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THE EFFECT OF ELEVATED Zn CONCENTRATIONS ON SEED GERMINATION AND YOUNG SEEDLING GROWTH OF *AILANTHUS ALTISSIMA* (MILL.) SWINGLE

Abstract: Some heavy metals such as Zn are essential micronutrients for plants. However, in a high concentration in soils they could cause repression of growth with toxicity symptoms. It is therefore very important to investigate the species that are tolerant to high concentration of zinc which have the ability to survive on soils contaminated with heavy metals. This paper presents an investigation of the effect of elevated Zn concentrations on the germination and seedling growth of the invasive tree species *Ailanthus altissima* (Swingle) Mill., aimed at a better understanding of its adaptation ability to heavy metal stress and potential use in phytoremediation. Seeds of *Ailanthus altissima* were treated with Zn concentrations of 25, 100 and 250 μM in form of zinc sulfate. After germination, the seedlings at the stage of first leaf development were transferred into a growing chamber in the hydroponic system where zinc sulfate was added in the concentrations of 100, 250 and 500 μM. The results showed that Zn treatments, including the highest concentration, have caused a reduction in seed germination parameters. Considering the hypocotyl and radicle length, there was no significant statistical difference among the treatments, but further growth of young seedlings in hydroponics under high concentrations of Zn, cause an inhibition of the root system growth and repress development of assimilating organs. Seeds and seedlings of *A. altissima* are tolerant to elevated Zn concentrations at early stages of development. However, later on with leaves development, the inhibitory effect of the strongest concentrations is expressed.

Key words: *Ailanthus altissima*, zinc, toxicity, seed germination, seedling growth, phytoremediation

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УТИЦАЈ ПОВЕЋАНИХ КОНЦЕНТРАЦИЈА Zn НА КЛИЈАЊЕ СЕМЕНА И РАСТ СЕЈАНАЦА *Ailanthus altissima* (Mill.) Swingle

Извод: Неки тешки метали, као што је цинк, су есенцијални микронутријенти неопходни биљкама за обављање важних физиолошких процеса. Међутим, повећане концентрације цинка могу имати негативне ефекте на раст и развој биљака и проузроковати појаву симптома токсикације. Због тога је врло важно испитати које дрвенасте врсте су толерантне на повећане концентрације цинка и које се одликују способношћу преживљавања на земљиштима контамираним тешким металима У раду је испитиван ефекат повећаних концентрација цинка на клијање семена и развој клијаваца инвазивне дрвенасте врсте *Ailanthus altisima* (Swingle) Mill. ради бољег разумевања адаптивних способности ове врсте на стрес од тешких метала и могућности коришћења у фиторемедијацији. Семена врсте *Ailanthus altissima* третирана су цинк сулфатом у концентрацијама 25, 100 и 250 μM. Након клијања, клијавци су премештени у комору за гајење у хидрокултуру где је цинк сулфат додат у концетрацијама од 100, 250 и 500 μM. Добијени резултати указују да третмани цинком, укључујући и највећу концентрацију, нису у потпуности инхибирали клијање семена иако је дошло до смањења показатеља клијања. Анализирајући дужину хипокотила и коренка, утврђено је да не постоји значајна статистичка разлика између различитих третмана. У даљем гајењу у хидропонском експерименту, цинк је инхибирао развиће листова и корена. Анализирани квантитативни параметри клијања и морфометрија клијаваца *A. altissima* показују толерантност на повећане концентрације цинка, у овим најранијим фазама развића, док се у фази формирања првих листова уочава инхибиторни ефекат већих концентрација.

Кључне речи: *Ailanthus altissima,* цинк, токсичност, клијање семена, раст младих клијаваца, фиторемедијација

1. INTRODUCTION

An increasing contamination of soils with toxic metals has become a great problem due to rapid industrialization, urbanization and intensive agriculture. These metals tend to accumulate in soils and cannot easily be removed or degraded by chemical or microbial processes. It is of great interest to find environmentally acceptable and sustainable ways to remove them from polluted soils. Zn accumulation in the environment is due to mining, chemical industry, burning of fossil fuels, and agricultural utilization of fertilizers and herbicides (Zn chloride and Zn sulfate) (Päivöke, 2003).

