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Original Research

Influence of Heavy Metals on Seed Germination and Growth of *Picea abies* L. Karst

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Abstract

This paper presents the influence of heavy metals on the process of seed germination and the survival rate of *Picea abies* L. Karst spruce seedlings. The experiments were conducted in laboratory conditions by adding solutions of compounds of zinc, copper, lead, and cadmium at three different concentrations (3 ppm, 33 ppm, and 100 ppm) to the media.

The observed heavy metals affected the germination of the spruce seeds in different ways. Although the seed was tolerant to the presence of all metals, the percentage of germinated seeds depended on the type of metal and its concentration. The lower concentrations of the heavy metals (3 and 33 ppm) partially inhibited seed germination, and the highest concentrations (100 ppm) of all metals caused total inhibition.

The *P. abies* L. Karst seedlings were very tolerant to the presence of all metals. The highest concentrations of cadmium and copper had a significant influence on the decrease of the number of the seedlings that survived, as well as on the decrease of biomass in comparison with lead. Zinc had the least adverse effect on the growth and survival of seedlings.

Keywords: spruce seed, germination, tolerance to heavy metals, survival and growth of seedlings

Introduction

Some heavy metals are essential to the plants as trace elements (Cu, Mn, Fe, Zn) since they are included in metabolic processes [1, 2]. In spite of their usefulness in small quantities, these elements become toxic to plants and microorganisms when they are present at higher concentrations in the soil. Heavy metals such as lead and cadmium are not essential to plants, i.e. are not elements that are necessary for the functioning of plant organisms [3, 4]. The presence of the heavy metals was reported as trace elements, and they are present in all non-polluted soils as a result of the decomposition of the parent rock. The heavy metals that accumulate in the soil are removed slowly, not only by wash-off, but also by the adoption by plants, or by erosion and deflation. As a result, they are widely spread in soil, plants, and animals. They affect microbial populations by changing size, diversity, and activity of microorganisms [5], and have adverse effects on seed germination and growth of plants, which is unfavourable to the forest ecosystem [6]. Accumulation in the upper soil layers could affect the establishment of seedlings and forest regeneration [7]. The behaviour of the heavy metals in the soil depends on the soil characteristics, mainly on the pH value, cation exchange capacity (CEC), content of the nutrients, content and type of clay, iron oxides, manganese, and aluminium [8, 9]. The layers of forest litter and surface organic miner-

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al layers, as a rule, contain the greatest concentrations of heavy metals. The main source of these elements in the surface soil layers is air pollution [10]. Kadovic and Knezevic [8] state that it particularly refers to lead, cadmium, copper, and zinc in the conifer forests, where, alongside an unfavourable degree of acidity, has an adverse effect on the process of seed germination and seedlings growth.

The tolerance of some tree species to the presence of heavy metals is the result of the peculiarities of these species [11, 12]. Knowledge of these peculiarities is necessary in order to make the proper selection of the species, particularly for the sake of the bioremediation of the polluted areas [13, 14] and regions that are degraded due to the anthropogenic excessive use of natural resources [15].

Some plants phytostabilise HMs in the rhizosphere through root exudate immobilization [7], while other species of plants incorporate metals into root tissues [16]. Some plant species are able to transfer metals to their above-ground tissues, potentially allowing the soil to be decontaminated by harvesting the above-ground parts of the plants [17].

Spruce, *Picea abies*, due to its modest requirements concerning the environment, alongside Austrian and Scots pines, is one of the most frequently used species for reforestation in Serbia [18]. Alhough it belongs to a species that favors acidic soils, it adapts even to limestone soils [19]. It is tolerant to unfavourable environmental conditions [20, 21], since this species also survives in areas where the acid rain or the deposition of nitrogen, potassium, or phosporous were reported [22, 23].

This paper studies the influence of heavy metals on the germination of spruce seeds and on the development of biomass of seedlings. The obtained results point out to the tolerance of this species in the phase of seedlings, since the high level of survival and the successful growth of biomass on the soils with heavy metals were reported. These results of laboratory studies can be applied in the process of reforestation of areas in whose subsoil the presence of the heavy metals was reported.

Experimental Procedures

The spruce seed used in the experiment was collected in Jastrebac Mountain, in the area managed by the Forest Enterprise Rasina from Krusevac, which is a part of Srbijasume State Enterprise. It was collected in the seed facility, registered under number C 01.02.01.10.

