PHENOTYPIC RESPONSE OF WILLOW CUTTINGS TO HEAVY METALS. A PRELIMINARY LABORATORY TEST

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ABSTRACT

The present paper analyses some biometric characteristics of willow cuttings in a comparative laboratory test with heavy metals. Six Swedish hybrids (Tora, Tordis, Klara, Olof, Stina and Sven) and one Romanian hybrid (1077) have been tested. As plant material were used one-year-old cuttings (5-10 cm long), with 2-5 buds each. There were ten experimental variants for each genotype: three concentrations of cadmium (1.0; 3.0, 6.0 ppm), nickel (50.0, 150.0, 450.0 ppm), lead (50.0, 150.0, 450.0 ppm) and Control (tap water). Biometrical observations on: the roots number and length, the shoots number and length, the leaves number/shoot in the days 7 and 15, were performed. All hybrids are quite resistant to heavy metals except hybrid 1077, for this one the shoots percent is much lower for all heavy metals concentration compared with the control one.

INTRODUCTION

There are more than 300 species of *Salix*, tree and shrubs. One of this species, with a high capacity of vegetative regeneration and biomass productivity is *Salix viminalis*. This is the reason why willow is used for energetic crops. Willow is important also for their capacity in phytoaccumulation and/or phytodegradation of contaminants from soils, sediments and water due to the high tolerance to metals. Because there is a permanent increase of energy and a large area with degraded soil, finding solutions for both, biomass production and soil phytoremediation is very important (Drzewiecka, 2012).

In order to evaluate the behavior of willow cuttings at different concentrations of heavy metals, has been installed an experiment whose results give us some basic information about the phenotypic response of plants to heavy metals. Three heavy metals were selected: cadmium, nickel and lead due to the fact that it causes a number of toxic symptoms in plants, e.g. growth retardation and severe chlorosis and necrosis (Mishra, 1974, Prasad, 1995).

MATERIAL AND METHOD

Biological material is made by one-year-old cuttings with 5-10 cm long and 2-5 buds per cutting. The experiment took place in the research laboratory of Department of Forestry. Six Swedish hybrids produced under license in Romania by REBINA Agrar and one Romanian hybrid produced by the Forest Research and Management Institute Bucharest has been tested. The Swedish hybrids are: Klara ((*Salix burjatica x S. viminalis*) *x S. burjatica*) x (*S. viminalis x* (*S. schwerinii x S. viminalis*)), Olof (*Salix viminalis* x (*S. schwerinii* x *S. viminalis*)), Sven (*Salix viminalis* x (*S. schwerinii* x *S. viminalis*)), Tora (*Salix schwerinii x S. viminalis*) Tordis *((*Salix schwerinii x S. viminalis*)* x S. viminalis).

The behavior of willow cuttings have been tested by three heavy metals (cadmium - Cd, nickel – Ni and lead -Pb) and three concentrations: *1.0 3.0, 6.0 ppm for cadmium,*

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50.0, 150.0, 450.0 ppm for nickel, 50.0, 150.0, 450.0 ppm for lead and one Control (tap water).

There were 28 experimental variants and measurements and observations have been made on 140 cuttings. The cuttings were maintained in solutions for 15 days. In the days 7th and 15^{th,} biometrical observations on: the roots number and length, the shoots number and length, the leaves number/shoot, viability of the shoots were performed One-way analysis of variance (Anova) and Duncan test were determined by STATISTICA 10 software. The differences were considered significant at a probability level of 95% ($p<0.05$)

RESULTS AND DISCUSSIONS

Phenotypic response of *Salix* genotypes to different concentrations of analyzed heavy metals is highlight in below graphs (Figures 1-4)

