 1979, Yurukov and Panayotov 2015). The systematics of three of them (*T. cordata*, *T. platyphyllos* and *T. tomentosa*) is not considered problematic, while the fourth species has undergone several nomenclatural changes, from *T. rubra* DC., (Černjavski et al. 1959, Jordanov and Peev 1979) through *T. corinthiaca* Bosc ex K.Koch, *T. caucasica* Rupr., and finally set as *T. begoniifolia* Stev. (syn. *T. platyphyllos* subsp. *caucasica* (V. Engl.) Loria) in Pigott (2012) and Raab-Straube (2017+).

Other *Tilia* species like *T. petiolaris* DC. have been reported from Bulgaria (Dončev 1964). Interestingly, it was described from a natural locality (Strandzha Mts) while Pigott (2012) considered this 'form' as only cultivated. Evidently, this needs further clarification. Raab-Straube (2017+) considers this taxon as synonym of *T. tomentosa* Moench.

There were some chromosome number reports of Bulgarian *Tilia* species (Petrova et al. 2007) confirming the previous findings that all European species are diploid (2*n*=82), with the exception of *T. dasystyla*, which is a tetraploid (2*n*=164) (see Pigott 2002, for review).

Interspecific hybridization between *Tilia* species was reported from different parts of the species' range; however, as pointed out by Pigott (2012), description of these hybrids in most cases was based on trees in cultivation. The topic is still scarcely studied in Bulgaria and adjacent regions. Our field studies revealed some populations (e.g., Golo Bardo, Vitosha (northern slope), Lozenska and Strandzha Mts., near the town of Trun, Pernik region, and in the valley of Yadenitza river, Pazardzhik region) with putative occurrence of *T. begoniifolia*, and with individuals expressing some intermediate characteristics between *T. platyphyllos* and *T. cordata*. These populations need further detailed investigations. Due to the existing taxonomic uncertainties, the inventory of lime species genetic resources was done only considering the three species – *T. cordata*, *T. platyphyllos* and *T. tomentosa*.

Genetic diversity

The studies on the genetic diversity of limes in Bulgaria to date are limited only to describing the variation among provenances and studies on the ecotype and phenotype variation (Peev 1970, Dobrinov et al. 1982; Kalmukov 1987, 1995). In fact, the studies on limes' genetic variation in Bulgaria by using genetic markers are still in their infancy. Some studies, employing genetic markers were performed in other parts of the natural distribution range of limes in Europe. Isozymes were applied to document genetic variation in the species and to study the interspecific hybridization (Maurer and Tabel 1995b, Fromm 2001, Fromm and Hattemer 2003). Fineschi et al. (2003) found relatively low differentiation among 17 European populations of *T. cordata* (Balkan populations not included in the set) by applying markers based on restriction fragments length polymorphism amplified by polymerase chain reaction (PCR-RFLP), and the patterns of diversity were attributed to human activity in the past.

Random amplified polymorphic DNA (RAPDs) were used for documenting genetic diversity of *T. tomentosa* in urban trees (Filiz et al. 2015) and natural populations (Gabur et al. 2019), and for studying the interspecific hybridization (Liesebach and Sinkó 2008, Mylett 2015).

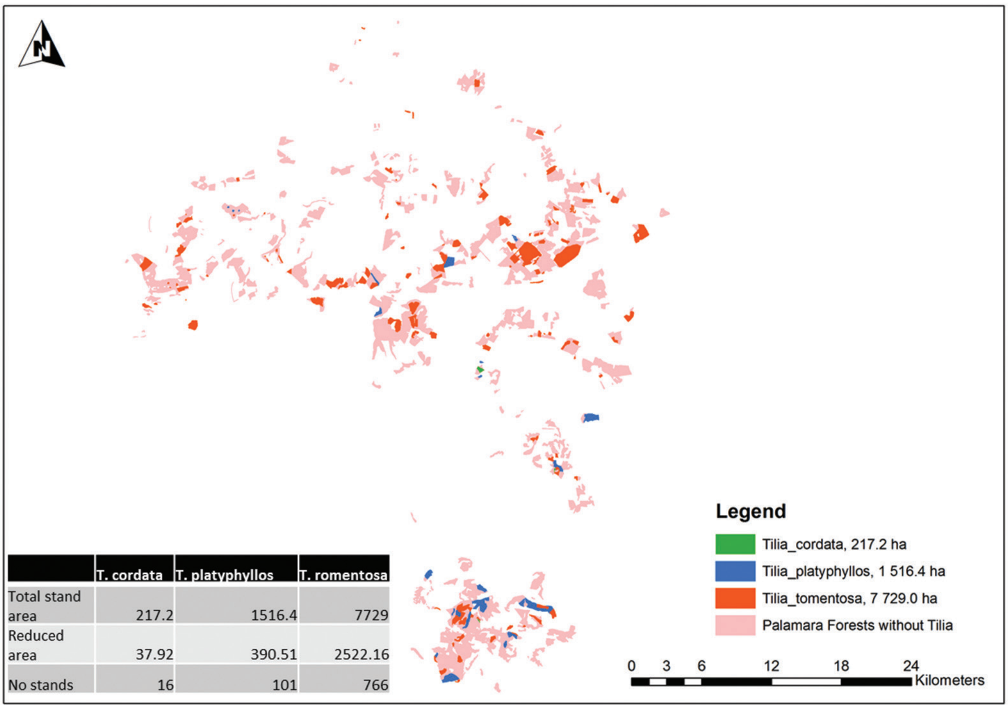
Barker (2017) studied fine-scale spatial genetic structure of *Tilia cordata* (microsatellite markers) and found that the species has a mixed reproductive system with approximately half of all individuals being of clonal origin. The studies on chloroplast genome of plants is considered very important both for studying the genetics of the species, and for systematic purposes. A complete sequence of the plastid genomes was performed of four Asian *Tilia* species (Cai et al. 2015). These approaches could be extended successfully to the European species.

Microsatellite markers have been successfully developed for *T. cordata* and *T. platyphyllos* (Phuekvilai and Wolff 2013, Phuekvilai 2014, Logan et al. 2015, Mylett 2015, Logan 2016, Vanden Broeck et al. 2018). These markers were applied to study genetic structure and differentiation of lime forests, hybridization and outcrossing. Lobo et al. (2018) found high genetic variation in phenology and fine-scale selection in favor of local growth conditions in *T. cordata*. Erichsen et al. (2019) detected high degree of diversity but low degree of differentiation among the Danish populations. These results could be instructive for similar future studies of the Bulgarian and Balkan populations. Furthermore, Logan (2016) reported thousands of simple sequence repeats, which can be used to extend the marker set for the two species.

Inventory of *Tilia* genetic resources using database searches

As indicated above, the database inventories rely on information obtained from forest management plans (FMPs). By regulation, the information in the FMPs is being updated every 10 years, and therefore, our database and the evaluations of the genetic resources based on it, reflect the current status of the genetic resources, a snapshot. Detecting finer temporal trends of their development requires additional studies and information.

The database contains information about all stands where species of genus *Tilia* occur including the location, administrative management unit (Forest service) and numerous stand characteristics. The database allows searches using different criteria. An illustrating example is provided in Figure 1, representing extraction of all stands with participation of *Tilia* species in one unit – Palamara Forestry Service and a map of the stands with occurrence of *Til-*



	T. cordata	T. platyphyllos	T. romentosa
Total stand area	217.2	1516.4	7729
Reduced area	37.92	390.51	2522.16
No stands	16	101	766

Fig. 1. Example of a database search of the total and reduced area covered by *Tilia* species in the Palamara Forestry Service. A spatial map of the distribution of the three species is also shown.

ia species (Palamara was chosen as an example because all three species occur in this district).

T. tomentosa is by far the most abundant species in Bulgaria (Table 1). The total area of the stands where it occurs, is 191,665.6 ha, 145,756.2 ha of which are natural stands, only 11.5 % of them being of seed origin and the remaining 88.5 % are coppice forests. The next species, in term of abundance by occupied area, is *T. platyphyllos*, with total area of the stands where it occurs, 15,842.8 ha, 92.7 % (14,701 ha) of them being indigenous, either of coppice (88 %) or of seed origin (12 %) (calculation based on stand number). The least distributed species is *T. cordata*, with only 3421.5 ha total area of the stands, and 2698.6 ha of natural origin. About 84 % of the stands are of coppice, and 16 % – of seed origin.

Table 1. Area and origin of *Tilia* species stands in Bulgaria.

Species	Total stand area, ha	Natural stands only, ha	Reduced area natural stands, ha	Origin: coppice stands; seed stands, %
Tilia cordata	3421.5	2698.6	528.59	84; 16
Tilia platyphyllos	15842.8	14701.6	5095.9	88; 12
Tilia tomentosa	191665.6	145756.3	46893.9	85.5; 11.5

Note: percentage calculations based on the stand number, not on area.

The species of genus *Tilia* are seldom dominant in the forest communities, which is due to their ecological and life-history characteristics. The only species tending to form pure stands is *T. tomentosa*; however, most of them are of secondary origin, formed after cutting the main oak stands. A more precise estimate of the amount of their genetic resources provides the reduced area, obtained by multiplying the respective share of the species by the stand area (Table 1).

The distribution of limes' proportion in mixed stands was calculated based on the number of stands and area. Results based on number of stands revealed that in 60 to 80 % of the stands lime species comprise up to 30 % of the total stand composition (Fig. 2A). This is especially true for *T. cordata* (83 % of the stands). The cases where the limes participate more substantially in the stands are few – in less than 10 % of the stands the lime species play dominant role, with more than 80 % participation ratio. This is valid even for *T. tomentosa*, which is more prone to form pure stands. However, these distributions changed when the calculations were based on occupied area (Fig. 2B). Then the distributions became more even, with greater *Tilia* share of the total stand areas, both where the species have dominant and sub-dominant position.

Distribution regarding the altitude and age classes was calculated based on the reduced area (Table 1). Generally, the species occur below 1000 m a.s.l., with some insignificant exceptions (Fig. 3). *T. cordata* is distributed mostly within the altitudinal range of 500–1000 m, *T. platyphyllos* within the range of 200–500 m and of *T. tomentosa* below 200 m. These patterns correspond well with the ecological characteristics of the species, even though some stands with lime species could reach up to 1300–1400 m a.s.l. (Fig. 3).

Distribution of genetic resources by

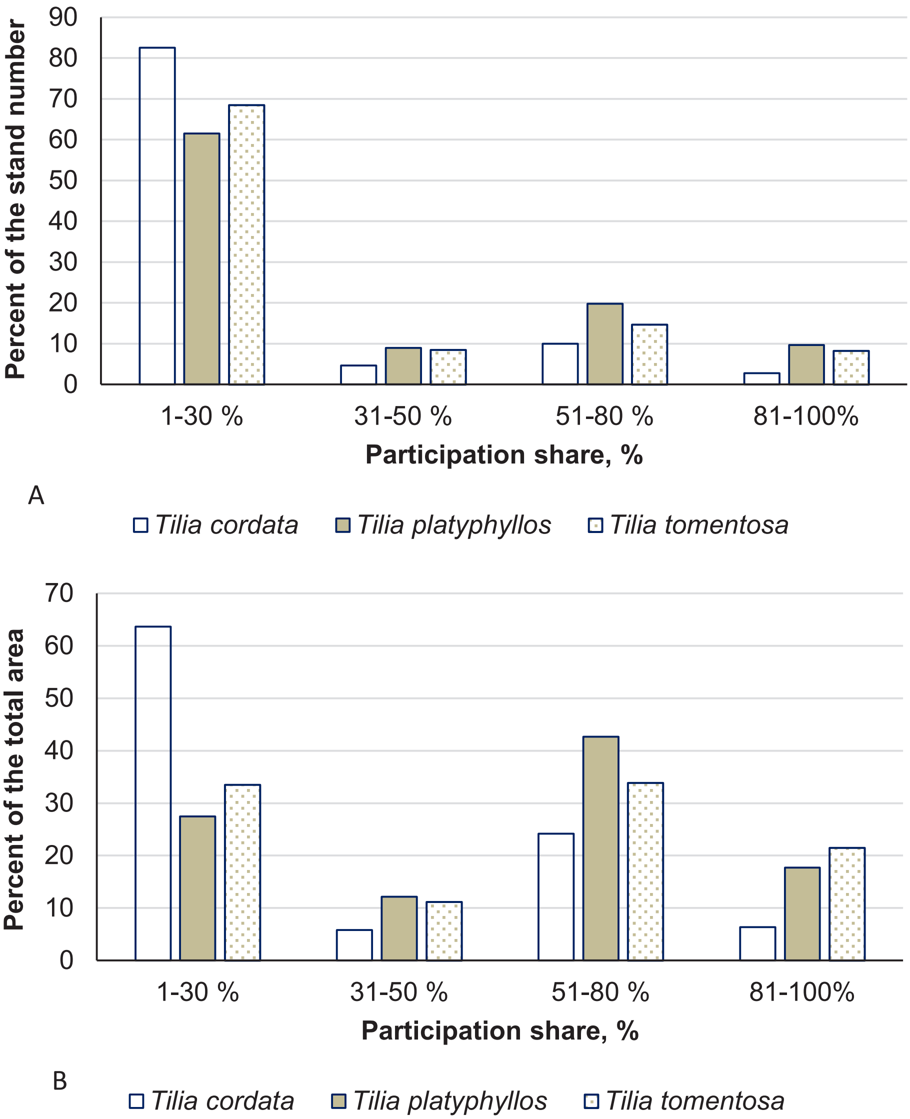


Fig. 2. Proportion of *Tilia* species in mixed stands: A – calculations based on the stand number; B – calculations based on the reduced area (see the text).

age classes revealed similar patterns in the three species. Predominant part of the area for all species is within the age classes 40–60 and 60–80 years (Fig. 4). It corresponds with the legacy of forest management in Bulgaria – there was a strong harvesting pressure during the 1950s and 1960s together with the large-scale afforestation occurring during the same period. Forests regenerated post this period now represent the two predominant age classes. Regardless, the age

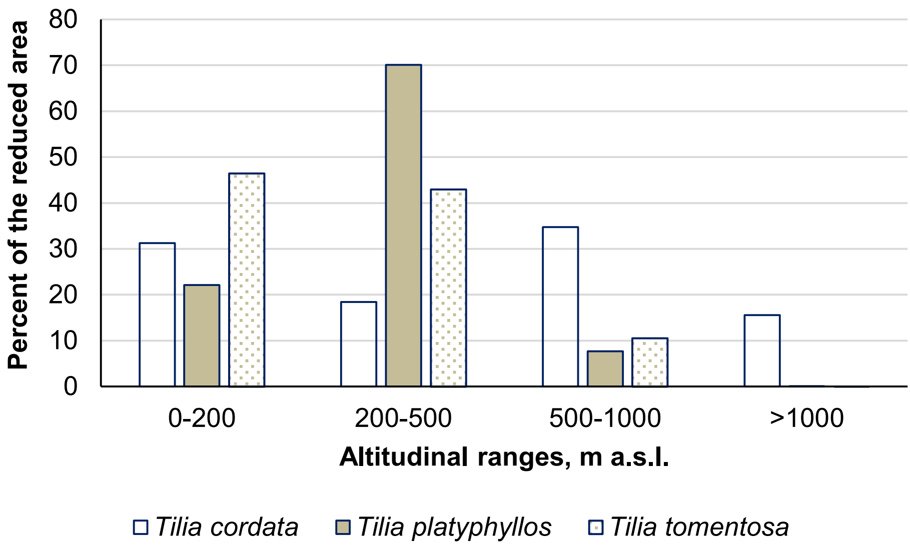


Fig. 3. Distribution of *Tilia* species by altitude.

