Effects of ph concentrations on germination and development of norway spruce (Picea abies (l.) Karst.) seed

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Vplyv pH na klíčenie a vývoj výsevov smreka obyčajného (*Picea abies* (L.) Karst.)

Effects of pH concentrations on germination and development of Norway spruce (*Picea abies* (L.) Karst.) seed

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Abstract

This paper presents the results of germination and development of spruce seeds in different pH solutions under laboratory conditions. Ten different pH concentrations were used with 4 repetitions each. Concentration values ranged from pH 1.7 to 6.7. Sulphuric acid (H_2SO_4) and deionized water were used to prepare the solutions. Seed germination and development were tested according to the ISTA Rules. Germination was tested in an illuminated incubator (Snijders model ECD01E, Tilburg, Netherlands).

The results provided some fundamental information on seed germination in different pH solutions for spruce and fir species. In addition, very useful information was obtained that will facilitate understanding of natural regeneration, particularly in changing environmental conditions (acid rain, soil acidity, etc.). The threshold pH value for normal seed germination was 2.6. Soil pH values in Croatia range between 2.7 and 8.10, with only 0.38% of soils having pH values lower than 3. Generally, natural pH concentrations of soils are favourable for spruce seed germination.

***Key words**: seed germination, pH – solution, seedling characteristics, Norway spruce Karst., decayed seeds*

Abstract

Práca prezentuje výsledky klíčenia a vývoja výsevov smreka pri rozdielnych hodnotách pH v laboratórnych podmienkach. Sledovaných bolo 10 rôznych koncentrácií pH v troch opakovaniach. Hodnoty pH sa pohybovali v rozpätí od 1,7 do 6,7. Roztoky boli vytvorené použitím kyseliny sírovej (H_2SO_4) a deionizovanej vody. Klíčenie bolo testované podľa pravidiel ISTA v osvetlenom inkubátore (Snijders model ECD01E, Tilburg, Holandsko).

Výsledky poskytujú niektoré základné informácie o klíčení smreka a jedle pri rozdielnych úrovniach pH. Okrem toho obsahujú informáciu, ktorá uľahčí pochopenie prirodzenej obnovy čiastočne aj v zmenených ekologických podmienkach (kyslé dažde a zakyslenie pôd). Hraničná hodnota pH pre normálny priebeh klíčenia bola 2,6. Reakcia pôd v Chorvátsku sa pohybuje v rozpätí medzi 2,7 a 8,1, pričom len 0,38% pôd má reakciu s nižšou hodnotou ako 3. Všeobecne sú pôdy z hľadiska ich kyslosti vhodné pre klíčenie semien smreka

Kľúčové slová: *klíčenie, pôdna reakcia, parametre semenáčikov, smrek obyčajný,*

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is a species whose distribution range extends over more than 200 million ha, and is therefore most widespread tree species on Earth (Oršanić, 2001). According to Vajda (1933), spruce occurs naturally in relief-conditioned depressions of high mountains of Gorski Kotar, Velebit and the rest of Lika. Its favoured sites are frost spots, where spruce does not face any serious competition from other tree species. Matić (2011) writes that Norway spruce is an important pioneer and transitional tree species which forms even-aged and multi-aged stands of high silvicultural form. Spruce participates in the composition mix with 52%, while silver fir, common beech and other autochthonous soft broadleaves account for the remaining percentage. At the level of Croatia, the growing stock of spruce amounts to 5.57 m^3/ha of the total forested area. In terms of participation by diameter classes, the largest part of wood volume belongs to the highest diameter and age classes, which indicates its over-maturity and physiological weakening. According to Čavlović *et al*. (2008), the total growing stock of spruce in the Republic of Croatia is 13 200 000 m^3, accounting for 2.4 % of the total wood volume.

Young and Young (1992) write that the seed of common spruce, unlike that of other species, has been thoroughly investigated and that it manifests high variability with regard to latitude and elevation. Seeds from northern latitudes and higher elevations have smaller mass than those from southern latitudes and lower elevations. The seed is minuscule, elongated, sharpened at the base with one well developed wing which is 2-4 times longer than the seed itself. The seed husk of a mature seed is brown or black and the cotyledons vary in number between 4 and 15. Ružić et al. (1998) examined the effect of a weak low-frequency magnetic field on spruce seed germination under low pH conditions. Their research showed that a weak, sinusoidal magnetic field (50 Hz, 26 and 105 µT, stimulation 12 h/day), which is computer controlled and generated by the Helmholtz coil, slightly shortened the length of the seedlings and postponed germination at low pH values. The application of auxin, ethylene, kinetin, GA_1 and GA_3 affects or even decreases the germinating vigour of Norway spruce and Scots pine seeds (Sandberg, 1988).

