Heavy metal tolerance in different willow genotypes cuttings. First stage development analysis

Corneanu Mihaela[1], Hernea Cornelia[1], Corneanu Gabriel[2], Sărac Ioan[1], Nețoiu Constantin[3], Petcov Andreea Adriana[1], Hollerbach Wilhelm[4]

[1]USAMVB Timisoara, Faculty of Horticulture and Forestry, Genetic Engineering Dept., Timisoara, Romania, e-mail: micorneanu@yahoo.com;
[2]University of Craiova, Faculty of Agriculture and Horticulture, Craiova, Romania;
d[3] Forest Research and Management Institute Bucharest,
[4]REBINA Agrar SRL; Timişoara, Romania

INTRODUCTION

The species of *Salix* genus, constitute a promising source in the action of fighting against the environment degradation, and offer remedy for about two third from the all degradation types. The majority of the willow species, present a good adaptation to hypoxic conditions, feature which suggest that they manifest a preference for mineral nutrition in comparison with organic one. Thus, many of willow species can be developed on soils with a big amount of minerals and/or radionuclides, being both phytoremediatory species, as well as pioneer ones, contributing to the soil restoration (Landberg and Greger 1994; 1996; Vervaeke et al., 2003; Meers et al., 2005; Wenzel et al., 2005; Borišev et al., 2009; Rowe et al., 2009; Borowiak et al., 2012).

In this paper are present some laboratory preliminary comparative tests of heavy metals tolerance on four *Salix* sp. genotypes. 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.

MATERIAL AND METHODS

❖**Biological material: clone 202 (*Salix alba*), hybrid 892 (*Salix alba*) (Romanian genotypes); Inger (*Salix viminalis*) and Gudrun (*Salix viminalis*) (Swedish genotypes).** As plant material were used one-year-old cuttings (5-10 cm long), with 2-6 buds each.

❖**Experimental design:** ten experimental variants for each genotype: three concentrations of Cd, Ni, Pb and Control (tap water).

❖**Methods:**

➢***Biometrical observations:*** the roots number and length, the shoots number and length, the leaves number/shoot, viability of the shoots (days 10th and 20th) .

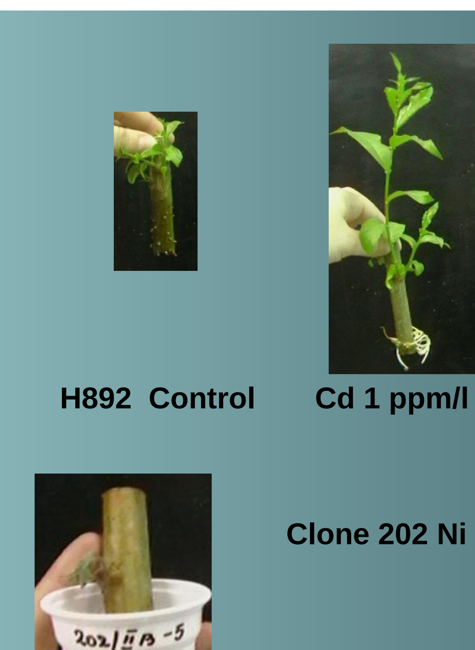
➢***Statistics***

All statistical analyses were performed with commercially available software (STATISTICA 10).The data were analyzed one-way analysis of variance (ANOVA) , Duncan test. The differences were considered significant at a probability level of 95% (P < 0.05).

Metal	A (ppm/l)	B (ppm/l)	C (ppm/l)
Cd	1.0	3.0	6.0
Ni	50.0	150.0	450.0
Pb	50.0	150.0	450.0

RESULTS AND DISSCUSIONS

Phenotypic response of Salix genotypes to different concentrations of analyzed heavy metals is dependent by metal, concentration and genotype, as reveals the analysis of variance (Table 1-4).



H892 Control Cd 1 ppm/l

Clone 202 Ni 150ppm/l

Table 1. Analysis of Variance (Marked effects are significant at p < 0.05000) Genotype x Metal x Concentration

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	3630.7	39	93.10	3725.5	566	6.5821	14.14376	0.000000
roots no./cutting 20 days	3625.1	39	92.95	5505.3	566	9.7266	9.55631	0.000000
roots length (mm) 10 days	278671.5	39	7145.42	194278.8	566	343.2488	20.81704	0.000000
roots length (mm) 20 days	342410.0	39	8779.74	194376.1	566	343.4206	25.56557	0.000000
shoots no./bud	112.6	39	2.89	64.5	566	0.1140	25.32367	0.000000
%active buds	274398.9	39	7035.87	157075.3	566	277.5183	25.35282	0.000000
shoots length (cm)	439796.3	39	11276.83	240671.6	566	425.2148	26.52031	0.000000
Leaves no./shoot	2582.7	39	66.22	2705.5	566	4.7800	13.85449	0.000000

