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Utricularia vulgaris L. AND *Salvinia natans* (L.) All. HEAVY METAL (Fe, Mn, Cu, Zn AND Pb) BIOACCUMULATION SPECIFICITY IN THE AREA OF BARDAČA FISHPOND

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Abstract

Maksimović T., Rončević S., Kukavica B.: *Utricularia vulgaris* L. and *Salvinia natans* (L.) All. heavy metal (Fe, Mn, Cu, Zn and Pb) bioaccumulation specificity in the area of Bardača fishpond. Ekológia (Bratislava), Vol. 38, No. 3, p. 201–213, 2019.

Paper deals with seasonal changes in heavy metal bioaccumulation (Fe, Mn, Cu, Zn and Pb) in *Utricularia vulgaris* L. and *Salvinia natans* (L.) All. of two localities (Necik and Sinjak – active fishpond basins) in the area of Bardača fishpond. According to our results, the better accumulator of Fe (3035 mg/kg) and Zn was *S. natans* (163.55 mg/kg), whereas *Utricularia vulgaris* better accumulates Mn (620 mg/kg) and Cu (10.18 mg/kg). Amounts of Pb in both investigated macrophyte were below the detection level (<0.51 mg/kg). The values of the bioaccumulation factor (BAC) of the tested metals were >1 or ~1 for both species, and the BAC values decreased in the following order in both investigated species: Fe > Mn > Cu > Zn. The results obtained indicated that tested macrophyte show very good characteristics as bioaccumulators and, thanks to this fact, they could be used in phytoremediation technique successfully in water-polluted ecosystem.

Key words: heavy metals, bioaccumulation, *Utricularia vulgaris* L., *Salvinia natans* (L.).

Introduction

Heavy metals are natural components of Earth's crust, but in many ecosystems, concentration of certain heavy metals reached toxic levels because of consequences of anthropogenic activities. In the recent years, pollution of environment with heavy metals has become a great global problem of modern society with characteristic cumulative effect (Prasad, 2003; Ghosh, Singh, 2005; Prasad et al., 2006; Liu et al., 2007; Rai, 2009; Nouri, Haddioui, 2016). Exposure of plants to toxic levels of heavy metals starts a wide spectrum of physiological and metabolic changes (Prasad, 2003; Prasad et al., 2006), which include the following: decrease in plant's growth, changes in cell metabolism, damage of cell membranes, inhibition of membrane proteins, changes mineral nutrition and inhibition of photosynthesis, which leads to senescence and, at the end, to plants death (Prasad, 2003; Prasad et al., 2006). Aquatic plants have a good ability to tolerate increased levels of heavy metals and accumulate them up to unusually high concentrations, causing accumulative effect without significant consequences to metabolic processes (Liu et al., 2007; Rai, 2009; Babović et al., 2010; Pant, Tripathi, 2014). Plants growing in polluted environment are very often under stress because of bioaccumulation through direct uptake by root, stem or sprouts (Prasad et al., 2006; Liu et al., 2007; Borišev et al., 2008; Rai, 2009; Babović et al., 2010).

Current technologies available for mitigation of pollution are very expensive, and in most of cases, they represent a cost for industry (Rai, 2009). Hence, lately, aquatic macrophytes are used as bioindicators of water quality and phytosanitors of water pools more often. There is a constant intention to incorporate waste water purification methods by these plants into directives. Different studies (Liu et al., 2007; Borišev et al., 2008; Rai, 2009; Babović et al., 2010) showed that aquatic macrophytes concentrate certain metals up to different degree and, hence, critical levels could vary among species.

Numerous studies of aquatic plants were performed in laboratory conditions, whereas small number of researches on potential of heavy metals bioaccumulation was made in the field environment. Owing to this, the aim of our study was to examine the ability of aquatic plants to accumulate heavy metals in their natural environment and the possibility of their use as bioindicators. Macrophytes can accumulate a considerable amount of heavy metals ($10–10^6$ times higher than their concentration in aquatic environments) in their tissue without disturbing their normal metabolism (Prasad, 2006). Plants chosen for investigation were different in position of sprout in air or water environment, as well as in relation of connection to the ground or free floating (emerged, submerged-not-rooted and floating-not-rooted) and per system of heavy metals bioaccumulation. *Utricularia vulgaris* (submerged-not-rooted) because of a thin cuticle absorbs heavy metals through the entire surface of the leaf from the water (Prasad, 2006), whereas *Salvinia natans* (floating-not-rooted) absorbs pollutants from the water and also from the air (Prasad, 2006).

Material and methods

Study area

Swamp-marsh ecosystem complex Bardača is located at the far North-East of Lijevče polje (Lijevče field in English), bordering the Sava river at North, Brzaja and Vrbas rivers at East, Matura at West, whereas perimeter canal Osorna-Borna-Ljevčanica (O-B-LJ) is located in immediate vicinity at South (Fig. 1) (Šarić et al., 2004).

On 02 March 2007, Bardača was declared as Ramsar area (number 1658), which confirmed its international importance as the 'Important Bird Area'. However, after gaining Ramsar area status, under the strong anthropogenic influence, there was a significant devastation of this sensitive ecosystem. Consequently, constant monitoring and assessment of the heavy metal load in this endangered area is one of the measures of revitalisation. Previous research (Maksimović et al., 2007; Maksimović, Ilić, 2008; Maksimović et al., 2016) and our results related to the content of heavy metals in aquatic macrophytes will determine the reliability of their use for water quality monitoring as well as the role of macrophyte vegetation in the remediation of pollutants, especially during the period of intensive fish breeding.

Plants samples (1 kg) were collected in shallow parts of fishponds. From each site, the collection and analysis of whole plants of *S. natans* and *Utricularia vulgaris* during the vegetation season were carried out at all stages of their

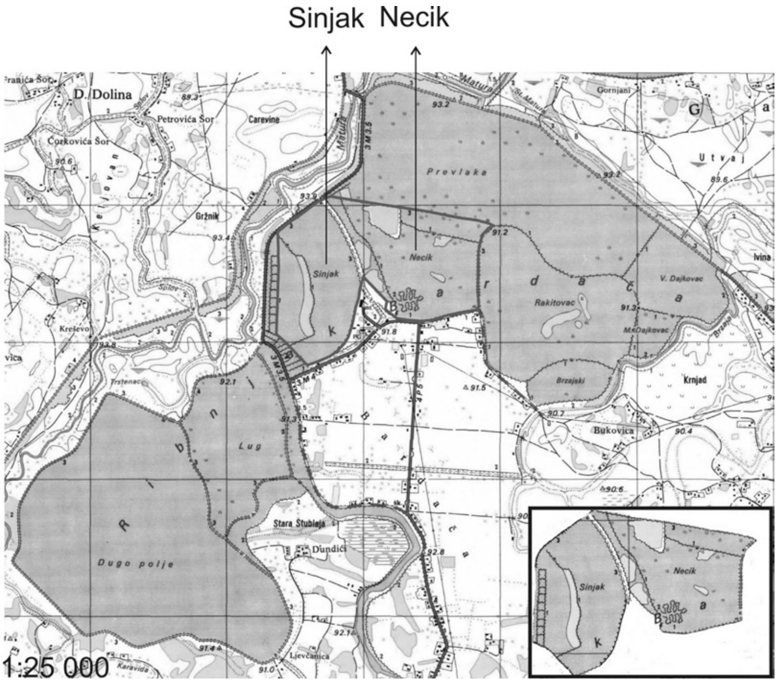

Fig. 1. Map of Bardača fishpond (a detail from topographic map 1:25,000), Nova Gradiška (Razboj-Ljevčanski) 4-4, Vojnogeografski institut, 1977 (Military Geographic Institute).

growth and development (May to September). At the same places, sediment samples were taken from a depth of 0–20 cm. Sediment was dried first at room temperature and then at 105 °C, milled to a powder and sieved through a fine sieve (≤500 μm). Sediment samples were prepared for further analyses by standard methods (APHA, 1995).

Sample analysis

In the laboratory, plant material was sorted, rinsed in tap and distilled water, dried in dryer at 105 °C and was prepared for further chemical analysis applied for water and water plants (APHA, 1995). Concentrations of microelements (heavy metals) were defined by atomic absorption spectrophotometry (AAS) directly from basic liquid. Samples were recorded on atomic absorption spectrometer Analyst 700, produced by Perkin Elmer, USA, using flame technique, standard method EPA 7000B.

Statistical analysis

The results were statistically processed using SPSS program (Statistical Package for the Social Sciences) 13.0 POZA 69. All the analyses were performed in three independent repetitions, and the analysed parameters were processed using variance method (ANOVA) factorial experiment. On the basis of the results obtained for the metal content in this article, water categorisation was made by the Decree on Water Classification and Categorization of Watercourses, Sl. glasnik Republike Srpske (2001) and classification of sediments according to the Netherlands (VROM, 2000) and Canadian recommendations (Canadian Council of Ministers of the Environment, 1999). Each analysis was repeated three times.

Data analysis

The values of BCF and BAC were used to evaluate potential plant species in phytoremediation techniques (Rahmani, 1999; Li et al., 2007). The ability of plants to accumulate heavy metals from water is estimated using the bioconcentration factor (BCF). Bioaccumulation coefficient (BAC) calculated as the ratio of heavy metal content in the above ground to the content of heavy metals in the sediment (Li et al., 2007).

$$BAC = \frac{[Metal]_{plant\ above-ground\ part}}{[Metal\ ]_{sediment}}\ .$$

The BCF value is calculated as the ratio of the concentration of heavy metals in the tissue of plants relative to its content in water (Rahmani, Sternberg, 1999). High BCF values involve a higher ability of the plant to bioaccumulate a particular heavy metal.

$$BCF = \frac{[Metal]_{part\ of\ plant}}{[Metal\ ]_{wather}}\ .$$

Results and discussion

Heavy metal concentration in water

The results of Maksimović and coworkers (Maksimović et al., 2007, 2016) showed increased concentrations of Pb and Zn in fishponds, Necik and Sinjak. On the basis of the concentrations of Pb and Zn and according to the Regulation on Classification of Water and Categorization of Watercourses (2001), investigated fishponds belonged to the class III/IV of water quality. On the other hand, according to the concentrations of Fe, Mn and Cu, the investigated fishponds belonged to class III/IV (Fig. 2). The authors emphasised that fishponds are waste water recipes: agricultural activities near the pool can cause pollution in an extent that is higher than the pools self-purification, which leads to an increase in the content of heavy metals.

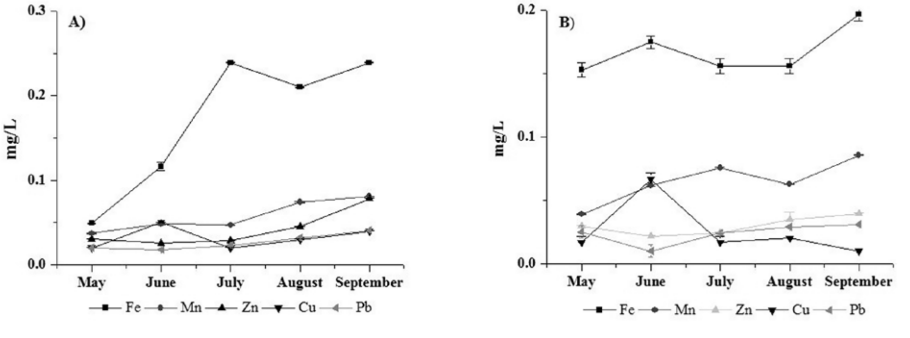

Fig. 2. Average values of heavy metal content in water at research sites (mg/L) for locality Necik (left) and Sinjak (right) during the period from May to September.

Heavy metal concentration in sediments

We measured the content of heavy metals (Fe, Mn, Zn and Pb) in sediments of fishponds, Sinjak and Necik, as well as their content in tissues *of U. vulgaris* and *Salvinia natans*. Fe content in the sediments on both the investigated fishponds was significantly higher compared to other metals (Table 1).

T a b l e 1. Heavy metal contents (Fe, Mn, Zn, Cu and Pb) in sediment on Necik and Sinjak locality (mg/kg). Values are expressed as mean ± SD (n = 3). The different letters in each row show a statistically significant difference ($p < 0.05$) for one heavy metal per month.

	Heavy metal	**Period (month)**				
		May	**June**	**July**	**August**	**September**
Necik	*Fe*	618.33^{a}	615.82^{b}	616.66^{b}	619.16^{a}	619.16^{a}
	Mn	206.66^{d}	257.50^{b}	226.66^{c}	189.99^{e}	224.99^{c}
	Zn	66.74^{d}	63.33^{e}	69.74^{b}	71.66^{a}	71.00^{a}
	Cu	6.08^{d}	7.16^{c}	17.72^{a}	10.50^{b}	6.91^{d}
Sinjak	*Fe*	620.00^{a}	618.33^{b}	617.49^{c}	618.33^{b}	618.33^{b}
	Mn	355.83^{a}	345.83^{a}	250.83^{c}	204.49^{d}	292.49^{b}
	Zn	67.25^{a}	60.00^{b}	55.16^{c}	51.66^{d}	60.08^{b}
	Cu	9.99^{a}	6.99^{b}	2.83^{f}	5.83^{d}	5.16^{e}

Concentration of Fe was in the range 616.66‑620 mg/kg, and in the fishpond Sinjak, higher Fe content was measured in comparison with the pond Necik. The values that we measured for Fe in the sediment of Sinjak and Necik were lower compared to that of Srebrno jezero (9.060 mg/kg) (Yurkova, Kochev, 1996), Begečka jama (1.2374 mg/kg) and Koviljski rit (16.154 mg/kg) (Štrbac et al., 2014). Mn content in the sediment varied more during the season (Table 1) and was two to three times lower than Fe contents. However, the measured concentrations of Mn was five times higher in relation to the previously obtained results on the same localities (Maksimović, Ilić, 2008) and also higher in comparison with other studies of similar localities (Yurkova, Kochev, 1996; Stanković et al., 2000; Pajević et al., 2002) where authors stated that Mn concentration varied depending on the adoption ability of aquatic macrophytes and chemical conditions of sediment. Zinc content in sediment of Necik during the research period was in the range 63–71 mg/kg, and that in Sinjak was 51–67 mg/kg, wherein the highest Zn concentration was measured in July in Necik sediment. Compared to localities Obedska Bara, Ludoš, Carska Bara (Prica et al., 2005), Tisa River (Štrbac et al., 2014) and Velenjsko jezero (Slovenia) (Grudnik, 2010), the detected values for Zn on the research localities were significantly lower. As was shown in previous research, the higher zinc concentrations in river sediments is in soluble form, associated with Fe and Mn oxide, carbonate and organic matter, whereas 30% of Zn is not dissolved (Tyler, Olsson, 2001), which probably resulted in lower content of Zn in the sediment compared to that of Fe and Mn. It is known that in acidic environment and in oxidising conditions, contents of soluble form of zinc are increased (Tyler, Olsson, 2001), so it could be assumed that changes in pH and oxygen contents during season affected the availability of Zn in sediment of researched basins.

Copper contents in the sediment of Sinjak and Necik (Table 1) were significantly lower comparing to those in other researched elements. So, Cu contents in Necik locality during the research period was in the range 6.08–17.72 mg/kg, and that in Sinjak locality was in the range 2.83–9.99 mg/kg, whereas in Necik locality, 34% higher contents of Cu was measured. Compared to localities Obedska Bara, Ludoš, Carska Bara (Prica et al., 2005), Palićko jezero (Raičević et al., 2011), Tisa River (Štrbac et al., 2014) and Velenjsko jezero (Grudnik, 2010), where Cu values exceeded the allowed limits, significantly lower values were detected in the basins of Bardača fishpond. Our data indicate that the level of Cu in the area of the fishpond Bardača not reached a critical level and that should not have a negative impact on the plant growth and development. Considering the fact that it is a mildly alkali soil, and because availability of copper decreases when pH values are more than 7 (Šarić et al., 2004), this could explain lower values of Cu in this area. Concentrations of available Pb in sediment are most often very low, because of its strong connection to organic substance, Fe–Mn oxides, clay and deposition in the form of carbonates, hydroxides and phosphates (Prasad et al., 2006; Tyler, Olsson, 2001). Lead values in the sediment of Sinjak and Necik were under the detected limit (<0.51 mg/kg) in contrast to previous studies in this area when the lead concentrations were in the range 27.62–32.26 mg/kg (Maksimović et al., 2007). If we compare concentrations of heavy metals (Table 1) in the sediment of the researched fishponds with *Netherlands* (VROM; 2000) and *Canadian* recommendations (1999), it can be concluded that the ponds were unpolluted by the investigated metals.

Heavy metal concentrations in plant organs

Depending on species, seasonal cycle of aquatic macrophytes is characterised by slower or faster growth in spring, peak of growth at the end of summer and smaller or bigger fall in autumn, so it is considered that macrophytes could affect seasonal storage and metal circulation in aquatic environment (Prasad et al., 2006). Degree of heavy metal accumulation in tissues of investigated macrophyte was not consistent with their contents in sediment. Therefore, the content of Fe and Mn in the tissue of *S. natans* and *Utricularia vulgaris* was significantly higher than that in the sediment.

In contrast, immersed, floating and submerged species accumulate metals in leaves and thus significantly contribute to the extraction of heavy metals from water basins (Prasad et al., 2006). The correction is fine The content of Zn in the tissue of the investigated macrophytes did not significantly differ from the content in the sediment. In contrary, the measured copper concentrations were lower in the tissue compared to those of Fe, Mn and Zn and did not correlate with the concentrations in sediments, indicating a lower translocation of Cu in plant tissues.

Accumulation of iron

The results obtained indicate different Fe concentrations in the tissue of examined plant species (Table 2).

Fe is one of the essential elements; however, high concentrations of Fe may be very harmful for the plants. Fe is a redox active metal that, through the Fenton reac-

T a b l e 2. Fe contents (mg/kg) in tissues of *Salvinia natans* and *Utricularia vulgaris* on two researched localities. The different letters in each row show a statistically significant difference ($p < 0.05$) for one heavy metal per month.

Plant species		Period (month)				
		May	June	July	August	September
Necik	*Salvinia natans*	3035[a]	1539[b]	1190[c]	866[d]	454[e]
	Utricularia vulgaris	1184[a]	938[b]	874[d]	893[c]	741[e]
Sinjak	*Salvinia natans*	-	1646[a]	720[d]	875[b]	758.26[c]
	Utricularia vulgaris	731[e]	747[d]	877[a]	865[b]	861[c]

tion, leads to the formation of hydroxyl radicals, which is highly reactive and can cause damage and death of plant cells (Dhir, Srivastava, 2013).

In the tissue of *Salvinia natans*, Fe contents varied from 453.74 to 3035.20 mg/kg. During season, higher values were measured in the beginning of vegetation period (May) and significantly lower (80%) by the end of research period (September). Fe content in the tissue of *S. natans* was in accordance with the research of other authors for Ribnjak Ečka (198–5979 mg/kg; Babović et al., 2010) and Jegrička River (2.527 mg/kg; Borišev et al., 2008). Fe concentrations in tissue of *S. natans* were twofold higher at Necik locality compared to Sinjak. Grisey et al. (2012) showed in their studies that seasonal changes in heavy metals contents depend on dynamics in plant growth and ability of metal translocation from roots to leaves, which could also be connected to the results obtained in this study.

The highest Fe value in the tissue of *Utricularia vulgaris* (1.184 mg/kg) was measured in May on Necik locality, whereas by the end of research period, the values were up to 60% lower. Stanković et al. (2000) showed that increase in Fe values in tissue of submerged species *Myriophyllum spicatum* and *Ceratophyllum demersum* (1.157–2.075 mg/kg) in Vrbas–Bezdan and Banatska Palanka–Novi Bečej canals results from surrounding agricultural surfaces as well as emission of metals from sediments, which most probably is one of the reasons for increased Fe concentration in this area. Submerged aquatic macrophytes (*Utricularia vulgaris*) accumulate significantly higher heavy metal concentrations, which was also supported by other researches (Borišev et al., 2008; Babović et al., 2010). During season, different distribution of Fe accumulation was detected; higher concentration was recorded at the beginning of vegetation period at Necik locality, whereas the opposite observation was observed at Sinjak locality. Also, during the season(May- September), Fe contents varied in tissue of *Salvinia natans* and *Utricularia vulgaris*: the highest level was measured in growing period (May–June), followed by a significant reduction at the end of vegetation period. Similar results have been demonstrated by Grudnik and co-workers (Grudnik, 2010) in their research of some submerged and floating plants.

Accumulation of manganese

Different adoption of Mn (Table 3) amongst submerged and floating species was also pointed out by other researchers (Yurkova, Kochev, 1996; Pajević et al., 2002; Borišev et al., 2008; Branković et al., 2009; Babović et al., 2010).

T a b l e 3. Mn contents (mg/kg) in tissues of *Salvinia natans* and *Utricularia vulgaris* at researched localities. The different letters in each row show a statistically significant difference ($p < 0.05$) for one heavy metal per month.

Plant species		Period (month)				
		May	June	July	August	September
Necik	*Salvinia natans*	350^{d}	399^{c}	579^{a}	481^{b}	266^{e}
	Utricularia vulgaris	620^{a}	440^{e}	495^{b}	497^{c}	453^{d}
Sinjak	*Salvinia natans*	-	374^{c}	446^{b}	479^{a}	325^{d}
	Utricularia vulgaris	382^{e}	410^{d}	520^{a}	424^{c}	472^{b}

T a b l e 4. Zn contents (mg/kg) in tissues of *Salvinia natans* and *Utricularia vulgaris* at researched localities. The different letters in each row show a statistically significant difference ($p < 0.05$) for one heavy metal per month.

	Plant species	Period (month)				
		May	June	July	August	September
Necik	*Salvinia natans*	163^{a}	60^{c}	81^{b}	44^{d}	39^{d}
	Utricularia vulgaris	55^{a}	54^{a}	45^{d}	48^{b}	47^{c}
Sinjak	*Salvinia natans*	-	54^{a}	42^{c}	43^{b}	32^{d}
	Utricularia vulgaris	39^{e}	52^{a}	47^{b}	45^{d}	46^{c}

Data on the ability of plants to adopt different types of metal could be very important in the selection of species in phytoremediation technique. In the tissue of *Salvinia natans*, the highest Mn concentrations were measured in July and August (vegetation peak), whereas in September, a significant decrease in the Mn content was observed. Research by Branković et al. (2009) showed that submerged plants had smaller Mn accumulation capacity compared to floating plants, which is not in accordance with our findings. The value of Mn was measured in the tissue of *Utricularia vulgaris* (620 mg/kg) was highest in May in Necik locality, whereas the Mn content in the tissue of *U. vulgaris* varied more during the (May–September) season compared to *Salvinia natans*.

Accumulation of zinc

The results presented in Table 4 show that, on an average, the Zn contents for investigated species was highest at the beginning of vegetation period (May–June) and decreased in autumn, which was in line with the previous results (Maksimović et al., 2014).

Such a seasonal distribution could be a result of antagonism with other elements, Mn and Fe, first of all, but it could also be connected to other environmental factors (pH, temperature, oxygen contents). Significant differences in Zn contents (accumulation capacity) were recorded between studied species: the highest accumulation capacity was expressed by floating *S. natans* in comparison with submerged *Utricularia vulgaris* species. Zn contents during the season (May–September) in *Salvinia natans* tissue vary from 32 to 163 mg/kg, where measured values were higher compared to the results for Ečka fishpond (Babović et al., 2010) and Jegrička River (Boriśev et al., 2008). Highest Zn content was measured in *S. natans* tissue in May (163 mg/kg), whereas Zn content decreased for 80% by the end of vegetation period. It is well known that all fertilisers and pesticides contain Zn (Prasad et al., 2006), so that their increased usage at the beginning of season consequently increases Zn accumulation in plants. Afterwards, Zn content in *S. natans* tissue decreases as its deposition in sediments increases. In the tissue of *Utricularia vulgaris*, 35% lower contents of Zn was measured compared to *Salvinia natans* at Necik locality, and 9% higher was measured at Sinjak locality (Table 4). The results obtained in this study show that Zn contents was higher in submerged leaves in comparison to floating leaves, which had been in accordance with the results of Stanković et al. (Stanković et al., 2000). Different studies (Liu et al., 2007; Tyler, Olsson, 2001; Maksimović et al., 2014) show that Zn adsorption is much better in alkali soil. As the investigated localities belong to the slightly alkali soil, it could be supposed that changes in Zn contents in our research could be because of mobility of Zn from sediment during the season (May- September) (Tables 1 and 4). As the investigated localities belong to the slightly alkali soil, it could be supposed that changes in Zn contents could be connected to mobility of Zn from sediment during the season (May–September).

Accumulation of copper

Contents of copper in *S. natans* tissue were at the detection limits during May, June and October at both the localities and increased contents (7.94 mg/kg) were detected in August and September (Table 5).

T a b l e 5. Cu contents (mg/kg) in tissues of *Salvinia natans* and *Utricularia vulgaris* at researched localities. The different letters in each row show a statistically significant difference ($p < 0.05$) for one heavy metal per month.

Plant species		Period (month)				
		May	June	July	August	September
Necik	*Salvinia natans*	<0.043	<0.043	3.00[b]	7.33[a]	7.94[a]
	Utricularia vulgaris	8.00[a]	6.03[c]	4.75[e]	5.87[d]	6.56[b]
Sinjak	*Salvinia natans*	-	<0.043	6.30[a]	6.25[a]	1.87[b]
	Utricularia vulgaris	10.18[a]	1.70[d]	3.87[c]	4.37[b]	4.37[b]

As a consequence of Cu antagonism with Fe, on the one hand, absorption of Cu from sediment is decreased, and, on the other hand, Cu toxic concentrations can be mitigated by adding Fe (Prasad, 2003; Rai, 2009). Owing to it antagonistic relations with Fe and as a consequence of increased Fe concentration at researched localities, both availability and intake of Cu by aquatic plants drastically decreased. Cu contents in the tissue of *Utricularia vulgaris* varied from 1.7 to 10.18 mg/kg, whereas higher contents were measured at Necik locality compared to Sinjak locality. Other authors also showed that Cu accumulated better in tissue of submerged plants compared to floating and emerged plants (Stanković et al., 2000; Pajević et al., 2002; Babović et al., 2010).

Accumulation of lead

Lead contents in the tissue of *Salvinia natans* and *Utricularia vulgaris* during the research period was under the detection limit (<0.51 mg/kg), which was significantly lower compared to the previous researches (Branković et al., 2009; Khellaf, 2009; Štrbac et al., 2014). In all plant species, contents of Fe, Mn, Cu and Zn were higher at Necik locality compared to Sinjak locality, which are most probably consequence of different physical–chemical environmental conditions, better availability of researched element and the presence of metals in soluble form, that is, its faster release from sediment.

Depending on the species, the seasonal cycle of aquatic macrophytes can mainly be characterised by faster growth in spring, maximum of growth at the end of the summer and more or less rapid growth decline in autumn, so macrophytes are thought to affect seasonal storage and circulation of elements in the aquatic environment (Prasad et al., 2006). The degree of accumulation of heavy metals depends on the plant species, locality and the sampling period. From the results obtained shown in the Tables 2–5, it can be seen that accumulation of Mn in *U. vulgaris* was increased during the period of flowering, whereas the content of Fe, Zn and Cu was lower compared to the beginning of the season (May–September). In *Salvinia natans*, the higher content of Fe and Zn was recorded at the beginning of the season (May–September) and Mn was recorded during the period from July to August, whereas Cu content increased more with the plant aging.

Phytoremediation potential of Salvinia natans and Utricularia vulgaris

The level of bioaccumulation is the result of the difference between the amount of metals that the plant adopts and its amounts in water and sediment and is specific to each plant species (Ghosh, Singh, 2005; Prasad et al., 2006). Also, the bioaccumulation coefficient depends on many factors of the environment in which the plant is located (pH, temperature and oxygen concentration).

The BAC is used as an indication of the potential of heavy metal accumulation for those plant species that have a well-developed cellular mechanism for detoxification and tolerance of heavy metals (Ghosh, Singh, 2005; Maksimović et al., 2008). The BAC values varied in relation to the type, metal, and sampling period (Table 6).

The BAC values for Fe in *Salvinia natans* leaves ranged from 1.40 to 2.29, 1.89‑2.30 for Mn; 0.73‑1.15 for Zn and 1.21‑1.23 for Cu. The BAC values obtained for Fe and Zn in *Utricularia vulgaris* were twice lower, whereas Mn was higher in relation to *Salvinia natans*. For Cu, BAC values similar in both the species. The average BAC values were higher at the Necik locality compared to Sinjak for all heavy metals. Therefore, the investigated species have mostly BAC >1 or, for Zn, BAC ~1, indicating that *S. natans* and *Utricularia vulgaris* have pronounced accumulation properties and can be successfully applied in phytoremediation techniques. It is very important to note that the results obtained depend not only on the total concentration of metals in the sediment but also on the physical and chemical characteristics of water, in particular the pH, as well as the ability to change the ions between the sediment of water and plant species (Yoon et al., 2006).

T a b l e 6. Average values of the BAC coefficient in relation to the content of heavy metals in sediment for Fe, Mn, Zn and Cu at the investigated localities in the area of Bardača fishponds during the period from May to September.

	Fe	**Mn**	**Zn**	**Cu**
Locality	***Salvinia natans***			
Necik	2.29	1.90	1.14	0.66
Sinjak	1.61	1.26	0.60	1.22
	Utricularia vulgaris			
Necik	1.40	2.30	0.73	0.79
Sinjak	1.32	1.60	0.79	0.84

T a b l e 7. Average values of the BCF coefficient in relation to the content of heavy metals in water for Fe, Mn, Zn and Cu at the investigated localities in the area of Bardača fishponds during the period from May to September.

	Fe	Mn	Zn	Cu
Locality	***Salvinia natans***			
Necik	16.902	7.918	2.901	215
Sinjak	5.769	3.358	1.515	271
	Utricularia vulgaris			
Necik	8.494	9.686	1.884	223
Sinjak	4.854	4.939	1.579	276

The plant accumulates heavy metals independently of their concentration in water and sediment, which is obvious from the results obtained (Fig. 2, Table 1). Differences between plants in bioaccumulation can be a consequence in the translocation of heavy metals through the plant because the bioaccumulation capacity of plants' tissue for certain metals is different. According to Zayed et al. (1998), plant is considered as good bioaccumulator if the BCF exceeds 1.000. BCF in relation to the water in the leaves of *Salvinia natans* and *Utricularia vulgaris*, shown in Table 7, indicates that the accumulation of heavy metals decreases in the following order: Fe > Mn > Zn > Cu at both the localities. *Salvinia natans* have a higher BCF for Fe and Zn, whereas *Utricularia vulgaris* showed a higher bioaccumulation capacity to Mn and Cu.

Conclusion

On the basis of the measured heavy metals concentrations (Fe, Mn, Cu, Zn and Pb) in sediment, we can conclude that investigated localities are not overloaded by these pollutants, which are dangerous for ecosystem. Taking into account all the results obtained, we can conclude that *U. vulgaris* and *Salvinia natans* accumulate Fe, Mn and Zn in a high degree. Both species proved to be insufficient bioindicators for Cu and Pb. The results acquired in this study at the area of Bardača fishpond imply that bioaccumulation of heavy metals and their availability to the plants had not reached critical level. Hence, it is important to set a constant monitoring to include monitoring of seasonal and spatial variations in the accumulation of heavy metals with the aim of estimating the capacity of researched species in phytoremediation techniques.

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Map of Bardača fishpond (a detail from topographic map 1:25000, Nova Gradiška (Razboj-Ljevčanski) 4-4, Vojnogeografski institut, 1977 (*Military Geographic Institute*).

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THE EFFECT OF THE SELECTED REMEDIATION MEDIUM ON THE CADMIUM BIOAVAILABILITY IN THE SELECTED ECOSYSTEM IN THE SOUTHWESTERN LOCALITY OF SLOVAKIA

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Abstract

Urminská J., Tóth T., Benda Prokeinová R., Ondrišík P.: The effect of the selected remediation medium on the cadmium bioavailability in the selected ecosystem in the Southwestern locality of Slovakia. Ekológia (Bratislava), Vol. 38, No. 3, p. 214–224, 2019.