An excessive level of heavy metals in soils can have a negative impact on seed germination, biomass production (Kukkola, et al., 2000) root growth (Arduini, *et al*., 1995), root morphology and architecture (Arduini, *et al*., 1994; Schmidt, 1997). It can also interfere with the activities of many key enzymes related to normal metabolic processes (Dhankhar, 2011 according to Ahssan, *et al*., 2007; Kuriakosa & Prasad, 2008; Kachout, *et al*., 2009; Zhang, *et al*., 2009; Rahoui, *et al*., 2010). Many researchers have examined the potential use of metal resistant plants, primarily herbaceous ones in the stabilization and reclamation of contaminated soils (Lidon &

Henriques, 1992; Hsu & Chou, 1992; Li, *et al*., 2005; Street, *et al*., 2007; Obratov-Petković, *et al*., 2008; Dhankhar, 2011; Drab, *et al*., 2011). However, a few studies have investigated metal resistance in woody species for the purpose of land reclamation (Punshon & Dickinson, 1997; Kukkola, *et al*., 2000; Lunáčková , *et al*., 2003; Lombardi & Sebastiani, 2004; Nikonov, *et al*., 2004; Fuentes, *et al*., 2007; Hemle, *et al*., 2007; Gatti, 2008; Durand , *et al*., 2010; Đunisijević Bojović, *et al*., 2012; Djukic, *et al*., 2013).

Zn is a trace element, essential for plants because of its presence in the enzymes composition catalyzing important life processes (Drab, *et al*., 2011 according to Alloway & Ayers, 1997). However, in a very high concentration, it can cause toxic effects and decline of a plants growth. In this respect, it is important to investigate which concentration of Zn is toxic to different plant species and how essential metal ions in high concentrations affect plant growth at different developmental stages (Li, *et al*., 2005). At the early stage of development, *A. altissima* has high biomass production under stressful environmental conditions such as urban pollution, drought stress, nutrient deficiency and metal toxicity and because of that it can be used in the research of toxic metal tolerance in trees (Đunisijević-Bojović, *et al*., 2012).

The aim of this study was to determine the physiological characteristics of *A. altissima* seed germination and early seedling development in the conditions of elevated Zn concentrations and to investigate the effect of zinc on the morphological traits of young seedlings.

This study can contribute to a better understanding of the adaptation ability of *A. altissima* to heavy metal stress.

2. MATERIAL AND METHODS

Seeds of *A. altissima* were collected from groups of trees in the forest park Košutnjak in Belgrade city. In total 450 (3 replicates of 150 seeds) seeds were treated with three different concentrations (25 μM, 100 μM and 250 μM) of $ZnSO_4$, while the control group was submerged in distilled water. Seeds were imbibed in continuously aerated solutions for 72 h. Three replicates of 50 seeds were germinated in Petri dishes on filter paper coated with fungicide PREVIKUR (fosetyl-aluminum, 310 *g/L*) under light conditions. Seed germination and seedling development were observed daily. The elongation of hypocotyls and radicles was measured after 14 days. After that, young seedlings were transferred into a growing chamber in the hydroponics system supplied with modified Hoagland nutrient solution containing (mM): 0.35 K_2SO_4, 0.05 KCl, 1.0 $Ca(NO_3)_2$, 0.25 $MgSO_4$, 0.05 KH_2PO_4, and (in μM): 5 H_3BO_3, 0.25 $MnSO_4$, 0.25 $ZnSO_4$, 0.1 $CuSO_4$, 0.005 $(NH_4)_6Mo_7O_{24}$, 20 Fe^{III}EDTA under controlled conditions: temperature regime of 20 – 25^0 C, humidity of about 70%, the photon flux density of ≈ 200 $\mu mol m^{-2} s^{-1}$, day-night light regime: 16/18. Zinc-sulfate was added at concentrations of 100 μM, 250 μM and 500 μM. The solution was continuously aerated. The main and lateral root length, number of lateral roots, shoot length, and number of leaves were recorded after 2 weeks of treatments.

Seed germination was determined by quantitative indicators: 1. *germination capacity* (GC), 2. *germination energy* (GE) and qualitative indicator 3. *germination intensity* (GI). *Germination capacity* (GC) is % of seeds that germinate during the test period. This is equal to a/b·100 (%), a = number of germinated seeds during the test period, b = complete number of tested seeds. *Germination energy* (GE) is % of seeds that germinate during a specified time interval determined by the peak germination rate. GE=a1/b·100 (%), a1 = number of germinated seeds up to the peak rate of germination, b=complete number of tested seeds. GE is determined based on the results obtained during the first five days of the test. *Germination intensity* (GI): $\sum(Z \cdot N)$, where Z = number of days' germination from the last to the first day, where the last day is marked with zero and N=number of seeds germinated in day Z. GI is expressed by seed/day (Schopmeyer, 1974; Grbić, 1997).