Acid rains and other acid precipitation form when polluted air mixes with rain, snow and fog. Distinctly acid rain leads to an increase in toxic matter in the atmosphere and the soil. The share of free lead, zinc, copper, chromium and aluminium increases. These toxic metals in the air, soil and water delay plant growth and reduce the population of nitrogen-fixing bacteria in the soil. Acid rains inhibit the absorption of biogenic elements from the soil. Sulphates and hydrogen sulphates in acid rain may lead to calcium and magnesium leaching from the soil. Lower pH values in the soil may reduce the population of microorganisms responsible for organic matter decomposition, thus impeding the restoration of nutrients to the soil. Acid rain that falls on the plants has a destructive effect on the leaves. The waxy protective coating is corroded and the path is opened for acids to enter the plant. Acids in the plants shift the water, inhibit the absorption of carbon dioxide and reduce or even halt the process of photosynthesis. The damaged leaves are highly susceptible to frost, which increases the danger of tree death during winter.

According to Schmidt (2000), the properties of growth media (pH, salt content and drainage) become exceptionally important during seedling growth and development. The same author states that seeds or seedlings are particularly vulnerable to physiological stress, mechanical damage and infections during the stage of germination and initial growth and development. Schmidt's thesis (2000) was corroborated by our findings, in which the seeds generally decayed at low pH levels, and all the seedlings were irregular (for one or more reasons).

Abrahamsen *et al*. (1976) stated that the application of acid rain on podzoloc soil in the quantity of 50 μmol^{-1} (pH 3) during two vegetation periods increased humus acidity and decreased base saturation.

In the past three decades, atmospheric pollution has caused severe problems in the nature and in numerous biological processes (Miquel Anglès Marín, 2004). Ho (1992) confirmed a negative effect of increased acidity on the growth of roots and the above-ground part of the seedlings.

Research by the author mentioned above confirmed that factors which control total soil chemism depend primarily on soil type and only then on acid rain. There is very little information in literature on the effect of pH or naturally occurring metal cation quantities on seed germination. Nitrates from the soil are well known stimulators of seed germination.

This research has the following goals:
- Determine threshold pH values for normal seed germination of Norway spruce under laboratory conditions.
- Determine the quality of seeds and seedlings at different pH values.
- Analyse the condition of natural pH levels in Croatian soils with regard to critical values for germination.

Materials and methods

Fresh seeds of Norway spruce were used in this research. A total of 1,000 seeds were weighted in accordance with the ISTA rules (2006). Ten different pH solutions ranging between 1.5 and 6.0 pH (an increase every 0.5) were used to treat the seeds. The solutions were prepared on the basis of sulphuric acid (H_2SO_4) and deionized water. (Deionized water was used as the control, whose pH was measured before the germination test). There were four repetitions with 100 seeds per every treatment. Laboratory seed germination and the initial seedling development in different pH solutions were tested in Czech germination incubators and on filter paper. Czech incubators were placed in a closed Snijders incubator (model ECD01E, Tilburg, the Netherlands) with artificial illumination and temperatures programmed at 20–30 ºC. According to a standard procedure (ISTA, International Rules for Seed Testing, Chapter 5: The Germination Test, 2006), laboratory germination of Norway spruce seed was tested on a filter paper medium and at changeable temperatures from 20 to 30 ºC. The seedlings were first counted on day seven (germination energy) and last on day 21, which is the length of the testing procedure. The seedlings were classified every week according to the ISTA Rules (ISTA Handbook on Seedling Evaluation). According to the ISTA Handbook on

Seedling Evaluation, species of the genus *Picea* L. belong to category B (trees and shrubs), section 22 (germinant type H and germinant group B-3-1-1-1). A digital camera was used to record all the irregularly grown seedlings and monitor the course of germination. Decayed seeds were taken out of the incubator and recorded during each counting session. At the end of the germination test, the vitality of non-germinated seeds was evaluated using the tetrazolium method. The ISTA Rules (Working Sheets on Tetrazolium Testing 2003, Volume II Tree & Shrub Species) were followed.