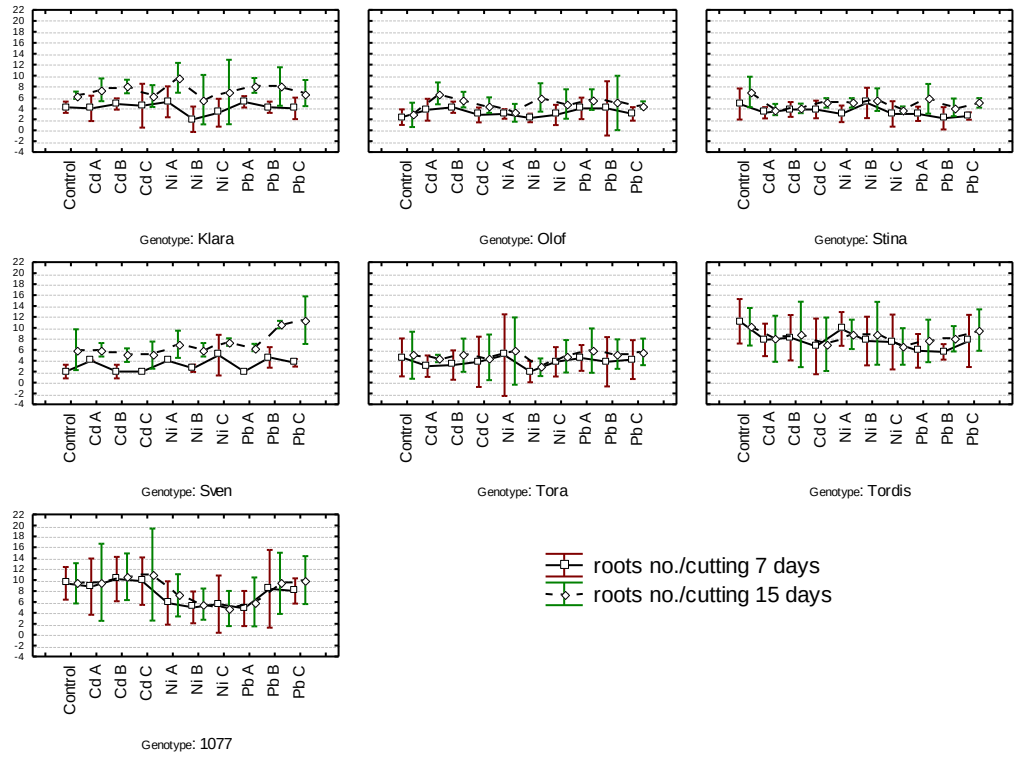

Figure 1 Phenotypic response of cuttings to heavy metals. Root number

Observations made about character “number of root” put in evidence an increase of these values for the both periods of observations. The highest variation but also the highest values of this character have been observed for Romanian hybrid 1077.

For all experimental variants it can be saw an increase of root length for *Salix* cutting. The highest variation but also the highest values of the character “root length” were also been observed for Romanian hybrid 1077. It also can be observed that as higher the metal concentration is as lower the roots length is.

For all experimental variant, the number of active buds per cutting is lower that the number of buds per cutting; this character seems not to be influenced by the nature and concentration of the metal.

In terms of “shoot number per bud”, the values are very close for all genotypes and all experimental variants. The character “number of leaves per shoot” show differences; it can be seen a decrease of the leaves number per shoot for genotypes Olof, Stina, Sven and experimental variants lead and nickel. Relative constant values have been observed

for genotypes Klara, Tora and 1077. The highest fluctuation it has been observed for genotype Tordis.