The coordinates are in the degrees of Gauss-Kruger Projection: φN 430 26', λEGr 190 02', the facility is located at altitudes ranging from 500 to 560 meters, and it is on the western exposure. The spruce stand is artificial-autochonous and even-aged (58 years old).

Laboratory analysis of the germination and seed health condition was conducted in the Institute of Forestry in Belgrade. Seed purity is 98.6% and there is 1.4% of the inert ingredient. Seed germination rate after 10 days is 75.6%, and viability of the germination seed is 81.0%. The content of moisture (using the method of drying at 105ºC) is 7.02%. The weight of pure seed in a 1,000-seed sample is 7.4 g.

The segment on the health condition was added in accord with the legally enforced rules on the health condition of seeds in Serbia:

- By studying surface infection, the presence of the following species was reported: 0.5% of *Aspergilus* spp., 0.3% of *Trichotecium roseum* Link. By studying the internal infection, the presence of neither saprophytic nor parasitic fungi was reported.
- By macroscopic and microscopic analyses of the internal and external infections, the damages made by *Cydia strobillela* L., *Dioryctria abietellia* Schiff., and *Ernobius abietis* F. (the types of pests the concentrations of which by the regulations of Serbia must not exceed 2%) were not reported

Study of the Influence of Heavy Metals on Seed Germination

The effect of the influence of the heavy metals on the germination of spruce seed was studied in laboratory conditions. The spruce seed was sterilized in 30% H_2O_2, for 20 minutes, and it was rinsed with sterile water and planted in Petri dishes. The double layers of filter papers soaked in solutions containing zinc, copper, lead, or cadmium in three different concentrations (3 ppm, 33 ppm, and 100 ppm) [24] were placed on the bottom of Petri dishes. These solutions are made from the salts of these metals in the form of the following chemical compounds: $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $Pb(COOH)_2$, and $CdSO_4{\cdot}8H_2O$, and before soaking the filter paper was sterilized in an autoclave at 120ºC under the pressure 1.5 at for 20 minutes. pH value of all suspensions was 5.5, except for copper, since its pH was 4.5. Petri dishes in which the filter paper was soaked only by the same quantity of the sterile water were used as the control variance. All Petri dishes with the seed were placed in the thermostat at 22±1ºC and the effectiveness of the seed germination by reporting the number of the germinated seeds and determining the period which is needed for it was monitored.

The Study of the Influence of Heavy Metals on Survival and Growth of Seedlings

The effects of the influence of the heavy metals on the survival and growth of seedlings were observed in controlled laboratory conditions. The spruce seedlings were obtained by placing seeds on WA medium (15 g of agar and distilled water up to 1.000 ml) in Petri dishes in the thermostat at 26ºC. Two weeks after placement the roots of the seedlings were about 2 cm long and were transported into the test tubes with thiamine-agar medium (15 g of agar, 100 mg of thiamine, and distilled water up to 1.000 ml) [25]. Upon the sterilization of thiamine-agar medium the solution of the salts of the heavy metals at concentrations 3, 33, and 100 ppm was added, and then one seedling on the upper, slanting part of the medium was added. The test tubes were closed by sterile screw caps and placed in an upright position in the stand so that the medium and roots are in the sheltered part of the stand, without the inflow of light.

In the laboratory conditions the studies were conducted in controlled conditions at 25±2ºC under flourescent light during the photoperiod – 16-hours of light and eight hours of darkness. The density flux of light to which the seedlings on the stands were exposed was 47 $\mu mol \cdot s^{-1} \cdot m^{-2}$ [26-28]. The light density flux was measured by the instrument Quantum Sensor Li-190 SA with Li-100 Datalogger (Lincoln-Nebraska, USA). The growth, development and survival of seedlings were monitored for two months.

Statistical Data Processing

The results of the measurement are synthesized in the suitable formats of Excel software, and their statistical processing was done in the software package Statgraph. The mean values and mean errors were determined, and analysis of variance (ANOVA-Duncan method) was used to determine the statistical significance of the difference between the variances.

Results

Seed Germination in the Presence of Heavy Metals

The percentage of germinated *P. abies* seeds was greatly affected by the type of metal and its concentration.

The presence of zinc at the lowest concentration did not influence the decrease of the number of the germinated seeds over a period of research (Fig. 1). The concentration 33 ppm caused a decrease of the number of germinated seeds. Only the highest concentration of this metal (100 ppm) completely prevented the seeds from germination.

The presence of copper at concentrations of 3 ppm and 33 ppm caused the greater decrease of the number of the germinated seeds in comparison to the presence of zinc at these concentrations, although the determined pH value was 4.5.