Soil is a sensitive ecological factor. Biodegradable materials from the environment can also be used to deal with serious ecological problems. Soil affecting by remediation medium - garden compost - was analysed for toxic cadmium (Cd) in terms of environmental protection. The objective of this research was to analyse soil and compost at foothill locality of the Tribeč Mountains (Southwestern Slovakia) in the years 2015–2017 to determine Cd contents in soil and compost, pH and to assess Cd bioavailability. The analyses were carried out using the Atomic Absorption Spectrometry with seven-step Selective Sequential Extraction methods. The results obtained were evaluated statistically using the SAS 9.4 software method by Spearman's correlation coefficient. The results showed that Cd contents in soil had reached 2.96 mg kg^{-1} and soil with compost (the ratio 1:1) 2.71 mg kg^{-1} dry matter. Cd contents in the soil exceeded maximum allowed limit of 196%. And deceased by 25% after adding compost. The pH in soil with compost varied from 6.78 to 7.98. The pH prevented the mobility of Cd about 8.3% in average. Statistical dependence was high, which was demonstrated for relationship between Cd in soil, pH and compost. Available Cd forms in soil were 53.3% and soil with compost were 45% in average. The garden compost as a remediation medium reduced Cd bioavailability.

Key words: fractionation, garden compost, high risk element, nature protection, seven-step selective sequential extraction.

Introduction

As environmental pollution is now a global phenomenon, the territory of the Slovak Republic is also not spared from this. At present, the ecological health quality is also affected by anthropogenic activities (Šimanský, 2015; Fazekaš et al., 2018). Growing of healthy foods is a priority for ecological awareness (El Rasafi et al., 2016). We still have to deal with increased and accessible forms of risk elements. It is justified to address the restriction of availability of toxic elements into organisms by means of easy to produce and economy remediation media. The agricultural production strives for sustainable management, which requires a lot of effort on the part of all those involved when it comes to maintaining a balance between income and nutrient expenditure in soil. Insufficient application of organic fertilizers leads to a gradual degradation of organic matter in soil, which contributes to the reduction of soil fertility and increase in risk of the toxic elements into soils, into all ecosystems (Fazekašová et al., 2013). Improper interventions on soil lead to gradual soil degradation, which reduce soil fertility and increase high risk of toxic elements in soil (Morais et al., 2012; Ying et al., 2018). In this case, the current low-utilizable biodegradable organic substances play an important role. In agricultural garden practice, when optimizing plant nutrition, pesticides are used in the long run; industrial fertilizers bring benefits but also dangerous risks. From the point of view of the demand of quality foodstuffs, which are not influenced by dangerous substances, cultivation with the use of alternative media is preferred in growing conditions too. Such a substance is a compost as a complementary alternative to plant nutrition, as well as a remediation medium that affects the bioavailability of risk elements in soil ecosystem (Belyuchenko, 2016; Khan et al., 2017). Composting technology is a naturally controlled biochemical and biological process, in which microorganisms and organisms decompose the substrates used for humus substances that are suitable for soil remediation. At present, when using garden compost as a remediation factor, it is necessary to take into account especially its quality. Important parameters are included in the concentration and the bioavailability of chemicals and potentially a soil exchange reaction. Composting is an aerobic process where biodegradable organic waste is converted to an organic-mineral fertilizer and a valuable remediation medium too (Belyuchenko, 2016; de O. Pinto et al., 2016; Khan et al., 2017). For the optimal course of biodegradation, it is important to formulate input substrates, which must provide the required parameters in terms of physical, chemical, biochemical, biological and the production process must be observed. Composting of biodegradable organic substances represents a technology of natural remediation and recycling based on natural, economic principles (de O. Pinto et al., 2016). Each component of the environment has a wide variety of chemical elements. At present, due to an increase in anthropogenic activities, there is a disproportionate increase in the concentration of risk elements in the environment (Morais et al., 2012; Ying et al., 2018). Such a highly toxic element is ubiquitous cadmium (Cd) (Liu et al., 2016; Shi-Wei et al., 2016; Peng et al., 2017). This work is focused on toxic Cd, which belongs to the element with a high risk potential for soil, plants and human health (Meharg et al., 2013; Das et al., 2014; Shi-Wei et al., 2016; Borgulat et al., 2018) and is focused on remediation medium - garden compost, which is usable in agricultural practices and has the potential to reduce the availability of toxic elements to the environment. It needs to be used more in the present practice. The main objectives of this paper were: to determine the Cd concentrations in topsoil and topsoil affected by remediation medium - garden compost (the ratio 1:1, the best scientifically proven division according to the valid agrochemical practice) by Atomic Absorption Spectrometry method during the long scientifically appropriate time; to determine potentially a soil exchange reaction in topsoil and topsoil affecting the garden compost as a key parameter in the assessment of mobility and bioavailability of risk elements in the environment; to do a detailed seven-steps fractionation of Cd in these accumulation mediums; to evaluate the statistical significance of dependencies of monitored parameters using by SAS Software; to find whether real garden compost as a remediation medium affects the bioavailability of Cd over a long time frame.

Material and methods

Agricultural land as a garden type is still not sufficiently explored for the contents of risk elements, while they are playing an important role in ecosystems.

Study area and creation of the garden compost

The monitored area was selected from the growing area at the foothills locality of the Tribeč Mountain, Southwestern of the Slovak Republic (48°20´15´´N, 18°06´30´´E, 236 m above sea level) in the years 2015–2017. Remediation medium - garden compost - was created on the basis of agrochemical practice and valid legislation of the Slovak Republic (Act No. 79/2015 Coll. of Laws) for the production and processing of biodegradable organic waste materials. Ingredient materials for compost as a remediation medium were: mowed grass, biowaste from households, vegetable waste, weeds, sawdust, straws, dry grass, shrubs, barks, leaves and needles tree, soil (Rendzic Leptosol), slaked lime (The materials used were applied in the exact percentage, the compost area was 4 m^2, the maximum height of one ingredient was up to 10 cm, the moisture, grain size and above all, process hygiene were maintained. The maximum composting height was 1.20 m). The composting process was put through the stages of decomposition, transformation, synthesis, stabilization and maturation processes.

Topsoil, remediation medium - garden compost and data analyses

The methodology for sampling the soil was governed by the valid the Slovak Republic legislation on agricultural land (Decree No. 59/2013 Coll.; NAFC, 2019). Sampling of soil was realized regularly at monthly intervals and was monitored over the years 2015–2017. It was carried out by a drilling probe placed in the centre of a circular shape with a radius of 10 m, the depth of 20 cm, and in addition, the sampling of 5 separate samples weighing 0.5 kg were randomly collected from the surface soil horizon from the sites for chemical analysis (NAFC, 2019). The samples were homogenized and dried at 40°C for 48 hours. Subsequently, they were ground with a VEB ThurmZG1 (an instrument VEB Elektromotorenwerke Kreis Zwickau DDR/GDR) soil grinder to a fine grain (2.0 mm). A potential soil exchange reaction soil exchange reaction was determined in aqueous extracts of 1 mol dm^{-3} KCl, according to the soil methodology (Fiala et al., 1999, an instrument InfolabMulti - WTW company). Analysis of leachate samples were carried out using *aqua regia* (HNO_3 + HCl 1:3) using the Atomic Absorption Spectrometry method in an acetylene-air flame on a Varian AA240FS instrument (Fast Sequential Absorption Spectrometer - Amedis company, CA). The Selective Sequential Extraction method was used for individual Cd fractions in soil and soil mixed with garden compost as a remediation medium (the ratio 1:1). Bioavailability was monitored according to the seven-step Selective Sequential Extraction methodology (detailed methodology in the Ziehen, Brümmer, 1991). The fractions analysed included (Ziehen, Brümmer, 1991):

- Label F1, representing a mobile bioavailable element form; extractant 1 mol dm^{-3} NH_4NO_3,
- Label F2, a lightly permissible form of element; extractant 1 mol cm^{-3} $C_2H_7NO_2$ pH 6.0,
- Label F3, is a part of the element bound to Mn-oxides; extractant 0.1 mol dm^{-3} $NH_2OH{\bullet}HCl$ + 1 mol dm^{-3} $C_2H_3NaO_2$ pH 6.0,
- Label F4, which is a part of the element bound to organic matter; extractant 0.025 mol dm^{-3} NH_4-EDTA pH 4.6,
- Lebel F5, which is a part of the element bound to amorphous of Fe-oxides; extractant 1 mol dm^{-3} $C_2H_7NO_2$ pH4.6,
- Label F6, which is a part of the element bound to crystalline Fe-oxides; extractant 0.1 mol dm^{-3} $C_6H_8O_6$ + 0.2 mol dm^{-3} NH_4-oxalate pH 3,
- Label F7, which is a part of residual element form; extractant 65% HNO_3 + 72% $HClO_4$.

Overall, the weather conditions during the complete research period did not disrupt the course of the research (Tables 1, 2). The observed locality of Southwestern Slovakia belongs to a warm and slightly dry climate region with a good level of diversity in its ecosystems.

T a b l e 1. The average monthly temperatures (°C) in the years 2015–2017.

A months	Year 2015	Year 2016	Year 2017
I.	1.6	-0.6	-9.1
II.	1.2	5.5	0.1
III.	6.3	4.3	8.7
IV.	10.4	9.3	9.7
V.	15.1	15.0	16.6
VI.	19.9	20.3	21.2
VII.	23.6	21.4	21.7
VIII.	23.5	19.5	22.4
IX.	17.5	17.5	14.6
X.	10.5	9.4	10.6
XI.	6.0	3.8	5.0
XII.	2.6	-2.7	1.6

T a b l e 2. Total monthly precipitation (mm) in the years 2015–2017.

A months	Year 2015	Year 2016	Year 2017
I.	52.0	28.6	12.8
II.	28.9	78.3	26.4
III.	35.4	32.4	9.9
IV.	25.0	24.8	39.5
V.	69.5	91.3	14.0
VI.	10.2	14.4	26.1
VII.	17.2	134.7	60.0
VIII.	57.7	35.0	23.2
IX.	33.2	36.9	93.0
X.	54.8	69.3	50.2
XI.	24.2	35.3	21.8
XII.	10.1	14.2	45.2

The results obtained were evaluated statistically using SAS 9.4 (North California USA) (Benda Prokeinová, 2014) method by the Spearman's correlation coefficient (Stehlíková, 1999). SAS provided all the activities related to performing risk analysis and selecting control samples. Spearman's correlation was used to check the relationship between selected observed parameters in soil and remediation medium.

Results and discussion

Each component of the environment has a wide variety of chemical elements. These also include heavy metals. Issues in their study include several important reasons: they are relatively expanded in environmental compartments, accumulate and form non-degradable waste; some are physiologically important for ecosystems (Poláková et al., 2011; Šillerová et al., 2011, 2012; Urminská et al., 2013); at high concentrations, they cause undesirable effects in biogeochemical cycle, and thus, have a direct relationship with agricultural production and human health (Morais et al., 2012; Meharg et al., 2013; Onistratenko et al., 2016; Peng et al., 2017). Their toxic effect may be manifested after an over concentration in the ecosystems, depending particularly on the contents of mobile and mobilizable forms (Bencko et al., 1995; Khun et al., 2008). The results showed that the proportion of fractions in the topsoil in the year 2015 were in the order: F7 > F2 > F3 > F6 > F1 > F5 > F4; in topsoil in the year 2016, these were in a little different order: F7 > F1 > F2 > F3 > F6 > F4 > F5; in the topsoil in the year 2017, these, were in the order: F7 > F2 > F1 > F3 > F6 > F4 > F5; in the topsoil affected by remediation medium - garden compost (the ratio 1:1) in the year 2015, these were in the order: F7 > F2 > F3 > F6 > F1 > F4 > F5; in the year 2016, these were in the order: F7 > F1 > F3 > F2 > F6 > F4 > F5; and in the year 2017, these were in the order: F7 > F1 > F2 > F3 > F4 > F6 > F5 (Fig. 1).

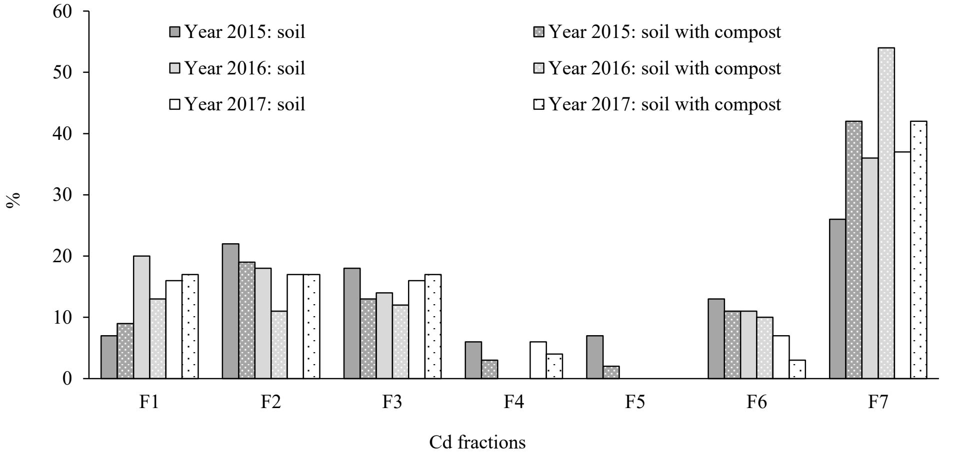

Fig. 1. Determination of cadmium for fractions in soil and soil with compost (the ratio 1:1) in the years 2015–2017.

The residual fraction F7 especially dominated, which is acceptable for the ecosystem. But was followed by bioavailable leachable fractions F1, F2. This fact confirm the findings of Makovníková et al. (2006), Khun et al. (2008), Liu et al. (2008) and Boriová et al. (2015). In our research, it was found that the bioavailable Cd forms (from the 1st to 4th fractions) reached 53% in the year 2015, 52% in the year 2016, and in the year 2017, up to 55%, of which, the most bioavailable form F1 and F2 in the year 2015 up to 29%, in the year 2016 up to 38% and in the year 2017 up to 33%. Bioavailable forms for organisms often exceed more than 50% (Liu et al., 2008). It was found that the bioavailability of Cd from soil for crops after the addition of garden compost significantly decreased by approximately 16% for accessible fractions F1 to F4. For the most bioavailable fractions, F1 and F2 were found to vary from 1 to 14%. It was shown that creation of compost positively affected the bioavailability of Cd over the research period under review from the soil for growing crops (de O. Pinto et al., 2016). It significantly reduced Cd availability. The Selective Sequential Extractions based on predefined procedures provided interesting information on bioavailability, and consequently, the risk of environmental contamination (Makovníková et al., 2006; Boriová et al., 2015). In the fractionation carried out by the Selective Sequential Extraction, the individual solid phases are elucidated from the accumulation media together with the elucidation of the elements associated with these phases (Cuske et al., 2017). Methods that use various extraction agents to determine the bioavailability of an element are useful in assessing real ecological risks (Antonkiewicz, Pełka, 2014; Boriová et al., 2015; Belyuchenko et al., 2016). Significant statistical correlations were found between mobile and potentially mobile heavy metal and organic amendments (Cuske et al., 2016; Khan et al., 2017) and pH too. The Cd contents, mean and standard deviation of the monitored parameters (mg kg^{-1} of dry soil) in the years 2015–2017 are documented in Table 3. The statistically highly significant correlation coefficients between the selected important parameters in the years 2015–2017 are documented in Table 4.

T a b l e 3. Variance analyses of Cd contents (mg kg^{-1} of dry matter) in soil and soil with compost (the ratio 1:1), pH in soil and soil with compost (the ratio 1:1) in the years 2015–2017 ($n = 12$).

Years	Analysed parts	Mean	Std. Dev.	Sum	Min	Max
Year 2015	Cd soil	2.45250	0.33374	29.43	1.98	2.96
	pH soil	6.27250	0.16510	75.27	5.98	6.51
	Cd soil + compost	2.33667	0.27424	28.04	1.89	2.71
	pH soil + compost	7.69083	0.31713	92.29	6.78	7.98
Year 2016	Cd soil	2.51000	0.33823	30.12	1.78	2.93
	pH soil	6.27833	0.21854	75.34	5.97	6.83
	Cd soil + compost	2.34500	0.25465	28.14	1.95	2.68
	pH soil + compost	7.67667	0.15245	92.12	7.31	7.87
Year 2017	Cd soil	2.49419	0.32609	29.93	1.82	2.89
	pH soil	6.14167	0.26683	73.70	5.74	6.56
	Cd soil + compost	2.37000	0.21076	28.44	2.04	2.68
	pH soil + compost	7.63750	0.20623	91.65	7.20	7.97

T a b l e 4. The statistically highly significant correlation coefficients between the selected important parameters in the years 2015–2017.

Relationship parameters	Cd S 2015	Cd S 2016	Cd S 2017	Cd S + C 2015	Cd S + C 2016
Cd S 2016	0.92542***				
Cd S 2017	0.92711***	0.96989***			
Cd S + C 2015	0.71983**	ns	ns		
Cd S + C 2016	ns	0.73557**	ns	0.97127***	
Cd S + C 2017	ns	ns	0.86483***	0.91779***	0.92995***
pH/KCl S 2016	ns	-0.71000**	ns	ns	ns
pH/KCl S 2017	ns	ns	-0.57724*	ns	ns
pH/KCl S + C 2015	ns	ns	ns	0.81161**	ns
pH/KCl S + C 2016	ns	ns	ns	ns	0.70110**

Notes: significance level $p_{value} < 0.05$*; $p_{value} < 0.01$**; $p_{value} < 0.001$***; ns - not significantly; S - soil; S + C - mixed soil + compost (the ratio 1:1).

Statistical dependence of the observed parameters was demonstrated for the relationship between Cd in soil : Cd in soil + compost, Cd in soil : pH in soil, Cd in soil + compost : pH in soil + compost. Especially high significance was determined for soil and garden compost, which correspondents to the long-term scientific patterns (de O. Pinto et al., 2016; Khan et al., 2017). Interesting data was found on the relationship between Cd contents in soil and pH/KCl in soil during the years - Spearman's correlation coefficient confirmed a negative correlation. It has been shown that the Cd contents is pH dependent, and where the Cd contents increases, there is a decrease in pH. For the relationship between Cd contents in soil during the years, the Spearman's correlation coefficients confirmed a positive correlation. This fact represents that Cd contents during the years were dependent. A similar fact applies to the relationship - observed years and soil affected by compost. A very important correlation was found between Cd contents in soil and Cd contents in soil affected by compost. Spearman's coefficients are high and dependencies are positive. Cd contents in soil is significantly positively affected by the addition of compost. Cd contents in soil varied from 1.78 mg kg^{-1} to 2.96 mg kg^{-1} dry matter and soil affecting by garden compost (relationship 1:1) from 1.89 mg kg^{-1} to 2.71 mg kg^{-1} dry matter. Cd contents in soil exceeded the limit up to 78% (in April in the year 2016) - 196% (in December in the year 2015) (limit for Cd in soil is 1.0 mg kg^{-1} of dry matter, Decree No. 59/2013 Coll.), in soil with garden compost up to 89% (in March in the year 2015) - 171% (in October in the year 2015) (Fig. 2).

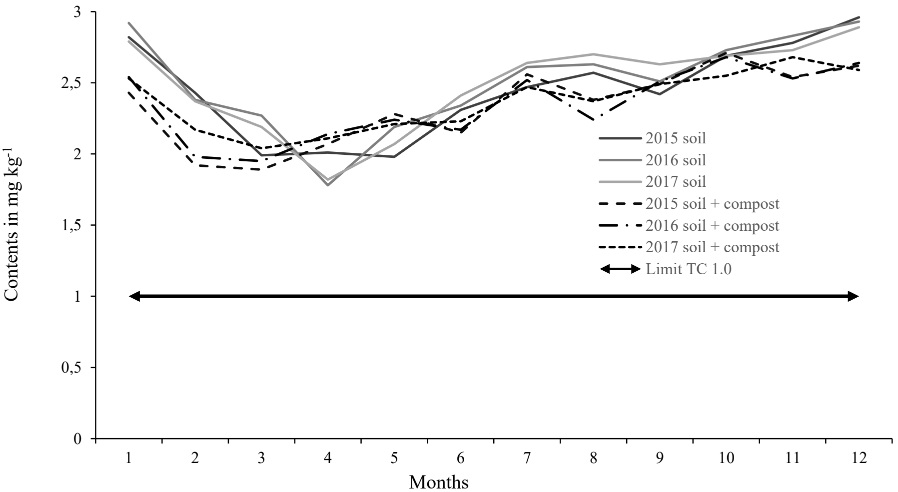

Fig. 2. Cd contents (mg kg^{-1} of dry matter) in soil and Cd contents in soil + compost (the ratio 1:1) in the years 2015–2017.
Notes: 1 January, 2 February, 3 March, 4 April, 5 May, 6 June, 7 July, 8 August, 9 September,10 October, 11 November, 12 December.

That contents of the soil exceeded the limit by 29.83% in average and after the addition of garden compost to the soil by 28.20%. In the over-all period, the average values were decreased by 1.1%. After the addition of garden compost into soil, the exceeded limit of Cd was lower (de O. Pinto et al., 2016; Khan et al., 2017; Urminská, 2017). The biocompatibility and high risk potential of Cd is influenced by pH and other chemical, physical and biological factors (Ying et al., 2018). We found that pH/KCl in the monitored soil varied from 5.74 to 6.83 and soil with garden compost (relationship 1:1) from 6.78 to 7.98. The pH is a key parameter in the assessment of mobility and bioavailability of risk elements in the environment (Makovníková et al., 2006). Uptake of Cd from soil into plants depends on the Cd contents in soil (Kabata Pendias, Pendias, 2011) and mobile bioavailable element forms. The most significant increase in the solubility of Cd occurs if pH of toxic Cd < 6.5 (Khun et al., 2008). Cd mobility and thus its bioavailability is highest in acidic soils with pH from 4.5 to 5.5, although Cd in alkaline soils is mostly less mobile (He et al., 2015). Acidity in soil is very dangerous for organisms, - especially plant organisms. If pH level is higher than 7.5, mobility of Cd is influenced by solubility of $CdCO_3$ or $Cd_3(PO_4)_2$ (Tlustoš et al., 2007). In this case, the mobility of toxic Cd is reduced. Our pH results showed that pH of the garden compost along with soil prevented the mobility of toxic Cd about 8.3% in average, at least partially too. Cd is highly toxic for all organisms (Tremlová et al., 2010; Mahurpawar, 2015; Rehman et al., 2017). According to Makovníková et al. (2006) and Tlustoš et al. (2007), the most commonly reported symptoms of Cd phytotoxicity are chlorosis of leaves, browning and root retardation, reduced photosynthetic activity, disruption of germination and plant transpiration, impairment of membrane systems, reddish-brown colouring of leaf veins, creation of purple-brown spots on leaves, necrosis of leaves and an overall decline in biomass due to leaf cast. If the growing agricultural crops are contaminated by Cd; it can get into human body by ingestion. Cd is a nephrotoxic and hepatotoxic chemical element. Carcinogenic and teratogenic effects of Cd are also known (Bencko et al., 1995, Kafka, Punčochářová, 2002; Khun et al., 2008). We found that at present, it is ecologically and economically potentially suitable to use the compost for the needs of biodegradability of hazardous chemical elements and increase of soil fertility (Khan et al., 2017). Many research studies have found that Cd as a hazardous element exhibits intense mobility and bioavailability in relation to soil - plants - humans (Wang et al., 2015; Zhang et al., 2015; Shahid et al., 2017). The compost that was made variously is useful as a remediation medium too (Wu et al., 2016; Khan et al., 2017). Raw compost (from 2 to 6 months old) contains active microorganisms. It is not suitable to be immediately incorporated into the soil (maximum in autumn), because it must not cause damage to agriculture; it must be sufficiently mature. It can be used appropriately in soil mulching. Mature compost (from 6 to 12 months old), the application is suitable in a volume of 10–15 kg of garden compost from 1 to 2 m^2 of soil to surface area. When planting, it is advisable to use compost mixed with 1:1 soil. For vegetables, the application rate is 5 litres per 1 m^2 and for flowers approximately 3 litres per 1 m^2 (Kmeťová, 2013). The result was that garden compost as a remediation medium is available, easy to produce, inexpensive to the conditions that helped to achieved a decrease of toxic Cd contents potentially and its bioavailability.

Conclusion

Study of risk of heavy metals as an ecological pollutant in every part of the environment are still present-day. Cadmium is a top element for actual study, because it is a cumulative poison for all the organisms. Its negative impact on the cosystem of plants is manifested by reduced photosynthetic activity and interferes with the transformation of nitrogen compounds and is also reflected by the impaired membrane systems. Moreover, it operates as a retardant on the root system and causes chlorosis, even necrosis with an overall decline in biomass and yields. At present, it is important to reduce the accessibility of risk elements to ecosystems. Compost is such a remediation medium, which is practical, economical and affordable. It is very important to look for suitable and inexpensive remediation media to ecological problems. Composting is an aerobic process, where biodegradable organic waste is converted to an organic-mineral fertilizer and a valuable remediation medium. The results showed that Cd contents in soil exceeded the maximum allowed limit of about 196%. And decreased by 25% after adding compost. The pH prevented the mobility of Cd by about 8.3% on average. Interesting data was found regarding the relationship between Cd concentrations in soil and pH/KCl in soil during the years and the relationship between Cd concentrations and Cd concentrations in soil affected by compost. Available Cd forms in soil were 53.3% and soil with compost were 45% on an average. The garden compost as a real remedial available and practical medium had reduced Cd contents.

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MACROPHYTES IN THE LITTORAL OF LAKE ARAKHLEY IN DIFFERENT STATES OF WATER REGIME

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Abstract

Kuklin A.P., Bazarova B.B.: Macrophytes in the littoral of Lake Arakhley in different states of water regime. Ekológia (Bratislava), Vol. 38, No. 3, p. 225–239, 2019.

The study of aquatic vegetation in the littoral of the dimictic water bodies of taiga is of particular interest in case of long-term observations carried out under conditions of climate fluctuations. During the low-water period, drying of the littoral with a decrease in the water level leads to the changes in phytomass of macrophytes, as well as in the composition of species and their distribution by depth. The area of littoral covered with ice in winter is also large in the low-water period; it affects the growth of perennial plants. When the water level decreases, the sand beach replaces the sand and pebble beach; it leads to the disappearance of epilithon and the predominance of rooting plants. The features of vegetation were determined for each period of water content. The low-water period is characterised mostly by grass-type vegetation; the high-water period is characterised by vegetation of mixed type.

Key words: littoral, aquatic vegetation, macroscopic algae, climatic cycles, Lake Arakhley.

Introduction

Climatic changes in the northern latitudes are characterised by continuing warming, whose rate is 2.5 times higher than that global warming (RosHydroMet, 2014). In Transbaikal region, the area of water bodies is decreasing, shallow waters are drying and ecotonal littoral ecosystems are adapting to new climatic conditions. The study of ecotones in the past decade is in the field of attention of UNESCO (Raspopov et al., 1998). Environmental factors such as storms or significant changes in water temperature have very strong influence on biological communities of littoral zones. One of the most influential factors is cyclical inter-annual fluctuations of the water level, which lead either to the drying of large areas of the littoral and destruction or the transformation of the hydrobiont complexes or to the flooding of the coastal areas with rising water and formation of new communities (Kurashov, 2011). Long-term observations of the state of ecosystems provide the most valuable data for the prediction of the effects of climate change (Sirakov, Slavcheva-Sirakova, 2015).

The littoral zone is characterised by the greatest biodiversity and bioproductivity in comparison to other parts of lakes. Biological and biochemical processes have the highest rates there. This zone is an exclusive area of distribution of higher aquatic plants; it extends from the shoreline to the lower boundary of the growth of macrophytes. Macrophytes, along with bottom sediments and water mass, are the main environment-forming elements of the littoral ecosystem. Long-term series of observations were carried out on the Narochansky Lakes (Mikheeva, Lukyanova, 2006), Lake Ladoga (Kurashov, 2011), Lake Chany (Bykova, 2013), lakes of Europe (Scheffer, 1997; Zębek, 2014), lakes of Asia (Atapaththu et al., 2017) and others. These studies characterise the climatic changes in conditions of temperate climate. However, there are only few works related to long-term series of observations, studying ecosystems of water bodies in conditions of extremely continental climate. Therefore, there is an urgent need to study the aquatic vegetation of water bodies in the areas of extremely continental climate.

Lake Arakhley is one of the large dimictic water bodies located in taiga of the Eastern Transbaikal region; it belongs to the Baikal basin. Arakhley is the largest lake in the Ivano-Arakhley system of lakes located in the southern part of the Vitim plateau. According to modern views, Lake Arakhley is the remainder of a large periglacial water body, which existed in the Transbaikal region at the time of maximum of the Samarovsky glaciation and connected Baikal Lake with the Amur River basin (Kuklin, Enikeev, 2017). The inter-annual fluctuations in the level of water surface of Lake Arakhley can reach 3 m in height. According to this value, Lake Arakhley takes an intermediate position between northern water bodies of the Transbaikal region characterised by low fluctuations of water level (Talovskaya, 1977) and the steppe water bodies characterised by pronounced fluctuations, which can reach 7 m in height (Ivanov, 1977). In the 1960-s, the theory about the cyclic development of the lake ecosystems in conditions of extremely continental climate of the Transbaikal region emerged (Shishkin, 1973). At the same time, stationary studies were started on Lake Arakhley, which made it possible to trace the dependence of the ecosystem of the lake on the dynamics of climatic parameters, in particular, the amount of precipitations and, consequently, on fluctuations in the water level (Bazarova, Itigilova, 2006). The information was summarised in monographs 'Biological productivity of Lake Arakhley (Transbaikalia)' (Bekman, Gorlachev, 1981) and 'The Ivano-Arakhley lakes at the turn of the century (state and dynamics)' (Obyazov, 2013).

Preliminary data on vegetation of the Ivano-Arakhley lakes were obtained in 1931 (Kozhov, 1950). In the 1960-s, Vladimirova and Dulepova carried out hydrobotanical studies of these lakes (Vladimirova, Dulepova, 1966; Vladimirova, 1970a, b). The annual production of macrophytes of Lake Arakhley was calculated for the first time in 1967 (Bondareva, 1974). In 1974–1976, geobotanical studies were carried out on lakes Arakhley and Shakshinskoe; the role of macrophytes in the creation of primary production of these lakes was determined (Zolotareva, 1981). In 1998, the study of macroscopic filamentous algae in the littoral of the lake was started.

The aim of the present work is to study the vegetation of the littoral of Lake Arakhley in different periods corresponding to different states of water regimes in order to understand the changes occurring in the ecosystems of dimictic continental water bodies under climate fluctuations. The research tasks included the establishment of environmental factors influencing vegetation in the littoral after the change in the water level and the registration of changes in the floristic composition and distribution of plant communities in the littoral in the high-water and low-water periods.

Material and methods

The article is based on the data obtained by the authors in the period from 1998 to 2017 in the course of stationary observations of the littoral of Lake Arakhley near Preobrazhenka village (Fig. 1). To characterise the littoral, the authors collected data on morphological structure, size and nature of the distribution of soil as well as on the chemical composition of water and the changes in depth, transparency and temperature. To characterise the macrophytes, more than 500 descriptions were given.

To construct a morphometric profile, the authors performed levelling on the drained part of the littoral of the lake near Preobrazhenka village (Fig. 2). The underwater part of the littoral was studied using an echo sounder HDS 5 Gen 2 with a beam of 50/200kHz (35°). At shallow depths, the bandwidth, whose data were compiled to calculate the bottom line, was small, which made it possible to use an echo sounder for terrain mapping. The authors applied Surfer 13 and DrDept 4.4 software for processing the obtained data.

As the connection between pieces of the sand samples was weak, the authors applied the sieve method (GOST sieve 6613–6673 mm) to study fractional composition of the soil. Sieve analysis is applicable for materials with particle sizes of 10–0.04 mm. For the samples that had high content of organic residues or the samples that lose more than 90% of their weight during drying (collected on depth of 2–6 m), granulometric composition was not analysed.

The hydrochemical samples were collected simultaneously with samples of macrophytes. At the same time, the authors measured the physicochemical parameters of water (pH, mineralisation, oxygen, water temperature) by AquaReader, a multi-parameter instrument for monitoring quality of water. The transparency of the water was measured using white Secchi disk. Hydrochemical analysis of the water samples was carried out by the method of atomic absorption, photometry and titrimetry in accordance with GOST 4389-72, 4192-82,3351-74, Federal Environmental Normative Document (PNDF) 14.1:2:4.138-98, 14.1:2:4.139-98 and Scientific Council on Analytical Methods of Research (NSAM) 335-g.