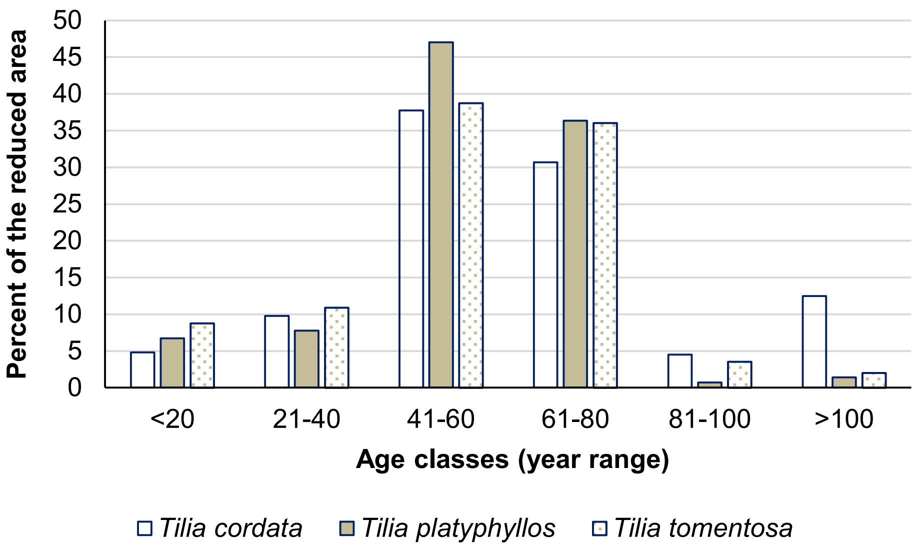


Fig. 4. Age distribution of *Tilia* species.

structure is far from optimal (Figure 4). However, having in mind the current undertaken measures aimed at sustainable and nature-oriented forest management, we could expect improving the age structure in the next decades.

Coppice management system is applied more frequently to the stands with participation of *T. tomentosa*, while *T. platyphyllos*, and particularly *T. cordata*, tend to be of seed origin more frequently (they have lower sprouting capacity).

Conservation of *Tilia* genetic resources

The measures for conservation of genetic resources of *Tilia* species have been usually part of larger-scale measures for conservation of forest ecosystems where *Tilia* species are part of and were the focus of the Noble Hardwoods network of the Euforgen conservation programme (Bozzano et al. 2006), with Bulgaria participating in the programme until 2014.

In situ conservation measures at national level include different kinds of protection and special management regimes. Typical example is the protection category known as a seed production stand. Such stands are selected based on phenotype superiority and are used for harvesting seeds for the afforestation purposes. They are managed in a way promoting flowering and seed production and not for timber. There are 121 permanent seed production stands of *T. tomentosa* with an area of 1433.8 ha. The respective figures for the other two species are: 22 permanent seed production stands of *T. platyphyllos* (239.7 ha) and only 2 of *T. cordata* (12.5 ha).

Some natural habitats where *Tilia* species play substantial role, are important from general nature conservation persepective. Two of them are included in the Red Data Book of Bulgaria, vol. 3 Natural Habitats: 23G1 Forest of Silver Lime (*Tilia tomentosa*) (Tzonev 2015) and 28G1 Ravine and slope woodlands (Tashev 2015). The same habitats are considered important for Europe and are included in the priority habitats of Natura 2000: 91Z0 Moesian Silver lime woods (Tzonev 2003, 2009) and 9180 *Tilio-Acerion* forests of slopes, screes and ravines (Dimitrov 2009). Numerous stands with participation of *Tilia* species fall into other protected territories (natural reserves, national parks) and/or Natura 2000 zones, but their amount and effectiveness of conservation measures still remain to be studied.

Ex situ measures are practically lacking and are undertaken for only *T. tomentosa*. There are three provenance trials, two clonal seed orchards (4.9 ha), and one seedling seed orchard (1.7 ha).

Knowledge gaps and needs of studies

Based on this review, we could identify and summarize some important knowledge gaps concerning Bulgarian *Tilia* species. The distribution of genetic diversity within and among populations is still largely unknown. This is true both concerning the application of genetic markers, and adaptive quantitative traits. There are still some unresolved taxonomic problems, whose investigation could benefit greatly from new DNA-based methods (Hörandl and Appelhans 2015, Soltis et al. 2018). The effectiveness of conservation measures is unknown, and the conservation units are not properly identified and described.

The identified knowledge gaps suggest some important and necessary activities and studies, briefly:

- documenting the existing genetic diversity by using a combination of neutral and adaptive genetic markers and characteristics;
- extending the ex situ conservation activities by establishing provenance/progeny trials and thus combining the conservation measures with studies on the adaptive quantitative traits;
- identification and detailed documentation of the existing gene conservation units;
- popularizing the economic, environ-

mental and conservation importance of the *Tilia* species among the experts and public.

Conclusion

Tilia species in Bulgaria are traditionally highly valued but still neglected by forestry professionals. The present review outlined the state of the art and existing gap knowledges and drew focus on areas that should be addressed in the near future, including detailed study on the genetic diversity of natural populations. These could be beneficial from both economic and conservation viewpoints.

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ENDOZOOCHORY ENHANCES SEED GERMINATION AND SEEDLINGS GROWTH OF BLACK CHERRY

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Abstract

The fruits of the black cherry are food for a variety of different species of animals. Therefore, animals have an important role in the spread of black cherries by zoochory. The aim of this study was to determine how the digested black cherry seeds by the badger (*Meles meles*) digestive system affects the germination efficiency of these seeds after voiding in the badger's faeces. It was hypothesized that seeds of black cherries collected from badger faeces will show greater germination efficiency in the first year after sowing than non-stratified seeds, stratified seeds and seeds with an exocarp. Black cherry seeds were collected in autumn 2018 at the Forest Arboretum in Zielonka from badger faeces, which comprised the first treatment group of the experiment and from standing trees, and among these seeds there were further variants of treatment. Seeds collected from standing trees were: without pericarp (treatment group), without pericarp and stratified, not cleaned but stratified and seeds untreated (control sample). The first seedlings to emerge were from seeds collected from badger faeces (206 days after sowing). The seeds with intact pericarp had the longest period of emergence, germinating 221 days after sowing. The highest seed germination capacity was noted for seeds from badger faeces (66.4 %), and the lowest one was observed for seeds sown with intact pericarp (15.2 %). Pericarp inhibits seed germination in the first year after sowing.

Key words: germination, invasive species, *Meles meles*, *Prunus serotina*, zoochory.

Introduction

One of the most invasive species in forest ecosystems in Europa is the black cherry (*Prunus serotina* Ehrh.) (Halarewicz 2011, Danielewicz and Wiatrowska 2014, Jagodziński et al. 2019). Its fruits serve as a food for a large range of birds and mammals (Starfinger 1997). The role of animals in the spread of black cherry should not be overlooked (Kurek 2011). The aim of this study was to determine the germination capacity of black cherry seeds collected from badger's faeces.

Material and Methods

In the summer of 2018 at the Forest Arboretum in Zielonka (17.10941 E 52.553975 N), the badgers (*Meles meles* L.) were observed to feed under black cherries. Numerous intact seeds of black cherries were found in the faeces of these animals, and these were used for this research.

Black cherry seeds were collected in the autumn 2018. In each experiment variant, the sample consisted of 500 seeds. Seeds were collected from badger faeces (i), which comprised the first treatment group of the experiment, and from standing trees, which seeds were used for the other variants of treatment. Seeds collected from standing trees were: without pericarp and stratified (ii), without pericarp (treatment group) (iii), not cleaned but stratified and seeds untreated (control sample; iv). Seeds were subjected to stratification (ii) by hot-cold treatment according to Krüssmann's (1978) recommendations consisting of 4 weeks at 20 ºC followed by 18 weeks in 3 ºC in the laboratory. Seeds from each treatment group were weighed before sowing. Then the seeds were sown in the autumn (3.10.2019) into cassettes (Alonet peat substrate) in the nursery of the Experimental Forestry Plant Murowana Goślina, 500 for each treatment group, except for the seeds subjected to stratification, which were placed in sand on that day. Stratified seeds were sown in early spring (18.03.2019).

The time of emergence of seedlings was observed in spring (beginning of May), and the number of emerging seedlings was counted once every three weeks (Table 1). Seedlings were removed and subjected to drying twice for eight hours at 60°C. Observations were carried out until September 2019. Seed cassettes were watered from June 2019 according to the Guidelines for irrigation of forest nurseries in open areas (Pierzgalski et al. 2002).

Results

The mean weight of 500 black cherry seeds ranged from 41 to 43 g (Table 1). The seeds with pericarp weighed 338 g/1000 pcs. The lowest weight was found in black cherry seeds obtained from badger faeces – 82 g/1000 pcs (Table 1). Results indicate that black cherry seeds did not differ in terms of the average weight. The lack of significant difference in weight concerned the seeds without pericarp.

Similar to the seed weight, there was no significant difference in the weight of seedlings. A single seedling weighed on average from 0.041 g (non-stratified seeds, treatment groups i and ii) to 0.052 g (stratified seeds, treatment groups iii and iv). Seedlings grown from badger faeces, similarly to seedlings with intact pericarps, had a mass similar to the average for the experiment (0.045 g).

The first emergence of black cherry seeds collected from badger faeces was observed on April 26, 2019 (Table 1) and the time that elapsed from sowing these seeds until the first seedlings appeared was 206 days. The first emergence of seeds with intact pericarps was not observed until May 11, 2019 (Table 1), 221 days after sowing these seeds. Hence, the seeds with pericarps were distinguished by the longest period of germination.

The seeds obtained from badger faeces showed the highest germination capacity. On May 21, 63.8 % of the seeds sown from this treatment group germinated, while by the last day of observation the germination capacity of these seeds in the growing season was 66.4 %. In contrast, the seeds sown with intact pericarps had the lowest germination capacity – 15.2 % of the seeds germinated (Table 1, Fig. 1).

Table 1. Observation results.

Variant	I badger		II stratified		III nonstratified		IV with a pericarp and stratified	
Date of observation (2019)	Total weight (seedlings), g	Total number (seedlings), pcs	Total weight (seedlings), g	Total number (seedlings), pcs	Total weight (seedlings), g	Total number (seedlings), pcs	Total weight (seedlings), g	Total number (seedlings), pcs
21.05	14.38	319	14.17	278	8.93	205	1.6	51
11.06	0.25	8	0.69	10	0.73	19	1.78	19
02.07	0.01	1	0.36	3	0.1	12	0.01	3
23.07	0	0	0	0	0.04	1	0.09	2
13.08	0	0	0	0	0	0	0	0
03.09	0.12	4	0	1	0.01	0	0.01	1
Weight of individual seedlings, g*	0.0445		0.0521		0.04134		0.0459	
First germination, date	26.04		16.05		29.04		11.05	
Mass of collected seeds, g	41		43		42		42 (169**)	

Note: pcs – pieces; * – average weight of seedlings 0.04597 (±SEM 0.00451); ** – mass of seeds harvested with pericarp.

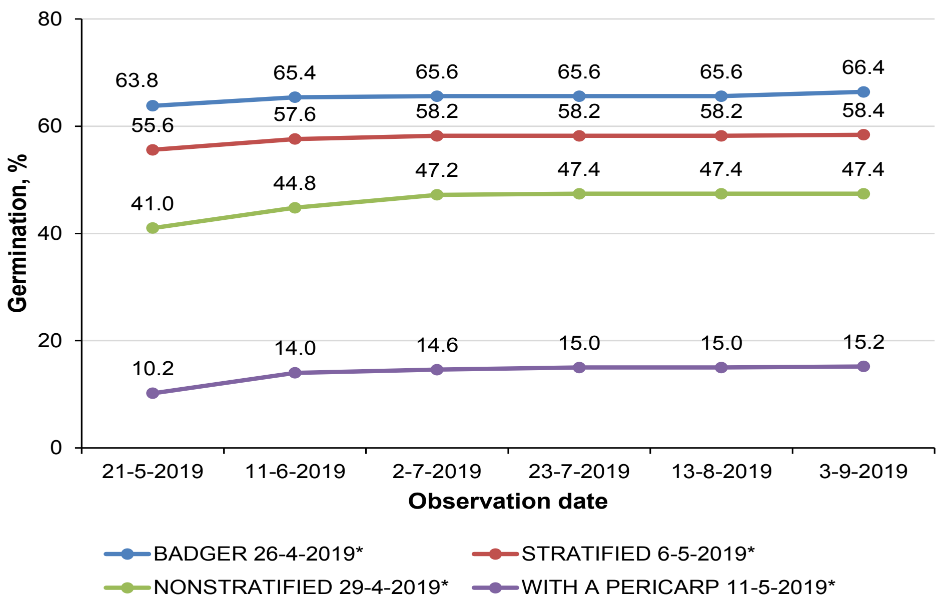

Fig. 1. Germination energy of black cherry seeds with observation date.
Note: * – date of first appearance of seedlings.

Discussion

This study showed that black cherry seeds collected from badger faeces (with destroyed pericarp) were characterized by greater germination capacity than untreated, as well as stratified seeds. Our results indicate that the process of germination of black cherry seeds is characterized by high violence at the initial stage, regardless of the variant. The vast majority of seeds (96 % badger, 95 % stratified, 86 % non-stratified, 67 % with pericarp) germinated one month after the appearance of the first seedlings. The acceleration of germination of seeds that have passed through the gastrointestinal tract of birds has been demonstrated in previous studies, which have also shown lower germination capacity of stratified seeds compared with seeds not subjected to digestion by passing through bird intestines (Krefting and Roe 1949, Smith 1975).

Various mammals also contribute to the spread of berry plants, including Black cherries, thereby increasing the effectiveness of their spread and invasive capacity in environments in which they are not normally distributed (Gosper et al. 2005, Deckers et al. 2008). The share of berries in the diet of forest mammals increases during the year (summer and autumn) (Baltrūnaitė 2001, Kurek 2011). Near the badgers' burrows, a range of different species of fruit trees and shrubs appear, facilitated by habitat conditions and badger endozoochory. Although the disturbance of habitats caused by these mammals is not extensive on a forest scale, they nevertheless directly increase forest community diversity locally and on a broader scale (Kurek et al. 2014). Therefore, the role of badgers in transforming plant communities should not be underestimated. Seeds transmitted by mammals germinate in the form of characteristic clumps (clusters) often composed of up to several dozen seedlings (Kurek 2011), and this was confirmed in our observations during the current work.

Black cherry seeds require stratification. In nature, they are subject to stratification on the forest floor during winter (Marquis 1990) and they may show delayed germination extending up to 3 years after being deposited (Marquis 1975). According to Krüssmann (1978) the germination capacity of seeds of the *Prunus* genus is 1–2 years, which is consistent with our finding that only a relatively small number of seeds with intact pericarps germinated (just over 10 %) whereas germination was 41 % in non-stratified black cherry seeds cleaned of pericarp.

Breaking of seed germination is an adaptation that allows plants to withstand the adverse environmental conditions (Starfinger 1991) and resume development when more favourable conditions return. Delayed germination results in the retention of significant amounts of seeds in the forest floor. It is estimated that hundreds or even thousands of black cherry seeds accumulate in the soil in any given year (Marquis 1975) and only about half germinate each spring (Uchytil 1991). The above reports are consistent with our findings, however, the mechanisms involved in the inhibition of germination of black cherry seeds are still largely unexplained and remain to be resolved by future research.