Table 2 Analysis of Variance (Marked effects are significant at p < 0.05000) Genotype

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	2488.8	3	829.59	4867.5	602	8.0855	102.6017	0.000000
roots no./cutting 20 days	1519.3	3	506.42	7611.1	602	12.6430	40.0554	0.000000
roots length (mm) 10 days	248259.9	3	82753.29	224690.4	602	373.2398	221.7161	0.000000
roots length (mm) 20 days	227448.0	3	75816.01	309338.0	602	513.8505	147.5449	0.000000
%active buds	226610.8	3	75536.93	204863.5	602	340.3048	221.9685	0.000000
no. shoots/bud	89.2	3	29.72	88.0	602	0.1461	203.4123	0.000000
shoots length (cm)	263777.1	3	87925.69	416690.9	602	692.1776	127.0276	0.000000
Leaves no./shoot	1263.2	3	421.08	4025.0	602	6.6860	62.9798	0.000000

Table 3 Analysis of Variance Marked effects are significant at p < 0.05000 Metal

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	212.15	3	70.72	7144.1	602	11.867	5.95906	0.000524
roots no./cutting 20 days	695.13	3	231.71	8435.2	602	14.012	16.53664	0.000000
roots length (mm) 10 days	1586.13	3	528.71	471364.1	602	782.997	0.67524	0.567442
roots length (mm) 20 days	19007.10	3	6335.70	517778.9	602	860.098	7.36626	0.000075
%active buds	4393.70	3	1464.57	427080.6	602	709.436	2.06441	0.103776
no. shoots/bud	4.12	3	1.37	173.0	602	0.287	4.77377	0.002689
shoots length (cm)	76402.52	3	25467.51	604065.4	602	1003.431	25.38043	0.000000
Leaves no./shoot	646.40	3	215.47	4641.8	602	7.711	27.94423	0.000000

Table 4 Analysis of Variance Marked effects are significant at p < 0.05000 Concentration

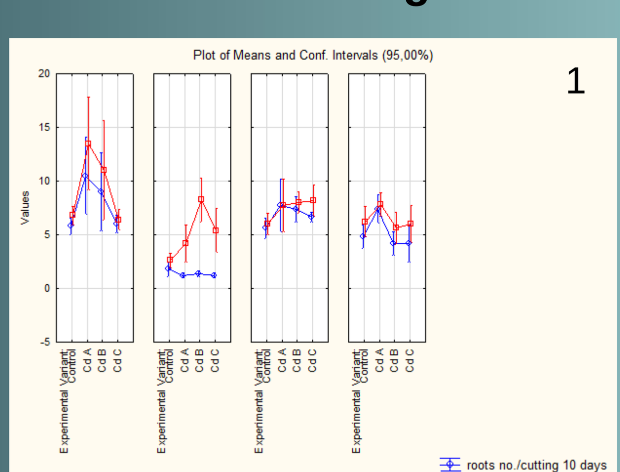
	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	373.11	6	62.18	6983.1	599	11.6580	5.33407	0.000022
roots no./cutting 20 days	749.37	6	124.90	8381.0	599	13.9916	8.92646	0.000000
roots length (mm) 10 days	4817.15	6	802.86	468133.1	599	781.5244	1.02730	0.406301
roots length (mm) 20 days	18228.20	6	3038.03	518557.8	599	865.7059	3.50931	0.002023
%active buds	10018.55	6	1669.76	421455.7	599	703.5989	2.37317	0.028332
no. shoots/bud	7.70	6	1.28	169.4	599	0.2829	4.53877	0.000163
shoots length (cm)	91385.41	6	15230.90	589082.5	599	983.4433	15.48732	0.000000
Leaves no./shoot	922.12	6	153.69	4366.1	599	7.2889	21.08488	0.000000

Cd stimulate significantly roots and shoots meristems differentiation, and slightly the cell division and elongation, as reveals the analysis of variance (Table 5, Figs 1-4) . Cd (1-3ppm/l) stimulate foliar organogenesis, in most genotypes. H 892 proved to be resistant, even at a concentrations over the alert limits, Inger was tolerant, while clone 202 and Gudrun proved to be sensitive.

Ni inhibited significantly the shoots development, inducing clorosis of the young shoots and finally necrosis (Table 6, Figs. 5-8). Clone 202 and Inger are most sensitive to Ni, with very few plants survived at the end of experiment.