The presence of lead (Fig. 2) in the medium had similar effects on seed germination as the presence of copper (Fig. 1), i.e. it caused the decrease of the number of the germinated seeds in comparison with the control variance and the variance to which zinc was added. The medium to which lead at a concentration of 33 ppm was added did not delay the beginning of germination, which was reported in the presence of copper at this concentration.

Cadmium had the most toxic effect on the spruce seed germination of all observed heavy metals (Table 1). At a concentration of 3 ppm (Table 2) the number of the germinated seed significantly decreased, and at the higher concentration (33 ppm) the adverse effect was reflected in both the decrease of the number of the germinated seed and in the delayed beginning of germination. In the presence of the highest concentration 100 ppm germinated seed was not reported.

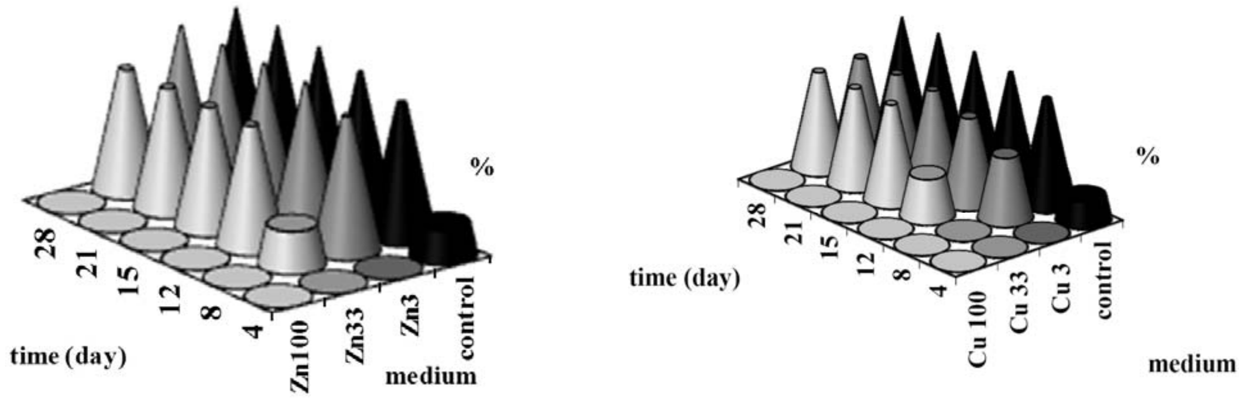

Fig. 1. Seed germination (%) *P. abies* in the control variance and at different zinc and copper (ppm) concentrations.

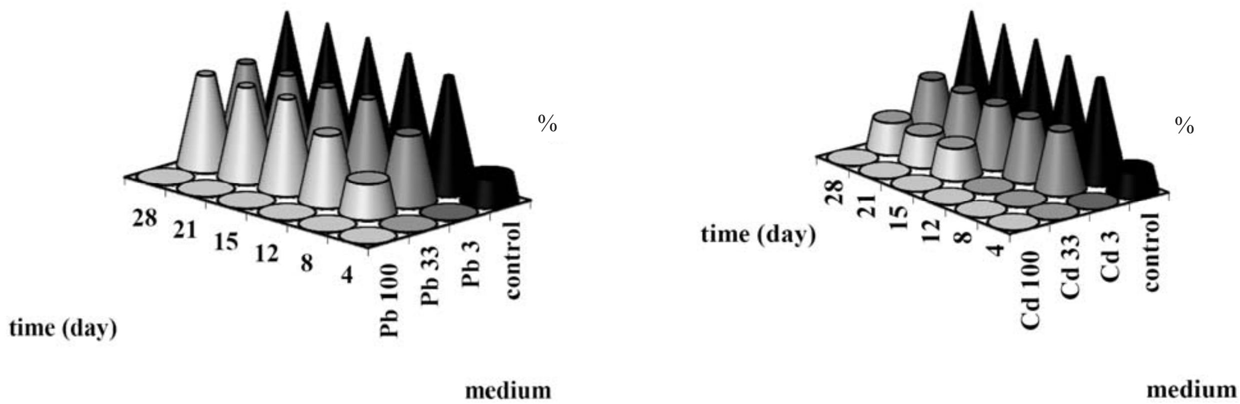

Fig. 2. Seed germination (%) of *P. abies* in the control variance and at different concentrations of lead and cadmium (ppm).