Some species of the *Prunus* genus require long periods of ripening, hot-cold stratification even before autumn sowing or they can be sown immediately after harvest (Grisez et al. 2008). Stratified seeds can be sown as early as possible in the spring, because high temperatures and drought can reduce the germination of black cherry (Huntzinger 1971). According to Hrynkiewicz-Sudnik et al. (2001) black cherry seeds should be sown in September or November after prior mixing of the seeds with sand and thereafter keeping them at low positive temperatures. This type of treatment according to Hrynkiewicz-Sudnik et al. (2001) is necessary because storing seeds without stratification until spring and followed by subsequent stratification, results in poor emergence.

Conclusions

Our findings show that endozoochory increases the germination capacity of black cherry seeds and contributes to their more effective germination. The pericarp inhibits the germination of black cherry seeds in the first year after sowing. Further observations will allow assessment of the ability of black cherry seeds with pericarp to germinate in the following years after sowing. Monitoring of badgers population and their participation in the spread of black cherry would be recommended.

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SOME PHYSICOCHEMICAL PROPERTIES OF PHAEOZEMS IN BULGARIA

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Abstract

The main components of the cation exchange capacity (CEC) are assessed in order to study the physicochemical properties in Phaeozems. These soils in Bulgaria usually have high humus accumulation, soil texture differentiation, high base saturation and soil reaction suitable for crops. They are spread in plains, valleys, forests and low hills with temperate continental climate. Soil-forming materials are Pliocene and Quaternary sediments, loess and loess-like clays. The soil reaction (pH) varies from slightly acidic to alkaline. Soil alkalinity increases in depth because of high carbonate mineral compounds that mainly contain Ca and Mg. Total acidity and exchange acidity are in very low values. Exchangeable Ca consists about 78 % of CEC. It is established that soil colloids are occupied by Ca and Mg ions, which determined the stability of clay minerals. Phaeozems have favorable physicochemical properties with high cation exchange capacity and base saturation. These soils are highly valued for their fertility potential.

Key words: base saturation, CEC, soil reaction, total acidity.

Introductions

According to WRB (IUSS Working Group WRB 2015), Phaeozems cover an estimated 190 million hectares world-wide. Some 70 million hectares of Phaeozems are found in the USA, in the sub-humid central lowlands and parts of the Great Plains. Fifty million hectares of Phaeozems are in the subtropical areas of South America and the third largest distribution area of Phaeozems (18 million hectares) is in Northeastern China. Smaller, mostly discontinuous areas are in Central Europe, notably in Danube region of Hungary, Serbia, Romania and Bulgaria.

Phaeozems are widespread between Chernozems and ordinary loess soils in areas with temperate continental climate in Northern Bulgaria. The relief on which they are located is flat and slightly hilly. Soil-forming materials are loess and loess-like clays. In Southern Bulgaria they are usually spread over different Quaternary materials. In the past most of these soils were under forest but now they are fertile and arable lands (Ninov 2002, Teoharov 2019)

In Bulgaria Phaeozems are characterized by thick surface horizon (A) of 30–40 cm and up to 80–120 cm Bt horizon, respectively, with fine granular (irregular) to small prismatic and large granular structure. These soils have a dark gray surface and a dark gray or dark brown luvic-metamorphic (cambic) horizon, with low textural differentiation. The base saturation throughout the profile is 70–90 %, the soil reaction is slightly acidic to medium acidic in the surface. The cation exchange capacity in A horizon ranges from 17–27 to 36 meq/100 g soil, and in Bt horizon from 14 to 31 meq/100 g soil. The soil texture could be loamy-clay, sandy-clayey and rarely clayey with a distinct process of *in situ* clay formation and a gradual decrease in humus (Ganev and Arsova 1980, Malinova 2010, Shishkov and Kolev 2014, Nikova et al. 2017).

According to Habel et al. (2007), Phaeozems have neutral reaction because of the presence in the parent material with different quantities of natural calcium carbonate. This confirms considerable buffer capacity and high 'natural 'resistance of these soils to degradation processes. A very high degree of base saturation should be assessed as exceptionally favourable for the growth and development of crop plants.

Phaeozems usually have neutral soil reaction at the surface. The optimum range for most agricultural crops is between 5.5 and 7.5, $pH_{(H2O)}$ and the most of nutrients are available. Soil alkalinity increases with weathering of silicate, aluminosilicate and carbonate mineral compounds that contain Na, Ca, Mg, NH_4 and K. The fore-listed minerals are usually added to the soil by the deposition of eroded sediments by water or wind (Oshunsanya 2018).

Similar research of effective cation-exchange capacity by low values is found in Romania (from 14.57 to 19.82 meq/100 g soil), and the degree of base saturation in surface horizon is (67.8 %), decreases abruptly (42.1 %) in deeper layers (Stanila et al. 2017, Stanila 2019). Researches about Phaeozems are made also in Eastern Qinghai-Tibet (Ma et al. 2020). The soils are similar with thick, dark-coloured surface horizon with high base saturation and a moderate to high content of organic matter.

The aim of this study is to determine main physicochemical indices of Phaeozems such as ability of soil to adsorb cations in exchangeable forms and to establish buffer capacity of soil. Some relationship would therefore be expected between soil pH, CEC and of base saturation.

Material and Methods

The main diagnostic soil characteristics were obtained using the following methods: soil pH was measured in water suspension 1:2.5. The exchangeable Al was determined titrimetrically after displacement of Al ions by 1 N $CaCl_2$. The total exchangeable hydrogen exch. $H_{8.2}$ (total acidity) and exchangeable bases (Ca and Mg) were determined titrimetrically and complexometrically, respectively, after saturation of the soil sample with buffer solution (Na-acetate and K-maleate, $pH_{(H2O)}$ = 8.2). CEC was calculated by the sum of $H_{8.2}$ and exchangeable bases in meq/100 g – method of Ganev and Arsova (1980). Base saturation is the percentage of CEC that is saturated with base exchangeable cations (Ca and Mg). It is calculated according to this formula (1).

$$V\% = 100 \times \frac{\sum sum}{CEC}, \quad (1)$$

where: *V*% is base saturation, Σ*sum* is the sum of exchangeable cations, CEC is the cation exchange capacity of the soil in meq/100 g. CEC is conventionally expressed in meq/100 g (Rengasamy and Churchman 1999) which is numerically equal to centimoles of charge per kilogram of exchanger (cmol(+)/kg).

For statistical analyses and graphics was used Microsoft Excel 2010 software. Six soil profiles are presented, one of them is from South Bulgaria – profile one (P1) close to Novi Han village, Sofia district. The rest of the soil profiles are from northern part of Bulgaria – profile 2 (P2) Volovo village, Rousse district; profile 3 (P3) Bezhanovo village, Lovech district; profile 4 (P4) Voidiza village, Turgovishte district; profile 5 (P5) Vladimirovtzi village, Razgrad district; profile 6 (P6) town of Isperih, Razgrad district. For soil profiles 3, 4, 5 and 6 the values are taken from Ganev et al. (1990).

Results

The obtained results show that Phaeozems are rich in bases in deep of the soil profile (Table 1). They have dark coloured surface horizon (figs 1 and 2) with favourable soil reaction. These soils are from neutral to moderately acidic $pH_{(H2O)}$ in surface horizon A (5.3–6.7) to medium alkaline reaction only in Ck (7.7–8.2). The mean value of soil pH is 6.55, which is favourable soil reaction for main crops (tables 1 and 2).

Table 1. Soil profiles with physicochemical properties.

Horizon/ Depth, cm	$pH_{(H2O)}$	CEC	ex. $H_{8.2}$	ex. Al	ex. Ca	ex. Mg	*V,* %
		meq/100 g soil					
			P1. Endocalcic Luvic Phaeozem				
Ap 0–24	5.5	37.5	11.0	0.9	23.0	3.2	70.67
Bt1 2–49	6.0	43.5	7.1	0.0	33.0	3.4	83.68
Bt2 49–67	6.5	43.4	5.2	0.0	34.8	3.4	88.02
Ck1 67–95	7.5	34.9	0.0	0.0	31.6	3.3	100
Ck2 95–150	7.7	38.0	0.0	0.0	34.5	3.5	100
			P2. Bhatycalcaric Luvic Phaeozem				
Ah 0–18	6.7	30.6	3.8	0.0	24.0	3.0	87.58
A2 18–40	6.8	30.0	3.5	0.0	23.2	3.0	87.33
AB 40–75	6.8	27.7	2.5	0.0	23.0	2.5	90.67
Bt1 75–108	6.8	31.0	1.6	0.0	27.0	2.4	94.84
Bt2 108–139	7.0	25.8	1.6	0.0	21.2	2.8	93.80
Ck1 139–160	8.1	25.0	0.0	0.0	22.0	3.0	100.00
			P3. Endocalcic Luvic Phaeozem				
Ap 0–29	6.3	33.6	4.5	0.0	27.2	2.1	86.6
Bt1 29–44	6.5	37.2	4.0	0.0	30.0	3.0	89.2
Bt2 44–71	6.6	37.0	4.1	0.0	30.1	3.0	89.0
Ck1 71–136	8.1	35.0	0.0	0.0	31.0	3.9	100
			P4. Bhatycalcaric Luvic Phaeozem				
Ap 0–32	6.1	26.6	4.9	0.0	18.4	4.0	81.6
AB 32–60	6.8	30.8	3.8	0.0	23.4	5.0	87.7
Bt1 60–89	6.8	30.9	3.8	0.0	24.3	3.9	87.7

Horizon/ Depth, cm	**$pH_{(H2O)}$**	**CEC**	**ex. $H_{8.2}$**	**ex. Al**	**ex. Ca**	**ex. Mg**	***V*, %**
		meq/100 g soil					
Bt2 89–128	6.8	30.6	3.9	0.0	23.1	4.3	87.3
Bt3 128–150	6.7	31.0	3.3	0.0	25.3	3.3	89.4
Ck1 150–180	8.2	37.2	0.0	0.0	34.8	2.4	100
P5. Bhatycalcaric Luvic Phaeozem							
Ap 0–28	6.1	25.1	5.5	0.0	18.0	2.3	78.1
A2 28–43	5.6	27.2	7.6	0.0	16.8	2.5	72.1
ABt 43–66	5.5	31.5	6.8	0.0	20.5	2.8	78.4
Bt1 66–98	5.8	25.5	5.4	0.0	20.3	3.4	81.7
BCk 98–130	7.2	30.6	4.4	0.0	24.9	1.9	85.6
Ck1 130–153	8.2	30.8	0.0	0.0	29.6	1.2	100
P6. Bhatycalcaric Luvic Phaeozem							
Ap 0–28	5.3	26.7	8.3	1.1	16.0	2.2	68.9
A2 28–40	5.4	26.7	8.2	1.2	16.8	1.5	69.3
Bt1 40–63	5.7	30.9	7.1	0.8	21.6	2.1	77.0
Bt2 63–100	5.7	30.5	5.7	0.4	23.0	1.3	81.3
Ck1 100–150	8.1	28.2	0.0	0.0	27.0	1.3	100

Note: CEC is cation exchange capacity; ex. $H_{8.2}$ is total acidity; ex. Al is exchange acidity; ex. Ca is exchangeable Ca; ex. Mg is exchangeable Mg; *V* is base saturation.

Fig. 1. Phaeozem from North Bulgaria – Dark grey forest soil (photo by Koinov et al. 1998).

Fig. 2. Phaeozem from South Bulgaria – Meadow cinnamonic soil (photo by B. Hristov).

High values of soil pH are due to soil profiles, are developing over calcareous sediments with alkaline reactions such as loess and similar soft Quaternary sediments (Hristov 2016).

Figures 1 and 2 show two deep, well-formed soil profiles of Phaeozems from Northern and Southern Bulgaria. The subsurface Bt horizon is commonly referred as clay rich layer significantly altered by pedogenesis, mostly with the formation of iron oxides and clay minerals. Typically clay formation in Phaeozems is *in situ*, but also clay illuviation from surface horizons could be found. This soil layer is usually brownish or reddish due to the iron oxides, which increases the chroma of the subsoil to a degree that it can be distinguished from the other horizons. Therefore, this is an argic horizon, in the middle of the profiles, with high clay content and high CEC.

In deep C horizon there are carbonates with white colour, respectively with high base saturation. The difference between them is reddish colour in the middle because of Mediterranean influence in Southern Bulgaria, and the Phaeozems from the north are deeper.

The cation exchange capacity of soil determines the total capacity of a soil to hold exchangeable cations. This can have a significant effect on its fertility management (Mengel 1993). According to Ganev et al. (1990) classification studied soils are moderately colloidal with average value of CEC about 31.59 meq/100 g soil (Fig. 3 and Table 2). In almost all soil profiles the highest values of CEC are in Bt horizon, because of high clay accumulation from leaching and lessivation in the argic horizon. P1 has the highest content of CEC up to 43.50 meq/100 g soil. The reason for that is parent rock of thick clay Pliocene and Quaternary sediments. All other soil profiles from North Bulgaria spread over clay loess.

Total acidity ($H_{8.2}$) and exchange acidity (Al) are in low quantities. The main reason for that is alkaline reaction and low amount exchangeable cations with acidic functions adsorbed on strongly acid and weakly acid ion exchangers of the soil colloids and lack of adsorbed exchangeable acid ions. The exchangeable acidity (ex. Al) is about zero in most horizons except in P1 and P6. The main presence of total acidity and exchangeable acidity is in surface horizons. This is because rainwater is slightly acidic

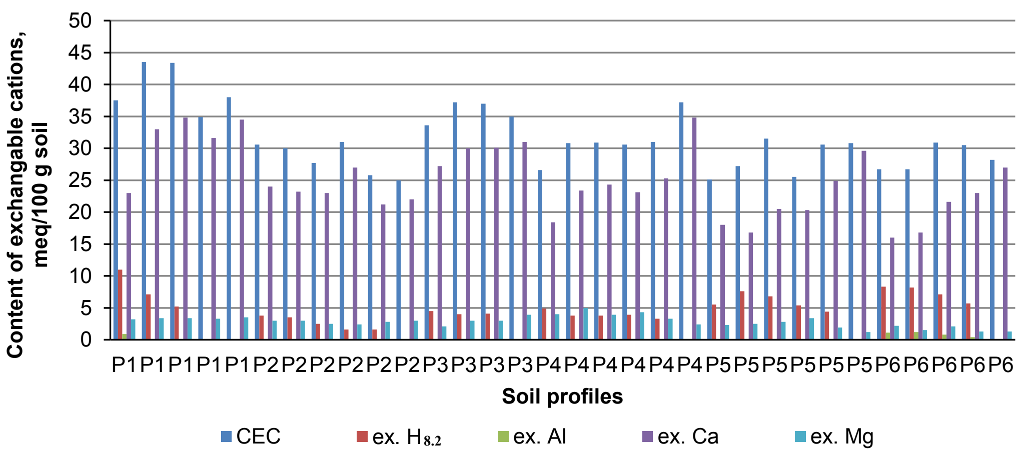

Fig. 3. Soil profiles with graphical values.

Table 2. Descriptive statistic of soil physicochemical properties.

Descriptive statistic	pH	CEC	ex. $H_{8.2}$	ex. Al	ex. Ca	ex. Mg	V%
Mean	6.65	31.59	3.99	0.14	24.98	2.84	87.11
Standard Error	0.16	0.88	0.51	0.06	0.96	0.16	1.68
Median	6.70	30.80	3.95	0.00	23.70	3.00	87.64
Mode	6.80	30.60	0.00	0.00	23.00	3.00	100.00
Standard Deviation	0.89	4.95	2.90	0.34	5.45	0.90	9.51
Range	2.90	18.50	11.00	1.20	18.80	3.80	31.10
Minimum	5.30	25.00	0.00	0.00	16.00	1.20	68.90
Maximum	8.20	43.50	11.00	1.20	34.80	5.00	100.00

Note: CEC is cation exchange capacity; ex. $H_{8.2}$ is total acidity; ex. Al is exchange acidity; ex. Ca is exchangeable Ca; ex. Mg is exchangeable Mg; V is base saturation.

