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THE INFLUENCE OF CADMIUM AND LEAD ON *ULMUS PUMILA* L. SEED GERMINATION AND EARLY SEEDLING GROWTH

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Abstract - The aim of this paper was to examine how the heavy metals cadmium (Cd) and lead (Pb) influence the germination and early growth of seedlings of the fast-growing tree species *Ulmus pumila* L. Seeds were germinated and seedlings were hydroponically grown in a solution with Cd-nitrate and Pb-nitrate at concentrations of 20 μM, 50 μM and 90 μM. Our results show that seeds can germinate in the presence of these two heavy metals at all of the applied concentrations with no significant reduction in qualitative (germination capacity, germination energy) or quantitative (germination intensity, mean germination period) germination parameters as compared to the controls. Early seedling development was also possible at higher concentrations of both heavy metals. Cd reduced hypocotyl length, but not significantly the length of radicles. Pb did not influence hypocotyl length and stimulated radicle length significantly (95%). These results could mark a step forward in defining the tolerance of *U. pumila* to the presence of Cd and Pb, and to the the possibility of using this fast-growing tree which is resistant to different abiotic and biotic stresses, for phytoremediation or soil reclamation purposes.

Key words: Ulmus pumila, seed germination, seedling growth, cadmium, lead, phytoremediation

INTRODUCTION

Investigations into the impact of numerous heavy metals on the growth of woody plants in polluted habitats has shown that different species have different tolerance levels (Farooqi et al., 2009; Đukić et al., 2013; Zeynep et al., 2011; Kwapuliński et al., 2012).

Cd and Pb are common pollutants in the environment and represent a very serious threat to human health due to their toxicity and non-biodegradable persistence in soil (Ferretti et al., 1995; Shi et al.,, 2008). The sources of Cd and Pb are industry, heating systems and traffic (Ferretti et al.,1995; Iqbal et al., 2000). Their toxic effects on plants depends on the tolerance of the plant species, other environmental conditions, concentration and length of exposure (Fargasová et al., 2003; Farooqi et al., 2009; Đukić et al., 2013). The most common toxic symptoms in plants are chlorosis, growth inhibition, low essential mineral absorption and other physiological disorders.

Ulmus pumila L. (Siberian Elm) is an outspreading woody plant that often grows near roads, railways or on other degraded land. It is highly resistant to various abiotic and biotic stress conditions. *U. pumila* and other Eurasian Elms (*U. parvifolia* Jacq. and *U. davidiana* Planch.*)* are also resistant to Dutch Elm Disease, unlike other species in the *Ulmus* genus (Sinclair et al.,2005). For this reason, *U. pumila* is used in researching Asian and American elms, with a view to breeding Dutch Elm disease-tolerant cultivars (Zalapa et al., 2008, 2009). In addition, *U. pumila* has been seen to harbor both strong and moderate accumulations of the trace elements Fe, Mn, Al, Zn, Pb, Ni, Cr and As from contaminated soil (Liu et al., 2005). *U. pumila* var. *pendula,* has a significant absorption capacity for Pb, Cd , Cr and Hg as pollutants in the atmosphere (Mu Li et al., 2004). It is also resistant to salt stress. The investigation of salt stress on the activity of superoxide dismutase (SOD) in *U. pumila* seedlings shows that the cell plasma membrane of leaves has a strong resistance to damage from salt ions (Song et al., 2006).

The aim of this research was to investigate some characteristics of *U. pumila* seed germination and early seedling growth in the presence of Cd and Pb with the view of using this fast-growing, resistant species for the purpose of phytoremediation or soil reclamation.

MATERIALS AND METHODS

Mature U. *pumila* seeds were collected in June 2012 in a Belgrade city park from a 15-year-old, 20 m high mother tree of good vitality. Material was stored for one month in plastic bags at 4°C to maintain seed viability (Grover et al., 1963).

In total, 100 seeds of each variant were treated with three concentrations of Cd $(NO_3)_2$ and Pb $(NO_3)_2$, 20 μM, 50 μM and 90 μM, while control groups were placed in distilled water. Seeds were soaked in solutions for 24 h with continuous aeration and set to germinate in light thermostat germination chambers on filter paper coated with the fungicide Previcur (fosetyl-aluminum, 310 $g \cdot L^{-1}$), at a constant temperature of 20˚C with a 12:12 h photoperiod of cold white light and darkness. After germination, young seedlings were transferred to a growing chamber and grown for 14 days in perlite under a photosynthetic light with a photon flux density of 200 $\mu mol m^{-2} s^{-1}$, photoperiod of 16:8 h, a temperature regime of 25/20°C and relative humidity of 70%. Seed germination was monitored every day for a period of 14 days and seedling development for 30 days.