For the statistical analysis of numerical data, computer program STATGRAPHICS Centurion XVI.I was applied. Germination and morphometric parameters of early seedling growth were analyzed using one-way analysis of variance (ANOVA).

3. RESULTS

3.1 Influence of Zn on *A. altissima* seed germination

Table 1. Effects of zinc (Zn) on *Ailanthus altissima* seed germination parameters - mean values.
Табела 1. Утицај цинка (Zn) на клијање семена *Ailanthus altissima*- средње вредности.

Treatment $ZnSO_4$ (µM) Третман ZnSO4 (µM)	Parameter / Параметар		
	Germinative capacity GC (%) Техничка клијавост (%)	Germinative energy GE (%) Енергија клијања (%)	Germinative intensity GI seed/day Интензитет клијања семе/дан
0 (control)	82.67 ^{a}	49.33 ^{a}	287.00 ^{a}
25	29.33 ^{c}	3.33 ^{b}	82.67 ^{c}
100	58.00 ^{ab}	8.66 ^{b}	171.33 ^{b}
250	49.33 ^{bc}	16.00 ^{b}	90.33 ^{bc}

Legend/Легенда: Different letters indicate significant differences between the means at a 95% level / Различита слова означавају значајне резлике између средњих вредности истог показатеља за три примењене концентрације и контролу на нивоу 95%.

The results of seed germination are presented in Table 1. The ability of a seed to develop into a plant is best expressed through its *germinative capacity* and *germinative energy*. Results show that Zn concentrations of 100 μM and 250 μM cause a reduction in the GC and GE compared to the control.

However, there was no statistically significant reduction in GC and GE between the treatments with 100 μM and 250 μM. The highest reduction was noted at the lowest concentration of 25 μM at which the germinative energy was significantly reduced by 46% compared to the control (Table 1). *Germinative intensity* was reduced at a statistically significant level in all treatments (Table 1).

3.2 Influence of Zn on *A. altissima* early seedling growth

At the early stage of seedling development in all treatments the elongation of the hypocotyl was significantly decreased, while there was no negative influence on the radicle length elongation (Figure 1).

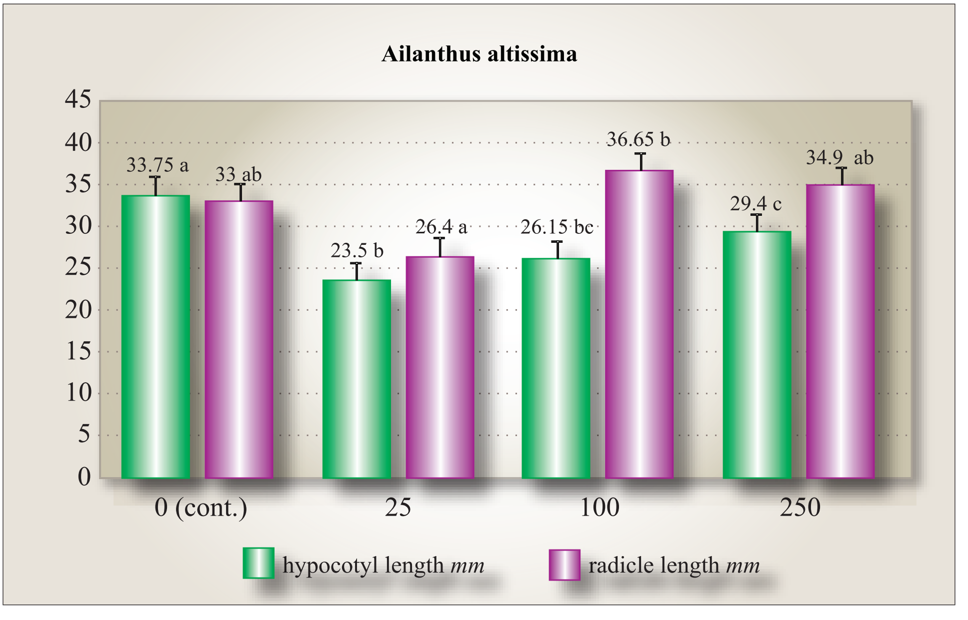

Diagram 1. Effect of zinc (Zn) on hypocotyls and radicle length (*mm*) of *Ailanthus altissima* early seedlings growth after 14 days after the treatment - mean values. The numbers 0, 25, 100, and 250 represent different concentration of $ZnSO_4$ expressed in μM