(about 5.7) due to a reaction with CO_2 in the atmosphere that forms carbonic acid.

The number of H and Al ions decreases and the quantity of Ca and Mg ions increases. Cations which are alkaline (non-acid) and therefore raise the soil pH and base saturation in studied soils are mainly Ca and Mg.

High amount of Ca cations in the soil is because it is part of many primary and secondary minerals content. It is usually the dominant basic cation in soil cation exchange reactions, typically accounting for more than 70 % of base saturation (Pagani et al. 2013). Exchangeable Ca reach maximum at 35 meq/100 g, with average content about 24 meq/100 g and averagely 78 % of CEC. It is adsorbed strongly as compared with other exchangeable cations. More clay minerals adsorbed more Ca (Ganev 1989, Sposito 1989, Pavlova et al. 2005b). There is positive correlation between Ca and CEC, pH, and base saturation (0.83, 0.6 and 0.69). The relationship is confirmed for the effective cation exchange capacity, which is due to its stability in different methods of analysis and evaluation (Arsova and Malinova 1995, Malinova 1996, Pavlova et al. 2005a).

Soil pH has good positive statistical correlation with exchangeable Ca (0.6) and base saturation (0.94) (Table 3). There is also negative correlation with total acidity $H_{8.2}$ (–0.93) and exhalable acidity Al (–0.55).

Table 3. Correlation matrix of variables.

Soil indices	$pH_{(H2O)}$	CEC	ex. $H_{8.2}$	ex. Al	ex. Ca	ex. Mg	*V*, %
$pH_{(H2O)}$	1						
CEC	0.12	1					
ex. $H_{8.2}$	**-0.93**	0.00	1				
ex. Al	**-0.55**	-0.13	**0.62**	1			
ex. Ca	**0.60**	**0.83**	**-0.53**	-0.43	1		
ex. Mg	0.05	0.25	-0.05	-0.33	0.14	1	
***V*, %**	**0.94**	0.21	**-0.97**	**-0.66**	**0.69**	0.11	1

Note: In bold, variables are positively correlated (>0.5) or negatively correlated (<–0.5).

The high levels of competing elements, such as Ca, reduce the availability of Mg. Although Ca and Mg share the same exchange processes, Mg sorbs less strongly than Ca to soil colloids and therefore it is more prone to leaching. The average content is about 2.8 meq/100 g with maximum value of 5 meq/100 g. The interesting fact is that Mg does not correlate with all other indices (Table 3).

As it was mentioned above base saturation (%) has high values. It is calculated as the percentage of CEC occupied by base cations Ca, Mg and K. All soil profiles are with Calcaric or Endocalcic qualifiers according WRB classification (IUSS Working Group 2015) with values of base saturation from 70 to 100 %. It is increasing in deepest horizons due to high carbonate content. Consequently, base saturation has positive correlation with soil pH and Ca and negative with total acidity (Table 3).

Conclusion

Phaeozems in Bulgaria are fertile soils with high CEC, high base saturation and favourable soil reaction for crops. These soils are neutral to moderately acidic in surface horizon and slightly leached with pH from 5.3 to 6.7 and alkaline reaction in depth up to 8.2.

CEC varies from 25 to 43 meq/100 g, that is high and similar to most fertile soils such as Chernozems and Kastanozems. The highest values of CEC are in argic – Bt horizon because of high clay content. Studied soils are moderately colloidal with average value of CEC about 31.59 meq/100 g. The content of total acidity and exchange acidity is low. The main reason for that is alkaline reaction of parent rock, because of high content of carbonates. Therefore, exchangeable Ca has high values, up to 35 meq/100 g, which is about 78 % of CEC. All other base cations are in low quantities, Mg varies from 1.2 to 5 meq/100 g. It is determined that variable charges on soil colloids are usually occupied by mainly by Ca and Mg ions. High base cations content means high base saturation. Studied soils have good base saturation from 70 to 100 %.

New statistical analyses were made that show good correlations between soil pH, ex. Ca, and base saturation. There is negative correlation between soil pH, ex. Al and ex. $H_{8.2}$, and also similar negative correlation between base saturation, ex. Al and ex. $H_{8.2}$.

Obtained results show that Phaeozems have good buffer capacity in whole soil profile, with favourable physicochemical properties and high agricultural potential.

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COMPREHENSIVE ASSESSMENT OF HERITAGE PARKS' PLANTATIONS OF ZHYTOMYR REGION, UKRAINE

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Abstract

In this article it is considered the current state of heritage parks of the Zhytomyr region. We conducted an ecological, geographical, phytosociological, and taxonomic analysis of trees and shrubs in 6 parks out of 18, according to Kolesnikov, Takhtajan, Vernander and others. In heritage parks, 71 species and 3 varieties of trees and shrubs were revealed, belonging to 44 genera, 20 families, which come from five floristic regions. The most abundant Magnoliophyta family is Rosaceae, which is represented by 21 species (28.2 % of the total number of species). We analyzed the correspondence of woody plants to the conditions of their growth. A lot of species that did not fit soil types were planted in heritage parks. About 20 % of megatrophs grow in soils with low fertility. More than 84 % of the tree species of the parks belong to the Red List of the International Union for Conservation of Nature, and only one species is endangered. The aesthetic evaluation of plantations is low. We recommend cutting diseased and weakened trees and shrubs, also planting highly decorative bio-groups. It is offered a list of trees and shrubs recommended for planting in old parks in the Zhytomyr region.

Key words: aesthetic evaluation, floristic region, woody plants, zonal type of forest conditions.

Introduction

Heritage parks are objects of historical, cultural, and architectural legacy. There are more than 400 old parks in Ukraine (Kuznetsov and Klymenko 2003), 18 of which are located in the Zhytomyr region. A significant part of them was created in the seventeenth and nineteenth centuries.

The old parks of the region are closely connected with events in the life of prominent people of the Russian Empire: an anthropologist, ethnographer, geographer N.N. Miklouho-Maclay, descendants of the Crimean War hero V.I. Istomin, a well-known family of patrons of art Tereshchenko, poet Count Gustav Olizar and others. In this regard, it is important to carry out reconstruction and restoration work, taking into account the intentions of their owners in the past.

In the second half of the twentieth century, in connection with the reconstruction, initiated by the authorities of that time, the structure of many parks has changed significantly. Mostly the work was carried out without taking into account the intentions of their founders. As a result, most of the objects lost their former landscape appearance. Quiet and calm in their functional purpose, the old parks were reformed into culture and recreation parks. Attractions, children's and sports grounds were installed on their territory (Markov 2015).

Today in Ukraine, architects are mainly engaged in the restoration of parkland, the main attention is paid to the restoration of architectural objects (buildings, fountains, arbours, etc.). At the same time, the overall historical appearance of the park is narrowed, because the main material of the architectural composition of the historical park is vegetation according to the Florence Charter (1982).

To carry out the reconstruction and restoration of plantations, it is first necessary to conduct an inventory of the composition of trees and shrubs, to assess their current condition. Issues on the restoration of plantings of historical landscape gardening objects are considered in the works of Kucheriavyi (2017), Rubtsov (1974, 1977), Kuznetsov and Bahatska (2011), Klymenko (2012), Dudyn and Bahatska (2012) and others.

A succession of plants of old parks is generally not investigated. There is no information about the taxonomic composition of vegetation, the compliance of plants with their environmental conditions, as well as the aesthetic value of landscape ensembles.

The aim of the work is a comprehensive analysis of woody plants of 6 ancient parks of the Zhytomyr region (Yulino, Miklouho-Maclay, Korostyshivskyi, Vilkhivskyi, Turchynivskiy and Chervonskiy), assessment of their compliance with environmental conditions, and aesthetic assessment of parks.

Material and Methods

Materials for the study were collected during 2015–2018. The taxonomic composition of trees and shrubs was studied by route surveys not only following the existing paths, plants were determined using atlases and reference books (Rubtsov 1974, Brodovich and Brodovich 1979, Kokhno et al. 2002). The distribution of trees and shrubs by ecological groups was carried out according to Kolesnikov (1974). The geographical analysis was performed by the botanical-geographical division of the world according to Takhtajan (1987).

Assessment of soil fertility was carried out according to the method of Vernander et al. (1951). Sod podzol, soddy gley sandy loam, and clayed sand soils are characterized by a small depth of the humus horizon (up to 25 cm) and low humus content (0.85 %). Grey forest podzolized clayed soils relate to soils with low fertility. Their upper layers are poor in organic and mineral colloids having a sprayed structure (Vernander et al. 1951).

The relationship between climate and types of forest conditions necessitated the creation of a forest typological classification of climates, which is based on the soil-hydrological classification of Alekseev-Pogrebnyak (Herushynskyi 1996). To determine the relationship between climate and types of forest conditions, Vorobiov (1953) proposed the empirical formula (1) used in our work.

$$W = \frac{R}{T^\circ} - 0{,}0286 \times T^\circ, \qquad (1)$$

where: W is an indicator of climate humidity; R is precipitation for the warm period, mm; T° is the sum of positive temperatures to estimate the amount of heat,

°C. The values of *R* and *T*° are average monthly from April to November.

Monthly average plus temperatures and monthly average precipitation are borrowed from the official website of the Ukrainian Hydrometeorological Center (2020).

Analysis of rare species was conducted according to the lists of the International Union for Conservation of Nature (IUCN) and the Red Book of Ukraine (Shaparenko and Shaparenko 2002). The IUCN Commission has developed the following categories for species survival: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Data Deficient (DD), Not Evaluated (IUCN 2020). The categories of the Red List of Ukraine are extinct (0), endangered (I), vulnerable (II), rare (III), unspecified (IV), unknown (V), and recovered (VI) (Shaparenko and Shaparenko 2002).

Aesthetic assessment of parklands was carried out on a three-point score scale of Kucheriavyi (2017). This scale has 2 parts: taxation and emotional (were provided by the authors). The first part takes into account landform, soil moisture, growth class, new growth, species composition and density of grass, stand age, forest density, clutter up the territory, signs of soil erosion, soil compaction. The second part takes into account crown closure, depth of perspective, the convenience of movement, colour scheme, wood texture, and shade density.

We selected research objects according to the geobotanical principle (Kuznetsov and Klymenko 2003). Of the 18 old parks of the Zhytomyr region, we chose six of them, which are located in various geobotanical areas, have a large area, and are less explored (Fig. 1).

Results and Analysis

Taxonomic analysis

Based on the collected materials and their analysis, the following was established: in heritage parks were revealed 71 species and 3 varieties of trees and shrubs belonging to 44 genera, 20 families (Table 1).

Pinophyta has 2 families Pinaceae and Cupressaceae, which are represented by four (5.6 %) and two (2.8 %) species of trees and shrubs, respectively. The first family includes *Pinus sylvestris* L., *Pinus nigra* Arn., *Picea pungens* Engelm. and *Picea abies* L. *Thuja occidentalis* L. and *Juniperus sabina* L. belong to the second family.

The most abundant Magnoliophyta family was Rosaceae, which is represented by 21 species (28.2 % of the total number of species). These include *Armeniaca vulgaris* Mill., *Cerasus avium* (L.) Moench., *Cerasus vulgaris* Mill., *Chaenomeles japonica* (Thunb.) Lindl., *Crataegus kyrtostyla* Fingerh, *Malus sylvestris* (L.) Mill., *Pyrus communis* L., etc. Family Salicaceae is represented by 8 species (11.3 %) and one variety: *Salix alba* L., *Salix alba* 'Vitellina Pendula', *Salix babylonica* L., *Salix caprea* L., *Salix fragilis* L., *Salix pentandra* L., *Populus alba* L., *Populus nigra* L. and *Populus tremula* L. The next in the number of representatives of the family, which has 6 species (8.5 %) and one variety, is Fabaceae. They are *Robinia pseudoacacia* L., *Genista tinctoria* L., *Gleditschia triacanthos* L., *Laburnum anagyroides* Med., *Chamaecytisus ruthenicus* (Fisch. ex Vorosch.) Klask, *Caragana arborescens* Lam. and *Robinia pseudoacacia* 'Decaisneana'. Representatives of Aceraceae count 6 species (8.5 %): *Acer platanoides* L. and *Acer*

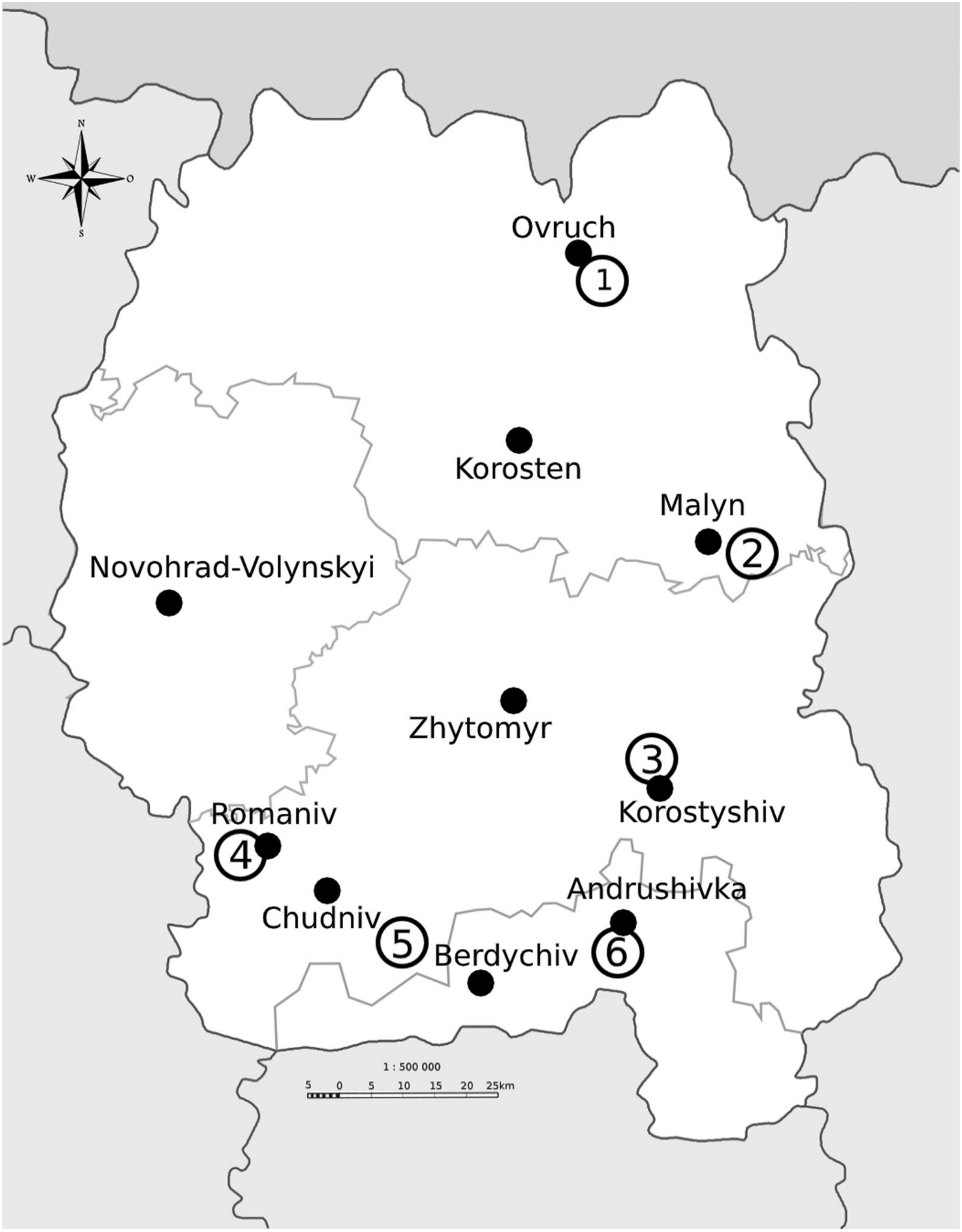

Fig. 1. Scheme of location of objects of research on the map-scheme of Zhytomyr region.
Parks: 1 – Yulino, 2 – Miklouho-Maclay, 3 – Korostyshivskyi, 4 – Vilkhivskyi, 5 – Turchynivskiy, and 6 – Chervonskiy.

negundo L. identified in all studied parks, in some parks found *Acer campestre* L., *Acer pseudoplatanus* L., *Acer saccharinum* L. and *Acer tataricum* L. Representatives of other families occupy less than 5 %.