Seed germination is determined according to several parameters: germination capacity (GC), or the percentage of seeds that germinate during the test period. This is equal to $a/b \cdot 100$ (%) = number of germinated seeds during the test period, with b = complete number of tested seeds (Schopmeyer, 1974).

Germination energy (GE), or the percentage of seeds that germinate during a specified time interval determined by the peak germination rate (Schopmeyer, 1974). $GE = a1/b \cdot 100$ (%), with a1 = number of germinated seeds up to the peak rate of germination, b = complete number of tested seeds. GE is determined based on 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, N = number of seeds germinated in day Z. GI is expressed by seed/day. The mean germination period (MGP) =: $\sum (Z \cdot N)/\sum N$, where Z and N are the same in terms of germination intensity. Elongation of hypocotyls and radicles was measured for 25 plants.

The tolerance indices (T.I.) were determined as T.I. = mean root (radicle) length in Cd or Pb solution/mean root length in distilled water × 100 (Iqbal et al.,1992).

The seedling vigor index was calculated according to ISTA formula (S.V.I.) = (hypocotyl length + radicle length) × germination capacity (GC) (1STA (1999).

Each value is the mean of three replications of 50 seeds each. The data were obtained by the program STATGRAPHICS ver. 2.1 that used for statistical tests: analysis of variance and multiple range analysis with a significance level of 95%.

RESULTS AND DISCUSSION

Seed germination of *U. pumila* was analyzed by quantitative indicators – germinative capacity (GC), germinative energy (GE), and qualitative parameters

Table 1. Effects of Cd $(NO_3)_2$ and Pb $(NO_3)_2$ treatments on *Ulmus pumila* on seed germination parameters expressed as mean values.

Treatments Cd and Pb	GC ± SE	GE±SE	GI ± SE	MGP
MM	%	%	seed/day	days
Cd-20	76.66 ±12.018 a	31.11 ± 5.879 ab	225 ± 25.83 a	9.93 ab
Cd-50	77.73 ± 2.942 a	32.20 ± 5.877 ab	237 ± 6.84 a	10.19 ab
Cd-90	83.30 ± 9.642 a	9.97 ± 1.934 c	227 ± 17.58 a	9.18 a
Pb-20	77.77 ± 6.780 a	43.30 ± 7.708 a	242 ± 19.94 a	10.40 b
Pb-50	79.97 ± 8.819 a	46.60 ± 8.819 a	247 ± 20.36 a	10.40 b
Pb-90	83.30 ± 6.925 a	36.60 ± 1.934 ab	254 ± 19.60 a	10.21 ab
Control	74.30 ± 6.831 a	24.40 ± 2.942 bc	214 ± 9.06 a	9.74 ab

Different letters indicate significant differences between means at 95% level.

Table 2. Effects of Cd $(NO_3)_2$ and Pb $(NO_3)_2$ treatments on *Ulmus pumila* seedlings growth – mean values of morphometric parameters.

Concentrations of Cd and Pb (MM)	hypocotyls length (mm)	radicle length (mm)	radicle / hypocotyl ratio	hypocotyl / radicle ratio
20 Cd	15.71 ± 0.908 b	17.58 ± 0.839 b	1.00 ± 0.097 b	1.29 ± 0.070 b
50 Cd	11.40 ± 0.595 c	19.67 ± 0.812 b	0.62 ± 0.034 d	1.86 ± 0.080 a
90 Cd	7.10 ± 0.315 d	11.66 ± 0.771 c	0.73 ± 0.046 cd	1.70 ± 0.092 a
20 Pb	23.59 ± 1.235 a	26.12 ± 0.870 a	0.93 ± 0.046 b	1.31 ± 0.085 b
Pb	22.58 ± 1.105 a	27.78 ± 0.946 a	0.85 ± 0.043 c	1.43 ± 0.087 b
90 Pb	23.01 ± 1.185 a	27.81 ± 0.938 a	0.89 ± 0.055 c	1.45 ± 0.090 b
Control	22.06 ± 1.158 a	17.89 ± 0.702 b	1.32 ± 0.785 a	0.96 ± 0.064 c

Different letters indicate significant difference between means at 95% level.

– germination intensity (GI) and mean germination period (MGP). Germination intensity (GI) is a combination of germinative capacity (GC) and germination energy (GE). Quantitative parameters show the results of a particular treatment. The most important parameters in terms of the quality of seed germination are germinative capacity (technical germination) and germinative energy.

Influence of Cd and Pb on U. pumila seed germination

Seed germination patterns are important as the first stage in a plant's development, correlating with both seedling survival and the distributional range of plant species (Brändle et al., 2003).