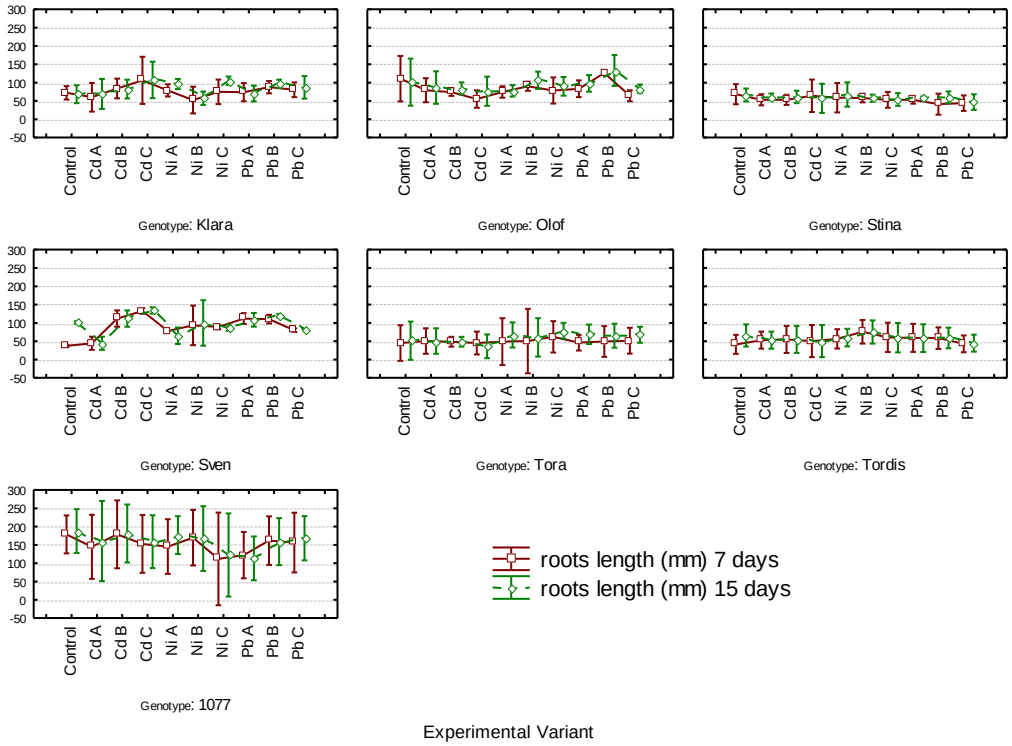

Figure 2 Phenotypic response of cuttings to heavy metals. Root length

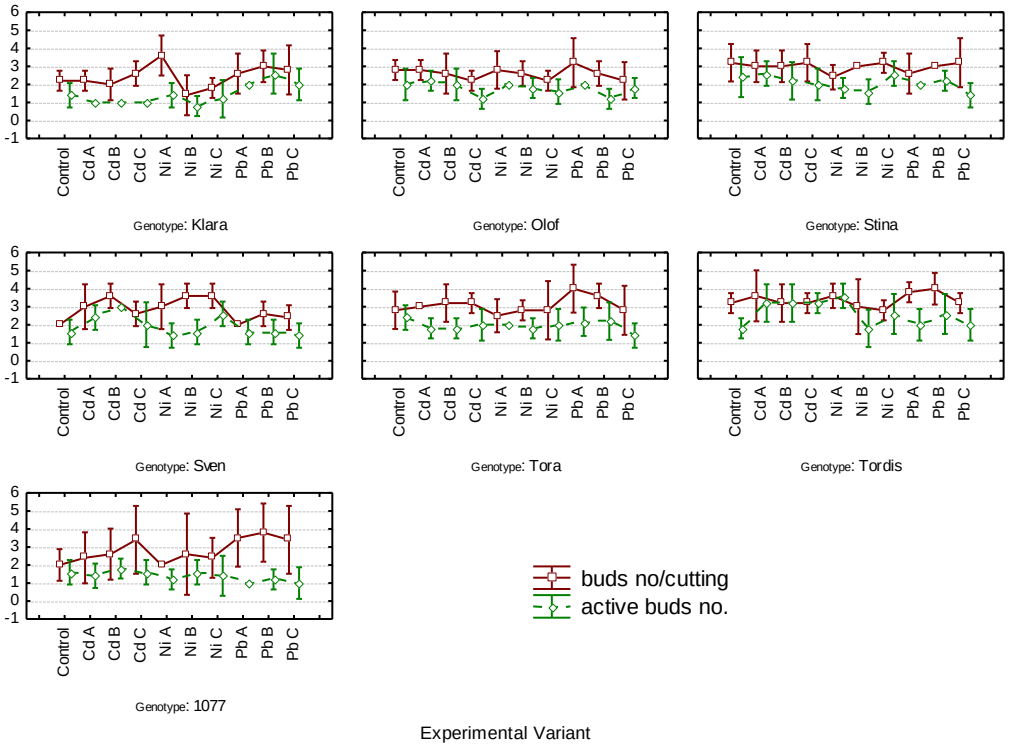

Figure 3 Phenotypic response of cuttings to heavy metals. Buds no. and active buds no. per cuttings