Results

Changed pH values of the solutions

The solutions prepared for the analysis of spruce seed germination ranged from extremely acid to weakly acid (Figure 1). An interesting feature was the occurrence of a low initial pH value in weakly acidic solutions and the control (deionized water), which increased towards more neutral values after two days (Figure 1). The reasons may be attributed to the occurrence of swelling and increased seed respiration, resulting in increased CO_2 concentrations. Since the test was done in closed incubators with no aeration, it is possible that CO_2 was dissolved in the solutions, which caused oscillations, especially in the solutions with lower pH values.

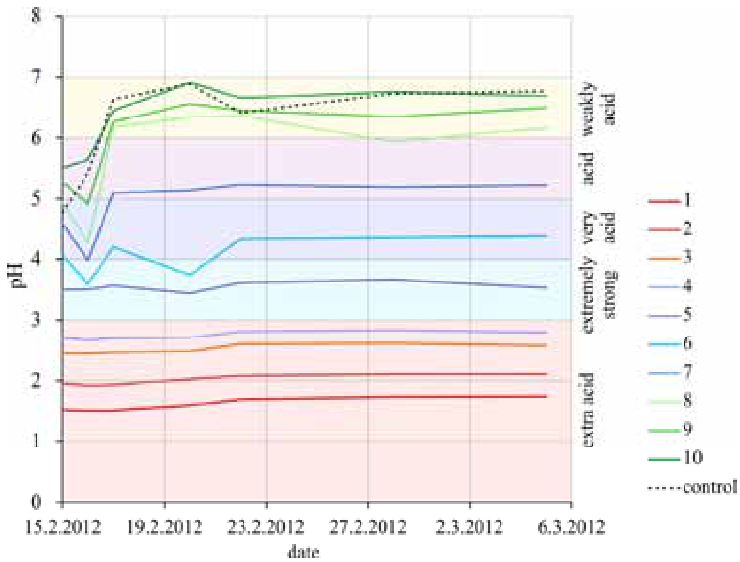

Figure 1: Measured pH values of the solutions during research

Germination energy, pH quantitative germination analysis

There were significant differences in average pH values among all the repetitions, except repetition 10 and the control. The established pH values ranged from 1.72 to 6.71. Overall germination after 21 days of testing showed significant dependence on pH values of the solutions. Thus, the repetition with a low average solution value manifested distinctly low germination percentage and very high percentage of dead and irregularly germinated seeds.

After 21 days, not one seed germinated (samples 1 and 2) in the repetitions with pH values up to 2.10. There were 44% of bad seeds on average. In general, pH values lower than 2.6 have an adverse effect on seed germination (repetitions 1, 2 and 3). They have distinctly low germination energy, a small number of normal seedlings and a large proportion of damaged or dead seeds. The largest number of normally germinated seeds was found at pH values between 3 and 6.7, 70 % on average, with only 20% of bad seeds, of which 9% were decayed.

Table 1: Average values of measured variables according to different pH values of the solutions (df = 4)

Sample	pH	Normal seedlings (%)	Non-germinated seeds (%)	Decayed seeds (%)	Bad seedlings (%)	Germination energy (%)
1	1,72	0,00	8,75	30,25	39,00	0
2	2,10	0,00	24,50	24,25	48,75	0
3	2,60	37,50	33,75	13,25	47,00	11,5
4	2,81	55,50	19,25	10,25	29,50	20
5	3,60	68,50	14,25	7,75	22,00	11,5
6	4,36	69,00	10,00	9,00	19,00	23
7	5,21	75,50	9,75	6,75	16,50	21,5
8	6,16	70,75	10,00	7,50	17,50	28
9	6,43	68,75	10,75	11,50	22,25	25,75
10	6,71	71,75	9,25	9,25	18,50	32,5
Control	6,64	69,75	12,25	9,50	21,75	16,75

The results of one-way variance analysis (One way ANOVA) indicate a statistically significant difference in the germination energy between repetitions 1 and 2, which have the lowest pH values (1.6 and 2.0) in relation to the rest (Table 2). A significant difference in the average values was also recorded for the percentage of normally germinated seeds between repetitions 4 to the control and the most acid solutions (sample 1, 2, 3).