According to Raspopov (1975), macrophytes are macroscopic plant organisms regardless of their systematic position, which does not require the use of optical instruments with high magnification to establish their genus (in some cases, their species). Modern studies of macrophytes in the littoral of Lake Arakhley were conducted from 1998 to 2017 at 5–7 stations in the period of open water, according to the depth of growth of plants, by applying the methods widely used in such studies (Kachaeva, 1974; Katanskaya, 1981).

To determine the phytomass of macrophytes at the depths of up to 1 m, the cuts were made using a standard counting frame with an area of 0.25 m^2. At the depths of more than 1 m, an instrument for counting gammarids was used with a capture area of 0.5 m^2 (Shapovalova, Vologdin, 1973). To count charophytes, the Petersen bottom grab was used with a capture area of 0.025 m^2. The sampling cuts were made on all profiles at depths of 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 m. At these depths, rectangular platforms with a length of 10 m were placed; the width of the sites depended on the outline boundaries of the communities. On these sites, 3–5 cuts were selected at a distance of at least 5 m from each other. The species collected in each cut were separately sorted and weighed in moist and air-dry state. To obtain an absolute dry weight, a weighed portion of one plant species (three replicates) was dried in an oven at 105 °C for 4–6 hours.

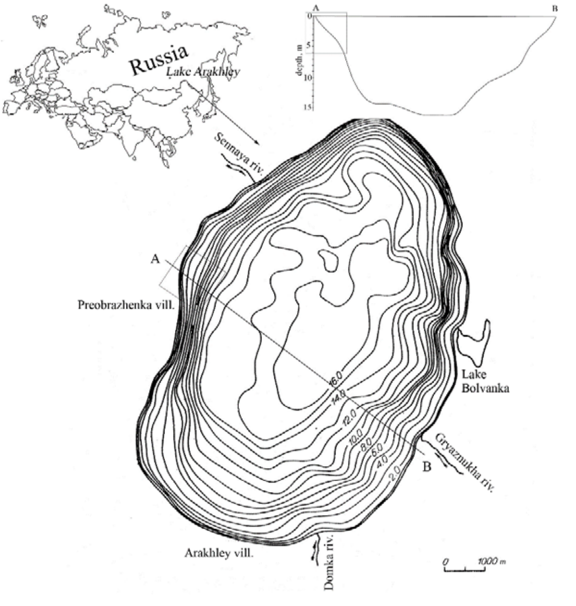

Fig. 1. Locations of sites that were used for studying the littoral of Lake Arakhley.

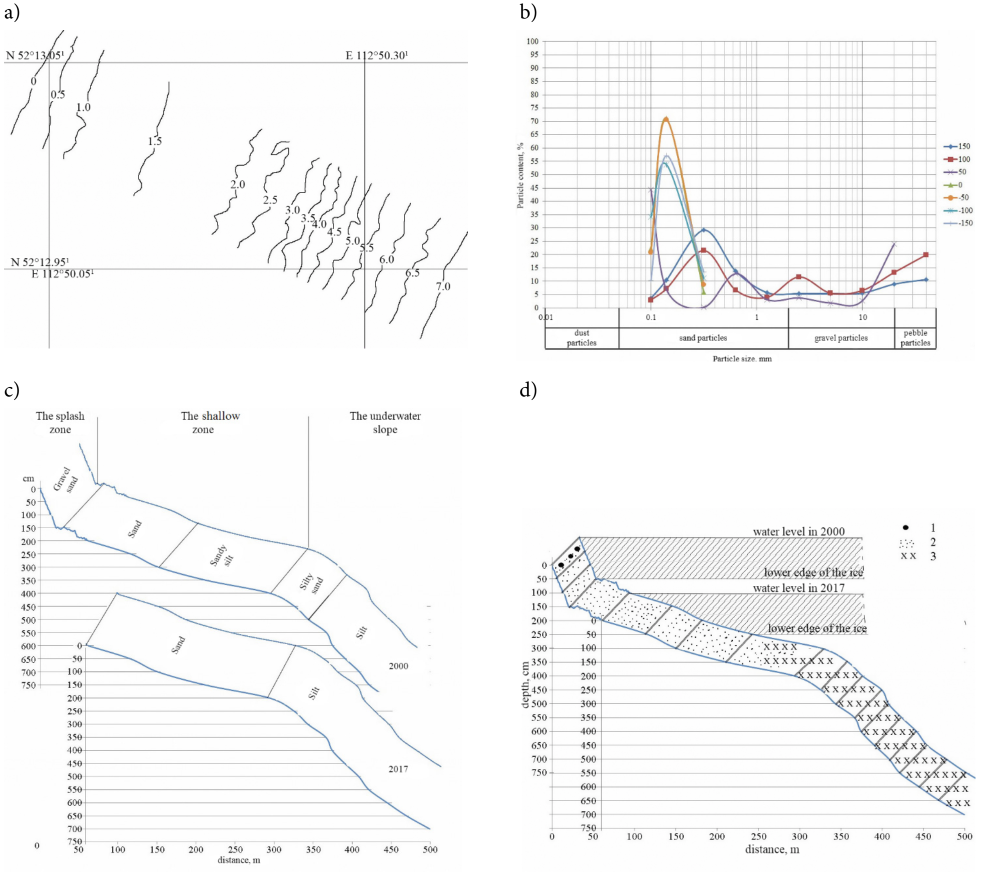

Fig. 2. Morphological structure and distribution of soils of the littoral of Lake Arakhley near Preobrazhenka village: (a) depth distribution as of 2017; (b) granulometric composition of bottom sediments; (c) distribution of various types of soils (according to Matafonov et al., 2006); **(d) position of ice during the winter period. The numbers designate the following:** (1) stone; (2) sand; (3) silt.

The authors selected and processed 427 cuts during the study. Macroscopic algae were collected by washing them from the substrates (pebbles, water vegetation); in this case, the authors measured the area where the substrates were collected. The authors also used a 10×10 cm frame to collect the algae, taking into account the algae projective covering of the bottom section. The algae were preserved using formalin (4%). The species composition was determined using microscopes Carl Zeiss Axio Scope A1 (1000´; Germany), MBS-10 (70´; Russia) and Mikmed-1 (400´; Russia), according to Russian and foreign algae identification guides (Gollerbakh et al., 1953; Vinogradova, 1980; Moshkova, 1986; Rundina, 1998). The species composition of higher aquatic plants was determined according to 'Flora of Siberia' (Kashina et al., 1988; Doron'kin et al., 1997; Timokhina et al., 1993). Herbarium samples are stored at the Institute of Natural Resources, Ecology, and Cryology (Siberian Branch of RAS).

In the process of classifying vegetation, the authors applied the dominant approach for determination of communities. Application of this approach makes it possible to reveal the leading role of plant dominants, especially edificators, in the processes of transformation of matter and energy in the community (Lavrenko, 1982). To distinguish the vegetation units, the authors used the principles of Shennikov (1935) applied in the classifications of Katanskaya (1981).

Results

Physical-geographical characteristics of the littoral

According to the work by Shishkin (1973), Lake Arakhley is located in an area of extremely continental climate. Obyazov (2013) gave ambiguous assessment of the manifestations of climate warming. Thus, he wrote, 'on the one hand, the temperature of summer water is rising, the number of days, when water temperature is above 10°C, is increasing, the duration of the period of ice phenomena is reducing, and the thickness of ice is decreasing. On the other hand, the facts show that water temperature falls below 4°C earlier, and the number of days, when the temperature is above 4°C, is decreasing, the period of ice phenomena begins earlier, and the time, when the lake is frozen over, is becoming generally longer' (Obyazov, 2013, p. 49). According to the same author, the thickness of the ice cover fluctuated between 125 and 170 cm during the studied period.

Climatic periods with high precipitation (wet periods) lead to an increase in the water level of the lakes (1960–1965, 1969–1974, 1995–2002). These years are characterised as high-water years. The periods of low precipitation (dry periods) lead to a decrease in the water level of the lakes (1980–1983, 2014–2017). These years are characterised as low-water years. The period considered in the article (1998–2017) is characterised by a decrease in the water level of the lakes. The data on the change in the maximum depth of the lake are presented in Table 1. With a decrease in the depth of Lake Arakhley, transparency of water decreases, but its mineralisation remains unchanged (Table 1).

The littoral zone of Lake Arakhley is an exclusive area of distribution of rooted aquatic plants. It extends from the shoreline to the lower boundary of their growth. Shishkin studied Lake Arakhley during high-water period in the early 1960-s. According to habitat conditions of benthic animals, he distinguished the following zones in the lake. The first zone – up to the isobath of 7 m (the lower boundary of the Potamogeton zone) – was defined as the littoral. The second one– up to the isobath of 11 m (the lower boundary of Charales and mosses) – was defined as the sublittoral. The last zone was the profundal. The author pointed out that with inter-annual changes in the level of lakes, the boundaries of the selected zones shifted (Shishkin, 1967). In the low-water year of 2017, the littoral boundary was at a depth of 5 m.

The position of water level in summer is different in low-water and high-water years. And the level of lower ice edge in winter is also different (Fig. 2d). With a relatively equal

T a b l e 1. Morphometric and hydrochemical characteristics of the littoral of Lake Arakhley in low-water and high-water phases.

Year	Parameter				
	S, km^2	D, max. m	k	Transparency, m	TDS, mg/l
1998–2000	58.481	15.5	0.92	7.0–11.0	115–150
2008	56.994	14.3	0.81	4.5–7.5	153–167
2017	55.962	13.5	0.93	3.7–5.8	131–167

Notes: D - depth; k - index of angularity of the coastline.

thickness of ice (at a maximum of 150–170 cm),there was a great difference between low-water and high-water years in the width of the area of the littoral, affected by the lower surface of the ice cover. Thus,in the winter of 1999–2000, the width of this area was 50–60 m near Preobrazhenka village; in the winter of 2016–2017, its widthreached 150 m (Fig. 2). Thereby, at low-water level, when ice thickness reaches its maximum during the cold period, the ice cover occupies a significant part of the shallowzone of the littoral. At the same time, during the high-water period, almost entire shallowzone was submerged throughout the winter.

The length of the littoral in the high-water period was 380–400 m. It consists of 30 m of the flooded shore, 270–300 m of the shallow zone and 90–110 m of the underwater slope. The higher values of bottom gradient are typical for the flooded shore (0.5‰) and for the underwater slope (0.13‰). The bottom gradient of the shallow zone is 0.083‰. Figure 2 shows bottom profile of the littoral near Preobrazhenka village.

The peculiarities of the morphometric structure of the littoral in 2017 included the appearance of temporary water bodies on the dried part of the littoral. In spring and early summer period of 2017, groundwater discharge formed temporary water bodies on the transitional area between the flooded shore and the shallow zone. They had shape of narrow strips (1.0–1.5 m) with a depth of up to 10 cm (mainly 5 cm). These water bodies were scattered and had no connection with each other. The number of such water bodies and their size were high in the shore areas near the gullies formed by runoff on the coastal slope. During the entire period of open water, additional temporary water bodies appeared in the splash zone because of the formation of sandbars from coastal sand and remnants of aquatic vegetation of the upper part of the littoral. The height of the sandbars determined the depth (10–15 cm) of these pools. Their area (5–10 m^2) determined the gradient of the shore. A significant difference between the spring temporary water bodies and the temporary pools in the splash zone is that the latter remained connected to the mother lake because of periodic erosion of the sandbars.

The studies of the composition and distribution of soil particles in the littoral were carried out on the dried and submerged parts of the littoral. It was found that the largest gravel and pebble soil particles compose the highest parts of the littoral. These particles are the product of erosion of the coastal slope (Fig. 2b). The shallow zone is composed of sands; the size of sand grains naturally decreases with an increase in the depth of the zone. The study revealed high amount of fine-dispersed organic sediments in the sands of the shallow zone in the high-water period. The data on the distribution of soils on the lake profile in high-water and low-water years are presented in Fig. 2c.

Special studies of wave activity in the littoral of Lake Arakhley were not carried out. However, it is undoubtedly high and its effect on macrophytes in the littoral of the lake is significant. It is obvious that during the transition from a high-water period to a low-water period, with a change in depth for 2 m, both the breaking zone and the area subjected to maximal wave influence moved towards the centre of the lake. According to the data on Lake Ladoga (Kurashov, 2011), the width of the breaking zone is limited by depths between the shoreline and the isobath, which is calculated as the maximum height of the storm wave increased 1.28 times.

Composition, spatial distribution, and dynamics of macrophytes

In the studied period (1998–2017), the authors observed 21 taxa of macroscopic filamentous algae of three divisions (Cyanobacteria, 9; Chlorophyta, 7; Charophyta, 7) in the littoral of Lake Arakhley. Seven species of vascular plants were registered (Table 2). In general, macrophytic filamentous algae, cyanophytes and vascular plants are represented by cosmopolitan species that are widespread in the Palearctic. The rarely observed species include *Aegagropila linnaei* Kützing, *Sirogonium sticticum* (Smith) Kützing and *Zygogonium ericetorum* Kützing, which have specific requirements for habitats.

T a b l e 2. Composition of macrophyte taxa in the littoral of Lake Arakhley near Preobrazhenka village in 1999 (according to Bazarova, 2002; Kuklin, 2002) and in 2017.

Taxon	1999	2017
Nostoc pruniforme C. Agardh ex Bornet & Flahault	++	++
Nostoc kihlmanii Lemmermann	+	-
Gloeotrichia pisum Thuret ex Bornet & Flahault	+	+++
Tolypothrix distorta Kützing ex Bornet & Flahault	+++	+
Tolypothrix distorta f. *penicillata* Kossinsk.	+++	+
Rivularia borealis P.G.Richter	++	-
Calothrix fusca f. *parva* (Ercegovic) Poljansky	+++	-
Calothrix kossinskajae Poljansky	+++	-
Calothrix parietina Thuret ex Bornet & Flahault	++	-
Chaetophora elegans (Roth) C. Agardh	+++	+
Stigeoclonium farctum Berthold	+++	-
Stigeoclonium tenue (C. Agardh) Kützing	++	-
Oedogonium sp. ster.	++	+
Bulbochaete sp. ster.	+	-
Cladophora fracta (O.F. Müller ex Vahl) Kützing	+	++
Aegagropila linnaei Kützing	+	-
Mougeotia sp. ster	+	-
Spirogyra sp_2 ster	+	+
Sirogonium sticticum (Smith) Kützing	+++	-
Zygnema cruciatum (Vaucher) C. Agardh	+	-
Zygogonium ericetorum Kützing	-	++
Chara arcuatofolia Vilh.	++	+
Nitella opaca L.	++	-
Potamogeton perfoliatus L.	++	++
Potamogeton compressus L.	+	-
Potamogeton praelongus Wulf.	++	+
Stuckenia pectinata (L.) Börner s.l.	+	++
Myriophyllum sibiricum Kom.	+	++
Ceratophyllum demersum L.	+++	+++
Lemna trisulca L.	+++	+

Notes: '+' - rare; '++' - common; '+++' - abundant.

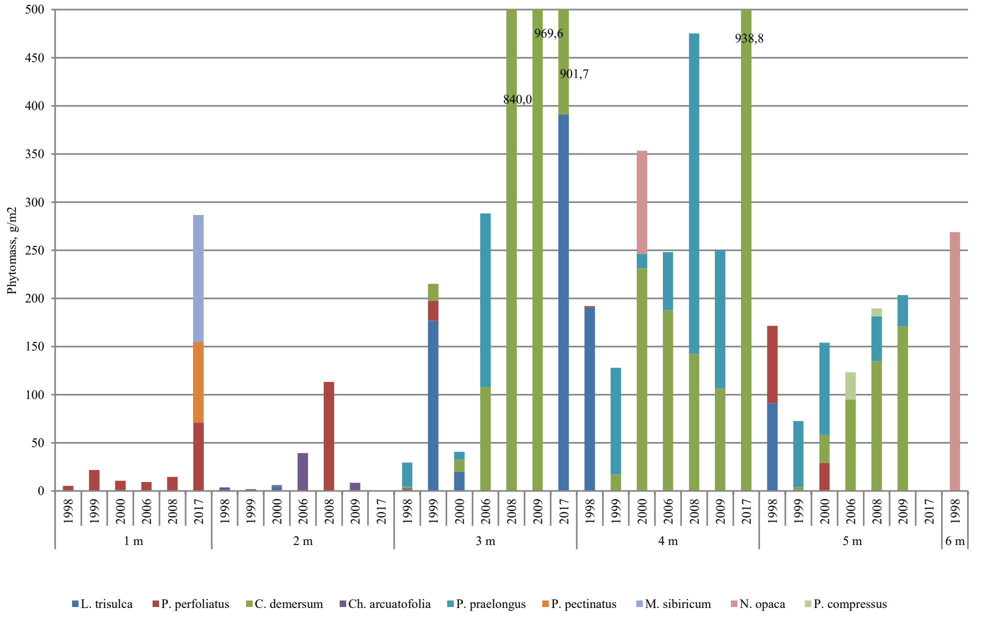

Fig. 3. Dynamics of hydrophyte phytomass distributed by absolute depth (Lake Arakhley near Preobrazhenka village).

In the high-water period of 1998–2000, vegetation of macrophytes in the littoral formed four ecological groups: epilithon, epiphyton, metaphyton and phytobenthos. In the epilithon, 14 species of macroalgae were identified, which were evenly distributed in the area adjacent to the shore. The most abundant vegetation on the territory from the shoreline to the 0.5 m isobath belonged to the rooted forms of the Rivulariaceae and Chaetophoraceae families. Seasonal changes in the species composition were manifested by the dominance of the *Calothrix kossinskajae* V. Poljansky during the summer period and its replacement by *C. fusca* f. *parva* (Erceg.) V. Poljansk in autumn. On some parts of the littoral in the epilithon, green alga *Stigeoclonium tenue* var. *tenue* (Ag.) Kütz grew and even dominated sometimes. In September, the number of *Chaetophora elegans* (Roth) Ag. increased. At a depth of less than 1.0 m in the metaphyton, *Stigeoclonium sticticum* f. *sticticum* grew on the littoral areas that were free of higher aquatic plants. In 1998, the vegetation of the alga continued until the last decade of September. In 1999, it continued until August. The phytomass of the alga was 1.8 g/m^2. The amount of phytomass largely depended on the presence of dead parts and rhizomes of higher aquatic or flooded terrestrial plants, which the alga used as substrates.

The macroalgae of the epiphyton were studied on *Lemna trisulca*, *Potamogeton praelongus*, *P. perfoliatus*, *Ceratophyllum demersum* and *Fontinalis* sp. The most intense growth of algae on *Potamogeton perfoliatus*, *Chara arcuatofolia* and *Lemna trisulca* was registered on the depths of 1.0–2.5 m. Within the vegetation period, the maximum species diversity was observed at the end of July and the beginning of August; the maximum of quantitative development was observed at the end of August and the beginning of September. The greatest number of algae colonies grew on the dying stems of *Potamogeton perfoliatus*. As the large colonies of *Gloeotrichia natans* and *G. pisum* were observed on *Lemna trisulca* in June, it was supposed that their single colonies could overwinter on the vegetation growing under the ice.

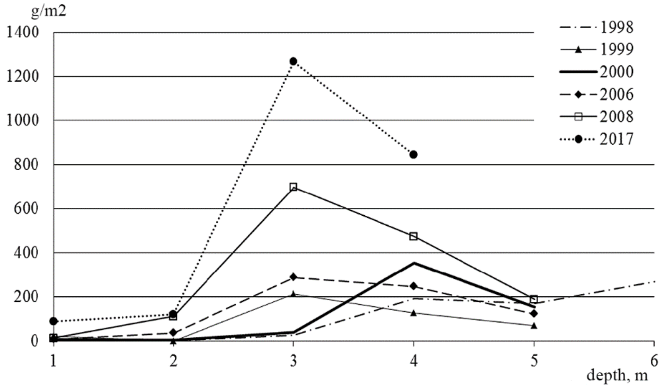

Fig. 4. Distributions of mean values of hydrophyte phytomass on the bottom profile of Lake Arakhley near Preobrazhenka village.

Vascular plants and charophytes dominated in phytobenthos, starting from the depths of 0.5–0.8 m; their communities had clearly defined boundaries. The ecological series represented according to the depths of 1.0–2.0–3.0–4.0–5.0–6.0 m had the following sequence: *Potamogeton perfoliatus* (a) – *Chara arcuatofolia* (b) – *Lemna trisulca* (c) – *Ceratophyllum demersum* (d) – *Potamogeton praelongus* (e) – *Nitella opaca*. It was characterised by the following percentage ratio of phytomass: a: b: c: d: e = 14.2:13.3:29.0:30.1:13.4. In 1998, the dense communities of *N. opaca* occupied maximal 6-m isobath. In 1999, the area of their distribution decreased sharply; they were represented by small clumps at 4-m depths. In 2000, *N. opaca* communities were not found in this area (Fig. 3).

The study of macrophytes in the low-water period was carried out in the littoral of the lake and in temporary pools on its dried part; three ecological groups were studied: epiphyton, metaphyton and phytobenthos.

The composition of species in each of the spring temporary pools was determined by a complex of factors specific to particular water body. Amongst the species, small colonies of *Nostoc pruniforme* were noted, as well as Nostoc commune Vaucher ex Bornet & Flahault. The latter is the soil alga widespread on the territory of the Transbaikal region. In early summer, flora of macroscopic algae of the splash pools was represented by cyanophytes (*Gloeotrichia pisum*, small colonies of *Nostoc pruniforme*). There were no filamentous (green and Charophyta) algae. By the end of summer, various filamentous forms appeared; their biomass reached 6.5–10.9 g/m^2 in wet weight. Along with filamentous forms, *Chara arcuatofolia* vegetated. Its phytomass was more than 250 g/m^2 in wet weight. Right before the lake froze over in autumn, vegetation of *Cladophora fracta* was noted amongst the aquatic plants in the splash pools and near the shoreline. Its phytomass reached 38 g/m^2 in wet weight. For the first time in the lake, *Zygogonium ericetorum* was registered. The alga grew on open areas of the splash pools with a depth of 1–3 cm. Its phytomass reached 28 g/m^2 in wet weight.

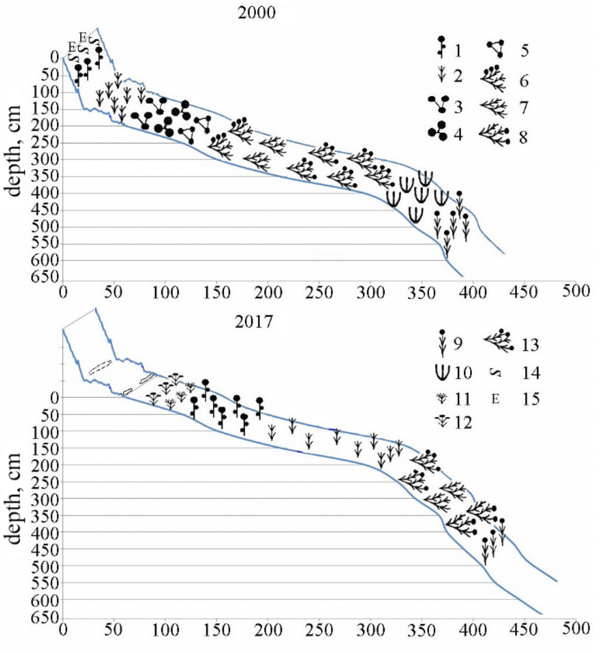

Fig. 5. Distribution of various types of vegetation in the littoral of Lake Arakhley near Preobrazhenka village. Associations: (1) *Potamogeton perfoliatus*; (2) *Chara arcuatofolia*; (3) *Lemna trisulca–Potamogeton perfoliatus*; (4) *Lemna trisulca + Ceratophyllum demersum–Potamogeton perfoliatus*; (5) *Lemna trisulca + Ceratophyllum demersum–Potamogeton praelongus*; (6) *Ceratophyllum demersum–Potamogeton perfoliatus*; (7) *Ceratophyllum demersum*; (8) *Ceratophyllum demersum–Potamogeton praelongus*; (9) *Potamogeton praelongus*; (10) *Nitella opaca*; (11) *Myriophyllum spicatumum*; (12) *Potamogeton pectinatus*; (13) *Ceratophyllum demersum + Lemna trisulca–Potamogeton perfoliatus*; (14) *Sirogonium sticticum*; (15) *Chaetophora elegans*.

Six species of macroalgae were registered in the epiphyton. They belonged to two divisions: Cyanobacteria (3) and Chlorophyta (3). Most of the plants growing in the area from the shoreline to the 5-m isobath were the rooted forms of the Rivulariaceae family. *Gloeotrichia pisum* was dominant. The composition of taxa is presented in Table 2. *Nostoc pruniforme* was the most 'noticeable' species in the metaphyton. In the phytobenthos, the dominant composition of hydrophytes did not change in the low-water period. However, the maximum depth of plant growth (measured from the water surface) reduced from 6.0 to 4.5 m; the quantitative ratios of the dominant species also changed (Fig. 3). The communities of hydrophytes in the part of the littoral near Preobrazhenka village began to grow starting from the shoreline. Up to the depths of 0.5–0.8 m, they were represented by well-developed communities of *Potamogeton perfoliatus*, *P. pectinatus* and *Myriophyllum sibiricum*. The values of their phytomasses are presented in Figures 3 and 4. At the depths of 1.5–2.0 m, the communities of *Chara arcuatofolia* grew as they did before. *Ceratophyllum demersum* communities occupied more deep zones of the littoral. At the depths of 3.0 m, the groups of *Lemna trisulca* were found in the hollows of the bottom relief. *Potamogeton praelongus* was registered at the depths of 4.0–4.5 m.

Discussion

The lower boundary of the littoral in freshwater reservoirs is the greatest depth of distribution of aquatic plants (Raspopov, 1975). In the absence of macrophytes, the lower boundary of the littoral zone is determined by the lower boundary of the trophogenic layer, that is, by transparency of water (Ruttner, 1962). In the littoral of Lake Arakhley, both these boundaries remained almost unchanged in high-water and low-water periods. In 2017, the level of the shore line corresponded to the depth of 2 m in 1998–2000 (Fig. 2d). However, the maximum depth of plant growth in 2017 (4.5 m) was only 50 cm deeper than that observed in 1998–2000. In the period from 1998 to 2017, there was a change in the boundaries of the littoral in the dried upper part of the lake; the lower boundary moved for 50–70 m towards the centre of the lake.

In the low-water period, the dried part of the littoral is a shore. Thus, it is related only in an indirect manner to the habitat of macrophytes of littoral. However, the splash zone of most Eurasian lakes is very poorly studied or not studied at all (Timoshkin et al., 2012). The study of macrophytes in the spring temporary pools and in pools of the splash zone revealed a high degree of similarity in composition of macroscopic algae of these water bodies and the main lake. Communities of *Chara arcuatofolia* grew both in the splash pools and at the depths of 1.5–2.0 m on sandy soils of the main lake. According to the literary data, the spores of charophytes can persist for several years in the layer (1–7 cm) of bottom sediments; under favourable conditions, they can grow again (Ozimek, 2006). The ability of spores of charophytes to remain viable for several years is evidenced by their appearance in the pools of the lakeside area. At the same time, the splash pools are a new environment inhabited by species that were not observed in the main lake (e.g. *Zygogonium ericetorum*).

High insolation, transparent crystalline ice and dry winters contributed to the formation of favourable conditions for subglacial vegetation of phytoplankton and a number of macrophyte species, for example, *Chara arcuatofolia*, *Lemna trisulca* and *Ceratophyllum demersum*. These factors also contributed to the reduction of harsh midwinter period to 2–3 months and the activation of spring growth processes and reproduction of organisms under the ice. On the basis of the peculiarities of the hydrobiological regime that formed in the reservoirs of Transbaikal region, Shishkin distinguished the Transbaikal-Mongolian limnic country (Shishkin, 1973). The processes described above were also noted in the high-water period of 1999–2000, when practically the entire shallow zone was flooded throughout the winter period, which also promoted subglacial vegetation of plants. The studies carried out in the low-water period with high snow cover of the ice showed that the heating of the subglacial waters started only after the melting of the snow cover. These studies also demonstratedthat the ice lying on the shoreline prevented the subglacial vegetation of the plants. During the period of ice breaking, the upper littoral was intensively ploughed, which also inhibited the development of the variety of rooting plants.

Vegetation of macroalgae in epilithon in the high-water period was possible because of the presence of pebbles in the composition of the soil (Fig. 2). Owing to the decrease in the water level in 2017, the shoreline near the village Preobrazhenka moved for 50 m towards the centre of the lake. Consequently, the zone with sand-gravel sediments disappeared from the submerged part of littoral. Owing to the absence of a fraction of gravel and pebbles on the shoreline, the epilithon community did not develop; a reduction in the qualitative composition of macroscopic algae (from 20 to 9 species) was observed. At the same time, in the area from the shoreline to the depths of 0.5–0.8 m, the diversity of hydrophyte communities increased (*Potamogeton perfoliatus*, *P. pectinatus*, *Myriophyllum sibiricum*). The increase in amount of organic fine-dispersed sediments in the sands of the shallow zone in the high-water period is a result of high density of plants, which retained the silt there. Mass development of communities of *M. sibiricum* and *Cladophora fracta* indicated that eutrophy of the upper littoral increased.

The diversity of aquatic vegetation in the upper part of the littoral (from the shoreline to the depth of 1.5 m) promoted the development of the epiphyton. The most 'noticeable' species in the metaphyton were species such as *Nostoc pruniforme*, *Aegagropila linnaei* and *Stigeoclonium sticticum*. The latter species used dead remains of aquatic and flooded terrestrial plants for the formation of cotton-like clusters. In general, the number of species of macro-epiphytic algae increased with an increase in depth. However, the number of algal colonies on higher aquatic vegetation declined sharply (Kuklin, 2002). Cyanobacteria predominated on submerged vegetation in shallow waters; with an increase in depth, the proportion of green algae increased. In 2017, there were no significant changes in the species composition of the epiphyton during the summer–autumn period.

Communities of *Ceratophyllum demersum* were greatly developed in the low-water period of 2017 at the depths of more than 2 m (Fig. 5). In comparison with 1998–2000, the role of *Lemna trisulca* and *Potamogeton praelongus* decreased. Plant associations with predominance of *Ceratophyllum demersum* occupied equal depths in low-water and high-water years. However, owing to the lowering of the water level, they moved deeper into the lake to the places where the association of *Nostoc opaca* had previously grown. Presumably, this was the reason that caused the reduction of the areas inhabited by *N. opaca*.

Both high-water and low-water periods are characterised by specific sets of conditions for the development of macrophytes in the littoral of the lake. A characteristic feature of the high-water period is the abrasion of the flooded coastal area. On the shoreline, the sandbars are formed from plant remains and sand. A significant gradient of the bottom prevents the formation of the splash pools. During the studied period, a decrease in the water level by 150 cm led to the displacement of the shorelinefor a distance of 20 m. The low-water period (from 2008 to the present) is characterised by erosion of the shallow zone and, because of a small gradient, formationof the splashwaterbodies above the shoreline. During this period,a decrease in the water level by 50 cm led to the displacement of the shoreline for a distance of 40 m (Fig. 2). Appearance of the splash poolsled to the appearance and development of species of macroscopic algae that previously were not observed in the littoral of the lake.

According to the analysis of the data on the composition and distribution of macrophytes in the littoral of Lake Arachley in the period from 2000 to 2017, there are two types of development of the littoral vegetation. In the high-water period, the higher aquatic vegetation is combined with the fields of charophytes, as well as with macroscopic algae of epilithon, epiphyton and metaphyton. The authors of the article proposed to call this type of vegetation development the 'mixed' one. A significant contribution to the biogenic nutrition of vegetationis provided, along with the supply from the drainage basin, by the organic matter accumulated during the low-water period and deposited in sandbars. By the end of winter, ice cover occupies less than 20% of the territory available for growth of algae. This leads to subglacial vegetation of charophytes, duckweed and so onand, consequently, to development of variety of epiphytes.

In the low-water period, the higher aquatic vegetation dominates in the littoral. The authors proposed to call this type of vegetationdevelopment the 'grassy' one. The absence of stone substrates contributes to intensive vegetation of vascular plants and filamentous algae. This process is also promoted by biogenic nutrition coming both from the central part of the lake in the form of silt sediments and from the splash pools in the form of decomposed organic matter. By the end of winter, the ice cover occupies half of the territory available for growth of algae, which inhibits vegetation of perennial plants.