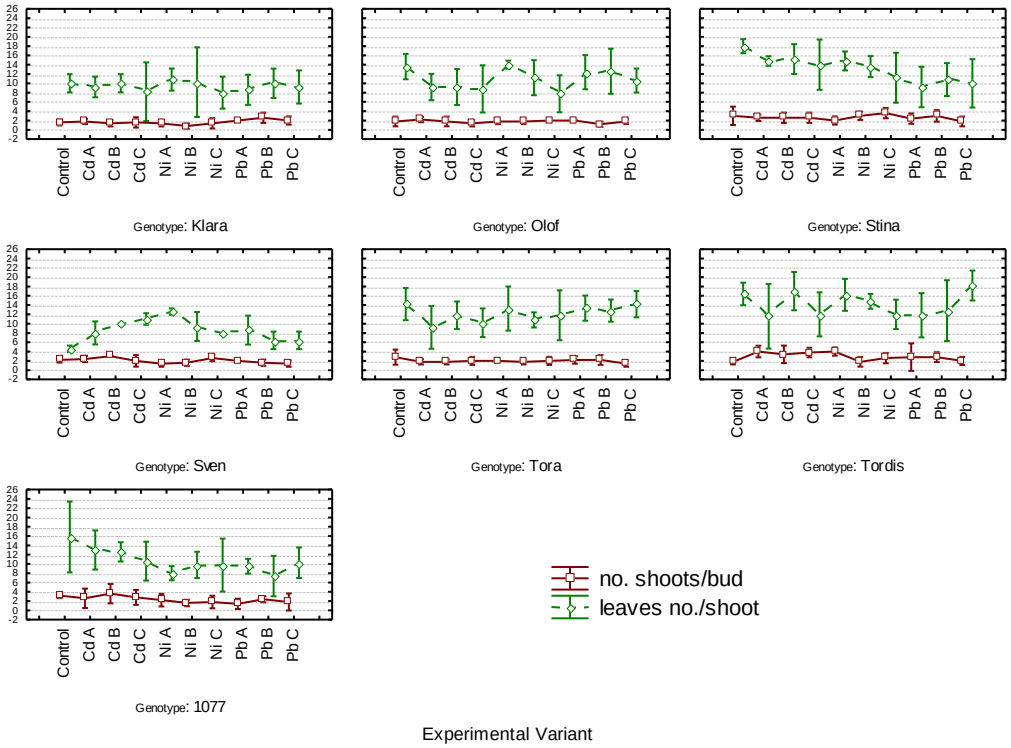

Figure 4 Phenotypic response of cuttings to heavy metals. Shoots no. /bud and leaves no. /shoot

One-way analysis of variance highlight significant differences for all analyzed characters (Table 1). In order to determine which experimental variant are particulary different from each other, Duncan test was applied for character „shoot lenght" (table 2.) For this character has been observed differences to genotype Sven. In this particular case experimental variant "Control" is very different for all other experimental variants with a much lower value.

Table 1

Analysis of Variance. Marked effects are significant at p<0,05000

Character		SS	df	MS	SS	df	MS	F	p
		effect			error				
roots no./ cutting 7 days	Cd	**929,5**	**27**	**34,42**	**575,3**	**108**	**5,32**	**6,46233**	**0,00000**
	Ni	**804,3**	**27**	**29,79**	**643,5**	**109**	**5,904**	**5,045734**	**0,00000**
	Pb	**695,5**	**27**	**25,75**	**561,60**	**109**	**5,152**	**4,999325**	**0,00000**
roots no./ cutting 15 days	Cd	**698,1**	**27**	**25,85**	**881,5**	**110**	**8,01**	**3,22655**	**0,00001**
	Ni	**528,8**	**27**	**19,58**	**716,4**	**111**	**6,45**	**3,034522**	**0,00002**
	Pb	**633,8**	**27**	**23,47**	**695,95**	**109**	**6,384**	**3,676446**	**0,00001**
roots length (mm) 7 days	Cd	**227636,8**	**27**	**8430,99**	**126807,7**	**107**	**1185,11**	**7,11405**	**0,00000**
	Ni	**170963,2**	**27**	**6331,96**	**147553,1**	**106**	**1392,01**	**4,548794**	**0,00000**
	Pb	**210004,2**	**27**	**7777,93**	**93860,52**	**109**	**861,105**	**9,032494**	**0,00000**
roots length (mm) 15 days	Cd	**249762,7**	**27**	**9250,47**	**128466,0**	**108**	**1189,50**	**7,77677**	**0,00000**
	Ni	**179605,2**	**27**	**6652,04**	**129987,3**	**109**	**1192,54**	**5,578029**	**0,00000**
	Pb	**181710,7**	**27**	**6730,02**	**84862,24**	**109**	**778,552**	**8,644279**	**0,00000**
% active buds	Cd	**23794,9**	**27**	**881,29**	**49613,6**	**112**	**442,97**	**1,98947**	**0,00671**
	Ni	**27950,3**	**27**	**1035,19**	**69913,6**	**112**	**624,22**	**1,658360**	**0,03544**
	Pb	**37341,3**	**27**	**1383,01**	**48729,15**	**111**	**439,004**	**3,150357**	**0,00001**
no. shoots/ bud	Cd	**73,2**	**27**	**2,71**	**94,8**	**112**	**0,84**	**3,20175**	**0,00001**
	Ni	**71,3**	**27**	**2,64**	**62,4**	**112**	**0,55**	**4,738841**	**0,00000**
	Pb	**40,2**	**27**	**1,48**	**90,00**	**112**	**0,803**	**1,851523**	**0,01363**
shoots length (cm)	Cd	**196493,9**	**27**	**7277,55**	**72249,8**	**111**	**650,89**	**11,18077**	**0,00000**
	Ni	**112760,8**	**27**	**4176,32**	**52473,8**	**108**	**485,86**	**8,595577**	**0,00000**
	Pb	**107660,9**	**27**	**3987,44**	**90058,20**	**110**	**818,710**	**4,870390**	**0,00000**
leaves no./ shoot	Cd	**1343,3**	**27**	**49,75**	**1042,8**	**112**	**9,31**	**5,34365**	**0,00000**
	Ni	**1300,3**	**27**	**48,16**	**751,4**	**108**	**6,95**	**6,921591**	**0,00000**
	Pb	**1560,7**	**27**	**57,80**	**954,95**	**110**	**8,68**	**6,658160**	**0,00000**