Table 1. Analysis of variance for seed germination (%), depending on metal type.

Metals	Number of samples	Mean values	Homogeneous groups
Cd	90	18.0389	A
Pb	90	32.9556	B
Cu	90	37.9444	BC
Zn	90	43.0756	C
Control	30	79.0667	D

Table 2. Analysis of variance for seed germination (%), depending on the treatments.

Treatment	Number of samples	Mean values	Homogeneous groups
4 (100 ppm)	120	0.0	A
3 (33 ppm)	120	42.3167	B
2 (3 ppm)	120	56.6942	C
1 (Control)	30	79.0667	D

The Study of the Influence of Heavy Metals on Survival and Growth of Seedlings

The influence of heavy metals was monitored in the test tubes with the thiamine medium to which the solutions of the heavy metals were added after sterilization by autoclaving. The influence of the metals was reflected in seed germination.

P. abies seedlings that developed on the slanting thiamine medium with three different concentrations of metals were considerably tolerant to the presence of all metals. The greatest number of the desiccated seedlings (above 70%) was reported in the variances in which the concentrations of cadmium and copper were 100 ppm. The smallest number of the desiccated seedlings (ranging from 7.1% to 16.7%) was reported in the variances in which the metals are present at the lowest concentration (3 ppm). On the control medium the desiccated seedlings were not reported (Table 3).

After the experiment the seedlings were removed from the test tubes with the medium and washed by the distilled water several times. After air drying the lengths of the aboveground part, tap-root, cotyledons, and primary needles were measured, and the mean values of these parameters were determined (Table 4).

The lengths of the aboveground part of *P. abies* is similar in all variances. The results of measuring of lengths of roots considerably differ. Since the presence of zinc in the quantities 3 ppm stimulated the seedlings, the highest value was reported. The length of cotyledons range from 11.2 to 16 mm, which insignificantly differs from the standard, which implies an average value ranging from 12 to 15 mm [29]. The primary needles are not developed in all variances, and the reported values, ranging from 3 to 9 mm, are considerably below the standard values, which range from 10 to 12 mm [30].

The needle chlorosis is very frequent in the variances (variants) with the presence of copper, cadmium and lead at all observed concentrations of metals, and is most expressed in the variances with the presence of cadmium. This phenomenon was not reported only in the variances with the presence of zinc.

Given the fact that in all variances the reported length of the tap-root is significantly greater or the same as in the control variance, it can be concluded that the presence of the observed metals stimulated the formation of the tap-root.

Discussion

The seedlings were more tolerant to the presence of heavy metals than the seeds. The seed germinated in all variances only at concentrations of 3 and 33 ppm. At the lowest concentrations the similar results were obtained on the media with cooper, lead, and cadmium, whereas in the presence of zinc the reported germination was even 92%. Since the highest concentrations (100 ppm) have adverse effects on germination, no germinated seeds were reported. The seedlings were more tolerant to the highest concentrations of the heavy metals than the seed as the greatest number of the seeds that survived in such conditions as well was reported.

The addition of zinc had the smallest adverse effect on germination, growth, and survival of seedlings. This metal is characterized by its high biological availability to the plants, is one of the ferment components, and has a role in the auxin synthesis [2, 3], due to which the highest concentrations of this element are mainly found in the root system and young leaves, whereas the considerably lower concentrations are found in the bark. When it is present in sufficient quantities it increases the resistance of plants to the lack of moisture and to diseases, which contributed to the survival of a great number of seedlings. The high percentage of this metal has a phytotoxic effect, by which the inability of seed germination in these conditions can be explained. The presence of copper at all concentrations had a similar effect on seed germination as the presence of zinc (Fig. 2).

Table 3. The influence of different concentrations of heavy metals on desiccation of *P. abies* seedlings.

Treatment (ppm)	Control	Zn			Cu			Cd			Pb		
		3	33	100	3	33	100	3	33	100	3	33	100
Desiccated seedlings (%)	0.0	7.1	12.5	23.1	16.7	16.7	72.7	10.0	20.0	71.5	8.3	33.3	50.0

Table 4. Mean values ($\bar{x}$) and standard errors (±SE) of the measured paramets of growth of *Picea abies* seedlings grown on the control medium and on media with different concentrations of heavy metals (ppm).