According to the data (Table 1), the GC and GI of seeds treated with Cd and Pb were not influenced to a significant level. The highest GC was in seeds treated with 90 μM either Pb or Cd. Germination energy (GE) had significantly lower values in seeds treated with 90 μM Cd. Germination intensity (GI) was most marked in seeds treated with 90 μM Pb, and least in the control group. The highest mean germination period (MGP) was required for seeds treated with 90 μM Cd, compared to the seeds treated with 20 μM and 50 μM Pb. This shows that higher Cd concentrations stimulated germination. Lead inhibited germination, while cadmium slowly accelerated it compared to control plants.

These concentrations of Cd and Pb did not inhibit or impair the seed germination of the Siberian Elm. Mild stimulation was evident, but not to a significant level. This confirms earlier observations that this species is resistant to obstructions to germination and can germinate in highly adverse environmental conditions. This could represent an advantage in terms inter-species competition in the processes of colonization (Donohue et al., 2010). The seed provenance is also important. It was reported that *U. pumila* seeds from the North American non-native range germinated significantly faster than native (Chinese) seeds. This could be a potential explanation for the invasion success of *U. pumila* in its new range, since it might provide a competitive advantage during colonization of new sites (Hirsch et al., 2012).

Influence of Cd and Pb on U. pumila seedling growth

The hypocotyl length was significantly decreased by all concentrations of Cd, but not with Pb, compared to control plants. Radicle length was significantly reduced by the strongest concentration of Cd and significantly elongated with all concentrations of Pb. The shortest hypocotyl and radicle length was observed at the treatment with 90 MM Cd. It was previously reported that the greatest reductions in leaf and root dry mass, as well as changes in leaf structure were obtained after treatment with Cd (1 mM), in comparison to Pb and Ni, for young wheat plants (Kovačević et al., 1999). Radicle/hypocotyl and hypocotyl/radicle ratios were significantly different in comparison to controls.

Root development is important in phytoextraction processes because the accumulation of toxic metals is often more intensive in roots than in aboveground parts, which can be a protection mechanism for plant development. There is a high variability in the translocation of toxic metals from root to shoot. Cd transfers rapidly to above-ground plant parts, but shows low translocation, unlike some other toxic metals such as Pb (Kastori, 1997).

In our research, seeds treated with 20 µM Cd properly germinated, with properly developed seedling radicles and hypocotyls. Some seeds treated with 50µ M Cd, developed into seedlings with stunted radicles, while the hypocotyls were better developed. In addition, some seedlings treated with 90 µM Cd, developed poorly in terms of both radicle and hypocotyls, with visibly chlorotic cotyledons. Similar symptoms of Cd phytotoxicity were observed in several plants, such as in the Cd hyperaccumulator *Pfaffia glomerata* Sprengel (Gomes et al., 2013), *Eucalyptus urophylla* S. T. Blake and in *E. maculata* Hook. tolerant to 90 µM Cd (Soares et al., 2005). This could be the result of the toxic effect of Cd on the synthesis and extensibility of cell wall components (Barcelo and Poschenrieder, 1990; Liu et al., 2004) and lack of some essential nutrients that are important for the formation and functions of chloroplasts (Breckle and Kahle, 1992). A decrease in chlorophyll content due to the toxic effects of Cd accumulation was also identified in Common Oak and Box Elder (Bayctailu and Ozden 2004). It was found that root-growth inhibition with Cd could be mitigated by calcium (Suzuki, 2005).

In our research, a few seeds treated by 20 µM Pb developed into seedlings with radicles bent upwards, while the hypocotyls were properly developed. For some seeds under the treatment with 50 µM Pb and 90 µM Pb, seedling radicles and hypocotyls were stunted and cotyledons were chlorotic. A similar appearance was noticeable for *Albizia lebbeck* L., *Thespesia populnea* L., *Ailanthus altissima* (Mill.) Swingle and *Acer negundo* L. (Farooqi et al., 2009; Đukić et al., 2013; Kabir et al., 2008).

Tolerance indices

Tolerance indices (Fig. 1) show that *U. pumila* seedlings were more tolerant to Pb than to Cd treatments. The least tolerance was recorded at the highest concentration of Cd (90 MM).

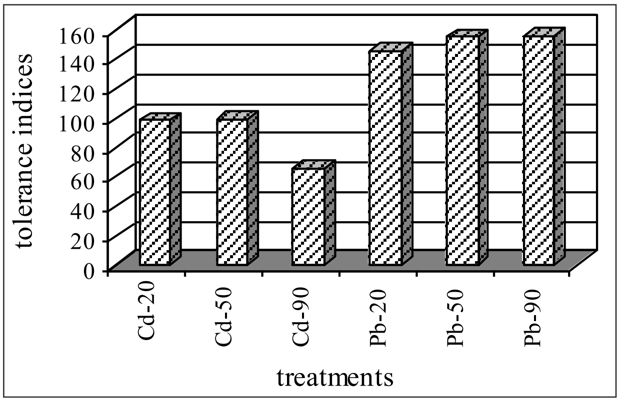

Fig. 1. Tolerance indices (T.I.) of *U. pumila* seedlings treated with different concentrations (20 MM, 50 MM, 90 MM) of $Cd(NO_3)_2$ and $Pb(NO_3)_2$ T.I. = Mean root (radicle) length in Cd or Pb solution / Mean root length in distilled water × 100.