Table 2: Results of Post-hoc test between repetitions with regard to pH values of the solutions

Samples	Normal seedlings	Non-germinated seeds	Decayed seeds	Ph	Germination energy	Bad seedlings
1	3-Cont	2,3	1,3,4,5,6,7,8,9,10, Cont	all different	4,6,7,8,9,10, Cont	
2	3-Cont	6,7,8,10	5,7,8		4,6,7,8,9,10, Cont	6,7,8,10
3	1	1,4,5,6,7,8,9,10, Cont	1		10	6,7,8,11
4	1,2,3	3			1,2	
5	1,2,3	3	2		10	
6	1,2,3	3			1,2	2,3
7	1,2,3,4	3	2		1,2	2,3
8	1,2,3	3	2		1,2	2,3
9	1,2,3	3			1,2	
10	1,2,3,4	3		No. Diff.	1,2,3,5	2,3
Cont	1,2,3	3			1,2	

The highest amount of non-germinated seeds, reaching 48%, was recorded in repetitions 2 and 3 (pH 2.10–2.60). In the most acid solution (repetition 1), the percentage of non-germinated seeds was low, but the number of damaged (decayed) seeds was 39%, which indicates physiological degradation of seed husk and tissue.

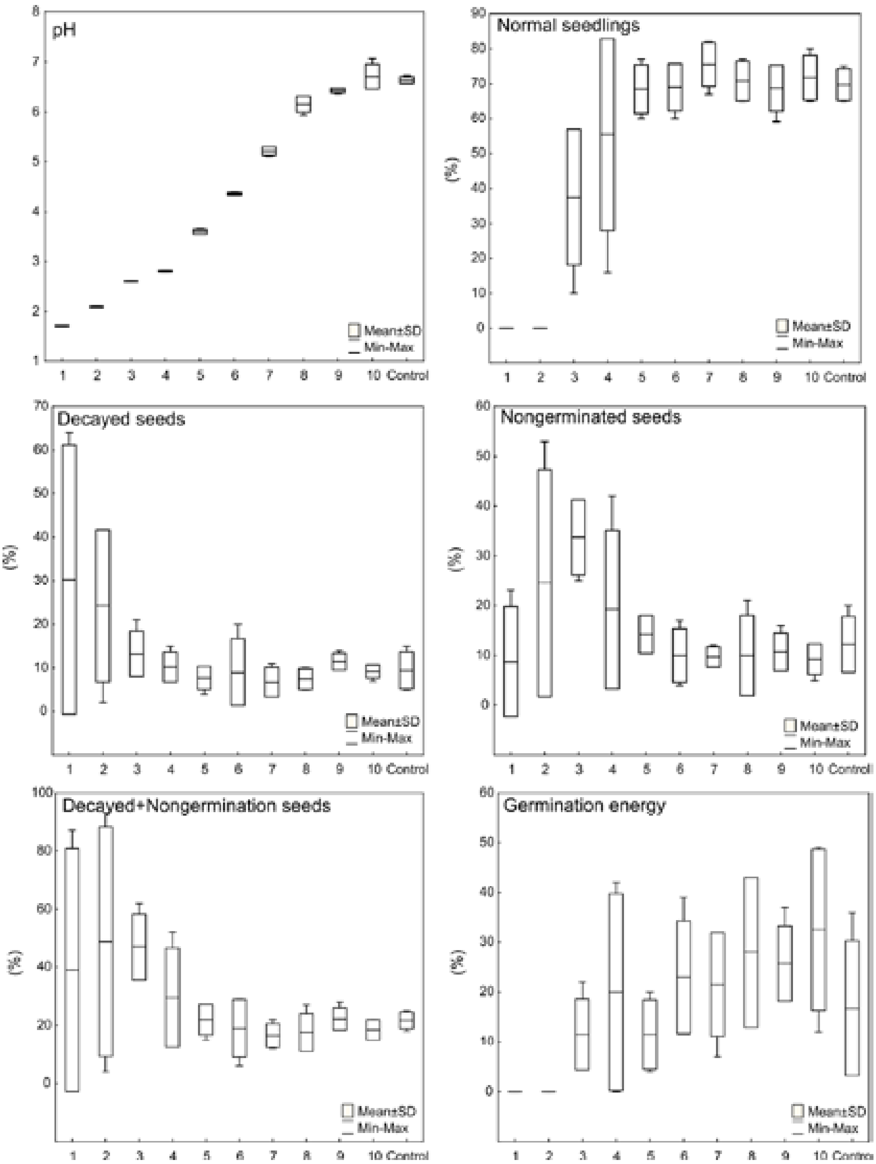

Figure 2: Box-Whisker plot according to the studied variables

Figure 3 shows the mean values and the range of the tested seed characteristics. Repetitions 3 and 4 manifested the highest range of normally germinated seeds, whereas repetitions from 5 up to the control had equal values.