Pb, in highest concentration, inhibited significantly both rhysogenesis and organogenesis, as well as shoot elongation. A tolerant behavior had H892 and Inger.

H892 Pb 50ppm/l 450 ppm/l



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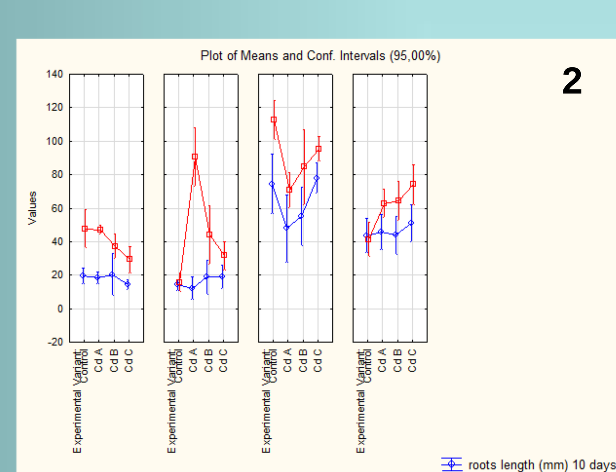

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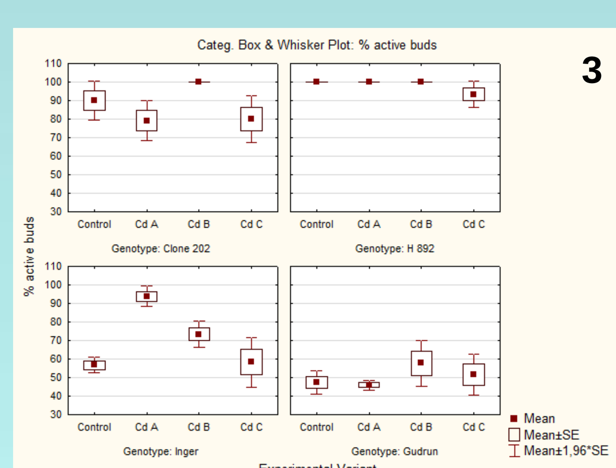

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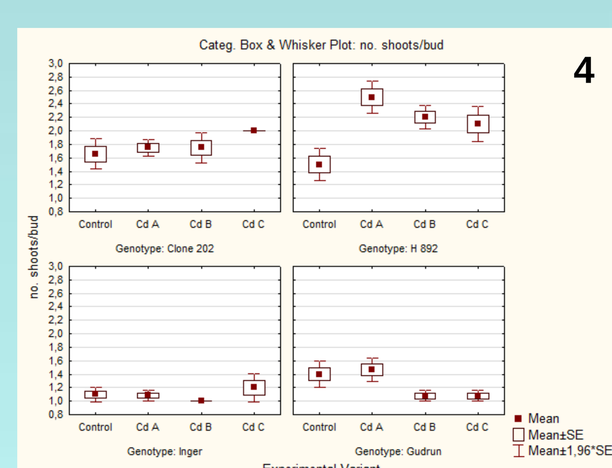

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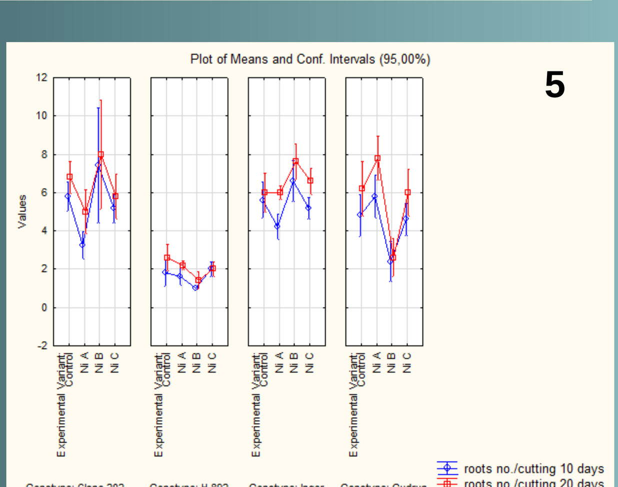
Table 5. Analysis of Variance Cd (Marked effects are significant at p < 0.05000)