Conclusion

The results of the studies show that it is necessary to perform long-term series of observations to detect changes in the vegetation of littorals of lakes. The authors of the article determined the factors (such as the position of shoreline and of lower edge of the ice cover and changes in the fractional composition of bottom sediments and in bottom morphology) that affect dynamics of vegetation in the littoral of a dimictic water body in conditions of extremely continental climate. Short-term series of observations do not give enough data to figure out the true reasons for the noted changes. In the low-water and high-water periods, the composition and distribution of macrophytes differ. For high-water years, a 'mixed' type of growth of macrophytes in the littoral is typical; this type is characterised by belt spread of vascular plants combined with the belts of charophytes and filamentous algae in the epilithon. In the low-water period, vascular plants predominate in the littoral. This type of growth of macrophytes in the littoral is called 'grassy'. In spring, temporary water bodies appear on the dried part of the littoral. Another temporary water bodies are formed in the splash zone. The species composition of these water bodies is determined by the spores buried in the bottom sediments. Long-term observations of the composition and distribution of vegetation in the littoral allowed the authors to determine the possible depth of distribution of macrophytes in the lake, thereby establishing the lower boundary of the littoral of the water body. The obtained data will make it possible in the future to predict the dynamics of plant communities, taking into account fluctuations of water regime.

Acknowledgements

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THE STATE OF BIO-ECOLOGICAL CHARACTERISTICS OF THE ONE-YEAR SHOOTS OF *Robinia pseudoacacia* L. UNDER THE CONDITIONS OF INDUSTRIAL POLLUTION

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Abstract

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The paper studies the influence of industrial pollution on bio-ecological characteristics of the one-year shoot of *Robinia pseudoacacia* L. in the conditions of the city of Dnipro, Ukraine. It analyses the state of biometric parameters of the shoot and anatomic indices of the stem of the studied species exposed to toxic gases. It was found that there are adaptive changes in the histological structure of the stem of *R. pseudoacacia* under the conditions of technogenesis. The study revealed that bio-ecological characteristics of the black locust are highly resistant to industrial emissions with big shares of SO_2 and NO_2. It was suggested to use *R. pseudoacacia* for greening of the technogenic territories.

Key words: anatomical indices, biometric parameters, toxic gases, greening, bioindication.

Introduction

The problem of environmental protection and rehabilitation is not losing its relevance in the 21 century. One of the most serious consequences of anthropogenic impact on the natural environment is pollution of atmospheric air, soil and water by man-made emissions that causes the reduction of biodiversity (Kozak, Didukh, 2014). Even such balanced ecosystems as forests change significantly as a result of technogenesis (Baciak et al., 2015; Masternak et al., 2015), and in many anthropogenic cases, the transformation of forest biogeocenoses leads to their degradation and the inability to perform ecological functions (Voron, 2011; Matkovskaya, Klymchyk, 2016).

However, it is the creation of artificial green spaces in the field of the impact of industrial waste and vehicle exhaust components that can significantly improve the environmental situation when properly selected for the planting of ornamental trees and shrubs (Levon, 2008;

Zhang et al., 2013; Nikolić et al., 2017). The main requirements for tree-bush species are good absorption capacity, resistance to certain classes of pollutants prevailing in the ecotopes of the planting area, and a wide range of adaptive reactions, from biochemical and anatomical-morphological to genetic ones, which allow maintaining at an appropriate level both the ontogenesis and the reproductive ability of individual components of phytocenosis, and the ability of the entire forest range to self-regulate, self-renew and self-reproduce (Levon, 2003; Kucheriavyj, 2005).

The study of tree species and shrubs also seeks to find sensitive bio-ecological indicators of the vegetative and generative spheres of a separate plant organism and tree stands in general, which can be used to diagnose plant conditions in megacities and industrial agglomerations (Hnativ, 2014; Grytsay, Miasoid, 2016) and set ecological norms of anthropogenic loads on forest ecosystems (Voron, 2011).

The response of the plant to the action of negative environmental stress factors can be determined by the appearance of plants and the changes in their morphometric indices (Ivanchenko, Bessonova, 2016). In addition to the intensity of vegetative growth and plant condition, the indices of the anatomical structure of the shoots are taken into account for the integral assessment of the viability and the promise of the technogenic introduction of tree-bush breeds as the processes of their formation are a structural reflection of the physiological and biochemical mechanisms of plant growth (Brajon, Chikalenko, 1992; Beck, 2005).

It is known that aerotechnogenic factors of the environment cause both functional and structural changes in tissues and organs (Arsenyeva, Chavchavadze, 2001; Albrechtova, 2003); therefore, stem histological characteristics can serve as informative criteria for monitoring anthropogenic environmental transformation (Kurteva, Stambolieva, 2007).

Taking into account all of the above, the purpose of the work is to analyse the state of bio-ecological characteristics of the one-year shoots of *Robinia pseudoacacia* L. in the conditions of industrial pollution of the city of Dnipro (Ukraine).

Material and methods

Study area

Dnipropetrovsk region is one of the most polluted areas in Ukraine, with high level of urbanization and concentration of industrial enterprises and highways. The forest cover of the region is 5.6% (General characteristics of forests of Ukraine, 2016); most of the forests are artificial and perform mainly sanitary, hygienic and protective functions. Dnipro is a powerful multi-industrial centre of Ukraine (The City of Dnipropetrovsk, Amending the general plan of the city development, 2015), which has four major industrial regions: Western, Left-bank, Southern and Eastern. The most contaminated areas are the Western and the Southern areas, the latter includes the CJSC 'Plant Dnipropres' (hereinafter 'Dnipropres') (Yemets, Serdyuk, 2003).

The chronic effect of aerotechnogenic loading is exacerbated by extreme climatic conditions, like hot summer with drylands, dry and frosty winter with frequent temperature fluctuations, strong winds, as well as soil-hydrological conditions, like frequent and prolonged droughts, which complicates the normal existence of the forest in the Dnipropetrovsk region (Zaitseva, Dolgova, 2010), located in the zone of the Northern steppe of Ukraine.

Monitoring points

The plant materials were selected in September 2011 in two sites. The test area is located in the artificial forest plantation, located along the highways at a distance of 2 km from the source of industrial emissions, Dnipropres, Dnipro

(48°38’00.8” N, 35°00’17.5” E), where the concentrations of SO_2 and NO_2 were 5.8 and 6 maximum permissible values accordingly (Peremetchik, Polischuk, 2011; Striletz et al., 2013). The relatively clean reference area is located in the village of Pryadivka of Tsarychanka area in Dnipropetrovsk region (48°89’81.3” N, 34°70’16.9” E), where the concentrations of sulphur (IV) and nitrogen (IV) oxides do not exceed the maximum permitted values (SO_2 – 0.05 mg/m^3, NO_2 – 0.04 mg/m^3) (Striletz et al., 2013). The forest conditions, the characteristics of the tree stand, the structure and composition of plantations in the test area and in the reference area were similar.

Plant material

The research object is the introduced tree species of black locust, or false acacia, – *R. pseudoacacia* L. (genus *Fabaceae* Lindl.). The species is widely grown in the steppe and forest-steppe zones, as it occupies an important place in amenity planting and phytomelioration. The widespread use of *R. pseudoacacia* for sylvatization in the steppe can be explained by its relatively rapid growth (Grimal’skij, 1951; Strojnaja, 1991), a small number of pests and diseases (Voloshyn, 1994), drought-and-wind resistance, and also by its ability to grow in very dry places (Zverkovskyy et al., 2018), including the steep slopes.

The samples of *R. pseudoacacia* one-year shoots in each of the test areas were taken from the southeast side of the crown of five model trees of the same age at a height of 1.7 m from the soil level. As bio-ecological indicators, the morphometric and anatomical characteristics of the shoots were studied. Biometric measurements were made in 100-fold repeat according to the generally accepted methods (Zlobin et al., 2009), after which the stems of 30 shoots were fixed in 96% alcohol. Cross sections of the stem were made at a distance of 2 cm from the stem base with the help of a manual microtome and stained with phloroglucinol (Albrechtova, 2003). The preparations were examined under a light microscope BRESSER Biolux LCD 40x–1600x and the thickness of the tissues was measured at 100-fold increase, the diameter of the vessels – at 400-fold increase in a 30-fold repetition.

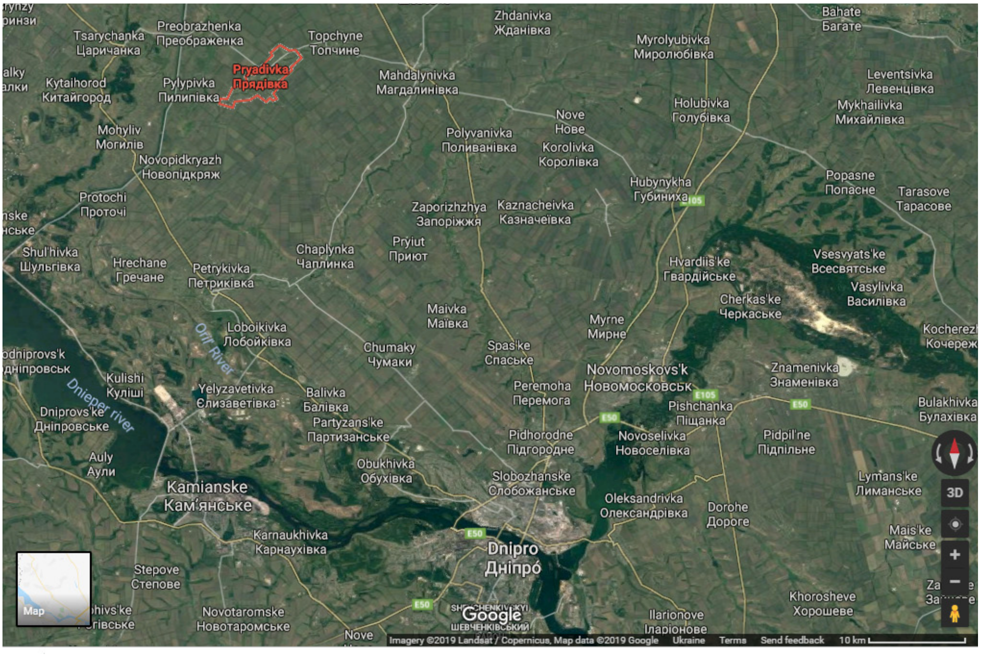

Fig. 1. The map of Dnipropetrovsk region and the sites of monitoring points. https://www.google.com.ua/maps/@48.7208385,34.6968211,10z?hl=en

Statistical analysis

The results of the study were handled using a multifunctional application software package *STATGRAFICS*. Mean absolute error was calculated. To compare the biometric characteristics of annual shoots and the stem anatomical parameters of reference and test samples we used Student's t-test ($p < 0.05$). Normality allocation of the sample had been preliminarily assessed.

Results and discussion

The state of biometric indices of the one-year shoots of Robinia pseudoacacia L. under conditions of industrial pollution

The research shows the ambiguous effect of industrial pollution of SO_2 and NO_2 on the biometric characteristics of the one-year shoots of *R. pseudoacacia*, in which the value of the part of the shoot morphometric indices decreases significantly compared with the reference one, whereas some other characteristics increase.

As can be seen from Table 1, the annual shoot growth of the *R. pseudoacacia* shoot under conditions of industrial pollution increases substantially compared with this indicator in the plants of the relatively clean zone and amounts to 146.5% of the control value. Probably, the stimulating effect of industrial emissions on the growth of shoots in technogenic zones can be explained by the increased content of nitrogen-containing compounds in industrial soil, as they can be a source of plant root nutrition (Korshykov, 1996).

Zverev (2008) showed a significant extension of the *Betula pubescens* subsp. *czerepanovii* (Orlova) Hämet-Ahti, which undergoes chronic emissions of SO_2 by nickel-copper smelter at Monchegorsk. Annual growth of the shoot of *Spirea* L. species representatives in the conditions of industrial pollution by SO_2, NO_x and heavy metals was 102–181% of the values of this index in the plants of the relatively clean area (Chernikova, 2009). Hryshko (2002) established an increase in the length of the shoots of *Ulmus pumilla* L., *Robinia pseudoacacia*, *Populus canadensis* Moench. due to the increase in the length of the internodes and their number; although *Aesculus hippocastanum* L. demonstrates a decrease in these parameters, which leads to a decrease in the length of the shoots in the plants of this species. However, Yakovleva-Nosar' (2017) found a significant decrease of the annual shoot growth of *Robinia pseudoacacia* shoots in the urboecosystem with a high level of technogenic loading.

T a b l e 1. The influence of industrial pollution on biometric indicators of annual shoots of *Robinia pseudoacacia* (M ± m, n = 100).

Indicator	**Control**	**Industrial area**
Annual shoot growth, cm	7.07 ± 1.13	10.36 ± 1.22*
Number of leaves produced on 1 year of growth shoot, items	6.57 ± 0.79	6.61 ± 1.32
Number of leaves on the model branch, items	92.93 ± 5.99	63.80 ± 2.44*
Number of leaflets in a model compound leaf, items	14.60 ± 2.16	13.77 ± 1.06
Area of one model leaflet, cm^2	7.66 ± 0.11	9.61 ± 0.26*
Area of one model leaf, cm^2	112.34 ± 10.29	135.97 ± 3.13*
The area of the assimilating surface on the model branch, cm^2	10435.29 ± 43.06	8679.37 ± 61.44*

Note: * – differ significantly from control ($p < 0.05$).

The state of growth and development of vegetative plant organs can be estimated using the biometric characteristics of the assimilation apparatus. Changes in the size of individual leaves and the leaf surface are generally associated with changes in the intensity of photosynthesis in the plant organism. The area of the assimilation surface in the studied tree species depends on a number of biometric characteristics of the shoots, namely, the number of leaves on the model branch, the area of the model leaf, which, in turn, depends on the other two parameters, the number of leaflets in the compound leaf and the area of the model leaflet, since the leaves of *R. pseudoacacia* are compound.

We found, the numbers of leaves produced in the one year shoots in the reference and test trees are almost the same, with insignificant difference between reference and test samples at $p < 0.05$ (Table 1). However, as the annual shoot growth is higher under the conditions of pollution, the number of leaves on the model branch (calculated per 1.0 m length) in the plants in the industrial site is significantly lower as compared with the relatively clean area and make 68.7% of the reference value. The number of leaflets in a compound leaf is a more constant value, probably programmed at the genetic level, and the study did not find the differences between the values of this index in plants in different growth conditions.

Regarding the area of the model leaf in *R. pseudoacacia*, there was a significant increase in the value of this parameter in plants in the sanitary protection zone around the CJSC 'Plant Dnipropres' (21.0% compared to the reference one), due to a significant increase in their area of the model leaflet by 25.5% compared to the reference value. When the leaf surface was studied, it was ascertained that this parameter in the research object decreases by 16.8% of this value in plants from the relatively clean area, which makes a significant change in the conditions of technogenesis. Despite the fact that the area of *R. pseudoacacia* model leaf increases significantly when exposed to the industrial emissions of SO_2 and NO_2, the value of the assimilation surface area goes down due to the slower number of compound leaves than in the reference area.

It should be noted, that the previous scholars have found that industrial emissions can both stimulate and inhibit the growth and development of leaves depending on the species of tree and shrub plants. Thus, the area of assimilation surface in the decorative shrubs increases under the influence of SO_2 and NO_2 toxic gases; the highest, compared with the control value, was the area of the leaf surface in *Ptelea trifoliata* L., and it grew in *Caragana arborescens* Lam. and *Cornus sanguinea* (L.) Opiz (Iusypiva, Minejeva, 2010). Kolmogorova (2013) found a decrease in the number of leaves on the annual shoots, the area and mass of leaves *Syringa vulgaris* L., *Betula pendula* Roth. and *Sorbus sibirica* Hedl., since they were exposed to the heavy car emissions in the city of Kemerovo. In the conditions of multicomponent pollution of SO_2, NO_2 and heavy metals, the area of the model leaf decreased significantly in *B. pendula* plants, but due to their bigger amounts, the area of the assimilation surface increased (Iusypiva, Zamorena, 2011). Yakovleva-Nosar (2017) has established a significant decrease in the leaf area of *Robinia pseudoacacia* exposed to the emissions of motor vehicles and metallurgical enterprises.

Parpan and Mylenka (2009) noted the decrease in the area of leaf plates and the growth of dichromatic and necrotic damages of leaves because of the industrial emissions near TPP. Krupenko and Kapelush (2014) revealed an increase in the fluctuating asymmetry of leaves and a decrease in the number of leaf plates in the plants of *Tilia cordata* Mill. in the conditions of the chronic action of the components of industrial emissions and emissions of vehicles in the city of Zaporizhzhia, as well as the representatives of *Betula pendula* in the city of Kryvyi Rih (Savosko, Katolichenko, 2014).

The previous research also found that near the highways in the city of Dnipro (Ponomaryova, Bessonova, 2009), environmental pollution causes a decrease in the morphometric indices of the assimilation apparatus *Tilia cordata* and *T. platyphyllos* Scop. compared to the reference value. In the conditions of urban parks in the city of Yaroslavl, it was ascertained that as concentrations of pollutants in the atmospheric air increase, leaf growth is suppressed and the number and degree of their necrotic damage to *Betula pendula*, *Tilia cordata* and *Populus nigra* L. increase (Marakaev et al., 2006).

Thus, the study of the influence of industrial emissions produced by 'Dnipropres' on the morphometric characteristics of *Robinia pseudoacacia* one-year shoots showed that the growth processes in plants of this species are suppressed in the conditions of technogenesis. In particular, the number of leaves on the model branch and the area of the assimilation surface decrease significantly, although three indicators increase. They are the annual shoot growth, the area of the model leaflet and the area of the model leaf. We view these reactions of *R. pseudoacacia* trees to the stress caused by industrial pollution as adaptive mechanisms of compensatory type.

The state of the anatomical indices of Robinia pseudoacacia *L. one-year shoots under the conditions of industrial pollution*

The study of the influence of 'Dnipropres' emissions on the state of the anatomical indices of *R. pseudoacacia* one-year shoot stem revealed their high sensitivity to the chronic effects of aerogenic pollutants (Tables 2,3, Figs 2,3). As can be seen from Table 2, in the conditions of chronic exposure to sulphur dioxide and nitrogen, the thickness of the primary cortex of the stem of the research object decreases by 11.9% compared with the reference value. This is due to a decrease in the size of such histological elements of the primary cortex as cork (phellem) and cortex parenchyma.

Phellem is an outer layer of the primary cortex, so it protects the underlying tissues from the stress caused by environmental factors. In plants growing in conditions of industrial pollution, the thickness of this tissue at the shoot base decreases by 11.8%, when compared to

T a b l e 2. The effect of industrial pollution on the thickness of the primary cortex tissues of the one-year shoot stem of *Robinia pseudoacacia*, μm (M ± m, n = 30).

Indicator	**Control**	**Industrial area**	**% of the reference value**
Cork Thickness	66.31 ± 1.49	58.47 ± 2.35*	88.2
Collenchyme Thickness	60.91 ± 2.97	57.88 ± 1.35	95.0
Primary Cortex Parenchyma Thickness	17.03 ± 0.49	12.16 ± 0.05*	71.4
Primary Cortex Thickness	144.27 ± 8.07	127.02 ± 3.46*	88.0

Note: * – differ significantly from control ($p < 0.05$).

the reference values. Due to the protective functions of the cork – like it does not infiltrate gases and liquids, protects against water loss and overheating and other adverse environmental factors (Brajon, Chikalenko, 1992; Beck, 2005) – reducing the cork thickness in technogenic conditions can reduce the resistance of *R. pseudoacacia* to toxic gases and negative impacts of climatic factors.

Collenchyma, that is, the mechanical tissue of the primary cortex of *R. pseudoacacia* stem is lamellar and placed under the cork in 3–5 rows. The analysis of Table 2 shows that the thickness of this histological part of the black locust stem in the technogenic environment is unchanged, with the insignificant differences between the reference and test samples at $p < 0.05$. The thickness of the parenchyma, which is located under the collenchyma and is an intrinsic layer of the primary cortex of the *R. pseudoacacia* stem, changes most significantly. It drops by 28.6% among the histological elements of the primary cortex of the stem in the shoot base.

It should be noted that the thickness of the primary cortex of the stems of woody and shrub plants can either increase or decrease depending on the type of plants, conditions of their growth, as well as the type and extent of the influence of anthropogenic environmental factors. Thus, Leppik and Bocharov (2007) found a decrease in the periderm thickness in the *Catalpa bignonioides* Walt. stems, which grew in the area of exhaust emissions. Nuzhyna et al. (2014) discovered that the number of collenchyma layers increased with the increase of the levels of technogenic loading on *Rosa centifolia* L., *R. rugosa* Thunb., *R. xanthina* Lindl, although the index of collenchyma thickness compared to the control values went up only in *R. rugosa* representatives. The authors explain this phenomenon as the protective reaction of the plant organism to the increasing effect of the irritant agents. The previous research showed that moderate doses of toxic substances stimulate the thickening of cork parenchyma more intensively in *R. centifolia* and *R. rugosa*, whereas severe contamination inhibits the development of this tissue.

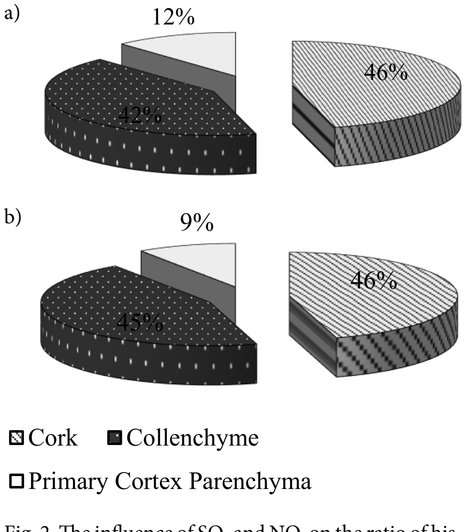

Fig. 2. The influence of SO_2 and NO_2 on the ratio of histological elements of primary cortex of annual shoot stems of *Robinia pseudoacacia*, %: a) – Control (reference area), b) – test area.

Bessonova and Kryvoruchko (2017) showed that in the shoots of *Quercus rubra* L. plants growing in roadside plantation, there was an increase of the cork and collenchyma thickness indices, 4 radial rows of cells in the reference area, 6 rows in the test area, which according to the authors, ensures better protection of internal tissues from the penetration of pollutants.

To analyse the effect of industrial emissions on the process of forming the external protective tissues of the stem, it is necessary to learn the thickness of the constituent parts of the cortex not only in absolute but also in relative values, that is, the proportion of each tissue in percentage as a share of the overall width of the shoot primary cortex. The analysis of Fig. 2 shows that due to the industrial emissions of Dnipropres, there are changes in the ratio of the histological elements of the primary cortex in the stems of *Robinia pseudoacacia*. Thus, the cork share remains unchanged, the thickness of collenchyma is increasing in the total volume of the cortex, and the share of parenchyma is decreasing by a quarter in comparison with the clean area.

The central cylinder (stele) of *R. pseudoacacia* stem consists of secondary cortex (phloem), wood (xylem), medullary sheath and pith (Table 3). It should be noted, that the phloem histological structure in the research object is specific; its hard bast forms a solid ring of strongly lignified bast fibres in the soft bast.

The analysis of the thickness of the histological elements of this part of the stele indicates that the chronic exposure of *R. pseudoacacia* plants to industrial pollution with the predominance of SO_2 and NO_2 leads to changes in its formation. Thus, the width of the bast fibre layer reduces by 18.2% under technogenic conditions compared with the control value (Table 3), while the thickness of the conductive elements of the secondary cortex and bast parenchyma increases by 11.9% relative to the size of the soft bast in the plants of black locust that grow in a relatively clean zone. We noted that under the influence of industrial phytotoxic pollutants on the studied plants, the changes in the radii of the phloem constituents are mutually compensatory, that is, they do not cause the changes in the width of the secondary cortex of *R. pseudoacacia* shoots stem base, with insignificant differences between the reference and the test samples at $p < 0.05$.

Bast formation is important for the normal functioning of the transport system in plants; it contributes to the creation of the donor-acceptor relations of the plant organism, which provides the outflow of photosynthesis products from the assimilation organs to other organs. As it stimulates the photosynthesis process, the soft bast volume growth in the conditions of technogenesis can increase plant productivity.

The literature analysis confirms the sensitivity of structural elements of trees and shrubs phloem to the action of negative biotic, abiotic and anthropogenic environmental factors. Thus, Abdussalam et al. (2015) recorded a complete damage to the secondary phloem in the

Table 3. The influence of industrial pollution on the size of the stele histological elements of *Robinia pseudoacacia* one-year shoots, μm ($M \pm m$, $n = 30$).

Indicator	Control	Industrial area	% of the reference value
Hard Bast Thickness	111.12 ± 3.25	124.34 ± 2.61*	111.9
Soft Bast Thickness	59.14 ± 1.92	48.39 ± 4.86*	81.8
Secondary Cortex Thickness	170.26 ± 16.13	174.74 ± 26.49	102.6
Wood Radius	284.96 ± 24.20	288.99 ± 44.82	101.4
The Largest Vessel Diameter	48.39 ± 10.20	47.18 ± 5.75	97.5
Medullary sheath Radius	84.23 ± 8.83	78.63 ± 5.99	93.4
Pith Diameter	913.14 ± 77.23	662.67 ± 48.15*	72.6
Stem Diameter	2283.81 ± 90.02	2159.95 ± 67.94	94.6

Note: * – differ significantly from control ($p < 0.05$).

stems of *Boerhavia diffusa* L. under the influence of heavy metals of cadmium, chromium and copper. Yusypiva and Rudenko (2009) found an increase of soft bast thickness in one-year shootings of *Ulmus pumilla* L. when exposed to SO_2 and NO_2 toxic gases. Under the conditions of urbocenosis, the thickness of the stem secondary cortex in *Acer negundo* L. and *A. platanoides* L. was almost unchanged in comparison to the values in the reference area; however, it increased by 25% in *A. pseudoplatanus* L. species (Pavlyukova, Legostayeva, 2016).

Xylem is an area of the central cylinder of the stem, located deep in the secondary cortex. It provides transport of water and mineral nutrients in plant, and it is the main supporting part of the stem (Brajon, Chikalenko, 1992; Beck, 2005). The structure of the wood and medullary sheath in *Robinia pseudoacacia* has certain features. Xylem looks like a solid ring formed by vessels of almost identical diameter. Medullary sheath in the form of a ring with uneven edges covers a pith that takes the form of a star.

In the conditions of industrial emissions of SO_2 and NO_2 (Table 3), the radius of xylem almost does not change compared with the value of this index in plants of a relatively pure zone; the differences between reference and test samples are insignificant at $p < 0.05$. The same can be concluded about the diameter of the vessels: the size of the largest of the tested vessels in *R. pseudoacacia* shoots is similar in value to the ones in the reference zone.

It should be noted that a large number of species of tree plants respond to industrial pollution by decreasing their vessel diameter and vessel wall thickness (Kaakinen et al., 2004; Mahmooduzzafar et al., 2010), the wood thickness (Griztay, Shupranova, 2015) and the annual rings diameter (Whitmore, Freer-Smith, 1982).

Therefore, the stability of these histological characteristics of the research object under exposure of chronic toxic gas impact testifies to the relative stability of *R. pseudoacacia* to the studied pollutants. That makes it possible to state that the anthropogenic introduction of the investigated tree species into the regions of the steppe Prydniprovya with priority pollution of SO_2 and NO_2 has been successful.

The pith of *R. pseudoacacia* stem consists of large diameter cells that perform a storage function. The reduction of this area of the stem central cylinder is significant and makes 27.4% compared with the reference value in the conditions of technogenesis. At the same time, the medullary sheath radius remains almost unchanged, at the insignificant differences between reference and test samples at $p < 0.05$. Since significant deviations in the thickness of the histological elements of the annual shoot stem stele of the investigated tree species were not found under the technogenic growth conditions, there are no significant changes in the stem diameter of *R. pseudoacacia* in the industrial site (Table 3).

The considerable amount of data obtained during laboratory and field experiments indicates the negative influence of toxic compounds on the growth of stems of woody and shrub plants (Whitmore, Freer-Smith, 1982; Kaakinen et al., 2004; Griztay, Shupranova, 2015, etc.) Thus, the long-term effects of high concentrations of CO_2 and O_3 reduced the distance between the stem pith and its cortex in *Populus tremuloides* (Kaakinen et al., 2004).

Table 3 presents data in micrometres. To analyse the effect of industrial emissions on the tissue formation of the stem central cylinder, we calculated the share of each tissue in percentages relative to the total width of the shoot stele, that is, the value of histological parameters in absolute values (Fig. 3).

It was found that the emissions of Dnipropres cause changes in the ratio of the components of *Robinia pseudoacacia* central cylinder; there is a significant increase of wood share compared to the indices of plants from the clean area, a less significant increase of the soft bast share and a significant decrease of the pith share. As for the shares of the hard bast and medullary sheath, their values in the test and reference areas are relatively similar.

In our previous study (Iusypiva, Miasoid, 2017), we found an increase in the shares of the hard bast and medullary sheath relative to the total volume of the stem stele in the undergrowth of *Acer platanoides* and *Fraxinus excelsior*, which increased when they were constantly exposed to sulphur and nitrogen dioxides in comparison with the values in the reference area. Notably, in the stems of the former species, the shares of the soft bark, wood and pith of the stem stele volume did not change in comparison with the control values, while in the latter species, the shares of the hard bast and medullary sheath increased, whereas the share of wood diminished.

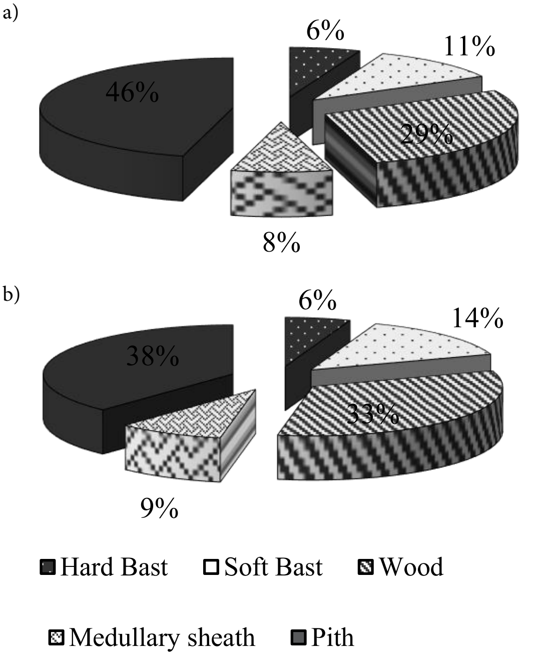

Fig. 3. The influence of SO_2 and NO_2 on the ratio of histological elements of the central cylinder of annual shoot stems of *Robinia pseudoacacia*, %: a) – Control (reference area), b) – test area.

Thus, the examination of the impact of industrial emissions with priority pollutants of SO_2 and NO_2 on the anatomical characteristics of the stem of *Robinia pseudoacacia* annual shoots showed that there are adaptive changes in the formation of histological elements of the stele under conditions of technogenesis in the plants of the species under study. This becomes evident in the fact that the shares of the transport tissues, soft bast and xylem, and the mechanical tissues, like collenchyma increase due to the decrease of parenchymal tissue of the stem (the pith and parenchyma of the primary cortex). Shcherbinina (2004) documented an increase of parenchymal tissues' shares in the representatives of *Betula pubescens* Ehrh. and *Tilia cordata*, which grew along the highway.

Conclusion

Technogenic pollution of the environment with sulphur and nitrogen dioxides leads to a change in the growth intensity of *Robinia pseudoacacia* aboveground vegetative organs. The values of some growth indicators, such as the number of leaves on the model branch, the area of the leaf surface goes down under technogenic conditions, while others increase, like the values of the annual shoot growth, the area of the model leaflet, the area of the model compound leaf.

It is ascertained that when the plants of *R. pseudoacacia* undergo the chronic exposure to SO_2 and NO_2 industrial emissions, the thickness of the cork and cork parenchyma decrease, which, in turn, causes the thinning of the primary cortex.

In technogenic conditions of plant growth, the secondary cortex thickness does not change significantly due to the compensation of the decrease of the hard bast width by increasing the soft bast thickness.

The pith diameter reduces due to the impact of phytotoxicants, while the wood and medullary sheath almost do not change with respect to the reference values. The stem diameter of the annual shoots of *R. pseudoacacia* plants growing in the area exposed to industrial phytotoxicants does not differ significantly from the stem diameter of the plants in the relatively clean area.