The correlation between normally developed seedlings and pH values is given in Figure 3. According to this research, the threshold value for spruce germination with regard to the laboratory value of pH solution amounts to slightly less than pH 3. The average amount of normally germinated seeds at pH values higher than 3 is about 70%.

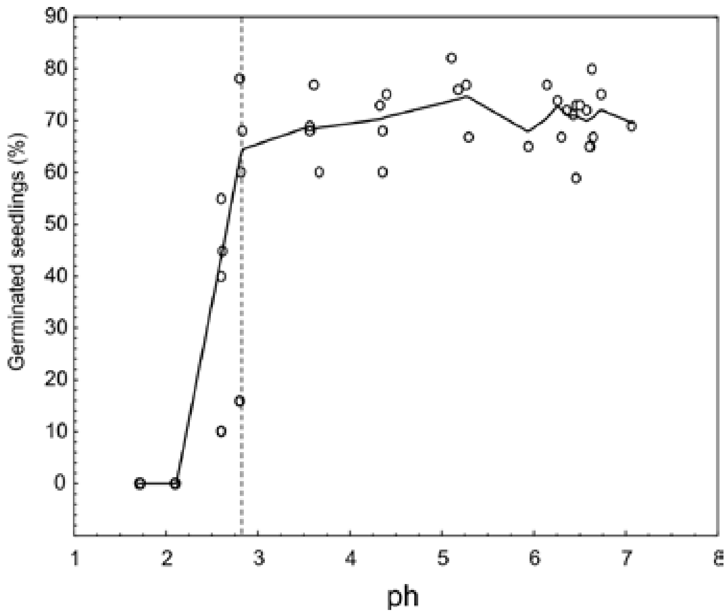

Figure 3: Correlation between germinated seeds and pH values. The broken line indicates the threshold pH value at which the number of germinated seeds is averaged with the control

Soil pH values in Croatia extend from 2.70 to 8.10. Lower pH values are mostly distributed in north-western Croatia, on Mt Bilogora, in the Pokuplje basin and in the Pannonian uplands. Higher values (more neutral soils) are generally distributed in the Dalmatian hinterland, the islands and in north-eastern Baranja. Of a total of 2,222 soil profiles in which soil values were measured, only 0.38% exhibited pH values lower than 3. The average pH value is 5.71±1.26, and the maximally measured one is 8.1. It should be stressed that these values are significantly affected by the anthropogenic factor, which includes forest management, forest and forest soil degradation and agriculture.