Table 1. Systematic structure of woody plants of heritage parks of Zhytomyr region.

Families	Genera		Species		Varieties, hybrids	Parks					
	n	%	*n*	%	*n*	Yulino	Miklouho-Maklay	Korostyshivskyi	Vilkhivskyi	Chervonskiy	Turchynivskiy
Pinophyta											
Pinaceae Lindl.	2	4.5	4	5.6	-	+	+	-	+	-	-
Cupressaceae F. Neger	2	4.5	2	2.8	-	-	+	+	-	-	+
Magnoliophyta											
Aceraceae Lindl.	1	2.3	6	8.5	-	+	+	+	+	+	+
Berberidaceae Torr. Et. Gray	1	2.3	1	1.4	-	-	+	-	-	-	-
Betulaceae Agardh	4	9.1	4	5.6	-	+	+	+	+	+	+
Caprifoliaceae Juss.	3	6.8	3	4.2	-	+	-	-	-	-	-
Celastraceae Lindl.	1	2.3	2	2.8	-	-	+	-	-	-	-
Fabaceae Juss.	6	13.6	6	8.5	1	+	+	+	+	+	+
Fagaceae A. Br.	1	2.3	2	2.8	-	+	+	+	+	+	+
Hippocastanaceae Torr.	1	2.3	1	1.4	-	-	+	+	+	+	+
Juglandaceae Lindl.	1	2.3	2	2.8	-	-	-	+	-	+	+
Moraceae Dc.	1	2.3	2	2.8	-	-	-	+	-	-	+
Oleaceae Lindl.	2	4.5	4	5.6	-	-	+	+	+	+	+
Rosaceae Juss.	13	29.5	20	28.2	1	+	+	+	+	+	+
Salicaceae Lindl.	2	4.5	8	11.3	1	+	+	+	+	+	-
Sambucaceae Link.	1	2.3	1	1.4	-	+	+	+	-	+	+
Tiliaceae Juss.	1	2.3	1	1.4	-	+	+	+	+	+	+
Ulmaceae Mirb.	1	2.3	2	2.8	-	+	+	+	+	+	+
Viburnaceae Dum.	1	2.3	1	1.4		+	-	-	-	-	-
Vitaceae Juss.	1	2.3	1	1.4	-	+	+	+	+	+	+
Total	44	100.0	71	100.0	3						

In the 1960s and 1980s, the local authorities of the USSR reconstructed the plantations of most of the ancient parks of Ukraine, including Zhytomyr region. Unfortunately, the planting of trees and shrubs was unsystematic and rarely relied on scientific research. Table 2 lists the plants that have been planted or have settled themselves, and in our opinion do not correspond to the creative ideas of the authors of old parks, because there are non-indigenous species. *Acer negundo* L. and *Acer pseudoplatanus* L. are potentially invasive.

Table 2. List of trees and shrubs in parks planted after 1960.

Species	Parks					
	Yulino	Miklouho-Maklay	Korostyshivskyi	Vilkhivskyi	Turchynivskiy	Chervonskiy
Acer negundo L.	+	+	+	+	+	+
Acer pseudoplatanus L.	-	-	+	-	-	-
Alnus glutinosa L.	+	+	-	+	+	-
Armeniaca vulgaris Mill.	-	+	-	-	+	-
Cerasus avium (L.) Moench.	-	-	-	-	+	-
Cerasus vulgaris Mill.	-	+	-	-	+	-
Malus domestica Borkh.	+	+	+	+	+	+
Populus nigra L.	-	+	+	+	+	-
Populus tremula L.	+	+	-	+	+	-
Prunus divaricata Ledeb.	-	+	-	+	+	+
Prunus domestica L.	+	-	-	+	+	-
Pyrus communis L.	+	+	+	+	+	+
Robinia pseudoacacia L.	+	+	+	+	+	+
Viburnum opulus L.	-	+	-	-	-	-

These species of plants were practically not planted in the parks at the beginning of the XX century, and if they were planted, then in small quantities (Regel 1896). Today, most of these species are dominant (*Acer pseudoplatanus* L., *Alnus glutinosa* L., *Populus nigra* L., *Populus tremula* L., *Robinia pseudoacacia* L.).

Geographical analysis

According to geographical analysis, the species of dendroflora of parks come from Boreal and Mediterranean zones, which included 5 floristic regions (Fig. 2).

The most widespread are species originating from the Circumboreal floristic region. There are 32 taxa (43 %). These are species that grow naturally in Europe, the Caucasus, Siberia, Alaska and Northern Sakhalin: *Juniperus sabina*, *Picea abies*, *Pinus nigra*, *Acer platanoides*, *Acer tataricum*, *Aesculus hippocastanum* L., *Betula pendula* Roth., *Caragana arborescens*, *Lonicera caerulea* L., *Malus sylvestris*, *Sorbus aucuparia* L. and others.

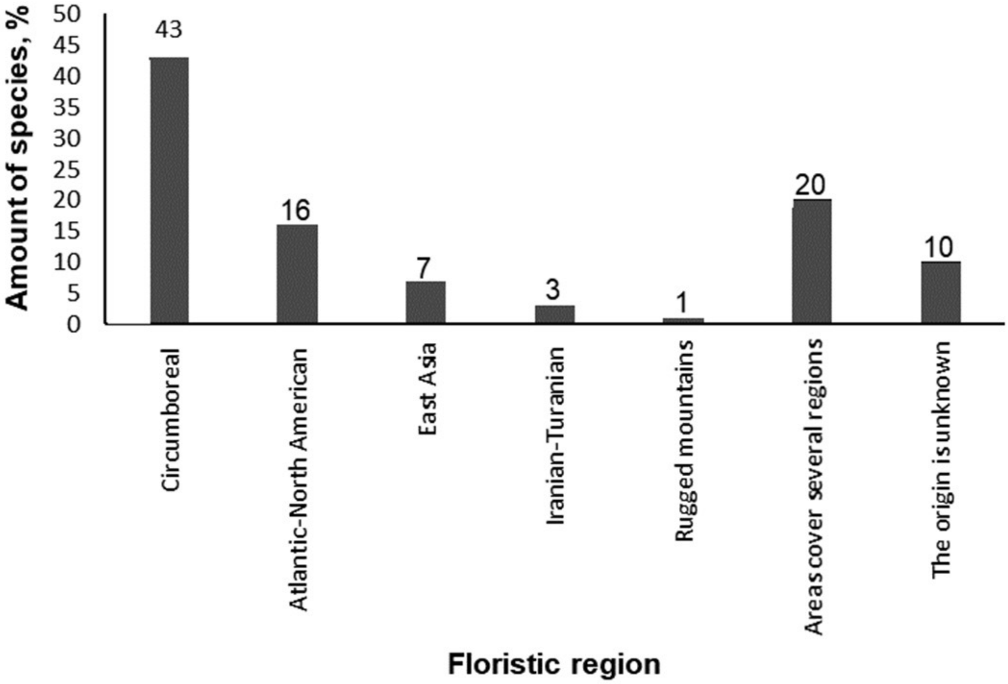

Fig. 2. Distribution of woody plants in parks by floristic regions.

The next place is occupied by species native to the Atlantic-North American floristic region (12 taxa). The natural range of these plants covers the area from the Atlantic coast of North America to the Great Plains and from the Gulf Coast to the southern regions of Canada. These are *Thuja occidentalis*, *Acer negundo*, *Acer saccharinum*, *Fraxinus pennsylvanica* Marsh., *Fraxinus lanceolata*, *Gleditschia triacanthos*, *Juglans nigra*, *Padus serotina*, *Parthenocissus quinquefolia*, *Physocarpus opulifolius*, *Quercus rubra,* and *Robinia pseudoacacia*.

There is a group of plants which ranges cover several floristic regions. *Pinus sylvestris*, *Cerasus avium*, *Corylus avellana* L., *Prunus divaricata* Ehrh., and *Rosa canina* L. belong to Circumboreal and Iranian-Turanian floristic regions. *Acer campestre*, *Berberis vulgaris* L., *Populus tremula* and *Salix alba* belong to Circumboreal and East Asia floristic regions.

A geographical analysis revealed a group of plants whose origin is unknown. These are *Cerasus vulgaris*, *Malus domestica* Borkh., *Malus prunifolia* Borkh., *Prunus domestica* L., *Robinia pseudoacacia* ‘Decaisneana’, *Salix alba* ‘Vitellina Pendula’ and *Spiraea vanhouttei* Zabel.

Ecological analysis

When assessing the state of green spaces of old parks, one should take into account the exactingness of tree and shrub species for certain growing conditions. According to the ecological principle of creating a composition, each plant bears the imprint of the geographical and climatic conditions in which this species was formed. Inconsistency of growing conditions with the needs of plant development is reflected in their appearance. Most plants reveal ornamental qualities completely only in certain conditions. In this regard, we analysed the correspondence of trees and shrubs of old parks in the region to their needs for land fertility (Table 3) and soil moisture (Table 4).

In terms of species, megatrophic plants growing on poor soils in the parks Yulino, Miklukho-Maklay, and Korostyshivskyi, prevail over oligotrophic plants (Table 3). We believe that in these parks, to create bio-groups, it is necessary to select plant species that will maximize their ornamental qualities on poor soils. Grey forest podzolized clayed type of soil is characteristic of Chervonskiy park (Table 3). Here we observe the dominance of plant species that do not require rich soils for their development and growth.

Chernozem-loess and degraded chernozem have sufficiently high fertility. According to the Table 3, the percentage of megatrophs and oligotrophs is distributed in favour of the first.

The zonal climate of forest conditions affects only the formation of loamy soils. All other soil types are intrazonal (Yulino, Miklukho-Maklay, and Korostyshivskyi parks). That's why we determined the zonal type of forest conditions for Vilkhivskyi, Turchynivskiy, and Chervonskiy parks, which are located in the south of the Zhytomyr region.

Table 3. Correspondence of woody plant species to soil conditions.

Park	Soil type	Plants trophotopes, %		
		Oligotrophic	Mesotrophic	Megatrophic
Yulino	sod podzol sandy loam	8.0	68.0	24.0
Miklouho-Maklay	soddy gley sandy loam	13.8	67.2	19.0
Korostyshivskyi	soddy gley clayed sand	20.0	56.7	23.3
Vilkhivskyi	chernozem-loess	12.9	64.5	22.6
Turchynivskiy	degraded chernozem	8.6	62.9	28.6
Chervonskiy	grey forest podzolized clayed	22.2	63.0	14.8

Table 4. Correspondence of park woody plants to humidification conditions.

Parks	Indicator of climate humidity (W)	Zonal type of forest conditions	Plants hygrotopes, %		
			Xerophyte	Mesophyte	Hygrophyte
Vilkhivskyi	1.6	slightly moist	6.5	87.0	6.5
Turchynivskiy	2.6	moist	5.7	88.6	5.7
Chervonskiy	2.6	moist	11.1	88.9	0.0

Table 4 shows the climate humidity calculation results and correspondence of park woody plants to humidification conditions.

Mesophytes are predominant. These plants grow under moderate conditions of moisture. In general, the plant assortment of the studied parks meets humidification conditions.

Phytosociological analysis

The studied parks have the status of parks, the monuments of landscape art, so it is important to identify rare species of woody plants. The phytosociological structure is represented in Table 5.

Table 5. Phytosociological structure of protected woody plants of parks.

Red list	Rarity category	Species number	%
International Union for Conservation of Nature	CR	2	2.8
	EN	1	1.4
	VU	2	2.8
	NT	1	1.4
	LC	48	67.6
	DD	6	8.5
	Total	60	84.5
Red Book of Ukraine	III	1	1.4
	Total	1	1.4

Aesthetic evaluation

The aesthetic assessment of the parks is given in Table 6.

Table 6. Aesthetic assessment of parks.

Parks	Assessment of signs, the average score			Aesthetic value class
	Taxation	Emotional	Average score	
Yulino	1.88	1.99	1.94	3
Miklouho-Maclay	1.87	2.11	1.99	3
Korostyshivskyi	2.01	2.01	2.01	2
Vilkhivskyi	1.93	1.96	1.95	3
Turchynivskiy	1.89	1.86	1.88	3
Chervonskiy	1.92	1.89	1.91	3

Table 6 shows that the studied parks have the lowest class of aesthetic assessment, only the Korostyshevsky park has second class. Some sections of the Miklukho-Maklay, Chervonsky and Turchinovsky parks have a second class of aesthetic value, but most territories are represented by a third class.

Discussion

None of the studied parks has the highest class of aesthetic value because care for parkland is almost not carried out. Also, during park reconstruction, plant species that did not fit soil types were planted there, like *Picea abies*, *Acer platanoides*, *Alnus glutinosa*, *Fraxinus excelsior*, *Laburnum anagyroides* Medik., *Populus tremula* L., *Quercus robur* L., and others.

When developing reconstruction projects, it is first of all necessary to eliminate the above mentioned disadvantages, thereby increasing the class of aesthetic value of parks. To increase the class of aesthetic value of parks, we recommend providing a reconstruction of the parklands. When carrying out works on the planting of trees and bushes it is necessary to provide a selection of a range of plants according to their demanding conditions of moistening and soil fertility (Table 7).

Table 7. Recommended list of plants for planting in old parks of Zhytomyr region.

Species	Parks					
	Yulino	Miklouho-Maclay	Korostyshivskyi	Vilkhivskyi	Turchynivskiy	Chervonskiy
Abies balsamea Mill.	-	-	-	+	+	+
Abies fraseri Poir	-	-	-	+	+	+
Abies nordmanniana Spach.	-	-	-	-	+	+
Abies sibirica Ledeb.	-	+	+	+	+	+
Chamaecyparis pisifera Endl.	-	+	+	+	+	+
Ginkgo biloba L.	-	+	+	+	+	+
Juniperus communis L.	+	+	+	+	+	+
Juniperus sabina L.	+	+	+	+	+	+
Larix gmelinii Rupr.	-	+	+	+	-	-
Larix decidua Mill.	+	+	+	+	+	+
Larix laricina K.Koch	+	+	+	-	-	-
Larix sibirica Ledeb.	+	+	+	+	-	-
Picea engelmanni Parry ex Engelm	-	-	+	+	+	+
Picea marianna Mill.	-	-	+	+	+	+
Picea rubens Sargent	-	-	+	+	+	+
Pinus pumila Regel	-	-	-	+	+	+
Pinus strobus L.	+	+	+	+	-	-
Taxus baccata L.	-	-	-	+	+	+
Betula ermanii Cham.	+	+	+	+	+	+
Castanea sativa Mill.	-	+	+	+	+	+
Crataegus nigra Waldst. Et Kit.	+	+	+	+	+	+
Fagus sylvatica 'Purpurea'	-	-	-	+	+	+
Fraxinus excelsior 'Pendula'	-	+	+	+	+	+
Gleditsia triacanthos L.	-	-	-	+	+	-
Gymnocladus canadensis L.	-	-	-	+	+	+
Liriodendron tulipifera L.	-	+	+	+	+	+
Phellodendron amurense Rupr.	-	-	-	+	+	+
Prunus virginiana L.	+	+	+	+	+	+
Quercus sessiliflora 'Longifolia Dipel'	-	+	+	+	+	+
Rhamnus cathartica L.	-	-	-	+	+	+
Salix alba 'Splendens'	-	-	-	+	+	+
Sorbus aucuparia L.	+	+	+	+	+	+

The full list of recommended plants can be seen in our work (Markov 2015). We also used the source of the renowned XIX century landscape architect Arnold

Regel to compile this list (Regel 1896).