The conducted research proves that the bio-ecological characteristics of one-year shoots of *R. pseudoacacia* are highly resistant to industrial emissions with predominance of sulphur and nitrogen dioxides; therefore, we propose to use this tree species in the landscaping of man-made territories contaminated with SO_2 and NO_2.

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THE EFFECT OF SOIL ON SPATIAL VARIATION OF THE HERBACEOUS LAYER MODULATED BY OVERSTOREY IN AN EASTERN EUROPEAN POPLAR-WILLOW FOREST

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Abstract

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The tree species composition can influence the dynamics of herbaceous species and enhance the spatial heterogeneity of the soil. But there is very little evidence on how both overstorey structure and soil properties affect the spatial variation of the herb layer. The aim of this study is to evaluate the factors of the soil and overstorey structure by which it is possible to explain the fine-scale variation of herbaceous layer communities in an Eastern European poplar-willow forest. The research was conducted in the "Dnipro-Orils'kiy" Nature Reserve (Ukraine). The research polygon (48°30'51"N, 34°49"02"E) was laid in an Eastern European poplar-willow forest in the floodplain of the River Protich, which is a left inflow of the River Dnipro. The site consists of 7 transects. Each transect was made up of 15 test points. The distance between rows in the site was 3 m. At the site, we established a plot of 45×21 m, with 105 subplots of 3×3 m organized in a regular grid. The adjacent subplots were in close proximity. Vascular plant species lists were recorded at each 3×3 m subplot along with visual estimates of species cover using the nine-degree Braun-Blanquet scale. Within the plot, all woody stems ≥ 1 cm in diameter at breast height were measured and mapped. Dixon's segregation index was calculated for tree species to quantify their relative spatial mixing. Based on geobotanical descriptions, a phytoindicative assessment of environmental factors according to the Didukh scale was made. The redundancy analysis was used for the analysis of variance in the herbaceous layer species composition. The geographic coordinates of sampling locations were used to generate a set of orthogonal eigenvector-based spatial variables. Two measurements of the overstorey spatial structure were applied: the distances from the nearest tree of each species and the distance based on the evaluation of spatial density of point objects, which are separate trees. In both cases, the distance matrix of sampling locations was calculated, which provided the opportunity to generate eigenvector-based spatial variables. A kernel smoothed intensity function was used to compute the density of the trees' spatial distribution from the point patterns' data. Gaussian kernel functions with various bandwidths were used. The coordinates of sampling locations in the space obtained after the conversion of the trees' spatial distribution densities were used to generate a set of orthogonal eigenvector-based spatial variables, each of them representing a pattern of particular scale within the extent of the bandwidth area structured according to distance and reciprocal placement of the trees. An overall test of random labelling reveals the total nonrandom distribution of the tree stems within the site. The unexplained variation consists of 43.8%. The variation explained solely by soil variables is equal to 15.5%, while the variation explained both by spatial and soil variables is 18.0%. The measure of the overstorey spatial structure, which is based on the evaluation of its density enables us to obtain different estimations depending on the bandwidth. The bandwidth affects the explanatory capacity of the tree stand. A considerable part of the plant community variation explained by soil factors was spatially structured. The orthogonal eigenvector-based spatial variables (dbMEMs) approach can be extended to quantifying the effect of forest structures on the herbaceous layer community. The measure of the overstorey spatial structure, which is based on the evaluation of its density, was very useful in explaining herbaceous layer community variation.

Key words: overstorey structure, soil properties, phytoindication, poplar-willow forest, scalogram, spatial eigenvector mapping.

Introduction

The greatest plant biodiversity in forest ecosystems is concentrated in the ground vegetation layer (Gilliam, 2007). Extinction rates in herbaceous plants are more than three times those of hardwood tree species and approximately five times those of gymnosperms (Levin, Wilson, 1976). The herbaceous layer has the potential to determine the overstorey species composition due to the fact that some herbaceous species may be superior competitors for soil nutrients, compared with tree seedlings (Lyon, Sharpe, 2003; Gilliam, 2007). The herbaceous layer plays an important role in the functioning of forest ecosystems by supplying high quality litter to the forest floor (Elliott et al., 2015). The herbaceous layer serves as habitat and food for other organism groups (Whigham, 2004). Herbaceous species can respond quickly to changed environmental conditions (von Oheimb, Härdtle, 2009).

There are two distinct explanations of community structure: niche assembly and dispersal assembly (Dallas, Drake, 2014). In accordance with niche assembly, community composition is controlled predominantly by environmental forces (Weiher et al., 2011). The scale of observation effects on the relative importance of specific environmental factors (Siefert et al., 2012) and the use of space across different scales in the community analysis could be useful (Gazol, Ibanez, 2010). Abiotic processes are generally considered as environmental filters, which select those species that match the specific habitat requirements (Silvertown et al., 2006; Lososová et al., 2015). Environmental filtering is probably not very important at finer scales (Chudomelová et al., 2017). Dispersal is the probability that a given patch will be colonized (King, With, 2002). Dispersal assembly suggests that community composition is forced by the ability of species to reach new habitats, making spatial variables stronger determinants of community composition than environmental variables (Weiher et al., 2011). Dispersal effects are important only within the confines of environmental gradients (Gilbert, Lechowicz, 2004). The influence of the environmental factors was revealed as progressively decreasing from broader to finer spatial scales (Laliberte et al., 2009). As scale becomes finer, dispersal or biotic processes such as interspecies interactions increase their importance (Gazol, Ibanez, 2010). Spatial distances may be considered as a surrogate for dispersal through space over time (Karst et al., 2005). Community composition and species distribution would be spatially structured independent of environment if dispersal limitation was a major assembly process (Jones et al., 2006; Aiba et al., 2012). Neutral processes become weaker as a consequence of the greater heterogeneity of environmental conditions (Nettesheim et al., 2018). By scaling down, the number of individuals within a given grain or extent is reduced, and individual-based stochastic processes become more important (Chase, 2014). As the size of the sampling plot decreased, the relative importance of environmental factors declined predominantly due to the reduction of environmental variability at finer scales (Frelich et al., 2003; Legendre et al., 2009).

Tobler's "first law of geography" says that everything is related to everything else, but near things are more related than distant things (Tobler, 1970). This law is the foundation of spatial autocorrelation. Spatial autocorrelation is the similarity between two observations of a measured variable based upon their spatial location (Griffith, 1992; Legendre, 1993; Lennon, 2000). Spatial autocorrelation considerably manifests itself at fine-scale level (Chudomelová et al., 2017). Spatial autocorrelation causes the problem of pseudoreplication. Pseudoreplication was defined as "the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent" (Hurlbert, 1984). Spatial autocorrelation of a species community is generated by intrinsic and extrinsic processes (Fortin, Dale, 2005). Environmental variables are the extrinsic forces that control species distribution (Legendre, Legendre, 2012). Dispersal, historical events and biotic elements are the intrinsic forces (Teng et al., 2018).

Explained variation in species composition can be separated into four parts: pure environmental variation, pure space variation, the variation explained both by environment and space, and unexplained variation (Borcard et al., 1992). Depending on the relative importance of niche explanations or dispersal processes, a community is considered to be controlled either by environmental or spatial factors (Cottenie, 2005). Variation explained purely by spatial variables represents partly unmeasured environmental variables with spatial structure (Legendre et al., 2009). The variation partitioning method results has been shown as being dependent on the quality of predictors. Spatially structured but unmeasured environmental variables may affect community composition (Jones et al., 2008). The degree of dispersal limitation contributes to both the pure environmental and pure spatial variance partitions. The assumption that a pure spatial component represents the role of dispersal limitation holds only in cases where all the relevant environmental variables are considered (Chang et al., 2013). The ratio of variation attributed to environmental variation depends not only on the strength of extrinsic processes, but also on the specific spatial patterns of the environmental factors (Smith, Lundholm, 2010).

The tree species composition can influence the dynamics of herbaceous species (Bratton, 1976) by changing light availability (Breshears et al., 1997) and enhancing the spatial heterogeneity of the soil (Andivia et al., 2015). When the spatial pattern in species composition of the overstorey forest stratum is significantly correlated with that of the herb layer stratum, the strata are said to be linked (Mölder et al., 2008). Herb-layer species are sensitive ecological indicators (Standovár et al., 2006). The herb layer and overstorey respond to different gradients in young stands but respond to similar gradients in increasingly similar ways as the stand matures (Gilliam et al., 1995). However, there is very little evidence on how both overstorey structure and soil properties effect herb layer spatial variation.

The aim of this study is to evaluate by which soil and overstory structure factors it is possible to explain the fine-scale variation of herb layer communities in an Eastern European poplar-willow forest. The first hypothesis is that the overstorey is able to spatially structure the soil properties, which in turn affect the variation of forest herb layer communities. The second hypothesis is that tree spatial patterns form complex structures whose effect may be best modeled on the basis of density dependent rather than distance dependent measures.

Material and methods

Study area

The research was conducted in the "Dnipro-Orils'kiy" Nature Reserve (Ukraine) (Fig. 1). The research polygon (48º30'51"N, 34º49"02"E) was laid in an Eastern European poplar-willow forest in the floodplain of the River Protich, which is a left inflow of the River Dnipro. The territory has a temperate-continental climate with an annual mean maximum decade temperature of 25.7 ºC, and a minimum of -10.0 ºC, and with a mean annual precipitation of approximately 565 mm (20 year average according to data of the Dnipro meteorological station).

The study site comprises 1.0 ha of deciduous woodland bordered by arena terrace above floodplain of the River Dnipro. Forests in the steppe zone of Ukraine have a very restricted distribution and usually have an island status. The soils are fertile sandy loam, the underlying geology comprises Quaternary Aeolian sandy sediments.

Data collection

The site consisted of 7 transects. Each transect was made up of 15 test points. The distance between rows in the site was 3 m. At the site, we established a plot of 45×21 m, with 105 subplots of 3×3 m organized in a regular grid. The adjacent subplots were in close proximity. Vascular plant species lists were recorded for each 3×3 m subplot along with visual estimates of species cover using the nine-degree Braun-Blanquet scale (Westhoff, van der Maarel, 1978). The projective cover of plant species was recorded at ground level, the understorey (up to 2 m height) and canopy (above 2 m height). We were able to make species level identification for all quadrats. Seedlings and saplings of woody species were later excluded from the analyses. Within the plot, all woody stems ≥ 1 cm in diameter at breast height were measured and mapped. Dixon's segregation index was calculated for tree species to quantify their relative spatial mixing. The measure of segregation describes the tendency of one species to be associated with itself or with other species (Dixon, 2002).

In syntaxonomic aspect, the vegetation can be identified as follows:

Class *Salicetea purpureae* Moor 1958,

Ordo *Salicetalia purpureae* Moor 1958,

Union *Salicion albae* R.Tx. 1955,

Ass. *Populetum albae* Br.-Bl.1931.

Based on geobotanical descriptions, phytoindicative assessment of environmental factors according to Didukh (2011) was made. Didukh phytoindication scales (2011) include edaphic and climatic scales. The edaphic phytoindication scales include soil water regime (Hd), variability of damping (fH), soil aeration (Ae), soil acidity (Rc), total salt regime (Sl), carbonate content in the soil (Ca) and nitrogen content in the soil (Nt). The climatic scales include the parameters of thermal climate (thermoregime, Tm), humidity (Om), cryo-climate (Cr) and the continentality of climate (Kn). In addition to these, the lighting scale (Lc) is highlighted, which is characterized as a microclimate scale. Thermal properties of soils are indicated by the scale of thermal regime, and hydrothermal is the scale of ombro mode. Phytoindicational evaluation of environmental factors was performed by the ideal indicator method of Buzuk (2017).

Measurement of soil mechanical impedance was carried out in the field using a hand penetrometer Eijkelkamp, to a depth of 100 cm with an interval of 5 cm. The average error of the measurement results of the device is ± 8%. The measurements were made by a cone with a cross-sectional dimension of 2 cm^2. Within each measurement point, the mechanical impedance of the soil was made in a single repeatability. To measure the electrical conductivity of the soil *in situ*, a sensor HI 76305 was used (Hanna Instruments, Woonsocket, R. I.). This sensor works in conjunction with the portable device HI 993310. The tester estimates the total electrical conductivity of the soil, that is, combined conductivity of soil air, water and particles. The results of measurements of the device are presented in units of saturation of the soil solution with salts as g/l. Comparison of measurement results of HI 76305 with laboratory data allowed us to estimate the conversion factor of units as 1 dS/m = 155 mg/l (Pennisi, van Iersel, 2002). The soil bulk density was estimated by Kachinskiy, soil moisture by weight method (Vadunina, Korchagina, 1986). The aggregate structure was evaluated by the dry sieving method according to Savinov (Vadunina, Korchagina, 1986). The percentage content of such fractions is established: < 0.25, 0.25–0.5, 0.5–1, 1–2, 2–3, 3–5, 5–7, 7–10, > 10 mm. Litter layer thickness was measured by a ruler at 5 sampling points per sample subplot.

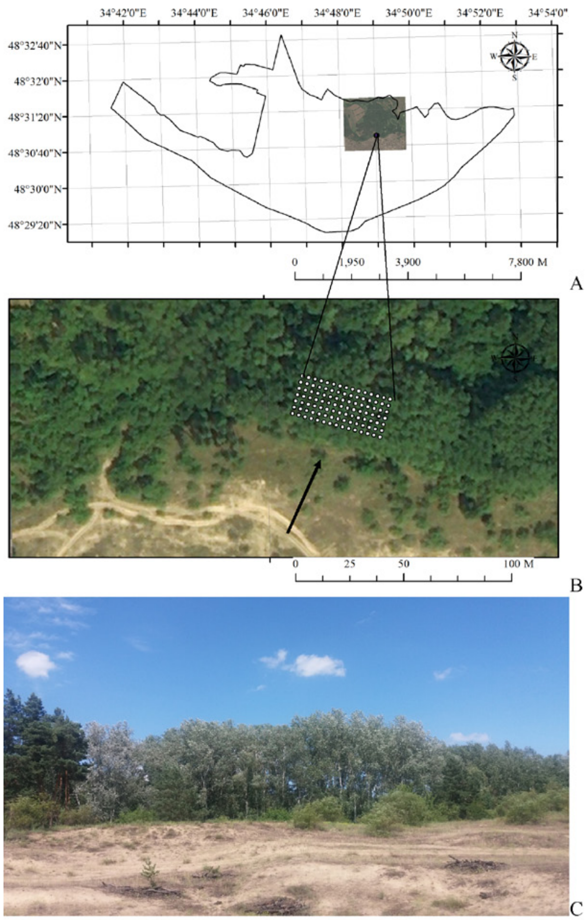

Fig. 1. Placing of experimental polygon and the sampling points. A – the map of the "Dnipro-Orils'kiy" Nature Reserve; B – the satellite image of the territory around the research polygon; C – photo of the research polygon (arrow shows the direction of the photographic shoot).

Data analyses

Soil aggregate structure is presented by the vectors of n (n = 9 in our case) strictly positive real components (x_1, x_2, ... ,x_n), such that $x_1+x_2+\cdots+x_n = \kappa > 0$, where κ is 100 (percentages) or 1 (proportions). Therefore, soil aggregate fractions are related to each other and any change in one fraction must affect the other fractions (Aitchison, 1986). Soil compositions were handled statistically using log ratio transformations (Parent et al., 2012). Isometric log ratios are orthogonal projections of compositional data (Egozcue et al., 2003). Due to there being D-1 degrees of freedom in compositional vectors (Aitchison, Greenacre, 2002), for the 9 soil aggregate fractions, there are 8 isometric log ratio variables (ILR-variables). The basis for isometric log-ratio transformation of the compositions data was computed by such methods as basic, balanced, optimal and principal balance methods (PBhclust, PBmaxvar, and PBangprox) (Egozcue et al., 2003). In order to select the optimal basis of the transformation, the matrix of distances of the sampling points in the space of the ILR-variables was compared with the matrix of distances of the sample points in the space of soil physical properties (electrical conductivity, soil mechanical impedance, bulk density and moisture), and with the distances matrix in the space of phytoindication scales and the matrix in the space of distances from tree stems. The evaluation was performed by using the Mantel test (ordinal and partial with matrix of the geographical distances as a conditional matrix). We assumed that transformation should lead to a solution that correlates best with the other properties of the environment and such correlation should not be spatially conditioned.

Redundancy analysis (RDA) was used for the analysis of variance in herb layer species composition (Rao, 1964). Before the analyses, percentage cover of species was Hellinger transformed to avoid problematic Euclidean distances used in the RDA (Legendre, Gallagher, 2001). Soil mechanical impedance, soil electrical conductivity, litter layer thickness, soil temperature, moisture and soil bulk density were log transformed. The significance of RDA global model including all soil variables was first tested. A soil model based on the forward selection of soil variables was built with double stopping rule (alpha significance level and the R^2_{adj} calculated using all explanatory variables) (Blanchet et al., 2008; Chudomelova et al., 2017). Variables were retained only with a significant relationship to community composition ($p < 0.05$, 9999 permutations). The models' marginal effect was computed, in which each selected soil variable was used separately as a predictor of community composition and the significance of all the models was tested and R^2_{adj} was extracted.

The geographic coordinates of sampling locations were used to generate a set of orthogonal eigenvector-based spatial variables (dbMEMs), each of them representing a pattern of particular scale within the extent of the sampling area (Borcard, Legendre, 2002). The forward-selection procedure on partial RDAs with previously selected soil factors as covariables was applied to the subset of spatial variables. The significance of soil models with selected spatial variables as covariables was tested by the Monte Carlo permutation test (9999 permutations).

Two measures of the overstorey spatial structure were applied: distances from the nearest tree of each species and the distance based on the evaluation of spatial density of point objects, which are separate trees. In both cases, the distance matrix of sampling locations was calculated, which provided the opportunity to generate eigenvector-based spatial variables (dbMEMs-tree and dbMEMs-density based respectively). A kernel smoothed intensity function was used to compute the density of the trees' spatial distribution from the point patterns data. Gaussian kernel functions with various bandwidths were used. The density of the trees' spatial distribution for each species was converted into the distance measure:

$$Dist = 1 - dens,$$

where *dens* – density of the trees' spatial distribution, *Dist* – measure of the distance. The coordinates of sampling locations in the space obtained after the conversion of the trees' spatial distribution densities were used to generate a set of orthogonal eigenvector-based spatial variables (dbMEMs-density), each of them representing a pattern of particular scale within the extent of the bandwidth area structured according to distance and reciprocal placement of the trees. The forward-selection procedure on partial RDAs with previously selected soil factors as covariables was applied to the subset of tree-structured spatial variables and the significance of environmental models with selected spatial variables as covariables was tested by the Monte Carlo permutation test (9999 permutations).

In the next phase of the study, the dbMEMs were forward-selected directly on community data to explore patterns in community variation by variance partitioning between environmental and spatial influence. The significance of pure spatial and environmental fractions was tested by Monte Carlo permutation tests with 999. The scalogram approach was applied to inspect in detail the spatial scaling of community variation (Legendre, Legendre, 2012). To do this, the two sets of RDA analyses were carried out with each of the dbMEM variables as a predictor. As a response variable, the first set of RDA analyses used raw (Hellinger-transformed) species data, while the second set used residuals of the environmental model in which forward-selected environmental variables acted as predictors (Chudomelova et al., 2017). From each RDA, we extracted R^2_{adj} for individual dbMEMs and plotted them into juxtaposed barplots (Chang et al., 2013). Phytoindication estimation of the ecological factors (Didukh, 2011) was used to find an ecological interpretation of spatial structures in community composition not explained by environmental variables.

All the statistical analyses were conducted in R (v. 3.5.0., R Foundation for Statistical Computing, Vienna, AT), using the following packages: vegan (v. 2.5-2, https://CRAN.R-project.org/package=vegan) for the multivariate analysis and for the computation of global and partial Moran's I. (Oksanen et al., 2018), adespatial (v. 0.3-2. https://CRAN.R-project.org/package=adespatial) for the forward selection and for the generation of spatial filters (Dray et al., 2018), compositions (https://CRAN.R-project.org/package=compositions) for compositional data analysis (Boogaart et al., 2018), dixon for testing the spatial segregation and association based on contingency table analysis of the nearest neighbor count (De la Cruz, 2008), spatstat for density estimation of the tree stems' spatial distribution (Baddeley, Turner, 2005).

Results

The forest overstorey includes *Populus alba* L. (41.5% of total tree stems), *Ulmus laevis* Pall (40.7 %), and *Crataegus fallacina* Klokov (4.4%). There are dead trees within the site (13.3 %) (Fig. 2). The distance from the sampling locations to *Populus alba* stems is 2.0±0.094 m (maximum – 4.6 m), to *Ulmus laevis* stems is 2.3±0.13 m (maximum – 7.6 m), to *Crataegus fallacina* stems is 12.8±0.81 m (maximum – 30.6 m), and to dead stems is 3.8±0.19 m (maximum – 9.1 m).

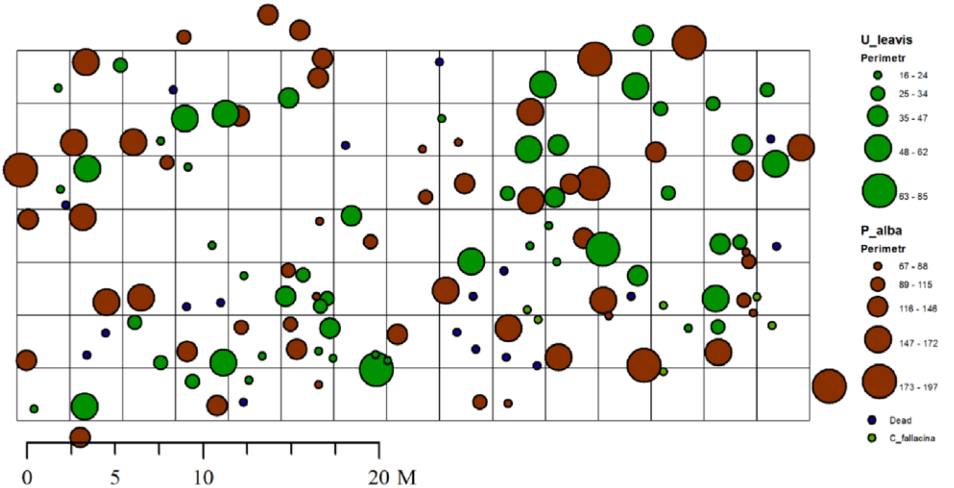

Fig. 2. Spatial locations of the three species individual within the site.

An overall test of random labelling reveals the total nonrandom distribution of the tree stems within the site (overall test of random labelling 21.1, *p*-value of the overall test from the asymptotic chi-square distribution with the appropriate degrees of freedom is 0.05). The species-specific test of random labelling shows the nonrandom distribution of the *C. fallacina* (11.1, $p = 0.01$), while other species are distributed randomly. The nearest neighbor contingency table indicates the aggregated spatial distribution of the *C. fallacina* stems within site (Table 1). There is no direct spatial connection between *C. fallacina* and *Ulmus laevis*. The dead trees are distributed randomly. The *Populus alba* stems are distributed non-randomly with a tendency to regular pattern. The *Ulmus laevis* distribution is random.

The simple Mantel test revealed that the soil aggregate structure is closely related to the variability of other physical properties of the soil, phytoindication estimates of environmental factors and varies depending on the distance to the tree stems (Table 2). The partial Mantel test showed that there is a spatial component in the soil aggregate structure data that is independent from spatial structure both in physical properties and distance from the stems as the Mantel statistic controlling for the effect of space is not significantly different from zero

T a b l e 1. The nearest neighbor contingency table and Dixon's spatial segregation test for tree species.

Species		*C. fallacina*	*Dead*	*P. alba*	*U. leavis*
C. fallacina	Obs.Count	2	1	3	0
	Exp. Count	0.22	0.81	2.51	2.46
	S	1.11	0.11	0.14	–
	p-value	0.00	0.82	0.68	0.04
Dead	Obs.Count	0	5	5	8
	Exp. Count	0.81	2.28	7.52	7.39
	S	–	0.42	–0.27	0.06
	p-value	0.35	0.12	0.23	0.77
P. alba	Obs.Count	4	8	15	29
	Exp. Count	2.51	7.52	22.99	22.99
	S	0.22	0.03	–0.28	0.19
	p-value	0.31	0.85	0.05	0.11
U. leavis	Obs.Count	0	8	25	22
	Exp. Count	2.46	7.39	22.99	22.16
	S	–	0.04	0.06	–0.01
	p-value	0.09	0.80	0.59	0.97

Notes: Obs.Count – observed nearest neighbor count; Exp. Count – expected nearest neighbor counts; S – segregation measure (values of S larger than 0 indicate that species is segregated; the larger the value of S, the more extreme the segregation; values of S less than 0 indicate that species is found as neighbor of itself less than expected under random labelling. Values of S close to 0 are consistent with random labelling of the neighbors of species); *p*-value – based on the asymptotic normal distribution of the Z statistic.

T a b l e. 2. Correlation (Mantel test) between distances matrix of the sample points in space of the soil aggregate fraction after log ratios transformation on the different basis and distance matrix in the space of the physical properties, phytoindication scales and distance from the stems.

Basis of the transformation	**Physical properties**				**Phytoindication scales**				**Distance from the stems**			
	Simple	**p-level**	**Partial***	**p-level**	**Simple**	**p-level**	**Partial***	**p-level**	**Simple**	**p-level**	**Partial***	**p-level**
Basic	0.20	0.01	–0.02	0.64	0.27	0.01	0.20	0.01	0.20	0.01	0.04	0.17
Balanced	0.22	0.01	0.05	0.17	0.19	0.01	0.12	0.01	0.18	0.01	0.05	0.15
Optimal	0.22	0.01	–0.01	0.57	0.30	0.01	0.24	0.01	0.21	0.01	0.04	0.14
Pbhclust	0.19	0.01	–0.02	0.65	0.27	0.01	0.21	0.01	0.14	0.03	–0.02	0.62
PBmaxvar	0.18	0.002	–0.03	0.70	0.24	0.01	0.16	0.01	0.20	0.01	0.05	0.20
PBangprox	0.25	0.01	0.04	0.21	0.25	0.01	0.17	0.01	0.13	0.01	–0.04	0.76

Notes: * – a partial Mantel statistic is calculated between the distance matrix obtained after soil aggregate fraction composition on the different basis and distance matrix in the space of the physical properties, phytoindication scales and distance from the stems controlling for the effect of geographic distance matrix.

(Legendre, Fortin, 1989). But the spatial structure in the soil aggregate fraction data is partly determined by the spatial gradient in the phytoindication scales and partly by other factors not explicitly identified in the study as the Mantel statistic controlling for the effect of space

T a b l e. 3. Spearman correlation between soil aggregate fraction and variables after log ratios transformation (presenting statistically significant correlation coefficients with $p < 0.05$).

Aggregate fraction, mm	Isometric log ratios variables							
	ILR_1	ILR_2	ILR_3	ILR_4	ILR_5	ILR_6	ILR_7	ILR_8
> 10	−0.78	0.87	0.84	0.78	0.76	0.71	0.70	0.62
7–10	–	0.65	0.65	0.75	0.77	0.72	0.62	0.48
5–7	–	–	0.40	0.40	0.34	0.45	0.29	0.26
3–5	–	0.46	0.28	0.65	0.62	0.45	0.38	0.35
2–3	–	0.19	0.21	–	0.21	0.28	0.31	0.19
1–2	–	–	–	−0.21	−0.40	–	–	–
0.5–1	–	–	−0.32	−0.33	−0.32	−0.55	–	–
0.25–0.5	0.19	−0.52	−0.61	−0.54	−0.56	−0.70	−0.88	−0.65
<0.25	–	−0.28	−0.40	−0.34	−0.31	−0.45	−0.64	−0.87

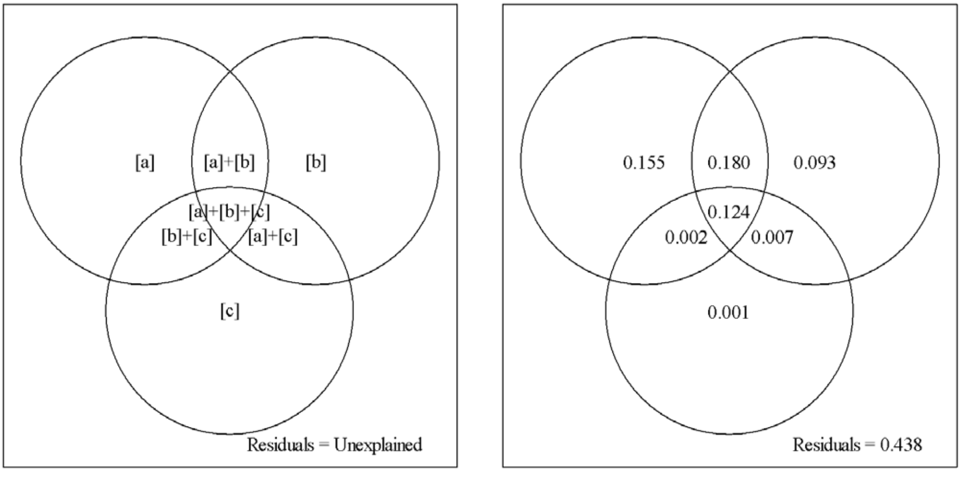

Fig. 3. Variance partitioning between spatial and soil explanatory variables.
Notes: [a] – variation explained solely by soil variables; [b] – variation captured by spatial (dbMEM) variables corresponds to pure space (residual spatial component); [c] – variation captured by distances from tree stems; [a]+[b] – variation explained both by spatial and soil variables; [a]+[c] – variation explained both by spatial variables and distances from tree stems; [b]+[c] – variation explained both by soil variables and distances from tree stems; [a]+[b]+[c] – variation explained by spatial, soil variables and distances from tree stems. All the variance fractions shown are significant ($p < 0.001$).

is significantly different from zero. The maximal Mantel test was obtained after optimal basis of the log ratios' transformation of the soil aggregate fraction data. The Spearman correlation matrix between soil aggregate fraction and variables after log ratio transformation allows for better interpretation of the ILR-variables (Table 3).

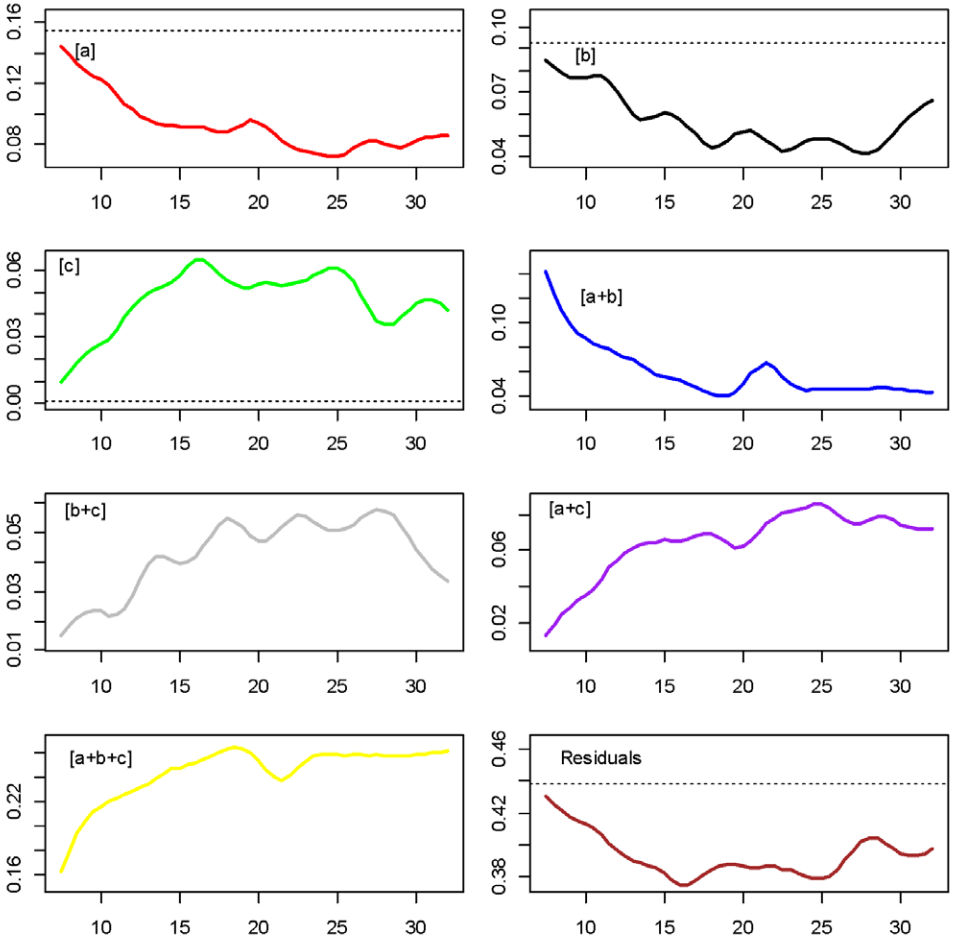

Fig. 4. Dependence of variation in herb-layer community composition from the bandwidth in evaluating the overstory density. Dotted line shows variation explained with tree-distance dbMEM variables. X-axis is a bandwidth, y-axis is variation explained by explanatory tables.
Notes: [a] – variation explained solely by soil variables; [b] – variation captured by spatial (dbMEM) variables corresponds to pure space (residual spatial component); [c] – variation captured by trees density; [a]+[b] – variation explained both by spatial and soil variables; [a]+[c] – variation explained both by spatial variables and trees density; [b]+[c] – variation explained both by soil variables and trees density; [a]+[b]+[c] – variation explained by spatial, soil variables and trees density.