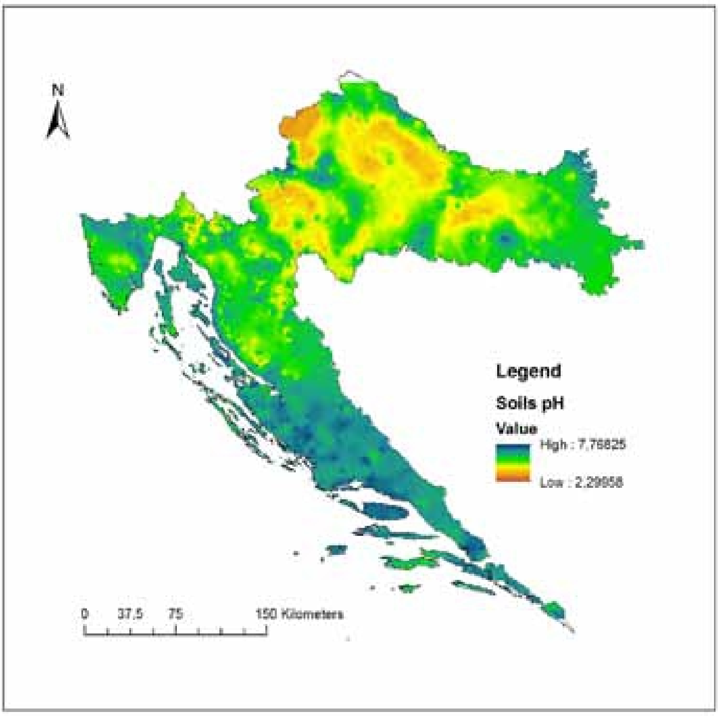

Figure 4: Spatial distribution of soil pH values in Croatia

Discussion

In his research of laboratory germination of spruce seed from the Velebit mountain range, Oršanić (2001) achieved average germination of 27% (min. 13%, max. 48%) from a sample of six experimental plots. He also states that natural germination is definitely lower than laboratory germination due to numerous adverse ecological factors occurring in the area of natural spruce distribution. Oršanić (2008) mentions the poor yield and quality of Norway spruce seeds in the investigated period. The author emphasises the correlation between population elevation and seed germination and concludes that seed germination is lower at higher elevations. According to Regent (1980), the average germination of spruce seed is about 70%. According to Frehner and Fürst (1992), germination ranges between 85–95%, whereas Dirr and Heuser (1987) report the germination capacity of 80%.

Leyton (1952) investigated the effect of pH and nitrogen forms (NH_4 iNO_3) on the growth of Sitka spruce (*Picea sitcbensis* Carr.) seedlings in a nutrient solution at a pH range of 3 to 7. In both nitrogen forms, the results showed an optimal growth of the aboveground and underground part of the seedlings at a pH between 4 and 5. Above and below this pH threshold, a progressive decrease was recorded in the production of dry matter, accompanied by an increase in the irregular growth of the root system. Nitrogen forms had a small effect on the overall growth at a certain pH, but the nitrogen form appears to stimulate root development better than the ammonia form. For this reason, the ratio between the root and the aboveground part changes.

Abouguendia a Redmann (1979) studied the effect of buffer solution (pH 2.2, 3.0, and 9.0) on seed germination and the initial seedling growth of the species *Pinus contorta* in the laboratory. Seed germination of *Pinus contorta* at low pH values was higher than in the control (distilled water, pH 6.5), seed germination of the species *Picea glauca* and *Pinus banksiana* at low pH values was equal to germination in distilled water, while germination of the species *Picea mariana* was significantly lower. It can be concluded that the results of this research into Norway spruce seed coincide with the research of the above mentioned authors only in the case of the seeds of *Picea mariana,* which evidently react to low pH of the solution similarly to the reaction of the seeds of *Picea abies*. In extremely alkaline conditions (pH 9.0), seed germination of the species *Pinus contorta, Picea glauca, Pinus banksiana* and *Picea mariana* was lower than seed germination in distilled water. Seedling growth of these tree species was statistically significantly lower at high and low pH values in relation to the control.

Seedlings of *Pinus contorta*, unlike the other mentioned species, grow best at a pH of 9.0, while seedlings of *Pinus banksiana* grow best at a pH of 3.0. In order to improve our research into seeds of Norway spruce, we should test germination and the initial seedling growth in a pH solution higher than 6.0.

Scherbatskoy et al. (1987) did not record any decrease in seed germination of the species *Picea rubens* Sarg., *Abies balsamea* L., *Betula alleghaniensis* Britt a *Betula papyrifera* Marsch at low pH values and in the presence of phytotoxic metal ions. Seeds of *Abies balsamea* L. and *Betula alleghaniensis* manifest significantly better germination at pH 3 compared to pH 4 or 5. These investigations confirmed that acid soils and soils contaminated with heavy metal ions in coniferous forest ecosystems growing at high elevations do not influence the germination of the studied tree species.