According to Klymenko (2012), no matter how carefully some landscape nodes of the park were restored, if in its main territory instead of those species that were in the heyday of the park, others will dominate, the reconstruction of the park cannot be considered satisfactory. In our earlier work (Markov 2015) we found out that the main park-forming species are not species-edificators of native forests, but their companions – *Fraxinus excelsior*, *Tilia cordata*, *Acer platanoides*, *Ulmus glabra* and others. Significant areas are occupied by plantations in which none of the species dominates. Dudyn, Bahatska (2012) and Kuznetsov (2011) make the same conclusion. Probably the reason for this is the degradation of plantations that once consisted of edificatory species of native forests.

Thus, for the territories of research parks, which were created based on natural vegetation or artificially, but with the use of edificatory species of local forests and their satellites, phytocoenotic degradation is characteristic. It is the most dangerous for the plantations of ancient parks, as it threatens to replace valuable plantations on a large area with low-value derivatives (Klymenko 2012).

In our opinion, the reasons for the replacement of dominant species are also the unsystematic planting of trees and shrubs during the reconstruction of parks in Soviet times without taking into account the compliance of plants with environmental conditions. As well as the use of fruit plants that are less stable in urban conditions (Table 2). As a result, we have the lowest class of aesthetic evaluation of parklands (Table 6). That's why we recommended a list of plants for planting in old parks of the Zhytomyr region (Table 7).

Conclusions

There are 71 species and 3 varieties of trees and shrubs in heritage parks of the Zhytomyr region. The largest family is Rosaceae – 20 species (28.2 %). Family Salicaceae is represented by 8 species (11.3 %) and one variety. Other families have a share of less than 10 % of species. In general, the taxonomic composition of parks is poor. Most species grow in inappropriate soil conditions. According to the geographical analysis of the parks' woody plants come from Boreal and Mediterranean zones, which included 5 floristic regions. This shows that Zhytomyr region belongs to the zone of wide introduced opportunities. One park has a second class of aesthetic value, the remaining five have the third class. To increase the class of aesthetic value of parks we recommend clearing from clutter, felling sick and weakened trees and shrubs, planting trees and bushes, which are represented in our work.

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THE POTENTIAL OF *FICUS* SPECIES AS FRUGIVOROUS FEED ON GENTONG HILL, MOUNT UNGARAN, INDONESIA

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Abstract

Ficus plays an essential role in the forest ecosystem and can be used as an indicator of success in the local environment. Its lateral rooting system binds with soil, thereby maintaining soil stability. The fig fruit of *Ficus* is essential food for frugivores. This study aims to determine the species richness and potential as frugivorous feed, focusing on Gentong Hill, Mount Ungaran. Mount Ungaran is a forest ecosystem with a variety of flora and fauna, including various species of *Ficus*, and was selected as a research location due to the habitat it provides for animals and plants. This research was conducted between January and May 2018. Data was selectively sampled based on *Ficus* accessibility and location. Its feed potential was determined using scoring based on fruit dimensions and colour. The research showed 24 species of *Ficus* in six sub-genera. According to our scoring results, these species were divided into four categories based on their frugivorous feed potential: high potential (4 *Ficus* species), potential (11), and less potential (9). This finding implies that Gentong Hill plays an important role in providing food for frugivores, and its maintenance is urgently necessary.

Key words: *Ficus*, frugivore, Mount Ungaran, species richness.

Introduction

Ficus L. (Figs) appears to be a genus that widely spreads throughout Southeast Asia and is considered a keystone resource for sustaining wild animals feed in the tropical forest (Caughlin et al. 2012). Indonesia is home to various species of *Ficus*, including 75 in six sub-genera in Java: *Urostigma*, *Sycidium*, *Sycomorus*, *Pharmacosycea*, *Ficus*, and *Synoecia* (Berg and Corner 2005). *Ficus* vary in morphology and can include trees, epiphytes, hemi-epiphytes, shrubs, creepers, and lianas. The 'fruit', more accurately a pseudo-fruit, is a receptacle that contains a large number of small fruits (achenes) inside (Poonswad 2012). *Ficus* produces a large quantity of fruit throughout the year and provides food for many frugivores (Tweheyo and Lye 2003), making it a major resource in the ecosystem. It is consumed by many frugivores, including 92 species of birds, 12 species of mammals, and 8 species of insects (Francis et al. 2013). Preliminary studies show that *Treron griseicauda* (Bonaparte, 1855) and *Psilopogon* spp. (Müller, 1836) have consistently been observed to consume *Ficus* in the Gentong Hill region of Mount Ungaran. Strangler figs species are the main resource for other species, as they support ecosystems and are capable of symbiotic mutualism with other species (Lu et al. 2001, Payton et al. 2002).

Gentong Hill is a forest area in the northeastern part of Mount Ungaran, located at 7°12′ S and 110°20′ E in Ngesrepbalong Village, Kendal. Gentong Hill is a habitat for endangered and protected animals, including *Aceros undulatus* (Shaw, 1811), (Rahayuningsih and Kartijono 2013), *Nisaetus bartelsi* (Stresemann, 1924), *Ictinaetus malayensis* (Blyth, 1843), *Lorichulus pusillus* (Gray, 1859), *Trachypithecus auratus* (Geoffroy, 1812), and *Hydrornis guajanus* (Statius Müller, 1776). Mount Ungaran is also classified as an Important Bird Area, as it is home to various species of birds which are protected and need to be preserved (Rahayuningsih et al. 2017) and is important for bird conservation due to its adequate resources and habitat (Manu et al. 2010).

However, bird hunting, deforestation, construction of tourist attractions, and settlements have threatened these species in Mount Ungaran. Central Java Province has a relatively high rate of deforestation, averaging 142,560 ha per year between 2000 and 2005. Meanwhile Mount Ungaran had 5413.94 ha of forest area in 1990, this number reduced by 28.43 % to 3874.79 ha in 2000 and decreased yet further to 1335.77 ha in 2006. In the past 16 years, it has lost 4078.17 ha (75.33 %) of forest area (Gunawan et al. 2010).

The forest area on Mount Ungaran is subdivided into natural and secondary forests (Rahayuningsih et al. 2015). Between 1991 and 2009, the natural forest area had a tendency to change. The distribution of natural forests decreased by 53.31 %, while other species of land increased, including secondary forests (63.98 %), plantations (38 %), gardens (82.29 %), and settlements (55.38 %). Various studies are currently being conducted to provide data in support of the protection of Mount Ungaran. The study of its resources is vital in evaluating the actual condition of feed for species inhabiting the area. Studies on *Ficus* diversity are very rarely carried out because the large number of species makes the identification difficult (Harrison 2005). As poor as research about the species of *Ficus* on Gentong Hillis, was limited. Caused those several reasons, we expected to provide profile data on *Ficus* species and protected areas on Gentong Hill, Mount Ungaran. Not only to determine the species richness, but also to evaluate various *Ficus* species that have potential as frugivorous feed and the frugivores attracted the kinds of fruits in this area.

Materials and Methods

Study area

This research was conducted between January and April 2018 on Gentong Hill, Mount Ungaran (7°12′ S and 110°20′ E), Ngesrepbalong Village, Limbangan District, Kendal, Central Java, Indonesia (Fig. 1).

Area division

The study area was further subdivided into three areas based on dominant vegetation: Area A (*Syzygium* sp. R.Br.

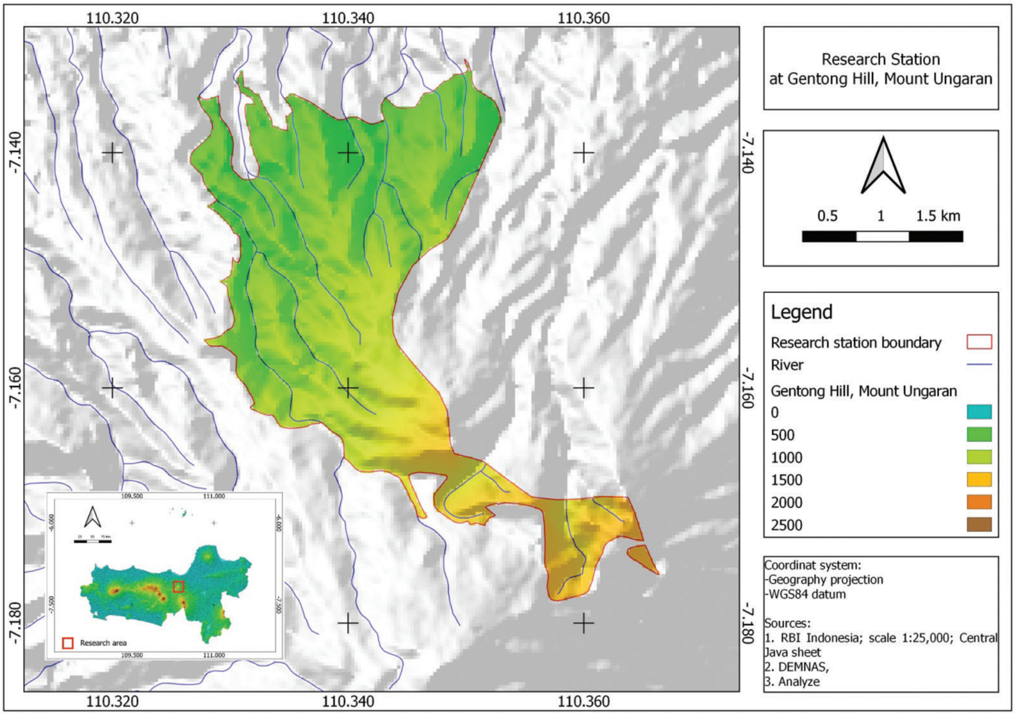

Fig. 1. Research area.

ex Gaertn. 1788); Area B (*Dacrycarpus imbricatus* (Blume) de Laub. 1969, and various species of Lauraceae family); and Area C (*Weinmannia fraxinea* (D. Don) Miq. 1856).

Collection and identification of specimens

Area A was divided into two observation areas, while areas B and C had one observation area. The species of *Ficus* were identified based on morphology, inflorescences, fig size, and scientific identity. The unknown species were carried out to record morphological data in the Biology Laboratory, Faculty of Mathematics and Science, Semarang State University. Identification was performed based on keys provided by Berg and Corner (2005).

Data analysis

Identification was performed descriptively based on the key determinants of *Ficus* Flora Malesiana by Berg and Corner (2005). Its fruits were divided into three groups based on the size in accordance with Lambert and Marshal (1991): small ($d \leq 1.5$ cm); medium (1.5 cm > $d \geq 2.5$ cm); and large ($d > 2.5$cm). The potential of *Ficus* adjusted to the animal's preference in selecting fig was determined via scoring.

The score was based on habits, the way how the species showed their fig, syconia size, and fig colour. The type of hemi-epiphytic fig has the highest value based on its fruit production which is abundant throughout the year (Poonswad 2012). Then followed by tree, liana and shrub types. Axillary fruit is very popular with various types of frugivores. In other hands, fruits on the trunk or branches affect mammals more than birds. Flagelliflorous fertilization is rarely a modification of cauliflorous with very long fruit stalks. Moreover, this is rarely the favor of frugivores (Shanahan and Compton 2001). Black/dark fruit color has the highest score, then the green color has the lowest score (Table 1). According to Shanahan and Compton (2001), the black fruit or dark fruit attracts all of types of frugivores, while the red, yellow, orange or pink were the main attraction for birds, and the green ones only attracts a few mammals, where there are far fewer species than birds. *Syconia* size is also determines the score. Medium fruit has the greatest score, while the large fruit has the lowest score (Table 1). It assumes by that all frugivores of various sizes can consume it, while large fruit can only be consumed by certain species with large body size, whereas such frugivores are very rare (Kinnaird and O'Brien 2007; Tan et al. 2014).

Table 1. List of morphological attributes for scoring of figs potential.

Observed attributes	Categories	Scores
Habits	Hemi-epiphytes	25
	Trees	12.5
	Lianas	6.25
	Shurbs/epiphytes	3.125
The way how the species showed their fig	Axillary	25
	Ramiflorous/cauliflorous	12.5
	Flagelliflorous	6.25
Fig color	Purplish/Black/Dark red	25
	Orange/yellow/pinkish	12.5
	Cream/greenish	6.25
	Brown	3.25
Syconia size categories	Large	6.25
	Medium	25
	Small	12.5

The score values were divided into four categories: no potential (≤25); less potential (26–50); potential (51–75); and high potential (≥76). Frugivores attracted by figs fruit were evaluated according to Shanahan and Compton (2001) who divided them into several categories including: canopy birds, arboreal mammals, understory birds, bats only, terrestrial mammals, and primates only.

Results and Discussion

A total of 24 fig species in six sub-genera were found in three areas of Gentong Hill, Mount Ungaran (Fig. 2).

The most common species originates from *Urostigma* sub-genus, followed by *Sycidium*, *Synoecia*, *Sycomorus*, *Ficus*, and *Pharmacosycea*. According to Berg and Corner (2005), Java Island contains 75 species of *Ficus* in six sub-genera. Our results showed that Gentong Hill represents all of *Ficus* sub-genera and 26.67 % of all *Ficus* species in Java. *Urostigma* sub-genus has the largest percentage of floristic constituents (42 %) compared to the other sub-genera. According to Berg and Corner (2005), 32 % of *Ficus* species in Java are of *Urostigma* sub-genus. This result is not surprising, as the genus *Ficus* is a species of cosmopolite (Berg and Corner 2005) and has a very wide tolerance range of environmental factors (Pociecha et al. 2016). In addition, *F. involucrata* is an endemic *Ficus* of Java island, Indonesia (Berg and Corner 2005). However, the reports are very rare to discuss about this species, even though this species is one of high potential types in providing feed for frugivores (Fig. 3).

In accordance with scoring results, 4 species were classified as 'high potential', 11 as 'potential', and 9 as 'less potential'

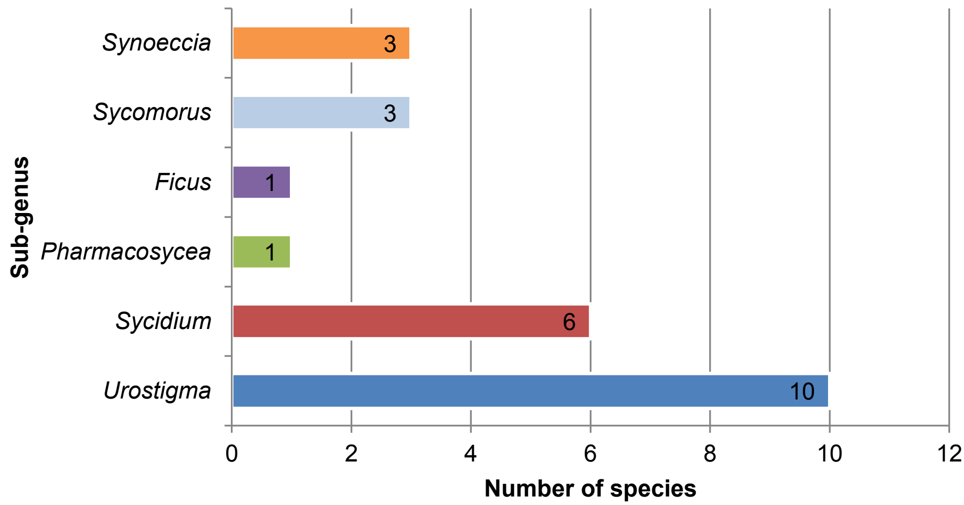

Fig. 2. *Ficus* species richness.