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	215.256	3	71.752	3927.5	241	16.297	4.402810	0.004890
roots no./cutting 20 days	380.746	3	126.915	4836.0	241	20.067	6.324705	0.000381
roots length (mm) 10 days	3325.882	3	1108.627	200569.0	241	832.237	1.332106	0.264532
roots length (mm) 20 days	6311.518	3	2103.839	266742.1	241	1106.814	1.900807	0.130069
%active buds	6274.347	3	2091.449	151522.6	241	628.725	3.326495	0.020358
no. shoots/bud	2.438	3	0.813	73.7	241	0.306	2.657760	0.048986
shoots length (cm)	5299.173	3	1766.391	311693.4	241	1293.334	1.365766	0.253871
Leaves no./shoot	142.598	3	47.533	1534.3	241	6.366	7.466266	0.000084



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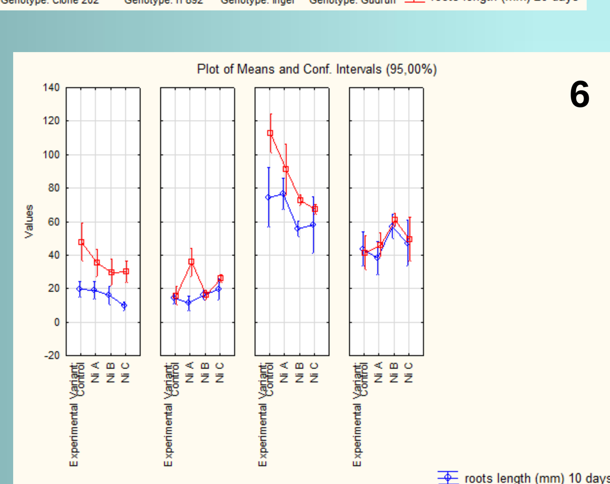

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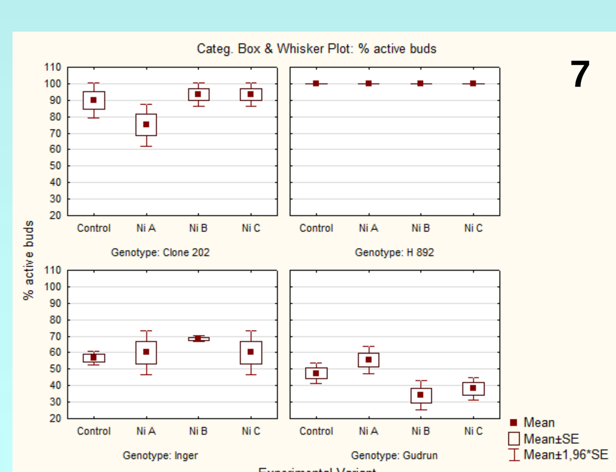

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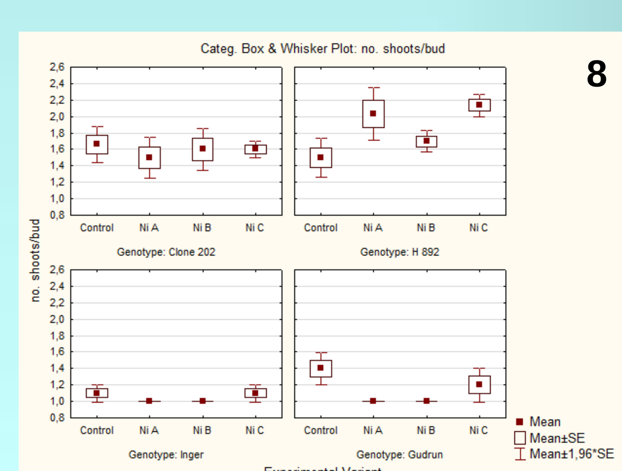

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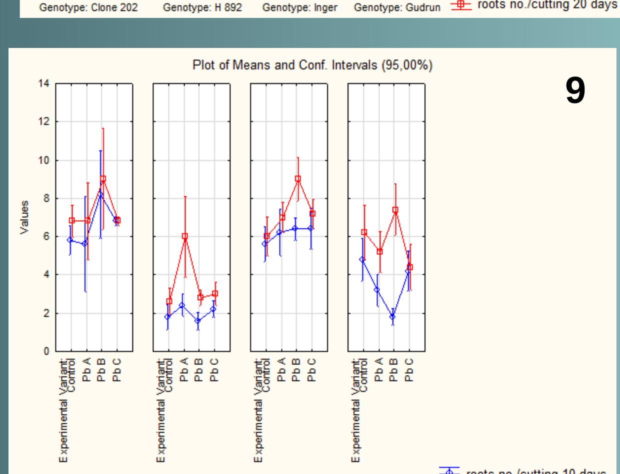
Table 6. Analysis of Variance Ni (Marked effects are significant at p < .05000)