The model of RDA including all soil variables was significant ($R^2_{adj} = 0.47$, $F = 3.84$, $p < 0.001$). The forward selection procedure allowed us to select 19 soil variables, which explain 46.1% of the variability of the community ($F = 5.94$, $p < 0.001$). The list of the important soil variables includes soil mechanical impedance (at the depth 0–5, 30–35, 55–60, 60–65, 65–70,

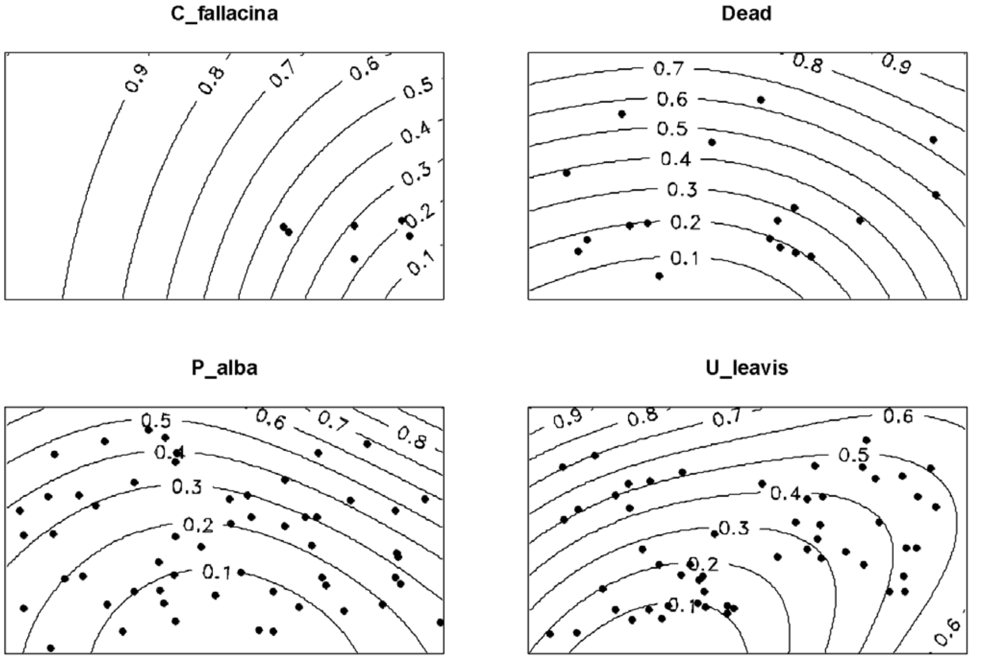

Fig. 5. Distance based on the density of the tree spatial distribution with bandwidth is equal to 17. The points show tree location. Distance is normalized to diapason 0–1.

75–80, 80–85, 85–90, and 90–95 cm), electrical conductivity, litter thickness, soil temperature, moisture, density and isometric log ratios variables (2, 3, 4, 6, 7).

There are 48 dbMEMs-spatial variables with soil variables as covariates, which together explain 41.1% of the plant community variability. The forward selection procedure allowed us to select 20 variables, which explain 40.3% of the variability of the community ($F = 11.04$, $p < 0.001$).

The model of RDA including all dbMEMs-tree distance variables with soil variables as covariates was significant ($R^2_{adj} = 0.15$, $F = 1.49$, $p < 0.001$). The forward selection procedure allowed us to select 1 variable, which explains 0.7% of the variability of the community ($F = 2.29$, $p < 0.001$).

The unexplained variation accounts for 43.8% (Fig. 3). The variation explained solely by soil variables is equal to 15.5%, while the variation explained both by spatial and soil variables is 18.0%.

The measure of the overstorey spatial structure, which is based on the evaluation of its density, enables us to obtain different estimations depending on the bandwidth. The bandwidth affects the explanatory capacity of the tree stand (Fig. 4). The lowest level of unexplained community variance obtained for bandwidth is equal to 17. The assessment of tree stand density was obtained with this bandwidth (Fig. 5). The model of RDA including all

T a b l e 4. Variation in plant community structure explained by the models with soil variables.

Variable	Marginal effect R^2_{adj}	*p*-value	Spatial partial effect R^2_{adj}	*p*-value	Tree partial effect R^2_{adj}	*p*-value	Tree density partial effect R^2_{adj}	*p*-value
Soil mechanical impedance at depth, MPa								
0–5 cm	0.030	0.001	0.007	0.121	0.012	0.038	–0.009	0.883
30–35 cm	–0.001	0.487	–0.002	0.616	0.010	0.045	0.039	0.024
55–60 cm	–0.002	0.607	0.010	0.045	–0.002	0.617	0.006	0.206
60–65 cm	0.018	0.014	0.003	0.239	0.027	0.004	–0.009	0.873
65–70 cm	0.009	0.061	0.009	0.069	0.001	0.366	0.051	0.012
75–80 cm	0.048	0.001	0.006	0.117	0.036	0.001	–0.010	0.974
80–85 cm	0.037	0.001	0.001	0.350	0.039	0.001	0.087	0.001
85–90 cm	0.028	0.001	–0.006	0.920	0.020	0.005	–0.003	0.406
90–95 cm	0.017	0.011	–0.005	0.853	0.023	0.004	–0.002	0.375
Other edaphic parameters								
EC	0.009	0.064	0.016	0.015	0.027	0.001	0.001	0.302
Litter	0.057	0.001	–0.003	0.657	0.041	0.001	–0.005	0.483
Temp	0.000	0.420	0.004	0.199	0.006	0.111	0.052	0.011
Moisture	0.025	0.004	0.016	0.012	0.033	0.001	–0.009	0.883
Density	0.015	0.025	0.004	0.179	0.033	0.001	0.039	0.024
Isometric log ratios variables								
ILR_2	0.013	0.023	0.008	0.067	0.027	0.001	0.006	0.206
ILR_3	0.005	0.130	0.003	0.241	0.024	0.003	–0.009	0.873
ILR_4	0.018	0.009	0.004	0.193	0.030	0.001	0.051	0.012
ILR_6	0.010	0.053	–0.002	0.596	0.031	0.001	–0.010	0.974
ILR_7	0.000	0.388	0.002	0.303	0.025	0.004	0.087	0.001

Notes: Marginal effect represents variation explained by a given variable without the effect of other variables in the model. *P*-value accounting for autocorrelation is a significance of soil model with selected eigenvector-based spatial variables (dbMEMs) as covariables; EC – Electrical conductivity, dSm/м; Litter – Litter depth, cm; Temp – soil temperature, °C; Wetness – moisture of soil, %; Density – soil density, g/cm^3.

dbMEMs-tree density distance variables was significant (R^2_{adj} = 0.46, F = 1.48, p < 0.001). The forward selection procedure allowed us to select 16 variables, which explain 11.8% of the variability of the community (F = 2.49, p < 0.001).

After accounting for spatial dependence in the model, considerable shifts in significance were detected for marginal effects of some variables (Table. 4). Variation explained by such variables as soil mechanical impedance at the depth 0–5, 60–65, 75–80, 80–85, 85–90, and 90–95 cm, litter thickness, soil density, ILR 4, and ILR 6 is spatially structured, meaning that there is an decrease in variation explained by these soil factors after including spatial variables as covariates in the models. Variation explained by some soil variables is structured by the influence of trees. Tree density creates a much stronger effect on the plant community than variables derived from distance from the tree stems. This became evident after a decrease in the variation explained by the larger number of soil variables, if one takes into account the effect of the dbMEMs-tree density variables than if one takes into account the effect of the dbMEMs-tree distance variables.

The majority of both the spatially structured and tree-distance structured variation in plant community composition was broad-scaled (captured by dbMEMs with lowest numbers) (Fig. 6). The soil models and pure spatial effect were able to account for mainly broad-to mesoscale variation.

We found that there is a range of values of bandwidth in which a higher level of explanation of the herbaceous layer community structure can be achieved than in the case of the use of distance to the nearest tree. The best explanation of the herbaceous layer community is due to the highest explanatory ability of the overstorey by means of density-dependent measure than tree-distance measure. Increasing the bandwidth up to the optimum value is accompanied by an increase in the number of density-dependent dbMEMs-variables (Fig. 7).

The significant relationship was found between soil induced community structure and phytoindication values of the soil acidity, carbonate content and aeration regime (Table 5). The significant rela-

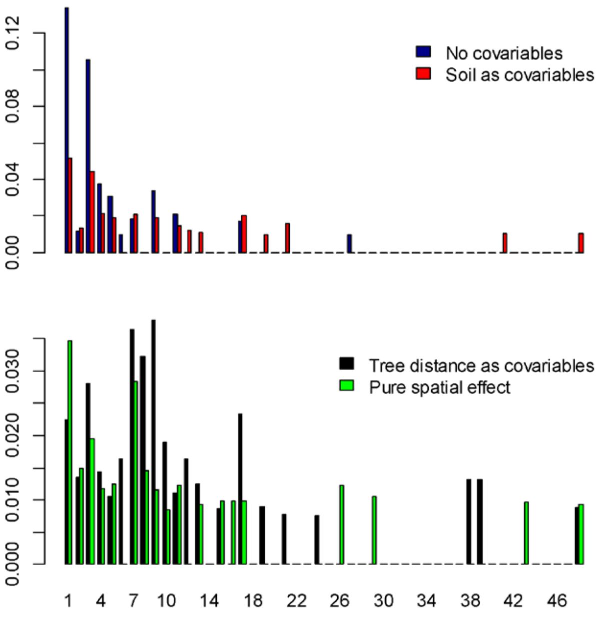

Fig. 6. Scalograms illustrating the scaling of spatially structured variation in community data (white bars) and residuals of the environmental models (black bars). The value of R^2_{adj} is the variation explained by individual dbMEM variables. The dbMEMs are ordered decreasingly according to the scale of spatial patterns they represent (dbMEM 1 represents the broadest scale, dbMEM 48 the finest scale).

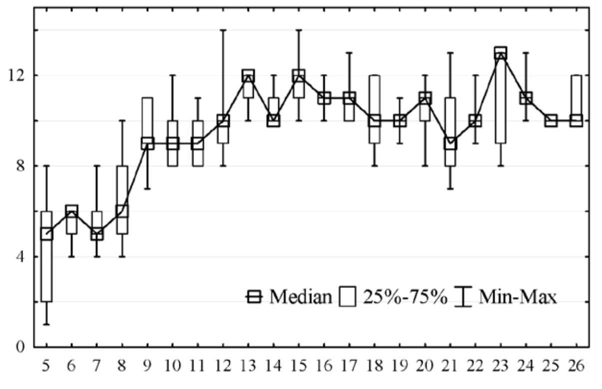

Fig. 7. Dependence of the number of the density-dependent dbMEMs-variables with a significant relationship to the community composition obtained after forward selection procedure from the bandwidth for tree density assessment.

T a b l e 5. Significance of regression between subplot-based phytoindicator scale values and soil effects in the species data (first two RDA axes with soil predictors as explanatory variables), pure space (first two RDA axes with spatial predictors as explanatory variables and selected soil and tree-distance variables as covariables), tree distance effects (first RDA with tree distance predictors as explanatory variables and selected soil and spatial variables as covariables), and tree-distance variables as covariables), tree density effects (first RDA with tree density predictors as explanatory variables and selected soil and spatial variables as covariables).

Phytoindicator scale*	**Soil effect R^2_{adj}**	***p*-value**	**Spatial partial effect R^2_{adj}**	***p*-value**	**Tree partial effect R^2_{adj}**	***p*-value**	**Tree density partial effect R^2_{adj}**	***p*-value**
Hd	-0.01	0.55	0.00	0.34	-0.01	0.88	-0.01	0.90
fH	0.01	0.17	0.00	0.40	0.04	0.02	0.04	0.02
Rc	0.06	0.01	0.00	0.48	0.01	0.21	0.00	0.38
Sl	-0.01	0.58	-0.01	0.96	-0.01	0.87	-0.01	0.79
Ca	0.06	0.01	0.05	0.01	0.05	0.01	0.09	0.00
Nt	0.00	0.25	0.01	0.21	-0.01	0.97	-0.01	0.80
Ae	0.04	0.02	0.02	0.07	0.09	0.00	0.08	0.00
Tm	0.01	0.14	0.00	0.27	0.00	0.41	-0.01	0.52
Om	-0.01	0.87	0.01	0.22	0.00	0.37	0.00	0.32
Kn	-0.01	0.66	0.02	0.08	0.00	0.30	0.00	0.46
Cr	-0.01	0.51	-0.01	0.93	0.00	0.48	-0.01	0.66
Lc	0.00	0.45	0.02	0.10	0.05	0.01	0.05	0.01

Notes: * Hd – soil water regime; fH – variability of damping; Rc – soil acidity; Sl – total salt regime; Ca – carbonate content in soil; Nt – nitrogen content in soil; Ae – soil aeration; Tm – thermal climate (thermoregime); Om – humidity; Kn – continentality of climate; Cr – cryo-climate; Lc – light.

tionship was found between the pure spatial component of the community variation and carbonate content. Tree distance and density effects are able to explain variability of damping, carbonate content, aeration and light regime.

Discussion

The small-scale variation of herb-layer community structure is influenced by the soil properties, structural features of the overstorey and neutral processes. The spatial factors may be considered as the markers of the neutral processes (Cottenie, 2005). The difficulty is that the variability of soil properties and effects of the overstorey also have a spatial component of its variation. There are reciprocal relationships between various components in the soil properties (Paluch, Gruba, 2012). Sunlight penetration through the canopy is directly related to the spatial pattern of the herbaceous layer (Blank, Carmel, 2012). The variability of the light caused by the stand density is often seen as the leading environmental regime, which determines the interactions between organisms and their physical environments (Stohlgren et al., 2000). This explains the widespread use of measuring light conditions under the canopy for assessment of the influence of the overstorey on the herbaceous layer (Chudomelova et al., 2017). It should be noted that the nature of influence of trees on herbaceous plants is much more complicated and involves the impact of trees on the habitat by modulating the availability of resources to the other species (Jones et al., 1994). Trees are capable of modifying the properties of the soil in their vicinity (Binkley, Giardina, 1998). The pattern of soil properties under single forest trees is generally developed with radial symmetry to the tree, varying with distance from the tree trunk (Zinke, 1962). Tree species have a significant impact on humus characteristics, which significantly explain the distribution of forest understorey species (Oijen et al., 2005). The nearest distance to the tree species can be considered as a very apparent measure of influence on the herbaceous plants. The arrangement of different tree species can significantly complicate the structure of ecological space. The distance of the nearest tree is an easy and obvious measure of the impact of the overstorey, both on the herbaceous layer and soil. But it does not take into account the role of the relative positions of the trees of the same species. Tree density is sensitive to the number of trees of the same species per square unit. Evaluation of density can be measured with different bandwidths, which provides another opportunity for the modeling of scale-dependent effects.

We proposed that the dbMEMs-approach can be a useful tool for modeling the spatial effects of the overstorey. The coordinates of sampling locations used to generate a set of orthogonal eigenvector-based spatial variables (dbMEMs) may be calculated in the space of the distances to the nearest tree species or in the space opposite to tree densities. The value inverse to density can be considered as the distance from or between clusters of trees of the same species. This distance can also be used to calculate the dbMEMs variables. It must be noted that the dbMEMs approach is usually applied for modeling the geographical patterns within the extent of the sampling area (Borcard, Legendre, 2002) or time patterns from the beginning to the end of the sampling period (Legendre, Gauthier, 2014). The trees constitute a specifically structured space, in which a gradient of the environmental properties is formed in relation to the distance from the tree trunk. In this case, the extent is defined by the distance from the tree trunk to the sampling location farthest from the trunk of the tree. In the process of modeling, the extent may be varied, but the reference point is a single tree (in the case of estimation of distances) or the point with the highest density of the stand (in the case of density estimation). Spatial patterns in the specified extent can be modeled using the dbMEMs-variables.

If variables derived from tree distances are considered as the reference for comparison, it can be concluded that the change of the bandwidth for computing a kernel smoothed intensity function from a trees' point pattern can significantly increase the explanatory ability in general of the model based on the dbMEMs-tree density dependent variables. Using dbMEMs-tree density dependent variables calculated with bandwidth is 17, which gives the possibility to reduce the unexplained component of the community variation from 43.8 to 37.5%. The increase of the explanatory ability of the model is mainly due to the dbMEMs-tree density dependent variables. A considerable part of the variation explained by soil variables is tree structured, meaning that an increase in variation explained by dbMEMs-tree density dependent variables decreases the variation explained purely by spatial variables. It is clear that the overstorey is important for spatial variability of the soil properties. This conclusion is also confirmed by the fact that the bandwidth increase of up to an optimal level leads to higher importance of the variation explained by spatial variables and shared with tree density and both by soil variables and shares with tree density.

The spatial heterogeneity of soil is mainly driven by the distribution of tree patches (Andivia et al., 2015). Our results revealed that the pure spatial component and the spatially structured soil properties are the most important factors that affect the herbaceous community. In turn, overstorey structure is more effective than pure effect of the soil properties. Soil variables are most important in explaining both broad-scaled and fine-scaled compositional patterns. In other studies, it was shown that environmental variables mostly explain broad-scaled compositional patterns (Laliberte et al., 2009; Legendre et al., 2009; Gazol, Ibanez, 2010; Chang et al., 2013; Chudomelova et al., 2017). We can explain this result by the ability of soil physical properties to form fine-scale patterns of an endogenous nature. Also, fine-scale morphological soil structure may be formed under the influence of trees. Morphological structure of soil on the fine-scale level is referred to as soil ecomorphs (Zhukov, Zadorozhnaya, 2016; Zhukov et al., 2018). These soil patterns influence the herb layer stratum. We found that understory species composition was best explained by the soil mechanical impedance, litter depth, soil temperature and soil moisture, and soil aggregate structure. It is also necessary to note the role of overstorey in the variation of soil properties. Mechanical impedance of the soil is a very important and ecologically relevant soil property, which is considered as a factor influencing the living conditions of plants (Zhukov, Gadorozhnaya, 2016; Zadorozhnaya et al., 2018). The general pattern of tree induced variability of soil properties is due to the difference between the effect of litter and the adjacent opening or neighboring tree (Zinke, 1962). The litter thickness is an important factor influencing the soil temperature (MacKinney, 1929) and can reduce the effects of soil temperature extremes and moderate minimum and maximum temperature values (Fekete et al., 2016). The soil moisture is positively dependent on forest litter thickness (Xing et al., 2018). On the other hand, soil moisture considerably effects leaf litter decomposition. In wetter conditions, a higher level of litter decomposition occurs in general (Yoon et al., 2014). Soil aggregate structure is critical to plant growth (Barthes, Rose, 2002; Canton et al., 2009). But the reverse is also true: vegetation affects soil structure at different scales and through a wide variety of mechanisms (Angers, Caron, 1998).

Conclusion

The local variation in the studied herb layer community was best explained by soil mechanical impedance (at the depth 0–5, 30–35, 55–60, 60–65, 65–70, 75–80, 80–85, 85–90, and 90–95 cm), soil electrical conductivity, litter thickness, soil temperature, wetness, density, and aggregate structure (isometric log ratios variables 2, 3, 4, 6, 7). A considerable part of the pant community variation explained by soil factors was spatially structured. The orthogonal eigenvector-based spatial variables (dbMEMs) approach can be extended to quantifying the effect of forest structures on the herb layer community. The measurement of the overstorey spatial structure, which is based on the evaluation of its density, was very useful to explain the variation of the herb layer community.

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MORPHOLOGICAL CHARACTERISTICS AND VARIATION OF WOOD, CONE AND SEED PRODUCTIONS IN THE REFORESTATION OF ALEPPO PINE IN NORTHEASTERN TUNISIA USING TERRESTRIAL AND SPATIAL INDEX APPROACHES

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Abstract

El Guemri R., Jaouadi W., Mechergui K., Alsubeie M., Naghmouchi S., El Ouellani S., Khouja M.L.: Morphological characteristics and variation of wood, cone and seed productions in the reforestation of Aleppo pine in Northeastern Tunisia using terrestrial and spatial index approaches. Ekológia (Bratislava), Vol. 38, No. 3, p. 273–291, 2019.

For several decades, forest management policies have encouraged land cover changes, with the establishment of tree cover such as Aleppo pine in natural or degraded ecosystems for soil protection and the firewood production. In order to investigate the importance of Aleppo pine trees in the ecosystem, this study was conducted in the reforestation of the Aleppo pine of Northeastern Tunisia. The production of wood, cones and seeds of Aleppo pine in the 6 date reforestation in Northeastern Tunisia were surveyed using 6 plots (20 tree/plot) spread over 6 different location. Our hypothesis was to determine the characteristics that can be decisive in estimating the production of Aleppo pine in reforestation in Tunisia using terrestrial and spatial measurements including (i) age, (ii) dendrometric characteristics, (iii) silvicultural treatments, (iv) stationary data and/or (v) remote sensing parameter (NDVI: Normalized Difference Vegetation Index). We found that the Aleppo pine in Northeastern Tunisia and at young age trees (14–35 years) were more productive than the regeneration or reforestation stand, either young or old, in Northwest of Tunisia. Wood, cone and seed productions were significantly different amongst the plots ($p < 0.05$). The NDVI was positively correlated with the production of wood, cones and seeds. Stand age, exposure and longitude were also positively correlated with the production. However, longitude and altitude were negatively correlated with the production, showing a negative effect on the morphological traits of trees and, subsequently, their growth. The regression analysis indicates that NDVI and age were the most determinant factors of seed production. This research suggests that reforestation planning, particularly the choice of altitude and latitude, may result in improved tree morphology that may increase Aleppo pine wood seed and cone crops.

Key words: reforestation stand, Aleppo pine, age, NDVI, cone and seed productions.

Introduction

The Aleppo pine (*Pinus halepensis* Mill.) trees are one of the most important trees in Mediterranean forests, with the largest ecological amplitude (Nahal, 1962; Pardé, 1957; Quezel, 1986; Mezali, 2003). The maximum development of this species is between 350 and 700 mm of annual precipitation (Quezel, 1980). The Aleppo pine is a native species of the Mediterranean zone, extending from Morocco to Spain via Lebanon, Syria and Jordan to the east (Fady et al., 2003). In Tunisia, the Aleppo pine occupies about 297,000 ha, around 56% of the total forest area (Sghaier, 2005), ranging from the upper arid to the subhumid Mediterranean bioclimate (El Hamrouni, 1978). It has great economic, ecological and social values in Tunisia (Khouja, 1997). Around 1 million Tunisians (i.e. 10% of the country's population) live in forest areas and benefit from its resources (Gader, 1999). The Aleppo pine forests play a crucial role in the reforestation of degraded environments and soil fixation, as well as the national economy and the human well-being (Ayari et al., 2012a). The Aleppo pine trees have multiple and diversified uses such as the exploitation of wood, tannin and seeds (Ayari et al., 2011a). The seeds of the Aleppo pine called 'zgougou' in Tunisia are considered amongst the main socio-economic non-timber forest products by the regional and local forestry services. In addition to their direct consumption by the population as pastry cream called in Tunisia 'Assida', there are several products based on the seeds of Aleppo pine, which are available on the market including the yoghurt with the aroma of 'zgougou', the aroma ice cream of 'zgougou', vegetable oil and so on (El Guemri, 2018). The cones of *P. halepensis* are also of great interest for decoration or extraction of seeds and their use in agrolimitation or extraction of essential oils and their medicinal and aromatic uses (Sghaier et al., 1997; Nasri et al., 2004; Khouja et al., 2006; Sghaier, Ammari, 2012; Ayari et al., 2010). The forests of the Aleppo pine have always been a staple food for the local population of forest areas in the recent decades and for the entire population of Tunisia (Ayari et al., 2012b,c). The annual exploited forest area ranges from 60,000 to 100,000 ha (DGF, 2010). The annual production of Aleppo pine seeds varies between 300 and 320 tons. Kasserine governorate is the main region producing Aleppo pines, with a production of 100–120 tons/year (DGF, 2010). To deal with the problems threatening the forests of the disappearing Aleppo pine and to increase their production of wood and seeds, it is necessary to adopt thoughtful management and planning techniques and policies to allow a better valorisation of the forest resources preserving their durability. The development of such techniques must imperatively be based on the knowledge of the potential for growth and production of the forest in relation to environmental conditions. Thus, the development of management and management models for the different stands of

Aleppo pine forests is currently one of the top priorities of foresters and forest managers. In this vein, the objective of our study is to determine the production of Aleppo pine in wood, cones and seeds in the different age categories and to look for the effect of station factors on the productivity of the stands as well as to rely on remote sensing in estimating forest production through the NDVI spatial index.

Material and methods

Study area

The present work was carried out in plantations of the Aleppo pine forest stand located in ' Melloul' and in ' Dar Chichou' forests in the Kelibia region of Nabeul governorate in northeastern Tunisia (Fig. 1). The forest of Melloul covers an area of 500 ha, of which 60 ha of Aleppo pine trees are planted. The plantation of the Aleppo pine in the forest of ' Dar Chichou' is in the order of 1,000 ha. The forest area under study has a Mediterranean type climate, belonging to the higher subhumid bioclimatic stage with warm winters. The annual average rainfall recorded in the area is around 500 mm. Rain in this area is characterised by a large irregularity on an annual scale. The average annual temperature in the study area varied from 17 to 18.5 °C. Autumn and winter are the driest seasons. January is the coldest month and August is the hottest month. The prevailing winds are from the northwestern sector; the mean wind speed is between 2.5 and 3.5 m/s, and the peaks can exceed 20 m/s.

Experimental field

We spotted 6 plantations of different ages of Aleppo pine in Melloul and Dar Chichou forests, and then we chose a representative sample of 20 trees in each plantation. Age, NDVI, geographic coordinates, elevation and plantation

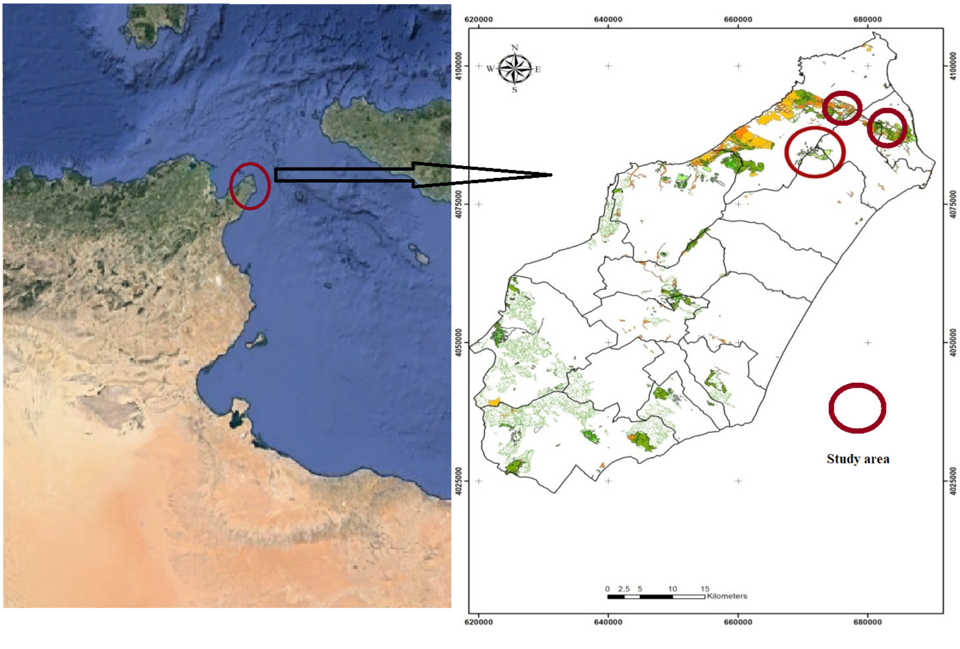


Fig. 1. Study area of reforestation of the Aleppo pine plot in northeastern Tunisia.

exposure were measured and presented in Table 1. We performed all the dendrometric measurements on the sampled trees, that is, diameter at breast height (DBH; 1.3 m), total tree height, tree pruning height and crown diameter in both north–south and east–west directions. To calculate the volume of the wooden tree, we used the formula of Matziris (2000). To estimate cone and seed productions, we performed a full harvest of all cones on the trees and we weighed the cones from each tree (i.e. measuring the height and width of the harvested cones). All measured cones are grouped into batches for each tree in craft paper packs. The packs bearing the numbers of the trees were placed in a heating oven set at 60 °C to trigger the opening of the cones. We recovered the seeds after opening the cones in the oven and for each tree we measured the weight of the seeds. Finally, we estimated the yields of cones and seeds per hectare.

Statistical analyses

Analysis of variance was calculated to determine associations between the measured traits using R package software (Oksanen et al., 2008). Principal components analysis with barycenter projection was made using measured data with SPSS program (IBM, 2009). Plot of circle correlation, principal components analysis and cluster analysis were conducted on the Euclidean distance matrix, and the different relationships amongst the parameters was made using measured data with the R program software. In order to find the main variation trends amongst the dendrometric parameters, the productive characteristics of the trees and the characteristics of the reforestation station and to evaluate their correlation, data were processed according to Pearson correlation coefficients with R package software. The histogram of the Pearson's correlation coefficients is presented within the colour key using R package software. The model equation models and Akaike information criterion (AIC) were used to study the relationship between the seed production and the NDVI, and the relationship between the seed production and the age of the Aleppo pine stand in reforestation has been realised by the past software (3.2).

Results

The characteristics of 6 reforested plots with 5 different dates, ranging in age from 14 to 60 years in our study are shown in Table 1. The altitude of the sampled plots varied from 50 to 210 m. Six different exposures were chosen to evaluate the effect of the exposures on the dendrometric parameters and the production of the Aleppo pine. The NDVI spatial index was determined for each plot by the Envi remote sensing software. Twenty trees from each plot were measured and harvested to estimate cone and seed productions. Analysis of the variance (Table 2) showed that the dendrometric parameters significantly varied amongst the plots of different ages of reforestation ($p < 0.001$). Statistical analysis showed that the morphological measurements positively evolved with the age of the trees, but cone production was more important in young aged trees (an average production of 576 cones per tree was re-

T a b l e 1. Characteristics of the study stations of the reforested stand of the Aleppo pine.

Plots	Age (years)	Altitude	Exposure	Latitude	Longitude	NDVI of plantation: Normalized Difference Vegetation Index	Number of trees studied
Plot 1	14	197	N	36.894	10.95	0.376	20
Plot 2	17	210	N-E	36.893	10.95	0.391	20
Plot 3	21	121	N-O	36.985	10.96	0.341	20
Plot 4	35	73	S	36.956	11.07	0.468	20
Plot 5	45	60	S-E	36.982	11.07	0.536	20
Plot 6	60	50	S-O	36.915	11.08	0.546	20

T a b l e 2. Analysis of variance and multiple comparison of means (Tukey test) of dendrometric characteristics, silvicultural operation and cone morphology of trees in the study stations of the reforested stand of the Aleppo pine (mean ± standard deviation).