Treatment		Shoot length (mm)	Root length (mm)	Length of cotyledons (mm)	Length of primary needles (mm)
		$\bar{x}$±SE	$\bar{x}$±SE	$\bar{x}$±SE	$\bar{x}$±SE
Control		43.4±0.43^{a}	27.0±0.51^{b}	12.5±0.02^{b}	6.2±0.03^{b}
Zn	3	44.2±0.56^{a}	45.2±0.24^{a}	14.6±0.04^{a}	9.5±0.45^{a}
	33	40.0±0.79^{b}	38.7±0.35^{a}	14.0±0.17^{a}	7.2±0.05^{b}
	100	33.0±0.45^{c}	23.5±0.42^{bc}	13.6±0.23^{a}	6.3±0.23^{b}
Cu	3	46.5±0.37^{ab}	26.7±0.51^{b}	13.5±0.19^{a}	9.7±0.19^{a}
	33	36.0±0.41^{b}	26.5±0.48^{b}	12.5±0.05^{b}	4.2±0.21^{c}
	100	32.5±0.39^{c}	22.0±0.39^{bc}	11.2±0.04^{bc}	3.0±0.23^{c}
Cd	3	35.5±0.42^{b}	30.0±1.2^{ab}	11.7±0.08^{bc}	4.5±0.31^{c}
	33	32.1±0.38^{b}	24.5±0.59^{ab}	11.5±0.09^{bc}	3.0±0.21^{c}
	100	28.5±0.56^{c}	22.1±0.55^{bc}	10.6±0.04^{c}	3.7±0.22^{bc}
Pb	3	36.2±0.45^{b}	26.1±0.47^{b}	12.5±0.03^{b}	5.8±0.25^{ab}
	33	34.8±0.47^{b}	25.5±0.58^{bc}	12.3±0.03^{b}	5.5±0.23^{ab}
	100	29.5±0.56^{bc}	23.7±0.66^{c}	11.5±0.02^{b}	5.3±0.21^{ab}

Each value is expressed as mean±standard deviation (n=10). In each row different letters mean significant differences ($P<0.05$).

In plants, cooper is an esential component of many enzymes and non enzyme proteins involved in electron transfer in many processes, including photosynthesis, respiration, and lignification [31]. Solubility, mobility, and availability to the plants to the greatest extent depends on pH of the environment, is drastically reduced when pH is above 7, and is available when pH is below 6 (particularly when it is below 5) [10, 32]. As in our research, pH value was 4.5, the morphometric qualities of the seedlings insignificantly differ from the control variance or from the presence of zinc.

The presence of lead in the medium had a similar effect on a seed germination as the presence of copper (Fig. 2), i.e. the number of the germinated seeds was smaller in comparison with the control variance and with the variance to which zinc was added. Since the differences over the period which is needed for the start of germination are significant, on the medium with lead at concentration 33 ppm the start of germination was reported in the shorter interval in comparison with the presence of copper at this concentration. Although lead belongs to the group of the least mobile metals, it is present in soil to a great extent. The natural content in the soil originates from the parent rock or from pollution and frequently decreases from the surface, owning to the ability to fix with the suspensions of the nutrient it migrates through the profile. Its low mobility in the soil increases owning to the increase of acidity as well, which is also reported for copper. This limited availability of lead to plants from the soil solution to the lower phytotoxicity is in comparison with copper and cadmium, although the plants are mainly very sensitive to the presence of lead [1, 33]. Lead tolerance is associated with the capacity of plants to restrict lead to cell walls, synthesis of osmolytes, and activation of the antioxidant dafense system [34]. In this experiment the considerably lower phytotoxicity of lead was reported, since the smaller number of the desiccated seedlings was determined at the highest concentrations.

Cadmium had the most toxic effect in comparison with cooper, lead, and particularly zinc [35]. The plants adopt cadmium in a passive way by the root system and assimilating organs [36, 37]. It is mainly accumulated in the root, but the plants adopt it more intensively and transport it to the aboveground plants than lead, and when there is increased content of cadmium in the air it is more intensively absorbed through needles. Cadmium uptake in *Picea abies* is a metabolically controlled proces [38] and it was reduced by low pH. The higher concentrations of cadmium inhibited iron metabolism, which caused chlorosis as a result of less intensive phytosynthesis [39], and led to the inhibition of transpiration [2, 40]. Owning to it, the needle chlorosis in the variances with higher concentrations of cadmium in this experiment occurred.

Conclusion

Although heavy metals affected seed germination and the growth and survival of spruce seedlings, this plant species is very tolerant. From the results of our research it can be concluded that the spruce should be selected for the reforestation in Serbia, particularly of the soils in whose subsoil the presence of these heavy metals was reported.

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