Графикон 1. Утицај цинка (Zn) 25,100, 250 у μM на дужину хипокотила и коренка (*mm*) клијаваца *Ailanthus altissima* након 14 дана третмана – средње вредности. Бројеви 0, 25, 100, и 250 представљају различите концентрације $ZnSO_4$ изражене у μM

Legend / Легенда: Different letters indicate significant differences between means at 95% level / Различита слова означавају значајне резлике између средњих вредности истог показатеља за три примењене концентрације и контролу на нивоу 95%

3.3. Influence of Zn on *A. altissima seedling* growth in hydroculture

When young seedlings grown in hydroculture were considered, there was no significant statistical difference among the treatments in the main root length compared to the control (Figure 2). Zinc concentrations of 100, 250 and 500 μM inhibited the development and lengthening of the lateral roots. The smaller number of lateral roots and leaves in all zinc treatments was noticed and their number decreased significantly with an increase in the $ZnSO_4$ concentration (Figure 3). The most pronounced inhibition of the formation of lateral roots and leaves were observed in seedlings exposed to the highest $ZnSO_4$ concentration (500 μM). However, there was no significant statistical difference between the concentrations of 250 μM and 500 μM (Figure 3).

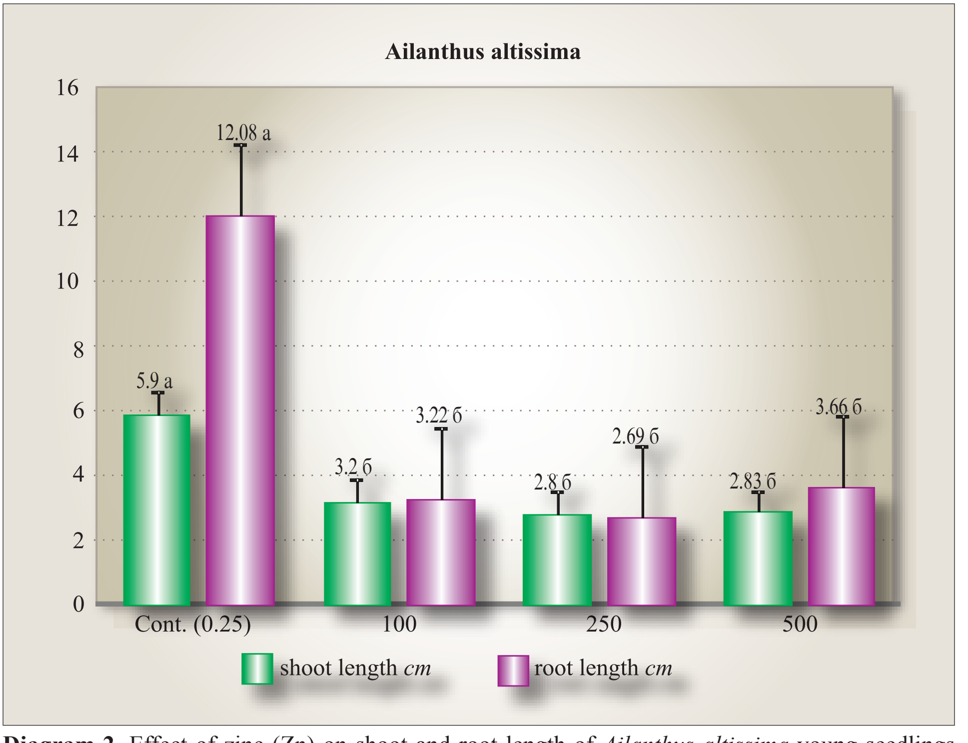

Diagram 2. Effect of zinc (Zn) on shoot and root length of *Ailanthus altissima* young seedlings two weeks after the treatment - mean values and standard errors. The numbers 0.25, 100, 250 and 500 represent different concentrations of $ZnSO_4$ expressed in μM