Dendrometric Parameters of tree	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**F value**	**Pr (>F)**
DBH: diameter at 1.3 m (cm)	13.623 ± 2.492 (ab)	13.495 ± 2.222 (a)	12.781 ± 2.470 (a)	17.156 ± 2.952 (b)	24.191 ± 3.838 (c)	32.460 ± 6.408 (d)	85.43	<2e-16 ***
Dcorw: diameter of tree crown (m)	5.222 ± 1.036 (a)	5.393 ± 0.938 (a)	5.579 ± 0.890 (a)	5.761 ± 0.512 (b)	7.081 ± 0.830 (b)	8.589 ± 1.410 (c)	35.28	<2e-16 ***
Heig: height of tree (m)	3.916 ± 0.650 (b)	3.208 ± 0.493 (ab)	2.610 ± 0.338 (a)	5.600 ± 0.804 (c)	6.656 ± 0.904 (d)	8.001 ± 1.161 (e)	141.3	<2e-16 ***
NCt: number of cones per tree	405 ± 201.552 (ab)	567.100 ± 205.140 (bc)	304.684 ± 150.471 (a)	381.900 ± 148.778 (a)	677.650 ± 171.554 (c)	900.700 ± 164.576 (d)	31.8	<2e-16 ***
SCrt: area of tree crown (m^2)	22.193 ± 8.422 (a)	23.491 ± 7.881 (a)	25.024 ± 7.922 (a)	26.256 ± 4.739 (a)	39.876 ± 9.358 (b)	59.395 ± 18.224 (c)	37.93	<2e-16 ***
STt: basal area of tree (m^2)	0.015 ± 0.005 (a)	0.014 ± 0.004 (a)	0.013 ± 0.005 (a)	0.023 ± 0.008 (a)	0.047 ± 0.014 (b)	0.085 ± 0.031 (c)	68.67	<2e-16 ***
Silvicultural operation	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**F value**	**Pr (>F)**
ThHe: thinning height (m)	1.632 ± 0.213 (c)	1.363 ± 0.239 (b)	1.101 ± 0.285 (a)	2.128 ± 0.193 (d)	2.306 ± 0.225 (d)	2.655 ± 0.328 (e)	108.1	<2e-16 ***
Cones morphology	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**F value**	**Pr (>F)**
WidC: width of cones (cm)	2.540 ± 0.206 (a)	2.415 ± 0.146 (a)	2.360 ± 0.444 (a)	3.070 ± 0.539 (b)	3.397 ± 0.338 (b)	4.301 ± 0.889 (c)	43.32	<2e-16 ***
lenC: length of cones (cm)	5.666 ± 0.519 (ab)	5.030 ± 0.584 (a)	5.094 ± 1.057 (a)	5.102 ± 0.487 (a)	5.186 ± 0.603 (a)	6.230 ± 0.718 (b)	9.184	000***

Notes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

T a b l e 3. Analysis of variance and multiple comparison of means (Tukey test) of tree and site productive parameters in the reforested stand of the Aleppo pine (mean ± standard deviation).

Stem volume and cone and seed productions	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**F value**	**Pr (>F)**
SVt: stem volume of tree (m^3)	0.023 ± 0.014 (a)	0.017 ± 0.005 (a)	0.012 ± 0.007 (a)	0.059 ± 0.030 (a)	0.141 ± 0.056 (b)	0.329 ± 0.154 (a)	59.55	<2e-16 ***
WCtKg: weight of cones per tree in kg	6.497 ± 2.393 (b)	5.869 ± 2.366 (b)	3.212 ± 1.794 (a)	3.652 ± 1.108 (a)	5.688 ± 1.840 (b)	7.224 ± 1.552 (b)	13.83	1.94e-10 ***
WStg: weight of seeds per tree in g	179.709 ± 131.287 (ab)	301.929 ± 119.486 (b)	97.070 ± 62.113 (a)	287.700 ± 128.033 (b)	551.9 ± 161.088 (c)	740.4500 ± 124.658 (d)	71.83	<2e-16 ***
Cone and seed productions per ha	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6		
Number of cones/ ha^{-1}								
Cone production kg/ha^{-1}	7,218.167	6,520.459	3,568.532	4,057.372	6,319.368	8,025.864		
Seed production kg/ha^{-1}	199.660	335.443	107.850	319.650	613.190	822.640		
Percentage of production (seed/ cone)	2.77	5.15	3.02	7.88	9.71	10.25		

Notes: 0 ‚***' 0.001 ‚**' 0.01 ‚*' 0.05 ‚.' 0.1 ‚ ' 1.

corded in plot 2, which is 17 years old; 405 cones per tree in plot 1, which is 14 years old; 304 cones in plot 3, which 21 years; 381 cones per tree in plot 4, which is 35 years old; 677 cones per tree in plot 5, which is 45 years old; and 900 cones per tree in plot 6, which is 60 years old). The production of cones was very important at the age of 60 years. The results showed that pruning on Aleppo pine trees varied significantly, depending on tree heights (Table 2). The length and average width of the cones also varied significantly between the plots, we found that at an age of 17, 21 and 35 years, the trees had similar cone lengths (from 5 to 6.2 cm). The analysis of the variance of the production of wood, cone and seed as well as the total production transformed into hectare were recorded in Table 3; this production varied significantly amongst plots of different ages. Wood production was low at early ages (14, 17, 21 and 35 years) but a little important at the age of 60 years. The production of cones at the age of 14 years (6.497 kg) was higher than that at the ages of 17, 21, 35 and 45 years. Seed production ranged from 97.070 g per tree at the age of 21 to 740.450 g per tree at the age of 60 years. We have had noticed that 17-year-old trees were more productive than 14- and 20-year-old trees. We converted the production to hectare and concluded that seed production was very important at the age of 60 years, but at a young age, 17-year-old trees had higher production than trees aged 14, 21 and 35 years. The correlation matrix (Fig. 2) of the different dendrometric, silvicultural, productive and spatial index (NDVI) parameters showed a strong positive correlation represented in blue and dark blue. The plot parameters, that is, longitude, exposure, NDVI and age were positively correlated with the different tree dendrometric parameters (between 0.2 and 0.9). Altitude and latitude were negatively correlated with dendrometric parameters and production of wood, cones and seeds. The results demonstrated that the production of Aleppo pine was influenced by the morphology of the tree. The principal component analy-

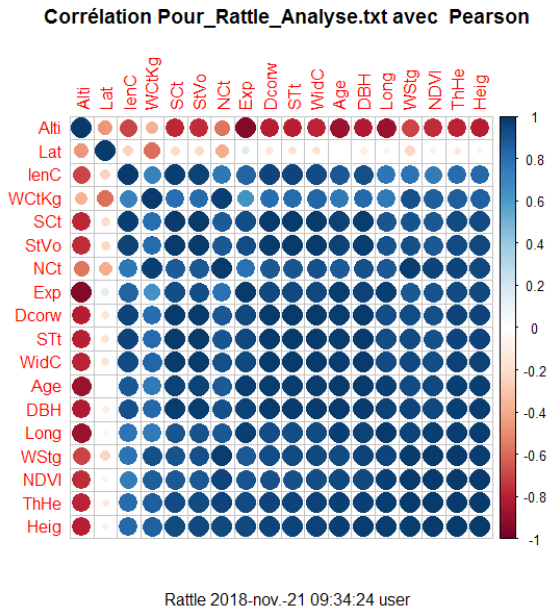


Fig. 2. Pearson's correlation coefficients of dendrometric characteristics, silvicultural operation, productive parameters and spatial index of trees and station variables (Alti, altitude; Lat, latitude; Long, longitude; Exp, exposure; Age, age; NDVI, normalized difference vegetation index; DBH, diameter at 1.3 m; Dcorw, diameter of tree crown; Heig, height of tree; NCt, number of cones per tree; SCrt, area of tree crown; STt, basal area of tree; SVt, stem volume of tree; ThHe, thinning height; lenC, length of cones; WidC, width of cones; WCtKg, cones weight per tree in kg; WStg, seed weight per tree in g). The histogram of the Pearson's (ρ) correlation coefficients is presented within the colour key.

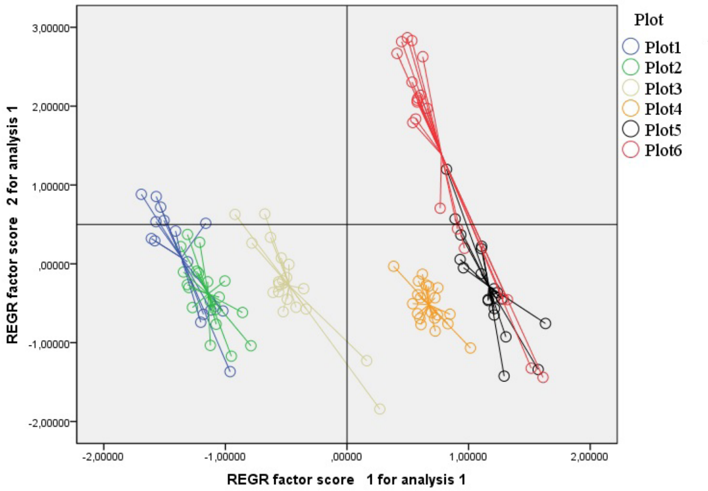


Fig. 3. Principal components analysis with barycenter projection of reforestation of six Aleppo pine plot based on dendrometric characteristics, silvicultural operation, productive parameters and spatial index of trees and station variables using the SPSS software.

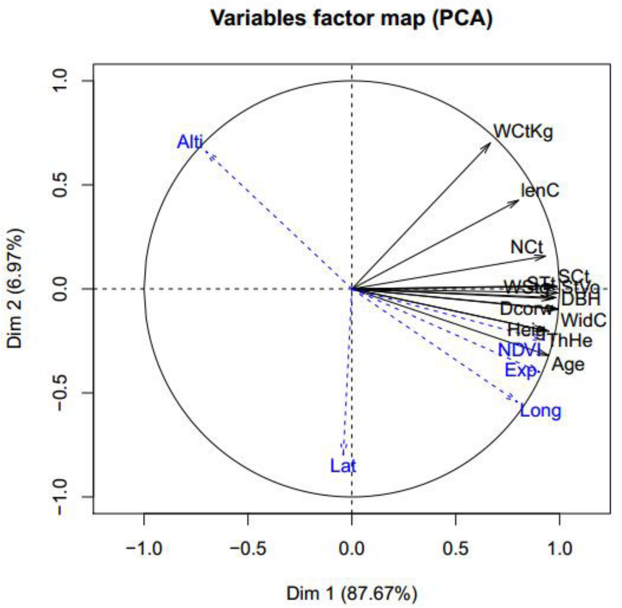


Fig. 4. Plot of correlation circle of reforestation of six Aleppo pine plot based on dendrometric characteristics, silvicultural operation, productive parameters and spatial index of trees and station variables using the R software (Alti, altitude; Lat, latitude; Long, longitude; Exp, exposure; Age, age; NDVI, normalised difference vegetation index; DBH, diameter at 1.3 m; Dcorw, diameter of tree crown; Heig, height of tree; NCt, number of cones per tree; SCrt, area of tree crown; STt, basal area of tree; SVt, stem volume of tree; ThHe, thinning height; lenC, length of cones; WidC, width of cones; WCtKg, weight of cones per tree in kg; WStg, weight of seeds per tree in g).

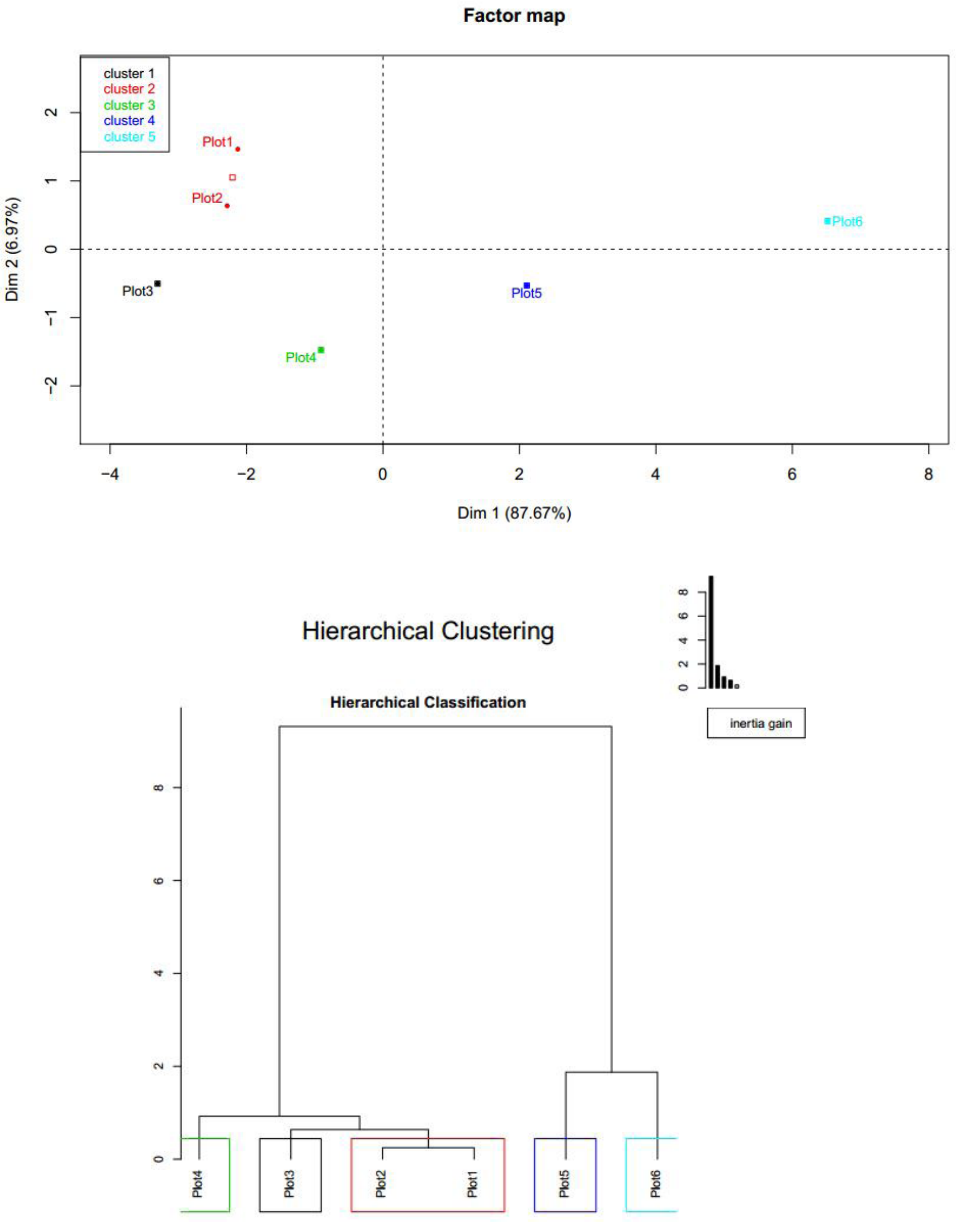


Fig. 5. Principal components analysis and Euclidean distance between the six Aleppo pine plot based on dendrometric characteristics, silvicultural operation, productive parameters and spatial index of trees and station variables using the R software.

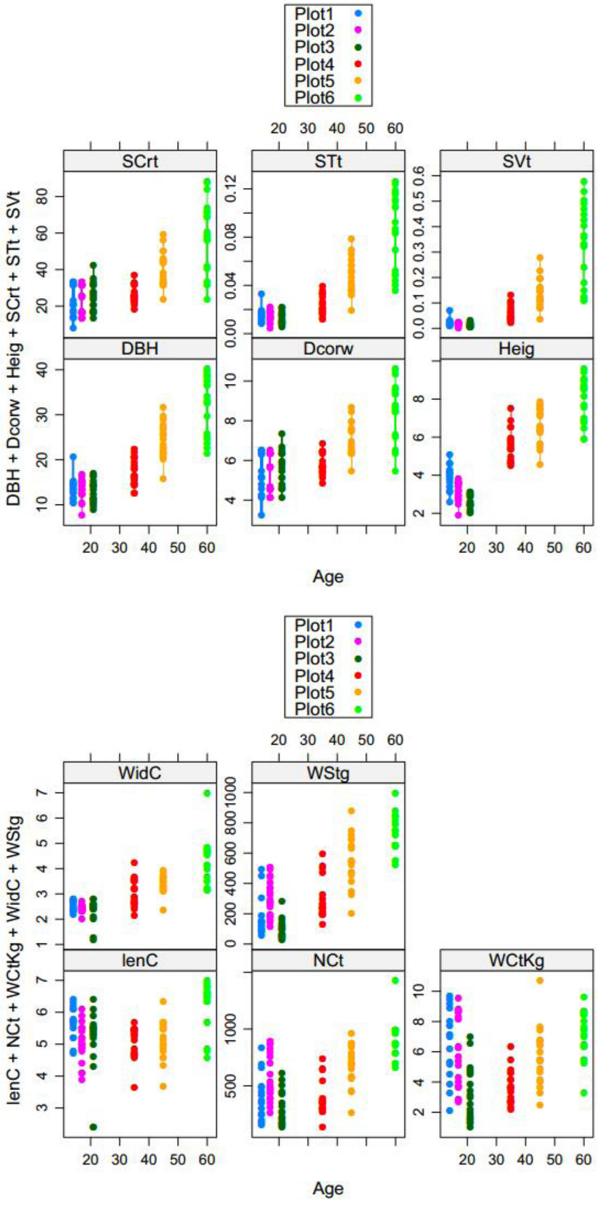


Fig. 6. Relationships between age and dendrometric characteristics, productive parameters of reforestation of six Aleppo pine plot (DBH, diameter at 1.3 m; Dcorw, diameter of tree crown; Heig, height of tree; NCt, number of cones per tree; SCrt, area of tree crown; STt, basal area of tree; SVt, stem volume of tree; lenC, length of cones; WidC, width of cones; WCtKg, weight of cones per tree in kg; WStg, weight of seeds per tree in g).

sis with the projection of the barycentre (Fig. 3) showed that plots 1 and 2 clustered together; however, plots 3 and 4 formed two distinct groups and plots 5 and 6 showed common characteristics. Figure 4 showed that altitude and latitude were not correlated with dendrometric and productive parameters in our study. However, longitude, NDVI, exposure and age were positively correlated with tree morphology and plot production. Our results also showed that the choice of plot exposure and longitude is decisive for the growth and production of Aleppo pine trees. The main component analysis and the plot classification (Fig. 5) showed two main clusters: the first cluster was formed by plots 1, 2, 3 and 4 and the second cluster was formed by plots 4 and 5. Cluster 1 is formed of three sub-classes: the first sub-class is formed by plot 4, the second subclass is formed by plot 3 and the third subclass is formed by plots 1 and 2, indicating that at a young age, the Aleppo pine had great variability in production and morphological characteristics explained by exposure and longitude. The relationship between age and various dendrometric and productive parameters are illustrated in Figure 6, showing that a 60-year-old adult production is maximal whilst at an age of 14, 17, 21 and 35 years, the Aleppo pine demonstrated variable production and different morphology. The relationship between NDVI and different dendrometric and productive parameters is illustrated in Figure 7 and showed that NDVI significantly explained the seed yield. Table 4 illustrates the models used to study the relationship between seed yield as a function of the NDVI spatial index and seed production as a function of reforestation age of the Aleppo pine stand. The most powerful model for estimating seed production according to the INDVI is that of Michaelis–Menten with Akaike IC = 1.9737 E06. The most powerful model for estimating seed production by age is the quadratic model with Akaike IC = 1.9737 E06. Figure 8 showed the shape of the curves of the relationships amongst NDVI, age and tree seed production. The most powerful model had weak Akaike information criterion (AIC). Here, a quadratic model was used and demonstrated a more powerful model for seed production according to the NDVI (AIC = 1.9737 E06). Regarding the relationship between age and seed production, the Michaelis–Menten model was the most powerful (AIC = 1.9737 E06).

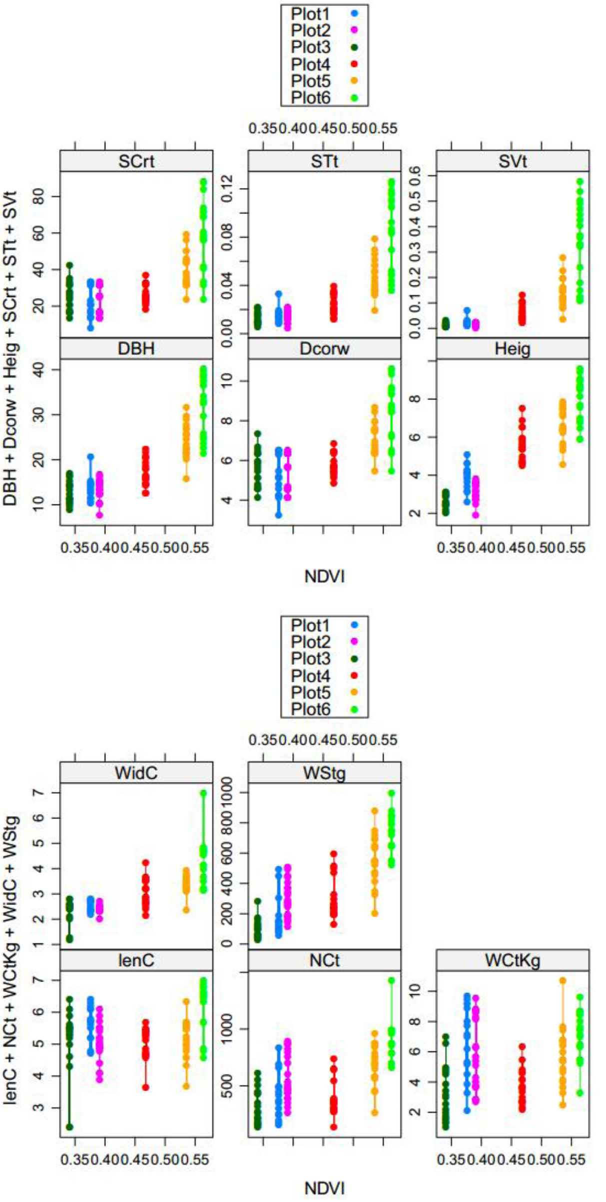


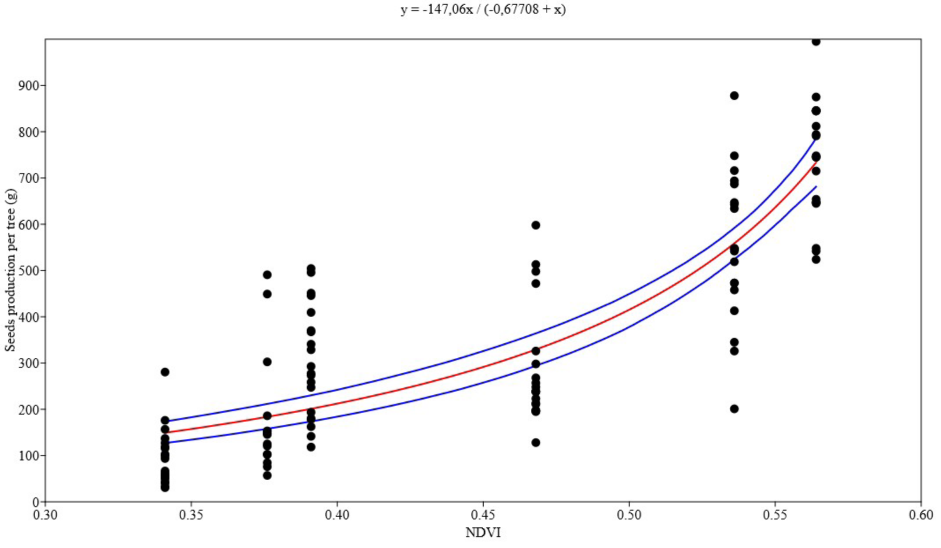
Fig. 7. Relationships between spatial index (NDVI) and dendrometric characteristics, productive parameters of reforestation of six Aleppo pine plot (DBH, diameter at 1.3 m; Dcorw, diameter of tree crown; Heig, height of tree; NCt, number of cones per tree; SCrt, area of tree crown; STt, basal area of tree; SVt, stem volume of tree; lenC, length of cones; WidC, width of cones; WCtKg, weight of cones per tree in kg; WStg, weight of seeds per tree in g).

T a b l e 4. Models used to study the relationship between seed production and the NDVI/seed production and the age of the Aleppo pine stand in reforestation.

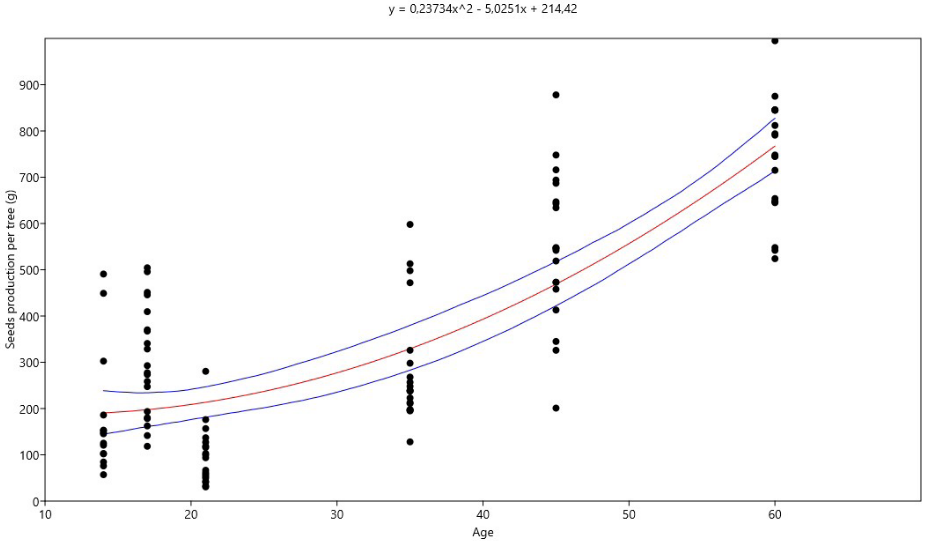
Model	**Equation**	**Akaike IC (seed production vs NDVI)**	**Akaike IC (seed production vs age)**
Linear	$Y = ax + b$	2.3004 E06	2.6212 E06
Quadratic	$y = ax^2 + bx + c$	2.0986 E06	**2.3459 E06**
Power	$y = ax^b + c$	2.0127 E06	2.3611 E06
Exponential	$Y=ae^{bx} + c$	1.9976 E06	2.4006 E06
von Bertalanffy	$Y = a(1 - be^{-cx})$	2.3015 E06	2.6226 E06
Michaelis–Menten	$Y = ax/b + x$	**1.9737 E06**	2.6478 E06
Logistic	$Y = a/1 + be^{-cx}$	2.0361 E06	2.3869 E06
Compertz	$_{Y=ae}be^{cx}$	2.0601 E06	2.3960 E06
Gaussian	$y = ae^{-\frac{(x-b)^2}{2c^2}}$	2.0487 E06	2.3946 E06
Hill's	$y = d + (a-d)\Big/\left(1+\left(\frac{b}{x}\right)^c\right)$	2.0128 E06	2.6967 E06

Discussion

Our study shows that the height and diameter of trees decrease significantly with latitude ; this result was confirmed by Ayari et al. (2011a) in Aleppo pine in dry areas of Tunisia and in pine populations in Spain (Moya et al., 2008a) and Israel (Goubitz et al., 2002). Dendrometric features allow us to quantify pine yields of wood, cones and seeds, and these results corroborate with those of Moya et al. (2008b) who found that dendrometric measurements are an approach for estimating seed yield and wood production in the same way as for other pine species. We found that basal area is positively correlated with pine production in this sense. Ayari et al. (2011a, 2012a), Mencuccini et al. (1995) and Arista and Talavera (1996) showed that basal area had a positive influence on seed and cone productions (Tunisia, Italy, Spain). Ayari et al. (2011c) showed that the seed and cone productions increased with an increase in tree height, DBH and age (Tunisia). Goubitz et al. (2004), Moya et al. (2008b) and Verkaik and Espelta (2006) found that the level of cone serotiny decreased with an increase in tree height (Israel, Spain). Ayari et al. (2012a), Girard et al. (2012) and Malcolm et al. (2001) showed that the seed and cone productions increased with an increase in tree crown height and tree crown diameter (Tunisia, France). Dendrometric measurements have been confirmed as easier and less costly estimators for Aleppo pine forests in the southern Mediterranean Basin, which have been described as more vigorous in growth and fruiting than the northern or eastern Mediterranean populations (Climent et al., 2008). Our study shows that the length of cones varies between 50.3 and 62 mm. Matziris (1998), Ayari et al. (2011a), Harfouche et al. (2003), Tapias et al. (2001) and Boulli et al. (2001) showed that the length of a cone was between 66 and 116



(a)



(b)

Fig. 8. (a) Relationship between seed production and the NDVI Aleppo pine stand (Michaelis–Menten model: AIC = 1.9737 E06) and (b) Relationship between seed production and age in Aleppo pine stand (Quadratic model: AIC= 2.3459 E06).