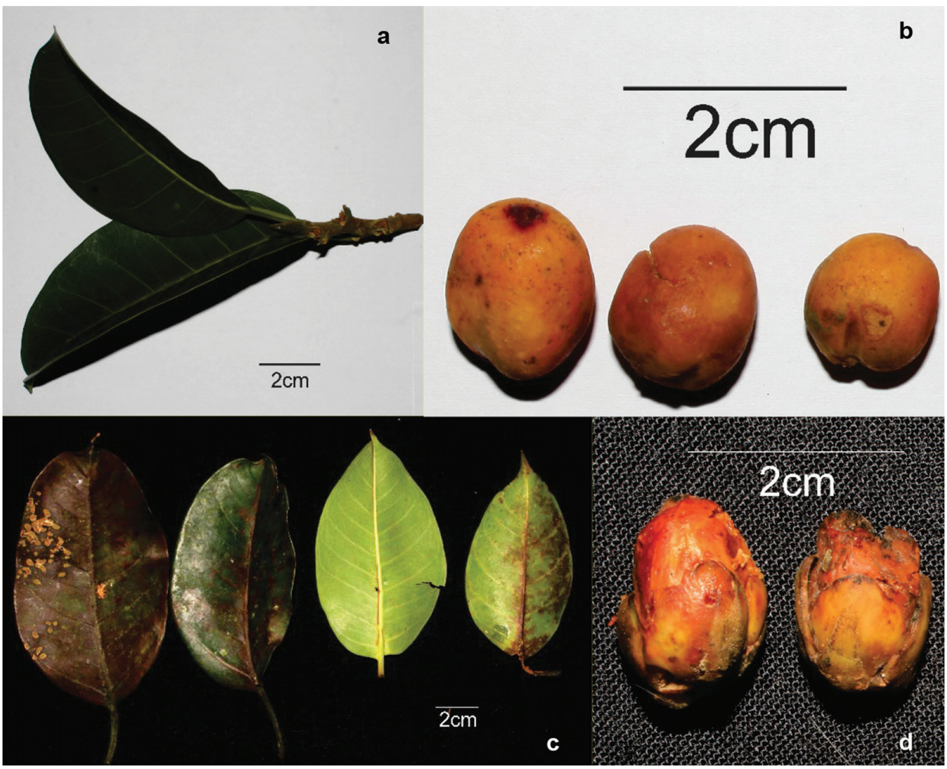

Fig. 3. Common *Ficus* species which has high potential value as frugivores feed at Gentong Hill, Mount Ungaran: *F. kerkhovenii*: a. leaves, b. ripe fruits; *F. involucrata*: c. leaves, d. ripe fruits.

(Table 2). High-potential species were morphologically classified as hemi-epiphytes with a large amount of fruit, medium-sized fruit, and a black or dark red color. The hemi-epiphyte species of *Ficus* generate a great deal of fruit production and were preferred by many frugivores (Naniwadekar et al. 2015). In addition, the species of frugivore has a tendency to select as fruit with a dark or black color.

Table 2. Species richness, characteristics, potential, and frugivores attracted of each *Ficus* species.

Subgenus	Species	Category	Frugivoes attracted[2]
Urostigma	*F. kerkhovenni* Valeton	High potential	CB, AM, UB
Urostigma	*F. benjamina* L.	Potential	CB, AM, UB
Urostigma	*F. binnendijkii* Miq.	Potential	CB, AM, UB
Urostigma	*F. caulocarpa* (Miq.) Miq.	Potential	CB, AM, UB
Urostigma	*F. crassiramea* subsp. *crassiramea* (Miq.) C.C.Berg	High potential	CB, AM, UB
Urostigma	*F. crassiramea* subsp. *stupenda* (Miq.) C.C.Berg	Potential	CB, AM, UB
Urostigma	*F. drupacea* Thunb.	High potential	CB, AM, UB
Urostigma	*F. involucrata* Blume[1]	High potential	CB, AM, UB
Urostigma	*F. microcarpa* L.f.	Potential	CB, AM, UB
Urostigma	*F. virens* Aiton	Potential	CB, AM, UB
Sycomorus	*F. fistulosa* Reinw. ex Blume	Potential	BO
Sycomorus	*F. ribes* Reinw. ex Blume	Less potential	TM
Sycomorus	*F. variegata* Blume	Less potential	PO
Synoecia	*F. lanata* Blume	Potential	CB, AM, UB
Synoecia	*F. punctata* Thunb.	Less potential	PO
Synoecia	*F. villosa* Blume	Less potential	CB, AM, UB
Sycidium	*F. ampelas* Burm.f.	Less potential	BO
Sycidium	*F. cuspidata* Reinw. ex Blume	Less potential	UB
Sycidium	*F. montana* Burm.f.	Less potential	TM
Sycidium	*F. obscura* Blume	Less potential	UB
Sycidium	*F. sinuata* Thunb.	Potential	UB
Sycidium	*F. tinctoria* G.Forst.	Less potential	UB
Ficus	*F. fulva* Reinw. ex Blume	Potential	CB, AM, UB
Pharmaco-sycea	*F. nervosa* subsp. *pubinervis* (Blume) C.C.Berg	Potential	BO

Note: [1]Java endemic species; [2]Frugivores attracted according to Shanahan and Compton (2001): CB=Canopy birds, AM=Arboreal mammals, UB=Understory birds, BO=Bats only, TM=-Terrestrial mammals, PO=Primates only.

Urostigma species is characterized as a high-potential (4 species) and potential (6 species). *Urostigma* is generally a hemi-epiphyte and known as strangler figs (Sastrapradja and Afriastini 1984). They have a large canopy (Harrison et al. 2003), and provide fruit throughout the year (Dew and Boubli 2005). The primary colors of this sub-genus are yellow, red, and black, which are highly favored by various species of frugivores (Kinnaird and O’Brien 2007). This explained why fig species from this sub-genus are frequently visited by various types of frugivores at fruiting in the study area. The types of frugivores that most frequently visit fruiting figs were the yellow-throated hanging parrot (*Lorichulus pusillus* Gray 1859), black-banded barbet (*Psilopogon javensis* (Horsfield, 1821)), and grey-cheeked green pigeon (*Treron griseicauda* Wallace, 1863). There is one additional piece of evidence which demonstrates that *Ficus* can support frugivores all year round, implying keystone status. It is the only known plant taxon upon which frugivorous birds in South-east Asia have specialized. *Treron* spp. (forest green pigeons) and probably some *Megalaima* (*Psilopogon*) sp. (barbets) are fig-eating specialists (Leighton and Leighton 1983).

On the other hand, the less potent species come from *Sycomorus* sub-genus (2 species), *Synoecia* (2 species) and most of *Sycidium* (5 species). The species of *Sycidium* generally are shrubs with low number of fruits. *Sycomorus* sub-genus such as *F. variegata* and *F. ribes*. *F. variegata* has a large fruit character which is only attractive to certain primate species, while *F. ribes* with flagelliflorous fruit type is only attracted by land mammals (Shanahan and Compton 2001).

Various morphological combinations of fig species at the study site showed the feed availability for all frugivore categories. Especially for medium sized fruit, it could be consumed by various frugivores with various sizes (Tan et al. 2014). Meanwhile, primates prefer large fruit with a size of more than 5 cm such as *F. variegata*. This is an evolutionary form of fig species forming a strategy for seed dispersal by developing the shape and fruit color in such a way as to attract the different types of frugivores that consume them.

Ficus on Mount Ungaran has potential as feed for frugivore groups, such as birds and mammals. They provide habitats for them (Rahayuningsih et al. 2017) and large trees for nesting (Rahayuningsih and Kartijono 2013). This study was able to explain that the various characteristics of fig from various categories could provide food for various types of frugivores. Even for the less potent species, figs still have a particular role to play for frugivores with special characteristics. This confirmed that the forest condition in Bukit Gentong, Gunung Ungaran, has good food availability, especially for frugivores. Forest areas with large trees and abundant feed have high potential as habitats for frugivores (Gomes et al. 2008).

A total of 24 *Ficus* with various fruit sizes have been identified, including 4 high-potential species, 11 potential species, and 9 less potential species. Strangler figs species are major resources (Kinnaird and O'Brien 2007), and losing them can lead to mass extinction (Watson and Herring 2012). Over all, *Ficus* protection is therefore essential to providing feed resources on Mount Ungaran to protect balance the ecosystem. However, less potential species does not mean that they do not have an ecological function, such as *F. variegata* which provides food for primates, where almost all species are scarce.

Conclusion

The research showed 24 species of *Ficus* species in six sub-genera recorded in Gentong Hill of Mount Ungaran. Those which has high potential value were *F. kerkhovenni, F. crassiramea* ssp. *crassiramea, F. drupacea, F. involucrate*. The various characteristics of figs from different categories were able to provide food for different frugivore groups. Even for the less potent species, figs on Mount Ungaran still have a certain role for the frugivore species with special characters.

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OPTIMIZATION OF THE COMPOSITION OF FOREST STANDS INCLUDING NORWAY SPRUCE ACCORDING TO THEIR RESISTANCE TO ADVERSE CLIMATIC FACTORS

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Abstract

The article considers the issue of establishment and formation of new plantations in forest areas, passing continuous sanitary logging. The evaluation of the reaction of European spruce in stands of various species composition to the different climatic factors impact by analyzing dendroclimatic information has been carried out. It was found out that the least meteosensitivity of spruce is observed in lime stands. Predicting the conditions of Norway spruce trees (*Picea abies* (L.) Karst.) and spruce stands in general after catastrophic drought of 2010 followed by eight-toothed bark beetle (*Ips typographus* L.) invasion is of immediate interest now. On the practical side optimization of stand composition according to the reaction to climatic factors is advantageous.

Key words: Norway spruce, Moscow region, drought resistance, tree rings, stand composition.

Introduction

Probably the first fundamental work about regularities of radial growth variation of Norway spruce trees was that of Norwegian dendrochronologist B. Eklund (Eklund 1957). Patterns, identified in it are of immediate interest now; for example the strong correlation between early wood width and late wood width oscillation requires further ecophysiological interpretation. In recent times the possibilities for research work in this domain have become much wider thanks to the introduction of new modern specialized equipment and software. Among the works of recent years many contain promising results (Ditmar and Elling 2004, Koprowski and Zielski 2006, Ribnicek et al. 2010, Hartl-Meier et al. 2014, Rumyantsev et al. 2016, Solomina et al. 2017).

For forestry practice in Norway spruce forests the main problem is the periodical death of the stands triggered by the effects of droughts. Drought resistance of spruce and predicting the conditions of Norway spruce trees and stands in general after catastrophic droughts followed by bark beetle (*Ips typographus* L.) invasion has been of particular interest then and now (Sukachev 1928, Tkachenko 1939, Pravdin 1975, Vorontsov 1978, Ditmar and Elling 2004, Rybníček et al. 2010). On the practical side optimization of stand composition according to their reaction to climatic factors is advantageous. Dendroclimatological investigations can help in achieving this goal.

Radial increment can be an indicator of a tree condition to some extent. It should be expected that short-time variation of radial increment will differ depending on forest stand composition where the recorded trees of a certain species grow (Ellenberg 1952). It is interesting to analyze the specific character of the influence of climate factors on the radial growth of spruce depending on the composition of the plant community. Stand-forming species have different ecological requirements. In the process of interspecific competition in Spruce stands, fluctuations in radial growth should take place differently depending on which species is the main competitor of spruce in the plant community. Competition for abiotic resources of the environment, as shown above, modifies the nature of fluctuations in radial growth from year to year.

The aim of this work was to assess the reaction of Norway spruce in stands of different species composition to the impact of different climatic factors.

Materials and Methods

To check the hypothesis about difference of Norway spruce radial growth fluctuations in stands differing in species composition, as initial objects we chose 13 forest stands with various species composition of Shcholkovsky Training and Experimental Forest Range (TEFR) belonging to Mytishchi branch of BMSTU.

The Scholkovsky TEFR is situated in the Moscow region, not far from Moscow (less than 50 km) (55.948165 °N, 38.127995 °E). The climate of the region is moderately continental and typical for the southern taiga zone of the Russian plain.

A core sampling was organized in the stands selected for investigation. One core was taken from each tree at a height of 1.3 m. The number of cores taken from each permanent sample area (PPP) varied from 7 to 13. The Inventory characteristics of experimental plots are presented in Table 1.

Lintab 5 equipment was used for tree ring measuring and Tsap-Win program was used to control the accuracy of measurement by the cross-date procedure (Palchikov and Rumyantsev 2009, Matveev and Rumyantsev 2013). Individual tree-ring chronologies were indexed by rationing radial annual increment to average radial increment over the past 5 years. On the basis of the indexed chronologies average chronologies for each sample plot were built.

To conduct the first part of our research we have selected forest stands with the following species composition: pure spruce (10 units), stands with spruce predominance (7 units), stands with aspen predominance (5 units), stands with birch predominance (7 units), stands with pine predominance (5 units), stands with lime predominance (7 units). We have conducted the analysis of the climatic factors impact on radial increment of spruce depending on plant community composition.

Table 1. Inventory characteristics of experimental plots.

