

ABSTRACT

Three willow clones were grown in a greenhouse pot experiment and were exposed to three treatments, one control and two Cd treatments (3 and 6 ppm). Different morpho-physiological characteristics were analyzed on *S. viminalis*, *S. matsudana* and *S. alba*. The aim of this study was to investigate the effects of elevated cadmium concentrations on three *Salix* clones. Results showed clone specific responses due to the applied Cd concentration. Exceed values of this heavy metal caused reduction of mineral elements (nitrogen, phosphorous and potassium) in combination with reduction of plant growth and changes in plant biomass. Obtained results of this study could be used in selection and improvement of *Salix* clones for phytoextraction. For enhancing the efficiency of mentioned technology-further analysis of morpho-physiological and biochemical traits should be preferred.

Keywords: willows, cadmium, mineral nutrition, biomass production, phytoextraction

REZIME

Tokom istraživanja praćen je uticaj kadmijuma (Cd) na morfo-fiziološke karakteriste tri klona vrba (*Salix* spp). Vrbe su gajene u polu-kontrolisanim uslovima (staklari) metodom zemljišnih kultura. Primenjeni tretmani su bili kontrola – 0 Cd, 3 ppm Cd i 6 ppm Cd. Korišćeni su genotipovi *Salix viminalis*, *Salix matsudana* i *S. alba*. Cilj istraživanja bio je proučavanje uticaja povišenih koncentracija Cd na morfo-fiziološke karakteristike kod različitih klonova vrba. Rezultati su pokazali specifične reakcije klonova u odnosu na primenjenju koncentraciju kadmijuma. Povišene vrednosti ovog teškog metala dovele su do smanjenja sadržaja mineralnih elemenata (azot, fosfor, kalijum), u kombinaciji sa smanjenim rastenjem biljaka i promenama u biomasi. Rezultati ovog istraživanja se mogu koristiti u izboru i unapređivanju klonova vrba za fitoekstrakciju. Dalje praćenje različitih morfo-fizioloških i biohemijskih karakteristika vrba je neophodno u cilju pobošanja efikasnosti pomenutog procesa.

Ključne reči: vrbe, kadmijum, mineralna ishrana, bioprodukcija, fitoekstrakcija



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MORPHO-PHYSIOLOGICAL TRAITS OF SALIX CLONES UNDER CADMIUM STRESS

Utica j kadmijuma na morfo-fiziološke karakteristike klonova vrba (Salix spp.)

Salix klónok morfológiai és élettani változásai kadmium stressz alkalmával

Introduction

In recent years the increase of heavy metals in the biosphere has been noticed. Heavy metals are elements with specific density over 5 g/cm^3 and relative atomic mass above 40 (Seregin and Ivanov, 2001). Among the heavy metals, cadmium (Cd) is considered as one of the leading toxic metal and can cause different morpho-physiological changes on plants (Lunacková et al., 2003; Arora et al., 2008). Plants adsorb Cd from the soil and water, and its transfer to particular organs induces chlorosis, necrosis, and root and shoot growth retardation, as well as reduced nutrient uptake (Irfan et al., 2012). A large number of research points to the influence of heavy metals on mineral nutrition in plants. Heavy metals affect the general metabolism of plants as they compete with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plants (Hassan et al., 2007). Moreover, they cause metabolic disturbance by altering essential biochemical reactions (Kebert, 2014). Cd can inhibit the absorption of necessary mineral elements, such as N, P, K, Mg, Ca, Zn, Cu and Na (Yang et al., 1996; Salt et al., 1998). Furthermore, elevated cadmium concentration in plants may have an effect on growth parameters.

According to official regulations, the maximum permissible concentration (MPC) of Cd in soil in the Republic of Serbia is 3 mg/kg. During the recent years, much research has been focused on selecting the plant species which can tolerate high concentrations of heavy metals. Plants have evolved different adaptive strategies with complex mechanisms that control the uptake and accumulation of heavy metals and detoxification (Hall, 2002). Several studies showed that, among the woody plants, Salix species showed a good potential to accumulate and tolerate high cadmium concentrations, and can be used in phytoremediation (Greger and Landberg, 1999; Marmiroli et al., 2011).

The phytoremediation potential of plants depends on both the biomass productivity rate and the concentration of the element (heavy metal) in the biomass. Previous studies have showed that *Salix* species have the capacity to tolerate high Cd concentration in plants tissue in combination with high biomass production, so they represent a good candidate for phytoextraction process (Landberg and Greger, 1996, Klang-Westin and Eriksson, 2003). In addition, *Salix* clones represent species adaptive to disturbed environments. Various traits useful for phytoremediation can be studied with the aim to identify variation within the species and clones. The objective of this study was to compare the responses different *Salix* clones gave to different cadmium concentrations. Different morpho-physiological traits were measured with the aim of indentifying species which are good for phytoextraction, as a subprocess of phytoremediation.