Графикон 2. Утицај цинка (Zn) на дужину изданка и коренка клијаваца *Ailanthus altissima* две недеље након третмана – средње вредности и стандардне грешке. Бројеви 0.25, 100, 250 и 500 представљају различите концентрације $ZnSO_4$ изражене у μM

Legend / Легенда: Different letter indicate significant difference between means at 95 % level / Различита слова означавају значајне разлике између средњих вредности на нивоу 95 %

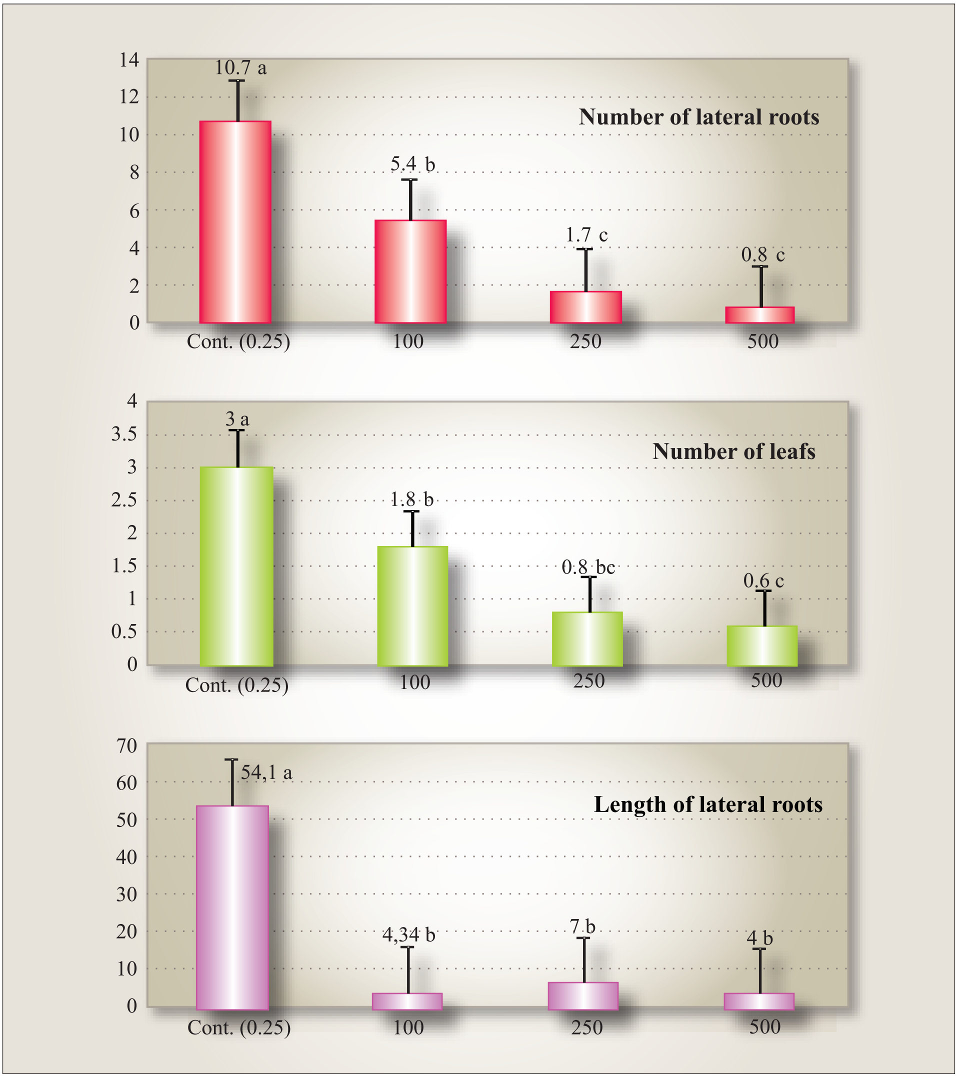

Diagram 3. Effect of Zink (Zn) on: a) number of lateral roots b) number of leaves and c) elongation of lateral roots of *Ailanthus altissima seedlings* (cm) two weeks after treatment - mean values and standard errors. The numbers 0.25, 100, 250 and 500 represent different concentrations of $ZnSO_4$ expressed in μM

Графикон 3. Утицај цинка (Zn) на: а) број бочних коренова b) број листова и c) дужину бочних коренова сејанаца *Ailanthus altissima* (cm) две недеље након третмана – средње вредности и стандардне грешке. Бројеви 0.25, 100, 250 и 500 представљају различите концентрације $ZnSO_4$ у μM

Legend / Легенда: Different letter indicate significant difference between means at 95 % level / Различита слова означавају значајне разлике између средњих вредности на нивоу 95 %

4. DISCUSSION

Seed is a stage in the life cycle of a plant that is well protected against various stresses. However, soon after imbibition and during the process of germination seeds become stress-sensitive in general (Weiqiang, *et al.*, 2005). It is important to determine which concentrations of toxic metals affect the ability of a seed to germinate.