Plot No	Year of last inventory		Species composition by tree layers	Average diameter by tree layers, cm	Average height, m	Cross-sectional area sum, m²/ha	Relative density	Average age, years	Growing stock, m³/ha	Site quality	Understory trees, thousands of individuals/ha (2013–2016)
10	2013		8S1As1B+O	19.2	15.5	23.3	0.54	58	181	II	5O4As1L (2.66)
	2016		8S1As1B+O	20.2	16.9	24.7	0.54	61	206	II	
100	2001		7S1As1B1L+P	20.3	21.4	33.3	0.65	47	341	Ia	9L1O (4.29)
	2013		7S1As1B1L+P	23.9	24.5	32.8	0.6	60	379		
106	2002		10S	14.2	15.2	40.6	0.96	44	310	I	8L2S+O (2.67)
	2013		10S	17.1	15.8	52.2	1.0	55	412	II	
119	2013	I	5L3As1B1S	26.1	25.0	32.5	0.9	55–65	364	Ia	7L2S1Al+O (5.7)
		II	9L1S+B	11.6	16.7	4.2	0.1	40–45	33		
120	2013	I	9S1B+As	21.5	20.4	25.9	0.72	127	242	III	6O4S (0.65)
		II	8S2B+O,As	12.7	16.5	10.0	0.29		78		
	2016	I	9S1B+As	22.4	23.1	29.2	0.66	130	273	III	
		II	8S1B1O+As	12.7	18.3	10.5	0.27		80		
121	2013		9S1B+O	19.8	15.4	38.6	0.89	55	298	II	10O (0.12)
	2016		9S1B+O	21.1	17.7	38.0	0.82	58	330		
122	2013	I	5As3L1B1S	24.5	23.0	35.6	1.0	60–75 (122*)	370	I	9L1S (7.31)
		II	7L3S	12.7	14.0	7.7	0.3	35–50	52		
123	2014		6L3S1B + P, O	32.2	28.7	40.3	1.0	75–90	511	Ia	9L1S+O, M (4.25)
127	2014	I	7L2S1B+O	23.6	25.0	29.5	0.9	65–70 (125*)	330	Ia	7S3L+O (5.74)
		II	8L2S+B, O	10.4	13.4	3.8	0.1	30–45	25		
129	2014	I	7B3L+As, S	16.1	23.3	12.7	0.5	30–35 (65*)	134	Ia	10L+As, O, S (4.14)
		II	5S5B+As, S	9.6	16.1	3.7	0.1	20–35	28		
131	2014	I	5P5B+S, L	26.1	27.3	31.3	0.7	65–80	379	Ia	9L1S+O (6.8)
		II	5S4L1B+P, M, O	11.2	13.7	8.3	0.3	30–55	55		
135	2015		4L3S3B+O, As	20.1	18.7	37.5	1.0	75	342	Ia	5L3S1B 1As+O (3.83)
140	2015		9S1B+O	21.4	24.8	45.7	0.83	66	534	I	10O (0.23)

Note: * – age of individual trees. Legend: S – Norway spruce (*Picea abies* (L.) Karst.); L – Small-leaved linden (*Tilia cordata* Mill.); As – Common aspen (*Populus tremula* L.); O – Common oak (*Quercus robur* L.); B – Silver birch (*Betula pendula* Roth.); P – Scots pine (*Pinus sylvestris* L.); Al – Grey alder (*Alnus incana* (L.) Moench.); M – Norway maple (*Acer platanoides* L.). Numbers symbolize the share out of 10.

Results and Discussion

The example of radial growth dynamic in spruce stands are shown in Fig. 1. Visual analysis of graphs says that the radial growth has significant variability from year to year and between different plots.

Tables 2 and 3 show the results of correlation analysis of the conjugacy of radial growth fluctuations and fluctuations in meteorological parameters of the current and previous year. Reliable values of correlation coefficients are shown in bold in the tables. At degrees of freedom = 53 and $\alpha = 0.05$, the correlation coefficient values from 0.27 and higher are significantly different from zero. The example chronologies from stands with spruce predominance more than 7 units are shown in Figure 1.

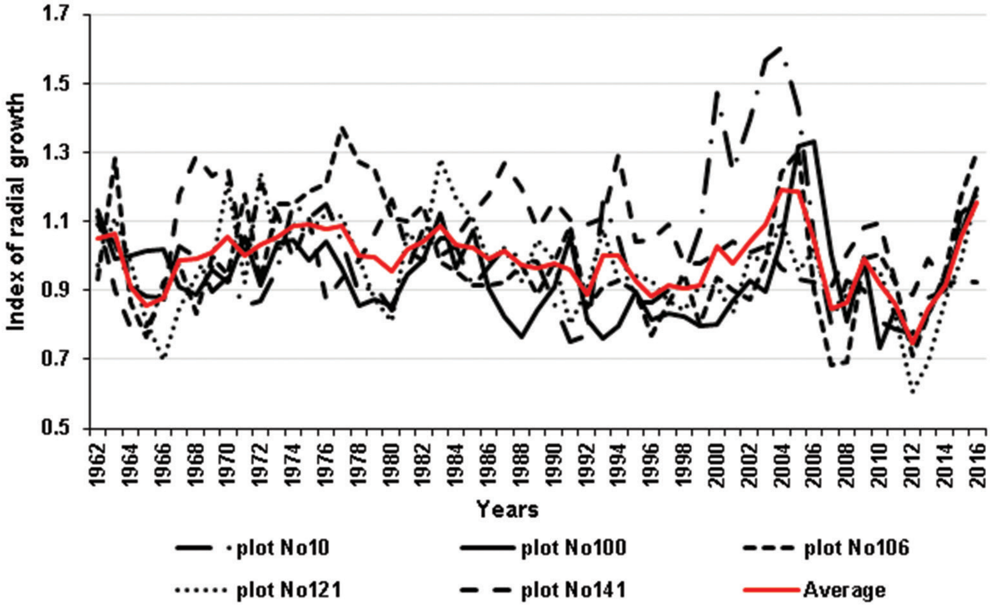

Fig. 1. Example chronologies from stands with spruce predominance more than 7 units.

Table 2. Correlation coefficients between increment indices and meteorological parameters of the current year.

Meteorological parameter	Chronology					
	Pure spruce stands	Stands with spruce predo-minance	Stands with aspen predo-minance	Stands with birch predo-minance	Stands with pine predo-minance	Stands with lime predo-minance
W in January	-0.01	0.15	-0.02	-0.08	**0.31**	-0.10
W in February	-0.05	-0.06	0.21	**0.30**	-0.13	-0.05
W in March	-0.19	-0.18	0.05	0.08	-0.06	0.05
W in April	0.03	0.20	-0.19	-0.18	-0.02	-0.17
W in May	0.17	0.18	0.22	**0.32**	-0.20	0.09
W in June	0.22	-0.05	0.08	0.22	**0.29**	0.00
W in July	0.16	0.10	0.19	0.21	-0.01	0.11

Meteorological parameter	Chronology					
	Pure spruce stands	Stands with spruce predo-minance	Stands with aspen predo-minance	Stands with birch predo-minance	Stands with pine predo-minance	Stands with lime predo-minance
W in August	-0.14	-0.14	-0.09	-0.01	0.15	-0.18
W in September	-0.12	-0.15	**-0.34**	-0.25	0.16	**-0.30**
W in October	-0.15	0.01	-0.02	0.04	-0.18	-0.11
W in November	0.21	0.00	0.01	-0.07	-0.13	0.09
W in December	0.16	-0.03	0.07	0.01	0.19	0.03
T_{av} in January	-0.14	0.06	0.12	0.13	0.19	-0.11
T_{av} in February	-0.01	-0.20	**0.37**	**0.37**	0.03	0.02
T_{av} in March	-0.14	-0.17	**0.32**	**0.29**	-0.03	0.03
T_{av} in April	-0.22	-0.10	0.06	0.15	0.09	0.01
T_{av} in May	0.06	0.03	-0.22	**-0.36**	-0.10	0.02
T_{av} in June	-0.24	-0.22	-0.07	-0.13	0.10	**-0.28**
T_{av} in July	-0.18	**-0.31**	-0.09	-0.14	0.08	-0.17
T_{av} in August	-0.22	-0.08	-0.21	**-0.32**	-0.04	-0.19
T_{av} in September	0.02	0.26	0.19	0.02	-0.07	0.07
T_{av} in October	**-0.30**	0.07	-0.05	-0.16	**0.29**	-0.04
T_{av} in November	0.18	0.16	-0.17	-0.06	-0.19	0.19
T_{av} in December	-0.09	0.24	0.17	0.03	-0.01	-0.14

Note: the correlation coefficients in bold are significantly different from zero; *W* – rainfall, T_{av} – average temperature.

Table 3. Correlation coefficients between increment indices and meteorological parameters of the previous year.

Meteorological parameter	Chronology					
	Pure spruce stands	Stands with spruce predo minance	Stands with aspen predo-minance	Stands with birch predo-minance	Stands with pine predo-minance	Stands with lime predo-minance
W in January	0.01	0.18	-0.03	-0.03	**0.36**	-0.08
W in February	-0.07	-0.11	0.01	0.18	-0.16	0.22
W in March	-0.06	-0.07	-0.12	-0.03	-0.04	0.06
W in April	0.08	0.15	0.05	-0.07	-0.08	0.09
W in May	**0.32**	**0.27**	**0.27**	0.24	-0.13	0.24
W in June	0.15	0.01	0.05	0.01	0.18	0.00
W in July	0.26	**0.28**	0.10	0.17	0.15	**0.34**
W in August	-0.04	-0.16	0.16	0.16	0.03	0.15
W in September	0.02	0.02	-0.14	-0.25	0.26	-0.04
W in October	-0.02	-0.15	-0.16	-0.06	-0.21	-0.05
W in November	0.10	-0.16	-0.24	-0.08	-0.13	0.21
W in December	0.13	0.09	0.10	0.00	0.07	0.19

Meteorological parameter	Chronology					
	Pure spruce stands	**Stands with spruce predo minance**	**Stands with aspen predo-minance**	**Stands with birch predo-minance**	**Stands with pine predo-minance**	**Stands with lime predo-minance**
T_{av} in January	-0.10	0.03	0.12	0.21	0.12	0.03
T_{av} in February	**0.29**	0.06	0.21	**0.45**	-0.08	0.16
T_{av} in March	0.14	-0.09	0.03	0.25	-0.07	0.08
T_{av} in April	-0.10	-0.01	-0.05	0.19	-0.11	0.15
T_{av} in May	-0.16	-0.04	-0.18	-0.20	-0.06	-0.07
T_{av} in June	**-0.33**	**-0.31**	-0.06	0.07	0.06	-0.18
T_{av} in July	-0.18	-0.07	-0.10	-0.11	-0.14	-0.23
T_{av} in August	-0.25	0.04	**-0.29**	-0.24	-0.13	**-0.41**
T_{av} in September	-0.15	0.25	-0.09	-0.10	-0.12	-0.22
T_{av} in October	**-0.37**	0.04	-0.05	0.05	0.05	-0.16
T_{av} in November	0.17	0.14	-0.14	-0.17	**-0.30**	0.06
T_{av} in December	0.03	**0.31**	**0.30**	0.25	0.00	-0.12

Note: the correlation coefficients in bold are significantly different from zero. *W* – rainfall, T_{av} – average temperature.

Based on the data in tables 2 and 3, it should be concluded that a specific set of reliable correlation coefficients was observed in chronologies or from stands of different species composition. However, the values of correlation coefficients were small and indicated only the presence of weak links. The maximum value of the correlation coefficient was observed for average temperature of February of the previous year (0.45) and spruce radial growth in stands with birch predominance. There was a relatively high correlation between spruce radial growth in stands with birch predominance and aspen predominance and average temperature of February of current year, too. The temperatures above average have positive effect for spruce radial growth. It may explain their influence on snowmelt and more rapid onset of the growing season. As a whole, even the particular ecophysiological mechanisms of the significant correlations are not understood, they are a biological fact, which was established by scientific methods and need further investigations. For example, the negative effect of high temperatures in May for radial growth of Spruce in the stands with birch predominance (correlation coefficient -0.36) may be related to the high reflective properties of birch bark for sun light, high light transmission capacity of the birch canopy in this period, and intensive cambial activity of the Spruce at that time, because the cambium of spruce is considered very sensitive for thermal damage (Tkachenko 1939, Vorontsov 1978, Hacura et al. 2011).

The results of correlation analysis of radial increment fluctuations and meteorological parameters (precipitation and monthly temperature) fluctuations for the current and previous year show that in the Spruce stands chronologies of various species composition revealed a specific set of significant correlation coefficients (scaling factors) (Table 4). Drought signal is the author's term introduced by D. E.

Table 4. Numbers of the significant correlation coefficients of the relationships between radial increment and meteorological parameters of current and previous year.

Chronology	Pure spruce stands (10 units)	Stands with spruce predominance (7 units)	Stands with aspen predominance (5 units)	Stands with birch predominance (7 units)	Stands with pine predominance (5 units)	Stands with lime predominance (7 units)
Drought signal	5	5	6	7	5	4

Note: Drought signal – the number of sound correlation coefficients values of radial increment and meteorological parameters (Solomina et al. 2017).

Rumyantsev (Solomina et al. 2017). It is defined as the sum of reliable correlation coefficients during the current and previous growing season. Each reliable value is assigned a score of ‘1’, but only positive correlation coefficients with precipitation and negative correlation coefficients with temperatures were taken into account.

The results of our research show that the highest sensitivity to climatic factors (meteosensitivity) of spruce was in small-leaved species stands (the majority of significant correlation coefficient values with meteorological parameters were observed at stands with birch predominance). Meteosensitivity of spruce in pure spruce stands and stands with spruce predominance was the same, that makes it possible to combine them into a generalized chronology (Fig. 2). In contrast, the lowest meteosensitivity of spruce was observed in lime stands. Based on the obtained results, for the next stage of our research we have chosen sample plots including 6 or more units of spruce and minimum 6 or more units of lime in species composition of a stand. The dynamics of radial increment indices for the last 15 years (in the considered chronology) is presented in Fig. 2.

During the studied period a well-defined drought was present in 2010. The

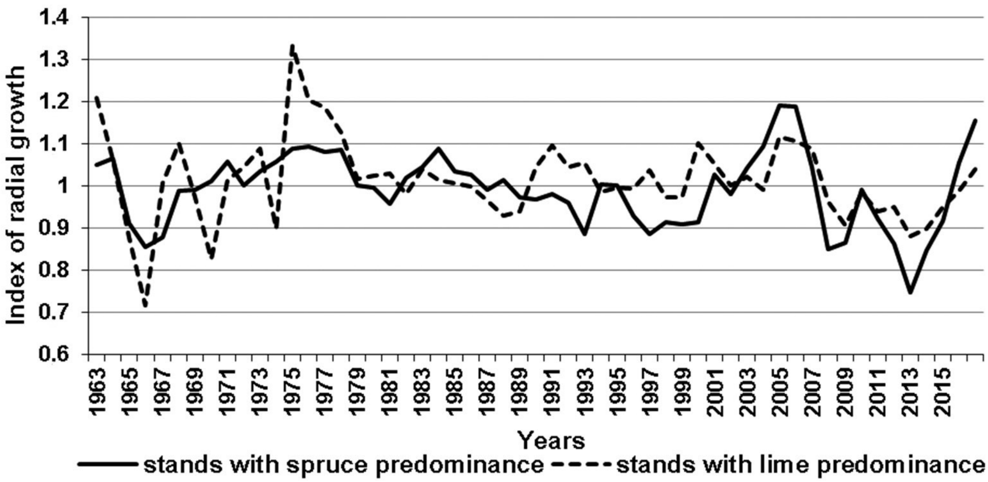

Fig. 2. Dynamics of radial increment indices of spruce in various species composition stands.

analysis of its impact on spruce increment in stands with totally different species composition is of some interest. Precipitation in July 2010 were significantly below the average (86.1 % less than long-term average annual rate). Moreover, July temperatures exceeded long-term average rates by 37.5 %. Analyzing the variation of radial increment indices for two selected groups of trees (Fig. 1), we should emphasize that in 2010 this indicator for the groups was the same, while in the following 2011 it went down dramatically for the group ‘stand with spruce predominance’ in comparison with the group ‘spruce in stands with lime predominance’. In 2012, 2013, 2014 there was still an excess of ‘spruce in stands with lime predominance’ over the group ‘stands with spruce predominance’ in terms of radial increment index. Thus, the 2010 drought probably has had more negative effect on the spruce growth in pure stands than in stands with lime predominance.

Conclusion

The increment value of spruce in stands with lime predominance turned to be less dependent on the climatic factors, which could be the evidence of higher spruce resistance in mixed lime-spruce stands in comparison to monocultures. Lower stability of homogeneous ecosystems is noted, and therefore, the establishment of pure spruce plantations, especially in protective forests, seems to be impractical. In the formation of mixed stands, it is necessary to select species that would be complementary to each other and compensate for the impact of certain environmental factors. As a secondary and satellite species, or in the zone of mixed coniferous-broad-leaved forests, the lime is proposed because of possessing natural mechanisms to withstand the dry periods, to which the spruce is vulnerable. Consequently, establishment of mixed lime-spruce plantations in Moscow Region could be recommended in order to increase spruce resistance to drought.

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