Material and methods

Plant growth and treatments

1-year-old woody cuttings were obtained from the Institute of Lowland Forestry and Environmental Protection in Novi Sad, Serbia. It concluded two autoctonuous willow species (*Salix alba* and *Salix viminalis*), and one alochtonuous species (*Salix matsudana*). Willows were grown by soil culture method under semi controlled conditions (greenhouse). Six woody cuttings per Mitscherlich pots were grown for one month. Cadmium was supplied as CdNO₃ solution as follows: 3 ppm Cd (MPC - maximum permitted concentrations) and 6 ppm Cd (2*MPC) (Official Gazette of the Republic of Serbia 2010).

Plant analysis

The plants were harvested after one month of applied treatments, in order to establish the impact of cadmium in short term exposure. Different morphological characteristics in various plant organs were measured. Leaves were divided into two groups, young and mature leaves. The division was carried out for testing the variation in cadmium impact depending on the age of leaves.

Morphological characteristics

Measurements of growth parameters were done before the harvest. The height of the plant was expressed in cm, while the diameter of the stem was calculated with digital caliper and expressed in mm². The total fresh weight of leaves, stem and root, and root volume were also measured. Weight of the leaves per plant (g/plant) was estimated and leaf area (cm²) was measured with an "ADC Bioscientific Ltd. AM350 Portable Leaf Area Meter".

Mineral content

The plant material was dried and milled. Organic matter was removed by high temperature (dry way) except in the case of determining N. Stock solutions were used for the determination of P and K. Phosphorus content was determined by spectrophotometer using the standard method (Gericke and Kurmies, 1952). The concentration of K was determined by flame photometry (PFP7 Flame Photometer, by Genway) directly from stock solutions. Additionally, N concentrations were determined with Kjeldahl's method (Kjeldahl, 1883; Nelson and Sommers, 1973).

Statistical analysis

The obtained data were expressed as mean of three replicates \pm standard deviation (SD). Data were subjected to the two-way analysis of variance (ANOVA) and followed by Fisher's multiple range test at a significance-level of $p < 0.05$. Different letters in the same column indicate significant difference between treatments.

Results and discussion

Mineral content

First, about the macronutrients content. Generally, the presence of cadmium in the nutrient solution at the concentration of 3 ppm and 6 ppm reduced the content of nitrogen (Table 1). As the average of nitrogen content shows it, the highest influence of cadmium was observed in young leaves, in comparison to other plant organs (Table 1). Values of N in young leaves were within the range 3.83-4.88%. N metabolism plays an important role in plant growth. Recent studies revealed that the addition of NO_3 and NH_4 supplement in nutrient solution (Xie et al., 2009) and availability of N (Finkemeier et al., 2003) affect plants sensitivity to Cd. There was no significant decrease in nitrogen content in mature leaves in *S. matsudana* clone in Cd treated plants in comparison to the control (Table 1). This might be due to the fact that Cd was applied as CdNO_3 , NO_3 supplement in nutrient solution can affect plants sensitivity to Cd and availability of N (Irfan et al., 2012). Kebert (2014) analyzed the influence of Cd to mineral nutrition to the *Populus* species. Results revealed increased nitrogen content in *Populus* plants in 3 ppm of Cd treated plants.

Present of cadmium in willows has different affect in phosphorous content among the analyzed clones and plant organ. Content of phosphorus in leaves varied between 0.16 (2MPC treatment in *S. alba*, and MPC treatment in *S. matsudana* clone) to 0.43 (control plant of *S. matsudana*). The highest P content among the analyzed clones was found in control plants of *S. alba*.

The increased amounts of cadmium in the medium caused disturbances in potassium level among the treatments of tested clones. The reduction of K, Ca and Mg in the tissue due to high concentrations of Cd has been reported in cucumber and tomato plants (Burzynski, 1988). Results obtained in mentioned study are in correlation with others works. Borišev (2010) showed similar results of mineral nutrition in analyzed *Salix* clones. *Salix alba*, *Salix viminalis* and *S. nigra* were grown in 10^{-5} M and 10^{-4} M Cd in nutrient solution. Plants showed decrease in content of N, P, K in various plant content, due to the applied concentration and selected clone. Souza et al. (2011) reported decrease in K content in leaves of *Genipa americana* induced by the presence of cadmium. This is contrary to our results which show the increase of K level in young leaves in *S. viminalis* and *S. matsudana*. Additionally, in young leaves of *S. alba* content of K was similar among the treatments. The potassium demands of individual plant species and genotypes are different (Kastori et al., 2013). In general, willows use considerable amounts of potassium from soil (Rosen et al., 2011). The main reason for no reduction of K content in Cd treated plants might be the relatively low toxic effect of applied Cd and high content of K available in soil.