In the current research the highest Zn concentrations did not inhibit the *A. altissima* seed germination much, while the lowest concentration (25 μM) had a more negative impact. In the research of Street, *et al.* (2007), seed of *Eucomis autumnalis* (Mill.) Chitt. had the smallest percentage of germination at even smaller Zn concentrations than 25 μM, which was used in this experiment. In addition, in experiments of Street, *et al.* (2007), with seeds of *Bowiea volubilis* Harv. ex Hook. and *Merwilla natalensis* (Planchon) Speta, high concentrations did not inhibit seed germination, but later influenced the development of the seedlings, which was the case with our experiment, too. Similar results were also noted in the experiment with *Zea mays* L. (Mahmood, *et al.*, 2005). Zn stress, in our experiment, influenced seed germination of *A. altissima* by reducing the values of GC, GE and GI, compared to control. The most significant reduction of all parameters was noticed at the concentration of 25 μM.

Considering the hypocotyl length there was no significant statistical difference among the treatments. Results indicate that at the early stage of seedling growth the elongation and development of a hypocotyl is more affected by a high Zn concentration than the radicle. It is interesting that the highest concentration of $ZnSO_4$ did not influence the radicle growth negatively. We can come to the conclusion that the highest concentration of 250 μM influenced the hypocotyl and radicle growth stimulatingly, while the lowest concentration (25 μM) influenced the reduction of the hypocotyl and radicle lengths. In the experiments of Weiqiang, *et al.* (2005), with *Arabidopsis thaliana* ecotype Columbia (Col-0) and Peralta, *et al.* (2001), with *Medigaco sativa* L., the positive effect on shoot and root elongation with an increasing concentration of Zn was noticed. However, in the present experiment with the growing of young seedlings in the hydroponics, elevated $ZnSO_4$ concentrations (100, 250 and 500 μM) inhibited the shoot and root growth. In the research of Dhankhar (2011) investigating *Vigna mungo* (L.), it was observed that the increase in the Zn concentration from 0.25 to 1.50 *mM* caused decreasing plumule and radicle lengths. The decrease of seedlings growth exposed to heavy metals can be explained by the effect of reduced development of meristematic cells and certain enzymes present in cotyledons and endosperms. During germination occurs the hydrolysis of the nutritional reserves regulated by hydrolytic enzymes. Heavy metals might negatively influence the activity of hydrolytic enzymes, which can have negative consequences on the relocation of the nutrients to radicle and cause a reduction in seedlings growth (Dhankhar, 2011).

Considering young seedlings grown in hydroculture, there was a significant statistical difference in the shoot and root lengths among different concentrations of zinc sulfate. The root elongation has been shown to be a sensitive parameter for assessing the toxic effect of heavy metals, and it is usually used to estimate the toxicity of certain metals to seedlings growth (Hassett, *et al*., 1976). In our experiment, zinc equally influenced the growth inhibition of both shoots and roots. This is in conflict with the results of Street, *et al*. (2007) and Dhankhar (2011), in which the inhibition of growth was stronger in roots than in shoots. Also, Godbold & Hüttermann (1985), established that the root elongation of *Picea abies* (L.) H. Karst is greatly inhibited by the Zn concentrations of 30 μM and 60 μM. The reasons for different response of the shoots and roots growth to heavy metals might be the fact that roots are the first point of contact with the toxic concentration of heavy metals, as well as the the rapid accumulation of heavy metals in the root or the faster rate of detoxification in the shoot (Manivasagaperumal, *et al*., 2011).