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	21.85	3	7.28	1690.6	237	7.1333	1.02110	0.383971
roots no./cutting 20 days	8.18	3	2.73	2082.5	237	8.7870	0.31021	0.818001
roots length (mm) 10 days	583.17	3	194.39	180767.8	237	762.7331	0.25486	0.857830
roots length (mm) 20 days	5234.98	3	1744.99	207765.6	237	876.6480	1.99053	0.116072
%active buds	62.26	3	20.75	179494.3	237	757.3597	0.02740	0.993866
no. shoots/bud	1.05	3	0.35	57.0	237	0.2403	1.45903	0.226417
shoots length (cm)	33183.53	3	11061.18	225057.6	237	949.6100	11.64813	0.000000
Leaves no./shoot	224.41	3	74.80	1838.7	237	7.7582	9.64201	0.000005

Table 7 Analysis of Variance Pb (Marked effects are significant at p < 0.05000)

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	10.01	3	3.338	1933.1	236	8.191	0.407465	0.747777
roots no./cutting 20 days	116.81	3	38.938	2004.2	236	8.492	4.585111	0.003851
roots length (mm) 10 days	3259.35	3	1086.451	192692.6	236	816.494	1.330629	0.265065
roots length (mm) 20 days	1977.83	3	659.277	223272.6	236	946.070	0.696858	0.554797
%active buds	934.14	3	311.380	166560.7	236	705.766	0.441194	0.723756
no. shoots/bud	3.05	3	1.015	59.6	236	0.253	4.019907	0.008152
shoots length (cm)	24964.09	3	8321.364	276481.9	236	1171.533	7.102968	0.000137
Leaves no./shoot	183.60	3	61.200	1897.8	236	8.042	7.610496	0.000070



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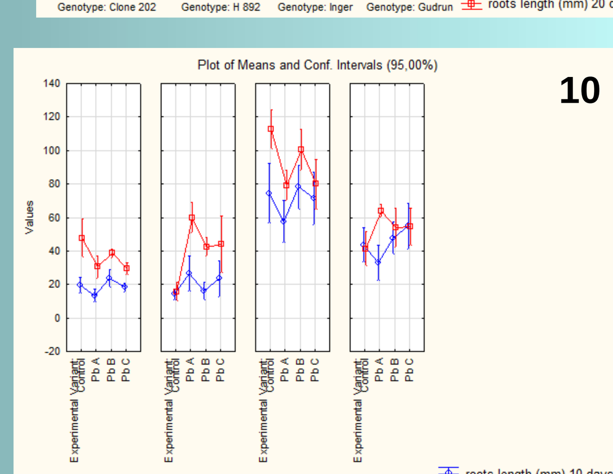

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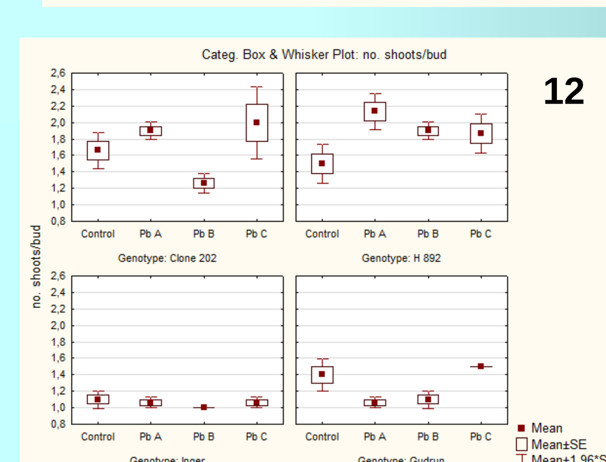

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CONCLUSIONS

➢**There are significant differences, regarding the developmental behaviour among the genotypes:**

❖**CLONE 202 – tolleant; Ni > Pb > Cd**
❖**H 892– rezistant ; Cd > Ni > Pb**
❖**INGER- tolerant Ni > Cd > Pb**
❖**GUDRUN– sensitive; Ni > Pb > Cd**

➢**The best results for all treatment variants were registered in hybrid 892. Clone 202, hybrid 892 and Inger are sensitive to highest concentration of lead (450.0 ppm) and tolerant to all other treatment variants, while Gudrun is sensitive to Ni, but all the concentrations of Cd and Pb presented an incentive effect on shoots development.**

➢**First stage development, in the presence of heavy metals may provide guidance in choosing proper genotypes for the field trial on degraded soil (industrial pollution).**

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Acknowledgements This paper was financially supported by MEN UEFISCDI, Programme PN II 2014- 2016, project no. 111/2014 SAROSWE.