Growth parameters and biomass production

Nutrient deficiency due to the presence of Cd in plants leads to the reduction of plant growth and biomass production (Prasad, 1995). Growth parameters and biomass production are shown in Table 4 and 5. Disorder in plant growth exposed to elevated concentration of cadmium is well documented (Landberg and Greger, 1996, Zacchini et al., 2009). Results in this work show that shoot growth was affected in all clones through stem height, significantly decreased in plants treated with 3 ppm Cd, while the diameter of the stem was not affected. Different studies have shown that growth inhibition is one of the most general symptoms caused by heavy metal stress. Analyses showed that leaves area of mature leaves in *Salix viminalis* was significantly reduced in both Cd treatments, while leaves area of young leaves was not affected (Table 4). Zacchini et al. (2009) found that total root length was significantly reduced in 50% of analyzed *Salix* clones treated with 50 μ M of cadmium. Transport of Cd within the plant occurs in the xylem through the cell membranes (Landberg and Greger, 1996).

Comparison of fresh weights of various plant organs among the clones (Table 5).

Fresh mass of stem was reduced in *S. viminalis* and *S. alba*, in both applied Cd concentration, in comparison with *S. matsudana* where negative effect of Cd was not recorded. Al-Yemens, (2001) reported marked decrease in root and shoot mass of *Vigna ambacensis* when treated with low concentration of Cd. Furthermore, in this research no chlorosis symptoms were recorded, which revealed that *Salix* clones have potential to tolerate high concentration of cadmium without toxic symptoms. Zacchini et al. (2009) showed that leaf area is parameter-sensitive to applied Cd in nutrient solution. Willow and poplar clones were exposed to Cd treatments in nutrient solution for 21 days. Authors

revealed that all poplar clones showed significant reduction in leaves area in contrast to willow clones, which showed smaller or no reduction in leaves area. Based on indicators of shoot and root biomass production, under the conditions of present cadmium, tested species showed good potential to tolerate contaminated soils. The root weight of all clones was increased under the influence of cadmium (Table 5). According to Gussarson (1994), increased root length in the presence of Cd could be a mechanism for protecting above-ground parts of the plant from the toxic effects of heavy metals. According to this hypothesis, increased root length achieves accumulation of greater quantities of heavy metals and isolates it from above-ground parts, where the expression of their toxic effects has the greatest consequences. Tolerance of heavy metals on root level, which indicates conservation of the selectivity of the root cell membranes, is the first prerequisite for a plant to be applied for phytoextraction (Borišev, 2010). Similar results were recorded in *Populus* species. Namely, Nikolić (2009) determined different anatomic characteristics of roots in poplar exposed to elevated concentrations of Cd. The results from the present investigation show that there is a high potential in *Salix* clones for phytoextraction. For enhancing the efficiency of this technology, further analysis should be carried out. The results of this study suggest that beside the metal concentration in plants and other morpho-physiological and biochemical traits the selection and improvement of *Salix* clones for phytoextraction should focus on biomass production as well.

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Table 1. Content of nitrogen (N) in different *Salix* clones in various plant organs

Clone	Treatment	Young leaves	Mature leaves	Stem	Root
<i>S.viminalis</i>	control	4.03±0.01	3.85±0.04	2.75±0.01	1.78±0.04
	MPC	3.89±0.06	3.46±0.18	2.08±0.04	1.47±0.01
	2MPC	3.83±0.01	3.44±0.19	1.58±0.01	0.71±0.03
<i>S.matsudana</i>	control	4.88±0.05	3.70±0.08	1.68±0.06	1.70±0.02
	MPC	4.66±0.08	3.57±0.07	2.03±0.01	1.99±0.02
	2MPC	4.06±0.05	3.62±0.17	2.21±0.02	1.70±0.05
<i>S.alba</i>	control	4.77±0.01	3.94±0.09	2.35±0.01	1.34±0.01
	MPC	4.52±0.08	3.94±0.09	1.79±0.03	0.94±0.03
	2MPC	4.39±0.06	3.50±0.02	1.97±0.02	1.15±0.11
	LSD	0.12	0.21	0.01	0.19

Excremental data were expressed as mean ±SD (n=3). Values followed by different letters within a column are significantly different at p<0.05

Table 2. Content of phosphorous (P) in different *Salix* clones in various plant organs