The number and elongation of lateral roots were reduced in the treatments with Zn. These effects could indicate high zinc toxicity for the root system of *A. altissima*. The same was observed in *Pinus pinea* L., where the concentrations of 100 μM and 150 μM Zn caused retardation of taproot elongation and reduction in the formation of secondary roots, their number and length (Arduini, *et al*., 1995). In the current research, plants of *A. altissima* grown under the conditions of 250 and 500μM $ZnSO_4$ were characterized by a lower number of developed assimilating organs and lower shoot length. The reason for this might be a decrease in the absorbing root surface, due to the inhibition of growth by an excessive zinc concentration and the competition for root transport system between zinc and other essential elements (Ivanov, *et al*., 2011). The reduced growth of root and shoot due to heavy metals was also noticed by other authors (Zhenguo, *et al*., 1998; Thomulescu, *et al*., 2004; Rajesh, *et al*., 2008; Zhang, *et al*., 2009; John, *et al*., 2009; Đunisijević-Bojović, *et al*., 2012, 2012a; Djukic, *et al*., 2013 (Đukić, *et al*., 2014)).

The early stages of plant development – seed germination and radicle and hypocotyls elongation under elevated Zn concentrations were slightly negatively affected in our experiment but the analyzed parameters were at a good level even at the highest concentrations. At later stages of development, growth inhibition of young seedlings under higher concentrations of Zn was more clearly manifested in an expressed inhibition of the root system growth and the repression of development of assimilating organs. The inhibitory effect was more pronounced as the Zn concentration increased.

The results of this research can be useful indicators of tolerance of the investigated species to metals for the purpose of phytoremediation.

5. CONCLUSION

This research has shown that the seed germination of fast growing species *A. altissima* is possible under higher concentrations of $ZnSO_4$ (100 and 250 μM) in contrast to the results of early seedling growth.

Zn reduced hypocotyls length, but not significantly the length of radicles. Plants treated with stronger concentrations of zinc grown in hydroculture showed a reduced growth of the shoot, main root length and proliferation of leaves and lateral roots, compared to the control.

According to these results, it can be concluded that *A. altissima* might probably be successfully used in the phytoremediation of the soils moderately polluted with zinc.

Regarding the results, further estimation is needed to determine the zinc concentrations under which the growth of this species is possible.

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УТИЦАЈ ПОВЕЋАНИХ КОНЦЕНТРАЦИЈА Zn НА КЛИЈАЊЕ СЕМЕНА И РАСТ СЕЈАНАЦА *Ailanthus altissima* (Mill.) Swingle

Резиме

Цинк је есенцијалан микронутријент неопходан биљкама за обављање важних физиолошких процеса. Међутим, у великим концентрацијама може имати токсичне ефекте на раст и развој биљака и проузроковати њихово сушење. Због тога је веома важно испитати врсте које су отпорне на високе концентрације цинка и које се могу адаптирати условима земљишта загађеним тешким металима, с обзиром да се овај токсични метал све чешће може наћи у животној средини због хемијске индустрије, сагоревања фосилних горива и због употребе ђубрива и хербицида у којима може бити саставна компонента (Zn хлорид и Zn сулфат). Циљ овог рада је био да се испита ефекат цинка на клијање семена и развој клијаваца дрвенасте врсте *Ailanthus altisima* (Swingle) Mill. ради бољег разумевања адаптивних способности ове врсте на стрес од тешких метала и могућности коришћења у фиторемедијацији. Семе врсте *Ailanthus altissima* третирано је цинком у концентрацијама 25, 100 и 250 µM у виду цинк сулфатне соли. Након клијања, клијавци су премештени у комору за гајење у хидрокултуру где је цинк додат у концетрацијама од 100, 250 и 500 µM.

Добијени резултати указују да семе киселог дрвета клија и при највећим примењеним концентрацијама цинка. Тиме се закључује да у овој фази развића биљака *A. altissima* токсични ефекат цинка није изражен. Анализирајући дужину хипокотила и радикуле, не постоји значајна статистичка разлика између различитих третмана са цинком, међутим, највиша концентрација цинка, од 250 µM је утицала инхибиторно на раст хипокотила. У каснијим фазама развића код биљака гајених у хидрокултури уочава се израженија инхибиција раста са повећањем концентрације цинка. Резултати показују да високе концентрације у каснијим фазама развића утичу негативно на раст младих биљака што се манифестује смањењем дужине кореновог система, инхибицијом пролиферације бочних коренова и асимилационих органа.

Истраживање клијавости семена и раног развића сејанаца *A. altissima* показало је толерантност у одређеном степену на повећане концентрације цинка тако да се потенцијално ова брзорастућа дрвенаста врста може користити за фиторемедијацију цинком контаминираног земљишта уз неопходна даља испитивања.