Raynal et al. (1982) tested seed germination of the species *Acer saccharum*, *Acer rubrum*, *Betula lutea*, *Tsuga canadensis* and *Pinus strobus* in differently acid substrates. Seed germination of *Acer rubrum* was inhibited at pH 4.0 and 3.0, and that of *Betula lutea* at pH 3.0. Control seed was tested at pH 5.6. For the seeds of the species *Acer saccharum* and *Tsuga Canadensis,* germination was not inhibited at low pH values. The lower the substrate pH, the higher the seed germination rate of the species *Pinus strobus*. Maximal germination was achieved at the pH values between 2.4 and 3.0. This research showed a broad range of susceptibility of certain tree species in terms of seed germination at low pH values.

Research of this kind is of great practical importance in the selection of tree species for phytoremediation of quarries, marginal soils and other soils with extreme pH values. Similar research is conducted in other fields of natural sciences (agronomy), where impacts of pH water solutions on germination of different sorts are examined. Grljušić *et al.* (2007) investigated soya germination and found that pH values of water solution, with the exception of germination energy, significantly affected the investigated properties. On average, germination was the highest at a pH of

5, and the lowest at a pH of 8. The root and the hypocotyl of the seedlings were significantly longer at pH 5 and 6. Demirezen Yilmaz and Aksoy (2007) found that pH 6.0 (98%) was optimal for seed germination of the species *Rumex scutatus* L. (*Polygonaceae*). Seed germination is inhibited at pH values lower than 6.0 (particularly at pH 4.0).

Hou a Wang (2000) investigated the effect of simulated acid rain adjusted to pH values of 2.0, 3.5, 5.0 and 6.0, and to the control (distilled water) on seed germination of five hardwood species (*Cinnamomum camphora* L., *Ligustrum lucidum*, *Castanopsis fissa* Rehd. et Wils., *Melia azedarach* L. and *Koelreuteria bipinnata* Franch.). Seed germination was remarkably inhibited by pH 2.0 treatment for three species. Significant foliar damage, decline in chlorophyll contents and retardation of growth for the seedlings of all the species were observed at pH 2.0. Seedling growth was stimulated at pH levels between 3.5 and 5.0. The authors found the critical threshold level for inhibition of seed germination and seedling growth. According to Puchalski a Prusinkiewicz (1990) and Schmidt-Vogt (1991), optimal conditions for seed germination of Norway spruce are in the substrates with good water-air ratios, pH values around 5.5 and temperatures between 15 and 25 ºC. These authors emphasise that light does not affect seed germination of Norway spruce, but is highly important at a certain stage of plant growth. In Norway, Abrahamsen (1976) found that pH values below 4.0 decrease seed germination of Norway spruce. Our research of Norway spruce seeds confirmed the threshold pH level of 2.5, below which no regular seedlings were recorded (pursuant to the ISTA Rules). We can improve research with seeds of Norway spruce by measuring the morphological parameters and physiological condition of the seedlings in different pH solutions. Hrabri (2002) records seed fullness of Norway spruce from 78.8 to 95.5%. According to the anatomical analysis, the content of non-vital seeds ranged between 1.2 and 6.7%, and according to the biochemical analysis it ranged between 1.9 and 11.2%. Seeds with several embryos were found in seven samples. Such seeds amounted to a maximum of 3.5%. This author recorded germination energy between 0.8 and 14.5%, or total germination between 43.3 and 67.5%. There were 8% of irregular seedlings found in 7 samples. Such seeds germinated with the green parts of the epicotyl. This research showed that seed germination energy in deionized water reached 16.75%, and the total participation of irregular seedlings was 12.25%. In both cases, this is slightly more than the values cited by Hrabri (2002).

Conclusions

Related to research on the effects of different pH concentrations on seed germination of Norway spruce in laboratory conditions, we may conclude the following:
The threshold pH value of the solution at which seed germination was recorded was 2.6. On average, high seed germination was recorded at pH values higher than 3. The highest germination energy was recorded in weakly acid repetitions, and the highest number of damaged and deformed seeds was found in extremely acid solutions.

Natural pH concentrations in the soils of Croatia are above the threshold germination value of 2.6, and only a small part of the soils (0.38%) have pH values lower than 3, which indicates favourable natural pH concentrations in the soils of Croatia. The conducted research also indicates changes in the pH values of deionized solution, which is as a rule used in close type incubators. This is related to the process of CO2 dissolution that occurs with seed respiration. Future research should be directed towards explaining the phenomenon of close type incubators.

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