mm (Greece), 66.6 mm (Tunisia), 73.7-87.4 mm (Algeria), 90 mm (Spain) and 31.28-37.62 mm (Morocco). Our results show that the width of the cones varies between 23.6 and 33.9 mm. Matziris (1998), Harfouche et al. (2003) and Ayari et al. (2011a) concluded that the width of a cone was between 32 and 46 mm (Greece), 45.1-56.2 mm (Algeria), 30.5 mm (Tunisia) and 63.50-90.13 mm (Morocco). We found that the number of cones per tree varies between 304 at the age of 14 years and 900 cones at the age of 60 years. Matziris (1997), Nathan et al. (1999), Ayari et al. (2011c) and Jaouadi et al. (2019) found that a cone number per tree was 168 (at the age of 9 years; Espagne), 155 (at the age of 10 years; Greece), 242 (Israel) and 113 (Tunisia). Verkaik and Espelta (2006) showed the number of cones per tree in control plot was 6202 (10 years) and 27,175 (22 years) and in thinning plot was 475 (10 years) and 10,892 (18 years) (Spain). In a study on the Aleppo pine in northeastern Tunisia, Ayari et al. (2016) found that the number of cones produced by a tree was, on an average, 8–97 cones/tree; the weight of cones per tree was 198.5–2018.6 g; the weight of seeds per tree was 7.28–82.94 g. Our study has shown that the Aleppo pine is well suited to the reforestation site in northeastern Tunisia in this sense; Vennetier et al. (2008) proved that this species is well adapted to the different climatic zones studied; the same results were found in Tunisia (Khouja et al., 2000), Algeria (Harfouche et al., 2003; Bentouati, Bariteau, 2005) and Morocco (Belghazi et al., 2000). In our study, we found that the crown surface is positively correlated with the productive capacity of Aleppo pine; the results were confirmed by Reid et al. (2003). Our results showed that silvicultural treatments (thinning) are positively correlated with the production of wood, cones and seeds; hence, the stand of Aleppo pine must have silvicultural interventions to increase their production in this way. The average number of mature cones per tree and their characteristics vary according to provenance, site quality, density, age and treatments (Tapias et al., 2001; Harfouche et al., 2003; Verkaik, Espelta, 2006; Moya et al., 2008b). The size of cones is positively correlated with seed production in this sense; Thanos and Daskalakou (2000) showed that larger cones without obvious damage caused by diseases and insects usually contained more seeds because the canopy seed bank depends on the abundance of cones and the size of the tree. Our study shows that the geographical effect (altitude, longitude and latitude) and age have a significant effect on the productivity of Aleppo pine; in these senses, several authors confirmed our results and showed that the production of Aleppo pine in cones and seeds showed great variability depending on the effect of many factors, such as geographical variables (Harfouche et al., 2003; Nasri et al., 2004), weather conditions (Messaoud et al., 2007), tree measurements (Moya et al., 2008b), site quality (Goubitz et al., 2002; Moya et al., 2008a) and genetic effects (Climent et al., 2008). Verkaik and Espelta (2006) have recently shown a relationship between the frequency of Aleppo pines for massive cone and seed productions and the climatic gradient in Spain. The longitude has a beneficial effect on the production of wood, cones and seeds of the Aleppo pine. On the contrary, longitudinal changes, that is, from east to west, have had different effects on several species such as those in North Africa (Boulli et al., 2001; Harfouche et al., 2003; Nasri et al., 2004). Harfouche et al. (2003), Boulli et al. (2001), Khouja, Sghaier (2000) and Nasri et al. (2004) showed a positive correlation between longitude and weight of 100 seeds (0.43, Algeria), weight of 1000 seeds (0.776, Morocco) and weight of 1000 seeds (0.71, Tunisia). Boulli et al. (2001), Khouja, Sghaier (2000) and Nasri et al. (2004) showed a negative correlation between latitude and weight of 1000 seeds (−0.820, Morocco) and weight of 1000 seeds (−0.500, Tunisia). Longitudinal effects strongly influenced the cone and seed productions of Aleppo pine. In this sense, Ayari et al. (2011a) showed that eastward direction of Tunisia was negatively related to cone size and mass and was a critical explanatory factor in the multiple regression analysis. Nasri et al. (2004) found similar results in Aleppo pine forests in Tunisia. In other forest areas of Aleppo pines, Harfouche et al. (2003) reported an increase in mean individual seed mass related to a coastal gradient on the Mediterranean coast of North Africa, but this was due to the confounding effect of latitudinal effects. A negative elevation effect on cone size was found in this study, confirming the previous results obtained by Ayari et al. (2011b) in the forests of northeastern Tunisia, Boulli et al. (2001) in Aleppo pine forests in Morocco and Climent et al. (2008) in many Mediterranean countries, including Tunisia. In this study, the production of wood, cones and seeds were altitude sensitive; these results were confirmed by Ayari et al. (2011c) who showed that the predictive models obtained showed that the elevation had an effect on the size of the cone. We believe that altitude effects may be greater in the absence of longitudinal or latitudinal gradients. In this sense, Turner et al. (2007) showed that in cold regions, previous results showed negative effects on pine cone production on elevation, as cold is known to kill flowers, abort cones and prevent maturation of trees seeds in conifers (Messaoud et al., 2007). The proximity of the coast has a negative effect on fruiting in the southern Mediterranean countries. This implies that an increase in the continental gradient has improved the production of cones and seeds of Aleppo pine (Harfouche et al., 2003). Boulli et al. (2001), Khouja and Sghaier (2000) and Nasri et al. (2004) showed a negative correlation between latitude and the weight of 1,000 seeds (0.820 in Morocco; 0.500, Tunisia). Unlike our results, Boulli et al. (2001) showed that the favourable influence of elevation on cone weight and size has been attributed to better moisture conditions and lower temperatures, leading to higher rates of photosynthesis and cones development. It is also important to note the implication of the geographical gradient, which could depend on the physiological implications of the zones, namely, a greater availability of nutrients, light and water with temperatures close to the optimum, are positively related to the quality and the amount of cones and seeds (Ayari et al., 2011a). Similarly, in North Africa, as in other areas of the Mediterranean Basin, a relationship between cone/seed characteristics and the elevation gradient are positively related (Harfouche et al., 2003; Nasri et al., 2004). Our research shows that altitude is negatively correlated with the production of Aleppo pine to sub-humid bioclimate; these results are not in agreement with Turner et al. (2007) who showed for a similar species, *Pinus contorta*, the positive effect of elevation on cone/seed production that was attributed to shorter drought periods associated with more abundant rainfall. Our study show that exposure north, north-east, north-west, south, south-east, south-west have a positive correlation with the production of wood, cones and seeds, which confirm the findings of Ayari et al. (2011b), showing favourable cone harvesting and seed yield in forests on favourable aspects, such as north-east, south-east south and north-west This could be explained by the higher availability of light and temperatures during the pollination period, which seems to play a major role in promoting conifer breeding success in the same way as other species (Hylander, 2005). Our results do not support the results of Schiller and Atzmon (2009) who concluded that higher egg abortions and lower pollen vigour and viability were found in the north, south, south-west and west aspects, favouring subsequent mortality from cone formation or leading to empty seeds (Schiller, Atzmon, 2009). Ferrio et al. (2003) concluded that favourable slopes (north-east, south-east, south and north-west) suggest better climatic conditions for plant growth in the Mediterranean climate (high temperatures and light exposure with moderate humidity), thus promoting the process serotinisation and promoting the development of Aleppo pines. Furthermore, Gracia et al. (2002) reported that stands of Aleppo pines grown in highly stressed Mediterranean areas (mainly drought periods in summer) depend on aspects that exaggerate it to the east and south, which could be more important in the expected climate change conditions. In our research, we showed that the subhumid bioclimatic zones resulted in greater cone harvest and seed yields, with a positive fruiting effect; these results are confirmed by Ayari et al. (2011a) who concluded that the subhumid zone showed higher average values characterising the amount of seeds and cones compared to other arid and semi-arid bioclimatic zones and longitudinal gradients were the most important geographical factor, resulting in net increase in cone size and seed content, mainly in the semi-arid zone. The effect of geographical distribution on fruiting was greater in drought conditions (Schiller, Atzmon, 2009). In addition, the slopes facing north–west, northwest, south and southeast were the preferred aspects of the stands for increasing the pine cone crop by taking into account the reforestation efforts in future. (Ayari et al., 2011c). The NDVI spatial index had a strong positive correlation with the production of Aleppo pine seeds as the plots showing the higher NDVI were the most productive. Consequently, NDVI represents an important indicator that can explain the productivity of wood, cone and seed drills and estimate the forest productivity. The NDVI can also give information on the age of the stand and its productive state. The age of the stand was strongly correlated with seed production. Our results have also shown that age is a good indicator to estimate the production of the Aleppo pine forests in the sub-humid bioclimate of Tunisia and plan for oriented strategies towards seed production in the first place in the forests of northern Tunisia. The mathematical models used to analyse the relationship between seed production/NDVI and seed/age production showed that the most powerful and successful model for estimating seed production/NDVI is that of Michaelis–Menten. However, the quadratic model is the most powerful to estimate the seed production/age. The results obtained should be of great value to Mediterranean foresters in choosing appropriate locations for future Aleppo pine plantations to maximise seed production. This research provides forest ecosystem managers with a simple means of recognising the best production sites for the Aleppo pine forest, thereby increasing the profitability of stands. This could help satisfy the growing consumption of Aleppo pine seeds, especially in Tunisia. The knowledge of the need for on-going security stability of forest ecosystems, and thus the existential basis of forestry, indicates the need for in-depth research and monitoring increment of forest trees as a complex reaction trees on stimulating and disruptive action of exogenous and endogenous factors (Šikanja, 2017).

Conclusion

The present work investigated the production of wood, cones and seeds of Aleppo pine trees in reforestation in northeastern Tunisia in relation to the characteristics of plantations and plots. We found as a first result a high variability amongst the six reforestation plots for several tree-specific dendrometric parameters. Our results showed that the quantity of Aleppo pine seeds harvested per hectare in the northeast is four times greater than that harvested in the northwest. In conclusion, the reforestation of Aleppo pine trees must be directed towards seed production in the Northeast of Tunisia and oriented towards wood production in the northwest. It is important to emphasise that the Aleppo pine forests constitute a forest heritage for Tunisian people and represents a natural resource because it plays major environmental, social, economic and cultural roles, especially with the considerable increase in the human consumption of the seeds of the Aleppo pines.

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TROPHIC CHARACTERISTICS OF *Lymnaea stagnalis* (MOLLUSCA: GASTROPODA: LYMNAEIDAE) IN TOXIC ENVIRONMENT

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Abstract

Pinkina T., Zymaroieva A., Matkovska S., Svitelskyi M., Ishchuk O., Fediuchka M.: Trophic characteristics of *Lymnaea stagnalis* (Mollusca: Gastropoda: Lymnaeidae) in toxic environment. Ekológia (Bratislava), Vol. 38, No. 3, p. 292–300, 2019.

The influence of ions of heavy metals (copper, cadmium, nickel, zinc, cobalt and manganese) was investigated on the basis of trophic characteristics: the average daily ration (ADR), and duration of food passage (DFP) of the *Lymnaea stagnalis* L. in various concentrations of toxicants in vivisection experiment. In addition to these indicators, the total amount of food consumed in the solutions with various concentrations of pollutants was found out during the chronic experiment and it was calculated for an individual (average monthly ration – AMR). It leads to the conclusions about the intensity of food consuming considering different levels of intoxication. In solutions with lethal concentrations, the death of animals occurs during the first day of its impact due to the damage of tissues and organ systems. Chronic lethal concentrations of toxicants inhibit the nutrition of pond snails dramatically. At the beginning of the experiment, solutions of heavy metals with sublethal concentrations give some stimulatory effect on the digestive system of molluscs that is replaced by its suppression in case of longer being in the toxic environment. The influence of toxicants within a subthreshold limit cannot be considered safe because of the cumulative properties of heavy metals – they become sublethal with prolonged exposure time.

Key words: *Lymnaea stagnalis* L., Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, toxicity, trophic characteristics, molluscs.

Introduction

The circulation of substances in reservoirs is closely related to the nutrition of animals (Abi-Ayad et al., 2018). Hence, there is a need to study the mechanism and nature of hydrobionts' nutrition (Sushkina, 1949). Selective food consumption of the most common species of hydrobionts, *Lymnaea stagnalis* (Linné, 1758) including, was established (Rodina, 1957). E. Frömming (1953) expressed the number of leaves of semi-submerged and submerged plants eaten by pond snails in their area. Pondweed is eaten only during starvation, but dead and macerated parts are consumed quite willingly. The same happens to elodea. According to some researchers (Wiktor, 1958; Kunakh et al., 2018), molluscs eat all the plants that are exposed to their radulae. There are other things except algae to feed molluscs, such as planktonic organisms, large invertebrates, plant detritus, animal corpses and feces. However, its favorite species are preferred if feed is sufficient and varied (Cihon-Lukanina, 1987; Yorkina et al., 2018). In laboratory conditions, pond snails eagerly eat carrots, cabbage, salad and dandelion leaves (Stroganov et al., 1977). The intensity of food consuming depends on its quality and quantity (Seifert, 1990), as well as on the physiological state of the organism. For example, a strong invasion of trematodes may increase it 81–93.6 times (Cihon-Lukanina, 1987). The average daily ration values the amount of food consumed, reflecting the ratio between the weight of feed taken in and the weight of a mollusc. The average daily ration depends on the age of the ponds (at a young age, it is 2.5 times that of the older individuals) (Stadnichenko, Kotsyuk, 1990) and on the temperature (young individuals react more slowly to changes in temperature in contrast to the old ones) (Sushkina, 1949). Laboratory studies have shown that the rate of eating does not increase after reaching maximum values with an increase in food quantity (Sushenia, 1975). Calculation of food consumed is carried out experimentally by means of analyzing the process of filling the digestive tract and the speed of food passing through it (Kerr, 1982).

Two ways of receiving inorganic ions constitute a distinctive physiological feature of hydrobionts' body. One of them is due to the absorption of ions directly from the water through the mucous membranes, and the other way is oral, connected with the passage of substances absorbed in the intestine to hepatopancreas (Romanenko, 1978). The nutrition process of herbivorous molluscs is characterized by intensity, regularity and labor intensity. During the nutrition process, a large mass of substratum is scraped off from the substrate, and only part of it is digested. Since plants are concentrators of heavy metal ions (Melchakov, 1989), it is possible to transfer heavy metals from the producers to further links (Morozov, 1983) in food chains, including phytophagous molluscs.

Particular attention should be paid to animals' nutrition in a toxic environment, as nutritive behavior (intensity or decrease in food consumption) may be the indicator of sensitivity to the effects of toxicants. At high concentrations of toxicants, there is always a suppression of nutrition. In sublethal concentrations, molluscs consume less nutrient during the first two weeks, and later more than it is normal (Stroganov et al., 1977). The reason for this is the need to withstand the pathological changes occurring in the animals' organism affected by toxicants (Pinkina, Pinkin, 2018). Therefore, establishing the main trophic parameters of *L. stagnalis* in the environment intoxicated by heavy metal ions is the purpose of our research.

Material and methods

As a material for comparative toxicological studies and for indicating the quality of water, any group of organisms can be used, large invertebrates among them are the most suitable research material. They are the most representative group in any reservoir, they do not need complicated equipment for extraction and microscopy and are relatively simple for systematization and experimentation. For toxicological experiments, gastropod pulmonary mollusks are very convenient objects (Bren, 1999; Zabory, 1986). The gastropod pond mollusc *L. stagnalis* L. was selected among them as the most common representative in the Central (Zhytomyr) Polissya. The choice of this object is determined by a small investment of time, labor and accordingly the cost of material resources for their collection, experiments and introduction into the system of hydromonitoring.

The same size pond snails (average height of the shell - 39.5 ± 1.1 mm) collected in the basin of the Middle Dniper River Teterev (Zhytomyr) served as the material for investigation. 2631 samples were used to study the peculiarities of molluscs' nutrition under the effects of toxicants; among them, 940 samples were used to determine the duration of food passing through the digestive tract, 1140 samples helped to determine the average daily ration and 551 items – to find out the average monthly ration.

Experimental conditions: water temperature - 19–23°C, pH 7.2–8.6, oxygen content 8.6–8.9 mg/dm^3. Toxicants - Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+} in liquid substance. The solutions were prepared on dechlorinated (1 day) water from zhytomyr water supply system.

Young dandelion leaves served as a feed object in our experiments. This plant is often used in laboratory studies and is eagerly eaten by pond snails.

Toxicological experiments were delivered according to the methods by Alekseev (1981). Two preliminary experiments were held for every toxicant before the main investigation. In the first one, concentrations were determined in which 100% of animals die or remain alive. The range between lethal and inactive concentrations was chosen as the starting point for the main experiment, and the concentration of the toxicant, in which 100% of the animals died, was the concentration of the mother solution. In the second preliminary experiment, which was as long as the main one, the ranges of the sharp lethal, chronic lethal, sublethal and subthreshold concentrations were established. In the main experiment, 4 concentrations were used, one for each range of concentrations (Table 1).

T a b l e 1. Concentrations of heavy metal ions (mg/dm^3) used in the experiments.

Ions	Concentrations			
	Sharp Lethal	Chronic Lethal	Sublethal	Subthreshold
Cu^{2+}	4	0,04	$4 \cdot 10^{-5}$	$4 \cdot 10^{-8}$
Cd^{2+}	5	0,05	$5 \cdot 10^{-4}$	$5 \cdot 10^{-6}$
Ni^{2+}	10	0,05	$5 \cdot 10^{-3}$	$5 \cdot 10^{-6}$
Zn^{2+}	15	0,5	$5 \cdot 10^{-3}$	$5 \cdot 10^{-5}$
Co^{2+}	25	2,5	0,25	0,03
Mn^{2+}	110	30	0,3	0,03

To determine the value of the average daily ration, the animals' shells were drained with filter paper, their total mass was determined and they were placed one by one in a separate tank filled with toxic stuff. The mollusks were immediately given feed preliminarily weighed on torsion weights (VLTK-500). The feed was placed between sheets of filter paper before weighing and kept under a weight of 1 kilo for 20 minutes. The duration of the experiment was 48 hours. At the end of the experiment, the remaining feed was drained to remove excessive moisture and weighed. By the difference in feed weight before and after the experiment, the amount of daily intake by each individual was determined. The value of the average daily ration – ADR (in % relative to the raw mass of the body of the pond snails) was determined by the formula:

$$X = (A.100)/P,$$

where X is the value of the average daily ration; A - the mass of food consumed by a mollusk, g; P - the total mass of his body, g.

To determine the length of feed passage along the molluscs' digestive tract, they were fed with thin slices of macerated (6 days) in water carrots. They were then fed with the green (dandelion leaves). The time for the appearance of the first excrements containing the remains of this feed was set, calculating the difference between this time and the beginning of consumption of the green feed. This difference is the duration of feed passing through the digestive tract (DFP).

In addition to these indicators, the total amount of feed consumed by molluscs (TAFC) was determined in solutions of various toxic concentrations during the chronic experiment and it was recalculated per an individual. It made it possible to draw conclusions about the intensity of feed intake at different levels of intoxication.

The received digital data are processed using standard methods of variation statistics (Lakin, 1990) and special computer programs *MS Excel* and *Statsoft Statistica 6.0*.

Results and discussion

It is well-known that feed is the main organisms' "channel of communication" with the environment. All the major aspects of their life depend on it: the cost of exchange, somatic growth, maturation of sexual products and reproduction. Therefore, the explanation of the peculiarities of the pond snails' trophic in the environment containing heavy metal ions is of considerable interest.

We investigated 3 main trophic indicators, namely: the average daily ration (ADR), the duration of feed passing (DFP) and the average monthly ration (AMR). ADR and DFP are the most important trophic characteristics for understanding the intensity of feed intake by the organism under different conditions and patterns of feed digestion and absorption of nutrients. Sushkina (1949) used these indicators in the study of trophic freshwater molluscs (*L. stagnalis* and *Planorbarius corneus,* Linne, 1758) for the first time. The functional relationship between the mollusc organism and its habitat concerning copper and zinc is partly studied (Vyskushenko, 2002). The values of ADR and DFP for *Lymnaea palustris* (O.F. Muller, 1774) under the influence of cadmium ions were determined by O.M. Vasilenko for the first time (Vasilenko, 2004). There is no data on the indicated trophic indices for *L. stagnalis* under the influence of nickel, cobalt and manganese ions, and the values of these indices have been established for the first time in this research, as well as the value of AMR for all six investigated metals, which indicate increasing or weakening of the trophic function of the pond snail in a long lasting poisonous environment.

The results of our research have proved that the average daily ration (Fig. 1) and the duration of feed passing through the digestive tract (Fig. 2) fluctuate within a rather significant range. The minimum and maximum values of these indicators are respectively: 1.55–2.73% and 350–440 min under the effect of copper ions; 1.65–3.45% and 341–483 min – cadmium ions; 2.52–4.94% and 396–477 min – nickel ions; 1.05–2.92% and 338–467 min – zinc ions; 1.30–1.74% and 360–944 min – cobalt ions and 2.18–4.65% and 302–423 min under the effect of manganese ions. According to the results of experiments by Cihon-Lukanina (Cihon-Lukanina, 1987), the range of nutrition indicators for the pond snails is also quite large (sometimes up to 85%).

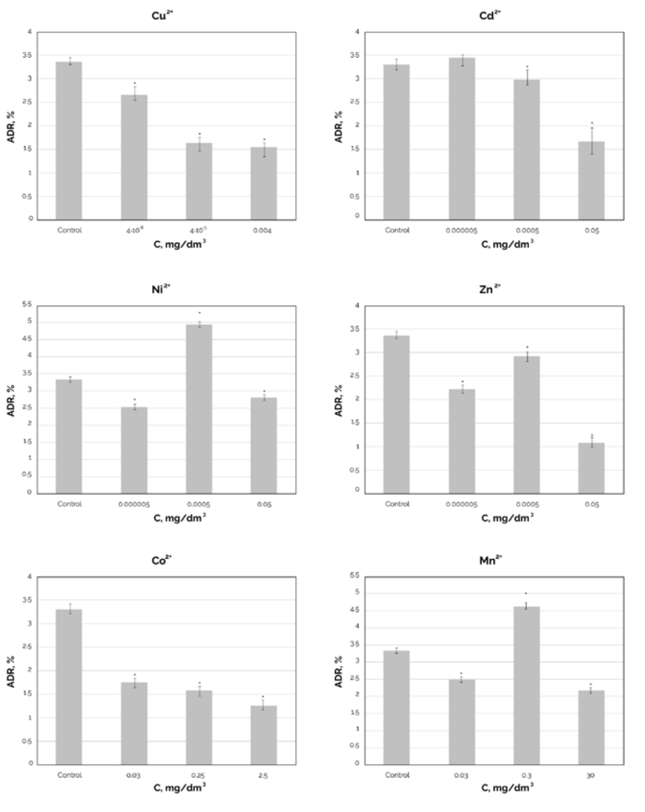

Fig. 1. The value of the average daily ration (%) *L. stagnalis* under the effect of heavy metal ions (Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2}) in liquid substance; $x \pm m_x$; n = 6. * – the differences compared with the control are probable.

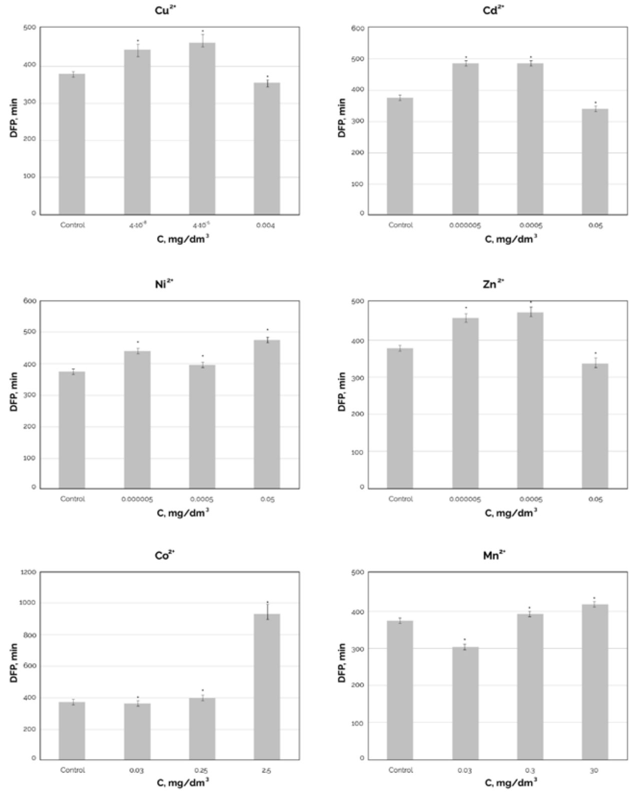

Fig. 2. Duration of feed passage (min) in *L. stagnalis* under the effect of heavy metal ions (Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}) in the liquid; $x \pm m_x$; n = 6. * – the differences compared with the control are probable.

In studying the nutrition peculiarities of the pond snails, the same concentrations as in the main experiment were used, namely, in one of the range of chronic lethal, sublethal and subthreshold concentrations (Table 1). Research on molluscs' feeding in sharp lethal concentrations was not carried out. It is caused by preliminary observations, stating that animals in solutions of these concentrations almost or completely do not consume feed. Only some representatives (about 7–9% of the total number of molluscs) out of 40 pond snails found in solutions of sharp lethal concentrations showed sporadically weak consuming activity. The main functions of molluscs' organism in such concentrations are completely suppressed, the regulatory mechanism that compensates changes in the body doesn't perform its function, thus leading to the death of animals in the long run.

In general, pond snails' eating activity (in particular, their refusal of food) under the effect of heavy metals in liquid substance varies. Thus, in subthreshold concentrations, only some individuals if any, refuse food. In sublethal concentrations, 7–10% out of the total number of molluscs follow this tendency. Up to 30% of animals almost do not consume food in solutions with chronic lethal concentrations.

Only at a concentration of 0.04 mg/dm^3, the decrease in the amount of food consumed as well as in the duration of its passage ($P < 0.05$) is observed. It indicates an increase in the toxic effects of copper at this concentration, which manifests itself in the suppression of digestive processes. The fact, that molluscs are sensitive even to the subthreshold non-active (according to other indicators of the investigated pollutant and animal ethology) concentrations, demonstrates a strong toxic effect of copper on the pond snails even at very low ($4 \cdot 10^{-8}$ mg/dm^3) concentrations.

The amount of daily consumed feed increases slightly, while the duration of food passage increases 1.3 times under the effect of ions of cadmium on molluscs at the concentration of $5 \cdot 10^{-6}$

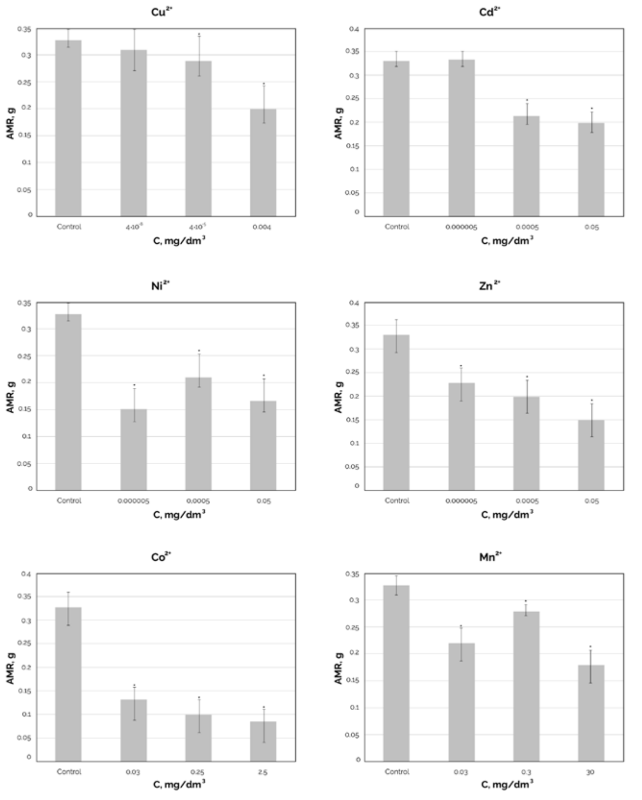

Fig. 3. The value of the average monthly ration (g) *L. stagnalis* under the effect of heavy metal ions (Cu^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}) in the liquid substance; $x \pm m_x$; n = 4. * – the differences compared to the control are probable.

mg/dm^3. As the toxicant concentration increases, ADR decreases (Fig. 1). DFP indices are significantly increased at concentrations of $5 \cdot 10^{-4}$ mg/dm^3, activating animals' metabolism to a certain extent, moreover DFP indices decrease considerably with increasing concentration of cadmium ions in the environment (Fig. 2). Consequently, molluscs are no longer able to withstand the pathogenic effects of the pollutant at concentrations of 0.05 mg/dm^3.

The information above corresponds to the data obtained by Vasilenko (2004), although the scientist demonstrated a larger range of the variations in the values of pond snails' ADR and DFP in solutions of cadmium ions. It can be explained by the difference in feed used in the experiments.

The same concentrations as in the study of the influence of cadmium ions (according to the results of the previous ecotoxicological researches, these metals belong to one toxicological group) are used in the analysis of the effect of nickel ions on the peculiarities of the pond snails' feeding. A different dynamic of changes in nutrition indicators is observed in this experiment. ADR decreases in 1.3 times and DFP increases almost as many times at concentrations of $5 \cdot 10^{-6}$ mg/dm^3 of nickel ions in the substance. There is a contrasting tendency at sublethal concentrations (0.005 mg/dm^3): the ADR value increases by 1.5 times, while the passage of food along the digestive tract is slowing down (Figs 1, 2). Feed consumption decreases again in solutions with higher concentrations of Ni^{2+} (0.05 mg/dm^3), indicating a strong depression of molluscs' digestive processes.

The value of DFP increases in solutions with nickel ions in response to the decrease in ADR, and although the animals are still able to withstand the influence of toxicants for a while, it is evident that their further presence in poisonous water will lead to the death of individuals.

The same pattern in reaction of the digestive system to the influence of the toxicant is observed as in the substance containing copper, cadmium and nickel ions if pond snails are in solutions with different concentrations of zinc ions. As the concentration of this toxicant increases, there is a decrease in the ADR values (Fig. 1). DFP values increase initially in response to it, compensating toxic effects to some extent, and then, they are greatly reduced with the development of the depressive phase of the general pathological process of intoxication, which is accompanied with the suppression of the protective and adaptive properties of these animals (Fig. 2).

Considering the change in DFP at different concentrations of zinc ions in water, it can be stated that the growth of its values in solutions of $5 \cdot 10^{-5}$ mg/dm^3 and $5 \cdot 10^{-3}$ mg/dm^3 can compensate the progressive fall of ADR of the molluscs under investigation only partly.

It was established that the value of ADR also decreases with increasing concentrations in solutions with cobalt ions (Fig. 1). The value of DFP grows rather slowly first with an increase in the concentration of toxicants and it gets higher than control by only 5.8% at the concentration of 0.25 mg/dm^3. The increase in the value of the investigated indicator continues at 2.5 mg/dm^3 of this ion in the substance, increasing by 151.6% (in 2.5 times) in terms of control (Fig. 2). It shows an extremely strong damage to the digestive system of the pond snail caused by high concentrations of ions of cobalt, which leads to almost complete paralysis of the intestines and ceasing of its peristaltic activity. Most animals generally refuse to consume feed in these solutions.

The change in the nutritional indices under the influence of manganese ions in water is similar to the effect of other investigated metals: there is a tendency to decrease ADR and increase DFP with the increase in the concentration of toxicant in the environment (Figs 1, 2) (Pinkina, Pinkin, 2018). These indices bear statistically significant difference to the control ($P < 0.05$) at all investigated concentrations of manganese ions.

Taking into account the cumulative properties of heavy metal ions, the values of the average monthly ration of molluscs (in terms of one person) were calculated, making it possible to more specifically talk about ruining the digestive processes of these animals. As a result of these studies, it was found that the amount of feed consumed by pond snails is progressively decreasing with an increase in the concentration of toxicants. The same pattern is established during the 48-hour exposition. However, the results of the chronic experiment show a sharper decrease in the amount of feed consumed with an increase in the concentration of metal ions in the medium (Fig. 3). It can be explained by the increase in the toxic effects of the indicated pollutants due to their accumulation in the body of molluscs.

The lack of statistically significant difference compared to control in solutions with copper ion concentration by $4 \cdot 10^{-5}$ and $4 \cdot 10^{-8}$ mg/dm^3 (Fig. 3) in the acute experiment (Fig. 1) indicates that animals submerged into a toxic environment, react more sharply on it initially. Adaptation mechanisms appear in some time, so the amount of feed consumed decreases insignificantly regarding control.

The amount of average monthly food consumed by an individual feeder at low concentrations coincides with it in the control and it is equal to 0.33 ± 0.03 g in solutions with cadmium ions. As the toxicant concentration increases, the amount of feed consumed decreases gradually ($P < 0.05$). The dynamics of changes in the investigated value is very similar to the same in solutions of copper ions.

The digestive system of molluscs is quite sensitive to the effects of nickel ions. If there is a slight stimulation of this function in the concentration of $5 \cdot 10^{-4}$ mg/dm^3 and consumption of food falls 1.5 times compared with control, then feed is reduced by half ($P < 0.05$) at lower ($5 \cdot 10^{-6}$ mg/dm^3) and higher (0.05 mg/dm^3) concentrations of this pollutant in the nutrition environment.

There is a pronounced depression of pond snails' trophic processes under the influence of cobalt ions at the concentration of the specified toxicant going high. At the concentration of 0.03 mg/dm^3, which molluscs do not react to with changes in ethology and other physiological systems at all, consumption of food is reduced by 2.5 times, and at the concentration of 2.5 mg/dm^3 even by 4 times (Fig. 3). Similar data were obtained even during the 48-hour experiment.

Since the trematode invasion complicates the course of the pathological process caused by the poisoning of molluscs with toxicants, the trophological indices of the pond snails infected with rediae and cercariae of the echinostomatitis trematodes (the family Echinostomatidae) were singled out from the general data obtained.

The results obtained in this research point to the importance of analyzing the trophic indices of animals in toxicological studies, since they clearly highlight the general physiological state of individuals that are in a toxic environment. According to the nutrition indicators, pond snails are quite sensitive to the influence of heavy metal ions.

In response to a decrease in feed intake, the body of the pond snail reacts with an increase in the time of finding food in the digestive tract of the mollusc. Such adaptation to the pathogenic influence of heavy metals only partially compensates for their negative effects, and at higher concentrations, pathological processes begin to prevail over protective and adaptive, which leads to a decrease in both ADR and DFP along the digestive tract of molluscs. Trophic indicators of pond snails are most sensitive to the effect of nickel and cobalt ions, and it differs from the overall toxicity of these substances, which is determined by the survival of individuals. Obviously, heavy metals having a general toxic effect on organisms, can have a specific (stronger or weaker) effect on certain functional systems. Such features can be used as test-reactions in the system of biomonitoring of the quality of natural water.

Conclusion

The results of our studies given above prove that the food consumption is sensitive enough to reflect the changes in the intensity and direction of *L. stagnalis* metabolic processes. It allows us to use the employed indicators as signs of the normal functional state of the organism and the functional state under the effect of extreme stimuli of chemical nature, including toxic substances.

Depression of nutrition and, consequently, strong growth retardation of animals are observed due to chronic lethal concentrations of pollutants. Under these conditions, pathological processes predominate over protective and adaptive ones, leading to a decrease in the average daily and average monthly rations and the duration of feed passing along the digestive tract. under the effect of sublethal concentrations of heavy metals. The reduction of feed intake is accompanied by lengthening of the time of feed remaining in the molluscs' digestive tract. Pond snails are most sensitive to the action of Ni^{2+} and Co^{2+} considering trophic indexes.

In most cases, animals infected with parthenitae and larvae of the trematodes undergo the toxicant effect faster than the uninfected representatives.

Adaptation to the action of heavy metal ions is relative, since large energy costs are compensated only partially, and in the end, it leads to a shortage of macroergic substances in the animal's body. Consequently, energy imbalance becomes one of the causes of death of animals in the toxic environment.

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