Clone	Treatment	Young leaves	Mature leaves	Stem	Root
<i>S. viminalis</i>	Control	0.35±0.04	0.23±0.04	0.03±0.04	0.12±0.01
	MPC	0.33±0.01	0.28±0.01	0.19±0.01	0.15±0.04
	2MPC	0.35±0.04	0.19±0.01	0.04±0.01	0.10±0.01
	control	0.43±0.01	0.20±0.01	0.15±0.03	0.05±0.01
<i>S. matsudana</i>	MPC	0.37±0.02	0.16±0.02	0.05±0.02	0.12±0.01
	2MPC	0.39±0.01	0.20±0.01	0.06±0.02	0.05±0.02
	control	0.39±0.02	0.35±0.02	0.29±0.06	0.14±0.02
	MPC	0.38±0.02	0.13±0.02	0.16±0.01	0.06±0.02
<i>S. alba</i>	2MPC	0.37±0.01	0.16±0.01	0.17±0.02	0.06±0.01
	LSD	0.04	0.05	0.01	0.01

Excremental data were expressed as mean ±SD (n=3). Values followed by different letters within a column are significantly different at p<0.05

Table 3. Content of potassium (K) in different *Salix* clones in various plant organs

Clone	Treatment	Young leaves	Mature leaves	Stem	Root
<i>S.viminalis</i>	control	3.57±0.04	3.61±0.04	2.54±0.21	0.48±0.03
	MPC	3.53±0.12	3.67±0.12	2.58±0.02	0.66±0.01
	2MPC	3.86±0.03	3.96±0.06	2.77±0.2	0.30±0.08
<i>S.matsudana</i>	control	3.62±0.09	3.88±0.09	2.19±0.04	0.62±0.05
	MPC	3.45±0.13	3.86±0.02	3.02±0.01	0.39±0.01
	2MPC	4.02±0.01	3.76±0.02	2.39±0.08	0.94±0.09
<i>S.alba</i>	control	3.76±0.03	3.36±0.31	3.68±0.06	0.81±0.09
	MPC	3.71±0.07	2.95±0.12	3.76±0.54	0.63±0.02
	2MPC	3.70±0.12	3.42±0.06	3.49±0.39	0.72±0.02
	LSD	0.14	0.22	0.42	0.10

Excremental data were expressed as mean ±SD (n=3). Values followed by different letters within a column are significantly different at p<0.05

Table 4. Growth parameters among the tested *Salix* clones

Clone	Treatment	Young leaves area (cm ²)	Mature leaves area (cm ²)	Stem height (cm)	Stem diameter (cm ²)
<i>S. viminalis</i>	control	39.08±13.24	92.59±27.9	28.30±2.1	2.29±0.33
	MPC	20.00±14.38	25.99±24.37	22.97±0.68	2.37±0.19
	2MPC	32.53±7.73	42.27±29.2	23.47±1.9	2.16±0.05
<i>S. matsudana</i>	control	41.34±16.18	23.51±18.77	19.57±2.05	1.89±0.17
	MPC	24.44±4.95	12.10±12.62	12.90±3.11	1.69±0.44
	2MPC	17.68±8.88	18.22±17.41	14.83±2.39	1.87±0.41
<i>S. alba</i>	control	47.06±12.37	93.32±42.93	23.33±3.59	2.00±0.43
	MPC	37.77±13.5	54.48±26.18	18.17±5.13	1.67±0.12
	2MPC	20.99±13.34	47.98±35.44	19.13±2.49	1.91±0.08
	LSD	23.94	47.24	4.9	0.49

Excremental data were expressed as mean ±SD (n=3). Values followed by different letters within a column are significantly different at p<0.05

Table 5. Fresh weight in different plant organs (g)

Clone	Treatment	Young leaves	Mature leaves	Stem	Root				
<i>Salix viminalis</i>	control	0.81±0.27	a	0.94±0.28	ab	0.88±0.19	a	0.15±0.02	e
	MPC	0.64 ±0.11	ab	0.69±0.11	bc	0.61±0.04	bc	0.28±0.01	bcd
	2MPC	0.59±0.06	b	0.75±0.18	abc	0.62±0.09	bc	0.31±0.13	bc
	control	0.63±0.05	ab	0.47±0.16	cd	0.49±0.03	cd	0.18±0.04	de
<i>Salix matsudana</i>	MPC	0.33±0.01	c	0.19±0.11	d	0.25±0.12	d	0.37±0.09	ab
	2MPC	0.38±0.09	c	0.33±0.08	cd	0.29±0.11	d	0.31±0.04	bc
	control	0.49±0.09	bc	1.17±0.52	a	0.76±0.32	ab	0.24±0.08	cde
	MPC	0.45±0.09	bc	0.61±0.34	bcd	0.50±0.12	cd	0.26±0.02	bcde
<i>Salix alba</i>	2MPC	0.45±0.03	bc	0.60±0.23	bcd	0.37±0.1	cd	0.46±0.03	a
	LSD	0.2	0.45	0.26	0.11				

Excremental data were expressed as mean ±SD (n=3). Values followed by different letters within a column are significantly different at p<0.05