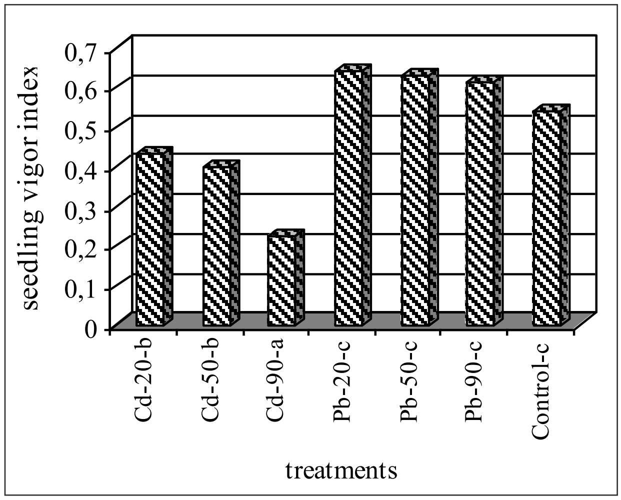

Fig. 2. Seedling vigor index (S.V.I) of *U. pumila* seedlings treated with different concentrations of cadmium Cd $(NO_3)_2$ and lead Pb $(NO_3)_2$ and control. S.V.I. = (hypocotyl length + radicle length) × germination capacity (GC). Different letters indicate significant difference between means at 95% level.

The amounts and interactive effects of some nutrients and toxic metals are very important. It would be useful to investigate lethal concentrations in the environment and in various parts of plant, as well as the interaction between Cd and Pb (Friberg et al.,1986). The reciprocal synergistic effect between Cd + Cu and Pb + Fe was also confirmed, but not between Cd + Zn, where an additive effect was observed. In a pure state, Pb and Cd were the metals that accumulated in roots in the highest amounts (Fargasová and Svetková 2003). Research of the influence of iron (Fe) deficiency on Pb accumulation in *Ailanthus altissima* (Mill.) Swingle seedlings showed that Fe-sufficient plants accumulated about 50% more Pb in leaves than Fe-deficient plants (Đunisijević Bojović et al., 2012). The influence of Fe nutrition on cadmium accumulation and toxicity in rice plants (Shao et al., 2007) and in *Ailanthus altissima* (Đunisijević-Bojović et al., 2012) have also been recorded.

Seedling Vigor Index

This indicator (Fig. 2) shows that Cd treatments had an inhibitory influence on seedling vigor, with the most significant for the 90 μM Cd. Pb treatments increased seedling vigor, but not to a significant level in comparison to the controls.

Heavy metals inhibit the growth and development of most plants, but tolerant plants like *U. pumila,* can grow and reproduce on contaminated soils. Producing seedlings in contaminated substrate may increase their tolerance to the presence of toxic metals. It was reported that *U. pumila* seedlings in medium and mild saline habitats had a stronger adaptability to a saline soil environment than *U. pumila* seedlings in a non-saline soil environment (Liu et al., 2012). In addition, the Cd-tolerant soybean Balkan genotype was apparently characterized by a more efficient control of the oxidative stress induced by Cd than the Cd-susceptible (L 608) soybean genotype (Dražić et al., 2009).

Although these results are observed at the very early stage of plant development, such research can be a useful indicator of plant tolerance to Cd and Pb. Very rapid growth with strong biomass production and tolerance to different values of soli pH (5-8) make this species suitable for phytoremediation and the reclamation of degraded soils and those contaminated by toxic metals.

CONCLUSION

Seed germination and early seedling development of the fast-growing woody species *U. pumila* was possible in the presence of Cd and Pb. None of the used concentrations of either of these toxic metals had any statistically significant impact on germination capacity as the most important parameter of quality for seed germination. During early seedling growth, Cd reduced hypocotyl length when present at 90 μM treatment, while Pb did not influence hypocotyl length and stimulated radicle length to a significant level (95%).

U. pumila seedlings show higher tolerance and seedling vigor to Pb than to Cd.

The Siberian Elm could be considered to be tolerant to the presence of these heavy metals; it could be successful in phytoremediation or soil reclamation technologies, however it needs to be tested further.

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