Differences have been observed for genotype Tordis also. In this case the value for experimental variant "Control" was much higher than others. It can be say that this genotype is not so tolerant to heavy metal compare with other genotypes. Differences have been also observed for hybrid 1077; the highest values have been registered for experimental variant "Control".

Table 2

Duncan test for character „shoot length"

Genotype	Experimental variant	Heavy metal					
		Cd		Ni		Pb	
		mean	Signif	mean	Signif	mean	Signif
Klara	Control	89.20	cd	89.20	cd	89,20	b
	Ni_A	85.80	cd	91.80	cd	77,80	b
	Ni_B	78.80	cd	71.00	de	77,80	b
	Ni_C	91.00	cd	87.25	cd	85,00	b
Olaf	Control	156.60	ab	156.60	ab	156,60	a
	Ni_A	164.20	ab	166.40	ab	155,00	ab
	Ni_B	162.20	ab	152.80	b	152,80	ab
	Ni_C	136.00	bc	130.75	bc	127,20	ab
Stina	Control	119.20	bc	119.20	cd	119,20	b
	Ni_A	81.80	cd	116.80	cd	95,00	b
	Ni_B	123.00	bc	90.20	cd	88,00	b
	Ni_C	114.20	bc	88.00	cd	96,40	b
Sven	Control	82.00	cd	82.00	de	82,00	b
	Ni_A	82.00	cd	137.50	abc	110,00	b
	Ni_B	106.00	c	102.00	cd	124,00	ab
	Ni_C	126.50	bc	91.50	cd	122,50	ab
Tora	Control	91.80	cd	91.80	cd	91,80	b
	Ni_A	56.40	d	82.80	de	83,60	b
	Ni_B	67.20	d	49.80	e	67,80	c
	Ni_C	45.20	d	81.40	de	84,60	b
Tordis	Control	123.00	bc	123.00	bc	123,00	ab
	Ni_A	65.00	d	81.00	de	88,80	b
	Ni_B	103.00	cd	115.00	cd	83,00	b
	Ni_C	59.00	d	96.00	dc	109,60	b
1077	Control	163.20	ab	163.20	ab	163,20	a
	Ni_A	151.20	ab	107.20	cd	111,00	b
	Ni_B	184.80	a	126.40	bc	146,40	ab
	Ni_C	147.40	b	102.75	cd	146,00	ab

CONCLUSIONS

The behavior of Salix hybrids to different concentration of heavy metal was analyzed in a laboratory research as a screening for future experimental crop culture.

In terms of phenotypic response of the hybrids (one Romanian and six Swedish) we can say that although there were lower values of analyzed characters at higher concentrations of heavy metals, especially Ni and Pb, the phenotypic response of the plants are good.

We must mention that experience has considered only the first two weeks of plant life and choosing the most tolerant genotypes for crop culture in areas with high concentrations of heavy metals can be taken only after testing in field culture.

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